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REVIEW

Polysaccharide Lyases

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ABSTRACT

Polysaccharide lyases (or eliminases) are a class of enzymes (EC 4.2.2.-) that act to cleave certain activated glycosidic linkages present in acidic polysaccharides. These enzymes act through an eliminase mechanism, rather than through hydrolysis, resulting in unsaturated oligosaccharide products. Acidic polysaccharides are ubiquitous and so are the lyases that degrade them. This review article examines lyases that act on acidic polysaccharides of plant, animal, and microbial origin. These lyases are predominantly of microbial origin and come from a wide variety of both pathogenic and nonpathogenic bacteria and fungi. The lyases discussed include alginate lyase (EC 4.2.2.3), pectin lyase (EC 4.2.2.10), pectate lyase (EC 4.2.2.2), oligogalacturonide lyase (EC 4.2.2.6), exopolygalacturonate lyase (EC 4.2.2.9), chondroitin lyases (EC 4.2.2.4 and EC 4.2.2.5), hyaluronate lyase (EC 4.2.2.1), heparin lyase (EC 4.2.2.7), heparan lyase (EC 4.2.2.8), and other unclassified lyases. This review examines the sources, regulation, purification, and properties of these polysaccharide lyases.

Index Entries: Polysaccharide; lyase; eliminase; alginate; pectin; pectate; chondroitin; heparin; heparan; hyaluronate; review; enzyme-catalyzed eliminative polysaccharide-cleavage; epimerization; glycosidic linkage, cleavage of; oligosaccharide.

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INTRODUCTION

Polysacchardie lyases are a class of enzymes that deploymerize certain acidic polysaccharides through an eliminative mechanism. This mechanism was first suggested by the isolation and subsequent characterization of an unusual unsaturated sugar formed by a microbial polysaccharidase acting on hyaluronic acid, an acidic polysaccharide (*1*). A generalized illustration of enzyme-catalyzed, eliminative polysaccharide cleavage is shown in Fig. 1.

Acidic polysaccharides are ubiquitous, being found in most plants, animals, and microorganisms. In plants, pectin and pectate (deesterified pectin) represent an important class of structural molecules contributing to plant shape and serving as a protective barrier (*2*). Similarly, in seaweed, alginic acid serves a protective function as an antidesiccant, preventing it from drying out during low tide (*2*). Animals incorporate most

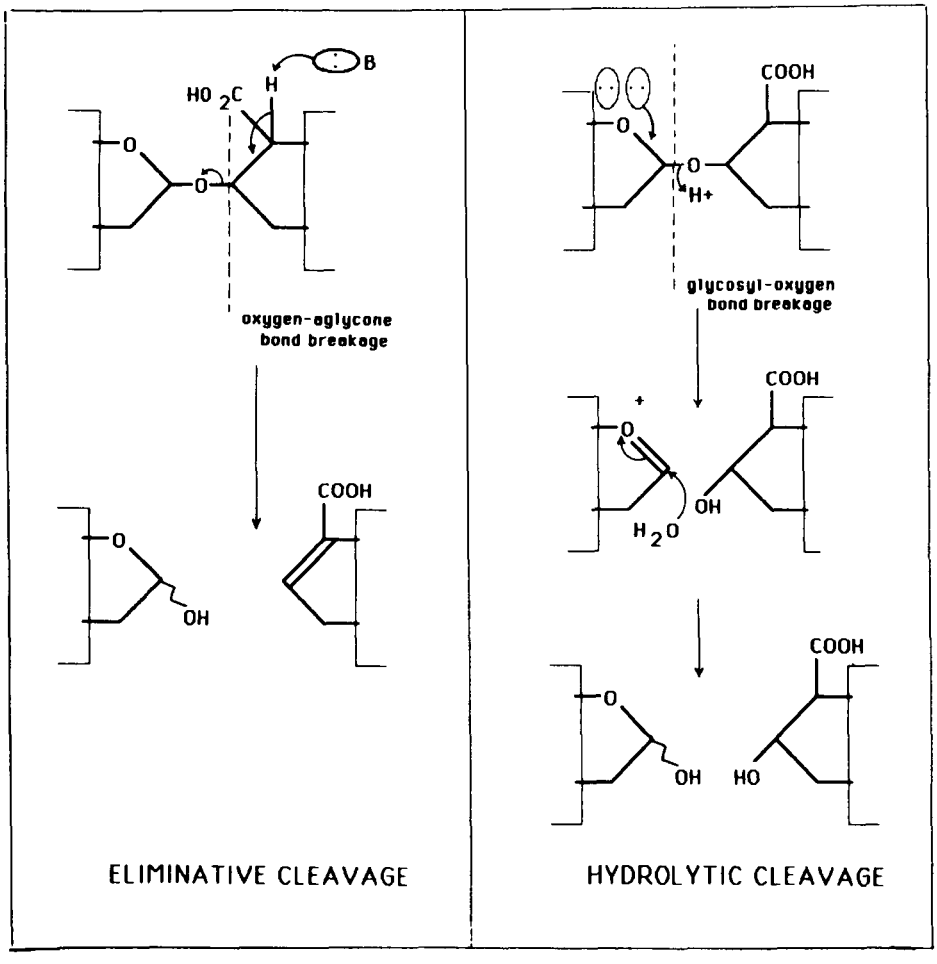


Fig. 1. A generalized illustration of enzyme-catalyzed, eliminative polysaccharide cleavage.

of their acidic polysaccharides in the extracellular matrix, such as that comprising cartilage (*3*). Unlike their plant counterparts, acidic polysaccharides from animals are often found linked to core proteins and hence, are called proteoglycans (*4*). These molecules help to shape the body and to support its movement (*3*). Finally, complex proteoglycans, such as heparin proteoglycan, also serve other important biological functions, including the regulation of blood coagulation (*5*). The function of acidic polysaccharides in microorganisms is less clear. In addition to contributing to microbial cell wall and capsular structure, extracellular acidic polysaccharides may serve a number of other very specific functions (*6*).

The structural complexity of acidic polysaccharides varies greatly, from simple linear homopolymers to homocopolymers and homocopolymers displaying structural variation and mixed branched polymers (*2*). Acidic polysaccharides are generally polydisperse and are often found attached to other biopolymers such as proteins (*7*) or lipids (*8*).

The study of the structure of acidic polysaccharides (*2*) has involved their depolymerization to smaller oligosaccharide fragments by either chemical or enzymatic catalysis (*6*). Enzymatic depolymerization has certain advantages over chemical methods, including high yields, mild conditions, and high specificity for the linkage cleaved (*9*). The glycosidic linkages that connect the sugar units in a polysaccharide are usually cleaved by the acid or enzyme catalyzed addition of water, termed hydrolysis. Hydrolysis involves the breakage of the glycosyl–oxygen bond in the glycosidic linkage; alternatively the glycosidic linkage can be broken at the oxygen–aglycone bond (*2*), with the loss of water through a process known as eliminative cleavage (Fig. 1).

This review will discuss the eliminase mechanism associated with polysaccharide lyases. It will also examine the known types of polysaccharide lyases, describe where these are found in nature, how they are prepared, and their characteristics. In addition, this review will speculate on what new types of polysaccharide lyases may yet be found and what advantages the lyase might have over the hydrolase in polysaccharide cleavage. The known lyases, the names by which they are commonly called, and the linkage at which they act are given in Table 1.

The Eliminase Mechanism

Polysaccharides susceptable to eliminative cleavage generally contain a carboxylate group on the carbon adjacent to the glycosidic linkage (Fig. 2). This electron withdrawing group enhances the acidity of the proton on this carbon. Abstraction of this proton either enzymatically (*10,11*) or by a chemical base, such as sodium hydroxide (*12,13*), results in a direct eliminative cleavage forming an α, β-unsaturated uronic-acid residue on the nonreducing side and a hemiacetal on the reducing side of the glycosidic linkage. The eliminase mechanism (Fig. 2), marked by the formation of unsaturated oligosaccharides, is characterisitic of the class of enzymes called polysaccharide lyases.

TABLE 1
Types of Polysaccharide Lyases (EC 4.2.2.-)[a]

Alginate eliminases

Alginate lyase (EC 4.2.2.3 also EC 4.2.99.4)
Specificity: →4)-α-L-GulUA-(1→4)-α-L-GulUA-(1→; or →4)-β-D-ManUA-(1→4)-β-D-ManUA-(1→; or →4)-α-L-GulUA-(1→4)-β-D-ManUA-(1→; or →4)-β-D-manUA-(1→4)-α-L-GulUA-(1→.
Other common names: alginase, polymannuronide lyase, polyguluronide lyase

Pectin eliminases

Pectin lyase (EC 4.2.2.10)
Specificity: →4)-α-D -GalUE-(1→4)-α-D-GalUE
Other common names: pectinase, pectin transeliminase, exopectin lyase

Endopectin lyase (EC 4.2.2.[3])
Specificity: →4)-α-D -GalUE-(1→4)-α-D-GalUE-(1→.
Other common names: pectinase, endopectin lyase

Pectate eliminases
Specificity: →4)-α-D -GalUA-(1→4)-α-D-GalUA-(1→.
Other common names: endopolygalacturonate lyase, endopectate lyase, polygalacturonate transeliminase, α-1,4-endopolygalacturonic acid lyase

Oligogalacturonide lyase (EC 4.2.2.6)
Specificity: ΔUA-(1→4)-α-D-GalUA-(1→.
Other common names: none

Exopolygalacturonate lyase (EC 4.2.2.9)
Specificity: →4)-α-D-GalUA-(1→4)-α-D-GalUA
Other common names: exopectate lyase

Chondroitin eliminases

Chondroitin ABC lyase (EC 4.2.2.4)
Specificity: →3)-β-D-GalNAc(4-SO_4^-)-(1→4)-α-D-GlcUA-(1→; and →3)-β-D-GalNAc(4-SO_4^-)-(1→4)-α-L-IdUA-(1→; and →3)-β-D-GalNAc (6-SO_4^-)-(1→4)α-D-GlcUA-(1→.
Other common names: chondroitinase ABC, chondroitin ABC eliminase

Chondroitin AC lyase (EC 4.2.2.5)
Specificity: →3)-β-D-GalNAc(4-SO_4^-)-(1→4)-α-D-GlcUA-(1→; and →3)-β-D-GalNAc(6-SO_4^-)-(1→4)-α-D-GlcUA-(1→.
Other common names: chondroitinase AC, chondroitin AC eliminase

Chondroitin B lyase (unclassified)
Specificity: →3)-β-D-GalNAc(4-SO_4^-)-(1→4)-α-L-IdUA-(1→.
Other common names: chondroitinase B

Chondroitin C lyase (unclassified)
Specificity: →3)-β-D-GalNAc(6-SO_4^-)-(1→4)-α-D-GlcUA-1→.
Other common names: chondroitinase C

(*continued*)

TABLE 1 (*continued*)

Hyaluronate eliminases

Hyaluronate lyase (EC 4.2.2.1 also EC 4.2.99.1)
Specificity: →3)-β-D-GlcNAc(1→4)-β-D-GlcUA-(1→.
Other common names: hyaluronadase, mucinase, spreading factor

Heparin eliminases

Heparin lyase (EC 4.2.2.7)
Specificity: →4)-α-D-GlcNSO$_3^-$ (6-SO$_4^-$)-(1→4)-α-L-IdUA (2-SO$_4^-$)-(1→.
Other common names: heparinase

Heparan eliminases

Heparan lyase (EC 4.2.2.8)
Specificity: →4)-α-D-GlcNAc(6-SO$_4$ or 6-OH)-(1→4)-β-D-GlcUA(2-SO$_4^-$ or 2-OH)-(1→ (heparitinase 1); or →4)-α-D-GlcNSO$_3^-$(6-SO$_4^-$ or 6-OH) (1→4)-β-D-GlcUA(2-SO$_4^-$ or 2-OH)-(1→4) or (heparitinase 2).
Other common names: heparan or heparitin monosulfate lyase, heparanase, heparitinase, heparin-sulfate lyase, heparin-sulfate eliminase

Miscellaneous eliminases

Emulsan lyase (unclassified)
Specificity: not known
Other common names: emulsanase

Acidic heteropolysaccharide lyase (unclassified)
Specificity: not known
Other common names: none

[a]Abbreviations: GulUA, gulopyranosyluronic acid; ManUA, mannopyranosyluronic acid; GalUE, methylgalactopyranosyluronic ester; GalUA, galactopyranosyluronic acid; ΔUA, 4-deoxy-α-L-threo-hex-4-enopyranosyluronic acid; GalNAc, 2-deoxy-2-acetamido-galactopyranose; GlcUA, glucopyranosyluronic acid; IdUA, idopyranosyluronic acid; GlcNAc, 2-deoxy-2-acetamido-glucopyranose; GlcNSO$^-_3$, 2-deoxy-2-sulfamino-glucopyranose; SO$^-_4$, sulfate; OH, hydroxy.

When examining the eliminase mechanism (Fig. 2) by which acidic polysaccharides are depolymerized, it is important to focus on the geometrical relationship between the C-4 (aglycone-oxygen) carbon–oxygen bond and the C-5 carbon–hydrogen bond. These bonds can be diaxial (in an *anti*-relationship), as in pectin/pectate, chondroitin sulfate B, and at certain linkages in heparin and alginate. It is easy to speculate on the mechanism for eliminative cleavage of polysaccharides having a diaxial (*anti*) relationship between the leaving groups. When pectin (*12*) or heparin (*13*) are treated nonenzymatically with alkali, unsaturated oligosaccharides are formed. This corresponds to a common class of chemical elimination reactions (Fig. 2) known as concerted (E2) eliminations (*14*), and it is likely that the lyase takes advantage of these properly oriented leaving groups through a similar concerted reaction mechanism.

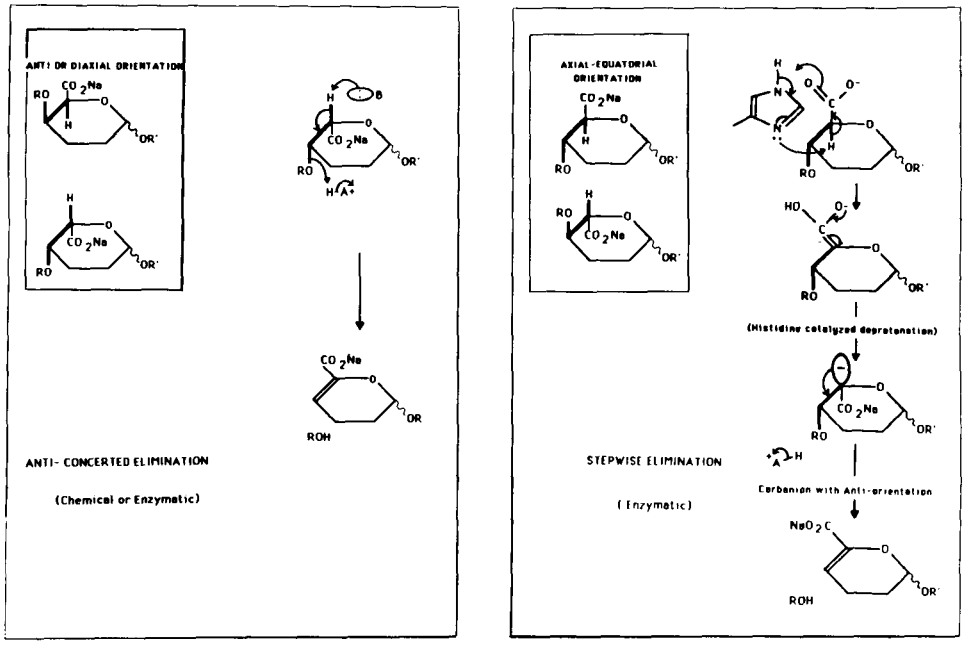


Fig. 2. Two types of eliminative cleavage: anticoncerted and stepwise.

Alternatively, these bonds can be in an axial–equitorial relationship, as in hyaluronate, chondroitin sulfates A and C, and at certain linkages in heparan and alginate (*15*). The treatment with a chemical base of polysaccharides having an axial–equitorial leaving group relation does not result in eliminative cleavage. The driving force for the abstraction of the C-5 proton cannot be the concerted breaking of the C-4 carbon–oxygen bond because of its improper orientation. Greiling and coworkers (*15*) have shown the importance of histidine to the catalytic activity of hyaluronate lyase and have proposed the role of this residue in elimination of the axial–equatorially related leaving groups in hyaluronate. The C-5 proton may be lost through the formation of the carboxylate dianion. Tautomerization to a carbanion with an *anti*-orientation to the C-4 carbon–oxygen bond would then facilitate its elimination. The involvement of a carbanion intermediate in this axial–equatorial elimination mechanism is substantiated by the analogous conversion of glucuronide to iduronide residues in heparin biosynthesis by a mammalian C-5 epimerase (*16*). When performed in ^3H_2O, tritium was incorporated into the C-5 position of the resulting iduronate residue (*17*). This suggests that epimerization may proceed through a mechanism similar to axial–equatorial elimination involving a carbanion intermediate. In epimerization, this intermediate is trapped by a solvent proton, whereas in the eliminative mechanism the intermediate collapses with breakage of the C-4 carbon–oxygen bond.

LYASES ACTING ON PLANT POLYSACCHARIDES

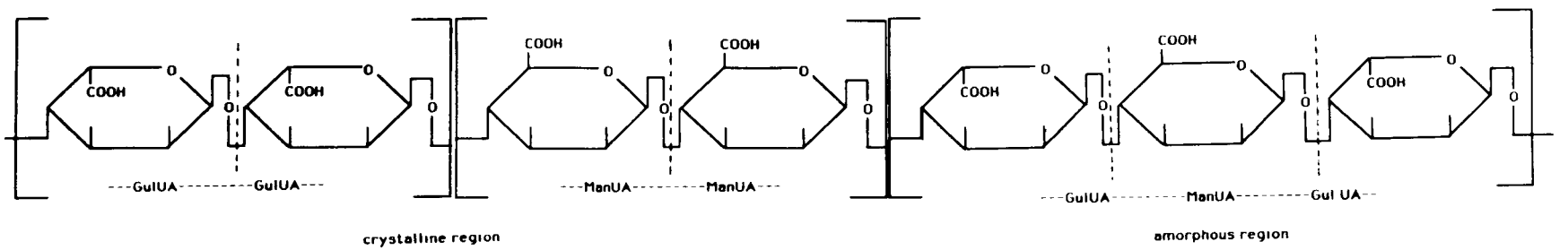
Polysaccharide lyases act on acidic polysaccharides present in plants. These polymers have the simplest structure among the acidic polysaccharides. Both alginate and pectin/pectate classes of polymers possess regular repeating linkages at which their respective lyase acts. None of these polysaccharides are covalently linked to proteins.

Alginate Lyases

Alginic acid [**1**] is a linear polymer comprised of 1→4 linked β-D-mannuronic and β-D-guluronic acid (*18*). These monosaccharide residues can occur in homopolymeric blocks and consist of either polymannuronic or polyguluronic acids comprising alginate's crystalline regions. Heteropolymeric sequences of alternating mannuronic and guluronic acid residues comprise alginate's amorphous regions (*2*). Alginic acid is produced by seaweeds, many species of marine algae, and certain bacteria (*19–21*).

Alginase has been isolated from both microbial and animal sources. Bacterial alginase is produced by several members of the alginate-producing *Pseudomonas* genus, including *P. mendocina* (*22*), *P. putida*, and *P. maltophila* (*23*). Additional bacterial sources include another alginate-producing bacteria, *Azotobacter vinelandii* (*19*), as well as *Beneckea pelagia* (*25*), *Altermonas sp.* (*26*), *Bacillus circulans* (*27*), and a group of unclassified bacterial isolates obtained from the recepticals of brown algae (*18*). In addition to the bacterial sources, mollusks (*Littorina sp.*) (*28*) and abalone (*Haliotus rufescens* and *H. corrugata*) (*29*) represent animal sources of alginate lyase.

Alginase is produced constituitively by alginate-synthesizing *Pseudomonas* sp. (*19*). Although alginate lyase from *P. mendocina* is intracellular, it is capable of acting on its own polysaccharide, markedly reducing the viscosity of the fermentation broth (*22*). This can be a significant limitation in processes for manufacturing alginate by fermentation. A second alginate-producing bacteria *A. vinelandii* (*19*) also synthesizes an intracellular alginase (*30*). Like the *Pseudomonas sp.*, *A. vinelandii* is incapable of utilizing alginate as a carbon source for growth (*19*), presumably because of the intracellular nature of these alginate lyases and their low-specific activities (*22*). *Beneckea (Vibrio) pelagia* produces an inducible alginate lyase (*25*) and is capable of growth on alginate as a sole carbon source. *Klebsiella aerogenes* also produces an extracellular alginate lyase (*31*). Aerotolerant and presumably pathogenic isolates from *Sargassum* tissue, classified as *Altermonas sp.* produce both cell-associated and extracellular alginate lyases when cultured on alginic acid (*26*). Several unclassified marine bacterial isolates produce both extracellular and cell-associated enzymes when induced by alginate (*18*).



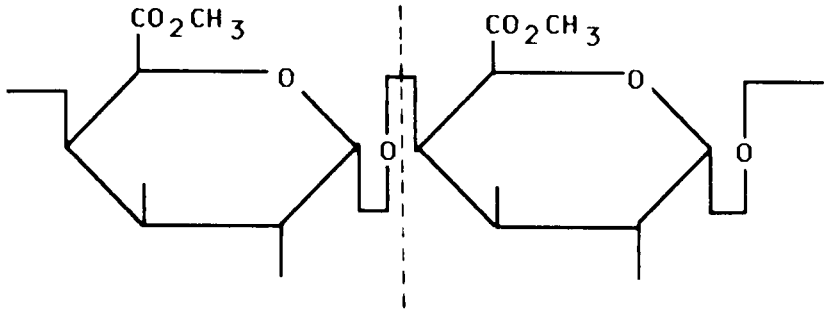
Scheme 1. Alginic acid.

Alginase from *Pseudomonas sp.* has been partially purified by gel permeation chromatography, following cell disruption. Two lyase fractions were obtained, having different substrate specificities and markedly different molecular weights (*32,33*). An alginase isolated from an aqueous extract of the mollusk hepatopancreas was purified to homogeneity by ammonium sulfate precipitation and ion-exchange chromatography. During the purification it appeared that as many as six alginase isoenzymes might be present in the crude extract (*28*). Two alginases were purified from abalone, with special precautions being taken to preclude the possibility of bacterial contamination and to confirm the animal origins of these enzymes (*29*). In this purification the hepatopancreas was homogenized and nucleic acids precipitated, and ion-exchange chromatography resulted in two alginases with different isoelectric points and substrate specificities.

The two partially purified alginases prepared from *Pseudomonas sp.* have been characterized (*32,33*). These alginases have very similar properties, but distinctly different molecular weights; the higher molecular weight enzyme also has considerably higher thermal stability. Both of these enzymes exhibit an endolytic-action pattern and are partially inhibited by EDTA. They act predominately on polyguluronic acid and the heteropolymer comprised of guluronic and mannuronic acid, hardly attacking polymannuronic acid. An extracellular alginase from *K. aerogenes* (*31*) acts predominantly on polyguluronic acid. Cell associated enzymes from several marine bacteria prefer polymannuronic acid, whereas extracellular enzymes act preferentially on polyguluronic acid, and both act on the heteropolymer (*18*). Extracellular alginase from *Altermonas sp.* is endolytic, acting preferentially on polymannuronic acid, whereas cell associated alginase from the same bacteria acts exolytically and equally at mannuronic and guluronic acid residues (*26*). *Littorina* produces as many as six cationic alginase isoenzymes (*28*). The most basic of these was purified, has a mol wt of 40,000, and acts endolytically on polymannuronic acid only. Abalone produces two alginases, one of which is endolytic, acting on polymannuronic acid, and the other an exolytic alginase acting on polyguluronic acid (*29*).

Pectin Lyases

Pectin [**2**] is a partially branched polymer containing homopolymeric blocks in the unbranched portion of its structure. These blocks consist of 1→4 linked α-D-methylgalacturonate (*2*). The carboxylate groups in this polymer are largely esterified, with Link pectin being up to 95% methylated (*34*). Enzymes capable of interconverting pectin and pectate, such as pectin methyl esterase, are found in many lyase-producing organisms; in addition, it is not unusual to find both pectin and pectate lyases being produced by a single organism (*35*). For this reason the production and characteristics of these enzymes are closely linked. Pectin lyases will be



Scheme 2. Pectin (methyl pectate).

discussed first because there are fewer reports of this enzyme than of the related pectate lyase (*36*).

There are two classifications of pectin lyases (Table 1), endopectin lyase (EC 4.2.2.3) and exopectin lyase (EC 4.2.2.10), and, in addition, there are a large number of unclassified enzymes described in the literature only under the generic name of pectin lyase. The nomenclature is further confused with the term pectin–*trans*-eliminase; this name refers to the *trans* or *anti* orientation of the groups being eliminated from the uronic acid residue (Fig. 2). Endopectin lyases have been inappropriately classified under the Enzyme Commission (EC) number assigned to alginate lyase, and, in addition, the exopectin lyases often act endolytically. Because of these problems, the classification of certain pectin lyases should be treated with skepticism.

The pectin lyases that have been classified by EC numbers 4.2.2.3 and 4.2.2.6 were all obtained from fungal sources (Table 2). In addition, the vast majority of the unclassified pectin lyases are fungal enzymes. Only the higher fungi of the Ascomycetes class (all the Euascomycetidaes are represented) and the Deuteromycetes-form class have been shown to produce pectin lyases. The majority of pectin lyase producers are Deuteromycetes and are either plant pathogens or associated with dead or dying plants.

The bacteria that produce pectin lyase (Table 2) cover the entire range from obligate anaerobes to obligate aerobes. Several of the pectin lyase producing bacteria are characterized as plant pathogens, including species of *Xanthomonas, Pseudomonas, Corynebacterium,* and *Acinetobacter.*

All of the characterized pectin lyases (Table 2) are extracellular enzymes. Four fungal species, all aquatic isolates, *Mycocentrospora, Articularspora, Tetrachaetum,* and *Tricladium,* produce inducible enzymes (*37*), whereas *Phoma exigua* produces a constitutive pectin lyase (*38*). The production of pectin lyase by *Penicillium digitatum* is reported to be under catabolite repression (*39*). In general, both the fungi and bacteria capable of pectin lyase synthesis also produce pectin and pectate hydrolases, pectate lyases, and pectin methyl esterases. The regulation of all of these enzymes may be coordinately controlled (*40*).

TABLE 2
Polysaccharide Lyases

Enzyme/Organism	Location and production	Molecular weight	Properties[a] pI	Act. opt., pH, T°C	Stab. opt., pH, T°C	Action pattern	Metal required	Other	References
ALGINATE LYASE									
Alginate lyase, EC 4.2.2.3									
				Bacterial sources					
Altermonas sp.	i, ex					Endo		Poly M and G	*26*
Azotobacter vinelandii	c, ca								*19*
Bacillus circulans	ex			7				poly M	*27*
Beneckea (Vibrio) pelagia	i								*25*
	i, ex								*25*
Klebsiella aerogenes	ex							Only poly G	*31*
Pseudomonas mendocina	c, ca					Endo			*22*
putida									*23*
maltophila									*23*
sp.	ca			8, 50	40	Endo		Only poly G and GM	*32, 33*
	ca			8, 50	30	Endo		Only poly G and GM	*32, 33*
Unclassified bacteria	i, ca					Endo		Poly M preferred	*18*
	i, ex					Endo		Poly G preferred	*18*
				Animal sources					
Littorina sp. hepatopancreas		40,000	7	5.6, 37	4–8, 40	Endo	No Ca^{2+}	Poly M only	*28*
Haliotus rufescens & corrugata			5.6	7.6	8	Endo		poly M only	*29*
hepatopancreas			5.6	4.0		Exo		at G	*29*
PECTIN LYASE									
Endopectin lyase EC 4.2.2.[3] (same EC # as alginate lyase)									
				Fungal sources					
Aspergillus fonsecaeus	ex				5.1	Endo	No Ca^{2+}		*43*
japonicus	ex	32,000	7.7	6–7, 55	4–5, 50	Endo	No Ca^{2+}		*36*
oryzae	ex		9.4						*42*
sojae	ex		9.4			Endo			41, 42

(*continued*)

TABLE 2 (*continued*)

Enzyme/Organism	Location and production	Molecular weight	Properties[a] pI	Act. opt., pH, T°C	Stab. opt., pH, T°C	Action pattern	Metal required	Other	References
Botrytis cinerea	ex			6		Endo			*152*
Collectotrichum altramentarium	ex					Endo			*152*
Glomerella cingulata	ex					Endo			*152*
Penicillium digitatum	ex							Catabrep	*39, 153*
italicum									*153*
Exopectin [poly(methoxygalacturonide)] lyase EC 4.2.2.10									
				Fungal sources					
Botrytis cinerea				8		Endo			*154, 155*
fabae						Endo			*155*
Fusarium oxysporum						Endo			*155*
Mycocentrospora angulata			4–7	9		Endo			*37*
Penicillum citrinum				8, 35					*156*
paxilli	ex			5–6, 35–40	4–6, < 40	Endo	No Ca^{2+}		*44, 157*
Phoma exigua	c, ex			9, 25		Endo			*38*
Sclerotinia fructigena						Endo			
trifoliorum						Endo			*155*
						Endo			*155*
Pectin lyase EC unclassified									
				Fungal sources					
Alatospora acuminata									*158*
Alternaria solani	c, ex		7–8			Endo			*61, 159*
tenuis	c, ex								*61*
Articulospora tetracladia	i								*37*
Aspergillus niger						Endo			*160*
sojae	ex	32,000		7, 40	4–7, < 40		No Ca^{2+}		*41*
Clavariopsis aquatica									*158*
Fusarium trincinctum	ex			8.5					*161*
Lemonniera aquatica									*158*
Tetrachaetum elegans	i, ex		4–7	9		Exo			*37*

Tetracladium marchalianum									*158*
Tricladium splendens	i								*37*
Trichothecium roseum	ex			8.5, 45	< 100				*45*
Verticillium albo-atrium	ex					Exo			*35*
dahliae	ex								*35*
nigrescens	ex								*35*
nubilum	ex								*35*
tricorpus	ex								*35*
				Bacterial sources					
Acinetobacter sp.									*162*
Bacillus megaterium									*162*
polymyxa									*162*
Corynebacterium sp.	ex								*163*
insidiosum									*163,164*
Clostridium sp.	ex								*163*
Micrococcus epidermidis									*162*
Pseudomonas cepacia									*162*
Vibrio sp.	ex								*163*
Xanthomonas sp.									*162*

PECTATE LYASE

Endopectate lyase (pectate transeliminase) EC 4.2.2.2 and 4.2.99.3

				Bacterial sources					
Aeromonas liquifaciens	c							Catabrep (EC 4.2.99.3)	*58, 59*
Arthrobacter sp.						Endo	Ca^{2+}		*48, 49, 50*
Azospirillum sp.	i, ex			7.5–8			No Ca^{2+}		*165*
Bacillus pumilus		20,000		8–8.5		Endo	Ca^{2+}		*54, 166*
polymyxa									*54, 167*
sp.	c, ex			9.5		Endo	Ca^{2+}	Alkalophilic	*48, 49, 50, 52, 53*
stearothermophilus		24,000		9, 70	7–10, 85	Endo	Ca^{2+}	Thermoph. anaerobe (EC 4.2.99.2)	*54*
subtilis	ex	33,100	9.85	8.5	6–10, 55	Endo	Ca^{2+}		*168*

(*continued*)

TABLE 2 (*continued*)

Enzyme/Organism	Location and production	Molecular weight	pI	Properties[a] Act. opt., pH, T°C	Stab. opt., pH, T°C	Action pattern	Metal required	Other	References
Bacteroides ruminocola								(Ec 4.2.00.3)	*169*
thetaiotaomicron	i, ex	74,000	7.5	8.7			Ca^{2+}	Membrane assoc.	*170*
Clostridium aurantibutyricum	ex					Endo			*171*
felsineum	ex					Endo		Same as *C.roseum*	*171, 172*
felsineum		105,000	5	8.5		Endo	Ca^{2+}	Mol wt by GPC (EC 4.2.99.3)	*69*
multifermentans						Exo			*173*
Erwinia aroideae			7	9.3	7, 35	Endo	Ca^{2+}	2 Isoenzymes	*174*
atroseptica	ex		9.7		< 30				*47*
atroseptica	ex		10.2		< 40				*47*
atroseptica	ex		10.35		< 40				*47*
carotovora	ex	42,500	9.8	8.5	< 60	Endo	Ca^{2+}		*55*
carotovora	ca	41,000	9.6			Endo		Catabrep	*55, 175*
carotovora	ca		9.4	8.5		Endo	Ca^{2+}		*46*
carotovora	ca		8.0	8.5		Exo, Endo	Ca^{2+}		*46*
carotovora	ca		6.3	8.5		Exo	No Ca^{2+}		*46*
chrysanthemi	i, ex	30–36,000	9.4	8–10		Endo	Ca^{2+}	Some constit.	*70*
chrysanthemi	i, ex	30–36,000	8.4	8–10		Exo	Ca^{2+}	Prod., but not	*70*
chrysanthemi	i, ex	30–36,000	7.9	8–10		Exo	Ca^{2+}	Under catabrep	*70*
chrysanthemi	i, ex	30–36,000	4.6	8–10		Exo	Ca^{2+}	(EC 4.2.99.3)	*70*
chrysanthemi	i, ex	30–32,400	8.8			Endo			*40, 46, 62, 63, 64, 65*
chrysanthemi	i, ex		9.8			Endo			*40, 46, 65*
rubrifaciens	ca	39–41,500	6.25	9.5, 37		Endo	Ca^{2+}	Essent. tyrosine	*67*
Klebsiella pneumoniae									*176*
Pseudomonas sp.						Endo	Ca^{2+}		*48, 49, 50*
Pseudomonas fluorescens	i, ex							Strong catabrep Nonpath. (EC 4.2.99.3)	*56, 58*

Streptomyces fradiae	ex		> 7	9.1	6–9	Endo	Ca^{2+}	Sdp 6	*177*
nitrosporeus	ex	41,000	4.6	10, 50	4–11, < 40	Endo	Ca^{2-}	Sdp 4–6	*71*
Streptococcus bovis						Endo			*178*
Treponema pectinovorum	ex					Endo			*51*
Yersinia sp.									*176*
Xanthomonas sp.						Endo	Ca^{2+}		*48, 49, 50*
				Fungal sources					
Aspergillus fonsecaeus								(EC 4.2.99.3)	*43*
Colletotrichum trifolli								(EC 4.2.99.3)	*179*
Fusarium (Hypomyces) oxysporum	ex		8.3						*180*
solani	ex			8.5				Catabrep (EC 4.2.99.3)	*60*
Phoma exigua	i, ex			9, 25–30		Endo		Some constit. prod.	*38*
Exopectate (polygalacturonate) lyase EC 4.2.2.9									
				Bacterial sources					
Butyrivibrio fibrisolvens	ex			8–8.5		Exo	Ca^{2+}	Sdp 4	*181*
Clostridium multifermentans						Exo	No Ca^{2+}		*75*
Erwinia aroideae						Exo	No Ca^{2+}		*174*
chrysanthemi	ca					Exo			*63*
Streptomyces massasporeus	ex	54,000	5.5	9.5, 40	3–10, < 50		Ca^{2+}	Sdp = 3	*72*
nitrosporeus		32–39,000	4.05	9.5	5–10	Exo		Glycpr, AAAn	*71, 73, 74*
				Fungal sources					
Botrytis cinerea						Exo			*154*
Oligogalacturonide lyase EC 4.2.2.6									
				Bacterial sources					
Erwinia aroedeae						Exo			*182*
carotovora	ca	31,000	6.5	7.5–8.5		Exo	Ca^{2+}		*46*
chrysanthemi	ca	30–32,400							*62, 63*
Pseudomonas sp.					Exo				*183*
Pectate lyase, EC unclassified									
				Bacterial sources					

(*continued*)

TABLE 2 (*continued*)

Enzyme/Organism	Location and production	Molecular weight	pI	Properties[a] Act. opt., pH, T°C	Stab. opt., pH, T°C	Action pattern	Metal required	Other	References
Clostridium butyricum	i, ex			8.5					*184*
				Fungal sources					
Alternia solani	c, ex								*61*
tenuis	c, ex								*61*
Penicillium oxalicum									*185*
CHONDROITIN LYASES									
				Bacterial sources					
Chondroitin ABC lyase, EC 4.2.2.4									
Bacteroides thetaiotamicron	i, ca	104,000 108,000	7.9 8.0	7				Unrelated by Peptide maping	*186, 187*
ovatus	i, ca								*82*
fragilis									*124*
melaninogenicus									*124*
Proteus vulgaris	i, ca	150,000		6–8, 37		Endo		Catabrep	*80, 84, 188, 189*
Chondroitin AC lyase, EC 4.2.2.5									
Aeromonas liquifaciens	ex		7	6.6, 37	6–9, < 45				*90*
Arthrobacter aurescens	ex	76,000		6, 50	5–7, < 45	Endo		Glycpr, AAAn	*81, 91, 95, 96, 190*
Beneckea (Vibrio) chitinovora	ex								*90*
labra	ex								*90*
Flavobacterium heparinum	c, ca	70–80,000	7	8, 40		Endo			*84, 86, 95, 96 189–191*
balustinum	ca								*90*
diffusum	ca								*90*
Microcossus varians	ca								*90*
Vibrio sp.	ca								*90*

Chondroitin B lyase, EC 4.2.2.?							
Flavobacterium heparinum	i, ca		> 7	8, 20			*86, 92*
Chondroitin C lyase, EC 4.2.2.?							
Flavobacterium heparinum	c, ca		> 7				*83, 92*
HYALURONATE LYASE							
Hyaluronate lyase, EC 4.2.2.1 or EC 4.1.99.1							
Bacterial sources							
Bacteroides thetaiotaomicron							*89*
ovatus							*89*
melaninogenicus							*89*
Propionibacterium acnes	c, ex	78–85,000		6.4	5–6, 50	Can prod. in 0_2	*76, 98, 99 100,117*
avidum	c, ex					Can prod. in 0_2	*98, 99, 100, 192*
granulosum	c, ex					Can prod. in 0_2	*98, 99, 100, 192*
Peptostreptococcus sp.	ex	160,000		7, 46		K_m 0.14 mg/mL hyal	*118*
Staphylococcus aureus	c, ex	84,000	7.4	5.2		Glycpr, AAAn	*93, 114, 193*
aureus	c, ex	84,000	7.9	5.2		Glycpr, AAAn	*93, 114*
Streptococcus pyogenes	ex	75,000	5.0	5.9		Eact, 15 Kcal/mol	*115, 194*
group A	ex	50–74,000	< 7	6		Glycpr, AAAn	*116*
equisimilis	ex	90–110,000		4, 95		Isoenzyme present	*105*
mitis	ex						*103*
Streptomyces hyalurolyticus	c, ex		< 7				*101*
Viral sources							
Streptococcus Bacteriophage							*106, 107 119*
Hyaluronidase (unclear classification, may be hydrolases)							
Animal sources							
Apis mellifera		41,000	9.0			Glycpr, blocked *N*-terminus	*108, 111*
Vespula maculifrons		46,000				Immunorelated to *A. mellifera*	*109*

(*continued*)

TABLE 2 (*continued*)

Enzyme/Organism	Location and production	Molecular weight	Properties[a] pI	Act. opt., pH, T°C	Stab. opt., pH, T°C	Action pattern	Metal required	Other	References
HEPARIN LYASE									
Heparin lyase, EC 4.2.2.7									
				Bacterial sources					
Flavobacterium heparinum	i, ca	42,800	8.5	6.5, 41	7, < 41	Endo	No M^+	AAAn, Sdp > 4	*129, 130, 195*
Bacteroides sp.								Unclr classif	*123, 196, 197*
HEPARAN MONOSULFATE LYASE									
Heparitin lyase, EC 4.2.2.8									
				Bacterial sources					
Flavobacterium heparinum	i, ca		> 7					Differ. substr.	*126, 128*
heparinum	i, ca		> 7					specificity	*136, 140*
MISCELLANEOUS									
Emulsan lyase, EC unclassified									
				Bacterial sources					
Zoogloea sp.	i, ex	89,000				Endo			*141, 142*
Acidic heteropolysaccharide lyase, EC unclassified									
Rhizobium trifolii	i	440,000		6.8, 32–40		Exo	Ca^{2+}	Bacteriophage	143
	i	540,000		5.4, 40		Endo	Ca^{2+}		

[a]i, inducible; c, constituitive; ca, cell associated; ex, extracellular; endo, endolytic; exo, exolytic; glycpr, glycoprotein; AAAn, Amino acid analysis reported; Poly G, M and GM, poly guluronic, mannuronic, and copolymer; unclr classif, unclear classification; catabrep, catabolite repression; GPC, gel-permeation chromatography; Sdp, Substrate degree of polymerization on which enzyme acts best.

A number of pectin lyases obtained from various *Aspergillus* species have been partially purified (*36,41–43*). Since these are extracellular enzymes, they were first precipitated from the fermentation broth. These enzymes were then purified by a series of ion-exchange (cation and anion) and gel-permeation chromatographic steps.

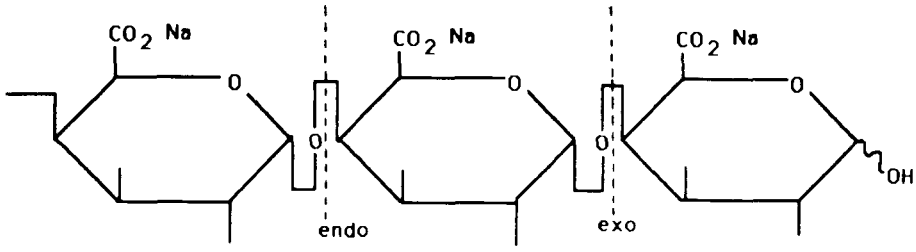
The properties of pectin lyases isolated from *Aspergillus japonicus, A. sojae,* and *A. oryzae* are remarkably similar (*36,41,42*). These are cationic proteins with a mol wt of 32,000 and neutral pH optimas and are most stable below neutral pH. These enzymes act endolytically and have no absolute requirement for calcium. A pectin lyase from *Penicillium paxilli* has similar properties (*44*). The only exolytic pectin lyases found were *Tetrachaetum elegans* (*37*) and *Verticillium albo-atrum* (*35*), and no pectin lyase showed an absolute requirement for calcium. A pectin lyase with unusually high thermal stability has been isolated from *Trichothecium roseum* (*45*).

Pectate Lyases

Pectate is a partially branched polymer containing homocopolymeric blocks in the unbranched portion of its structure [**3**]. These blocks consist of 1→4 linked α-D-galacturonate (*2*). Unlike pectin, the carboxyl groups exist as the free acid. Enzymes capable of interconverting pectate and pectin, as well as hydrolases (such as polygalacturonidase) acting on pectate, are found in many pectate lyase producing organisms (*46,47*). Pectate lyase has been investigated and its production and properties characterized more frequently and in more detail than any other type of lyase (*48–50*).

Bacteria are the main producers of pectate lyases, in contrast to the dominance of fungi in the production of pectin lyases (Table 2). Again, the diversity here is striking, with producing organisms ranging from strict aerobes to strict anaerobes and containing both gram-negative and -positive bacteria. These bacteria have been isolated from the soil, dead or decaying plants, and the rumen of various animals. Unusual pectate lyase producers include a spirochete *Treponema pectinovorum* isolated from the human oral cavity (*51*) and an alkalophilic (*52,53*) and a thermophilic *Bacillus* (*54*). The predominate pectate-producing genus, *Erwinia,* is well known as a plant pathogen that causes plant vascular disease (*55*). The *Xanthomonas* species, widely known as a plant pathogen, also produces pectate lyase (*49*).

Fungal pectate lyases are also known (Table 2), and these are often accompanied by other lyases and hydrolases acting on pectin as well as pectate. Again, here, as with pectin lyase, pectate lyase production is only seen in the higher fungi Deuteromycetes and Ascomycetes (specifically, the Plectomycetes), and these are all considered to be plant pathogens.



Scheme 3. Pectic acid (sodium salt).

The regulation of pectinolytic enzyme synthesis, including the production of pectate lyase, is an important factor in determining the virulence of plant pathogens. Synthesis of a basal level of pectinolytic enzymes is important for pathogenic organisms because they allow the organism to attack exposed pectic materials in a wounded plant before these layers are sealed, typically within 24 h (*56*). *Pseudomonas fluorescens*, a nonpathogenic saporophyte, has an inducible pectate lyase and when induced by growth in the presence of pectin this bacterium becomes virulent (*56*). Similarly, *Erwinia carotovora* strains that have lost their ability to synthesize pectinolytic enzymes are no longer virulent (*57*). The most virulent strains of *P. fluorescens* synthesize large quantities of inducible pectinolytic enzymes (*58*). Although *Aeromonas liquifaciens* produces a constitutive pectate lyase and would be expected to be virulent, this is not the case because it is under strong catabolite repression (*58,59*). *Fusarium solani* is under catabolite control, but it produces a basal level of pectate lyase, and hence is pathogenic (*60*). Finally, pathogenic organisms generally require enzymes with endolytic action patterns because these are more successful in macerating plant tissue (*40*).

Regulation of pectinolytic synthesis has been extensively studied in pathogenic fungi, such as *Alternia* species, and pathogenic bacteria, particularly the *Erwinia* species. *Alternia solani* and *A. tenuis* produce constitutive pectin and pectate lyases (*61*). The production of these enzymes is stimulated when these fungi are grown on pectin. Growth on pectate, however, only stimulates *A. tenuis* production (*61*). *Erwinia carotovora* produces a basal level of both intra- and extracellular pectate lyases, and their production at high levels can be induced with pectin and controlled (particularly the intracellular lyase) by catabolite repression (*58*).

Stack and coworkers (*46*) have proposed a model for the synthesis and regulation of pectinolytic enzymes in *E. carotovora*. They suggest cAMP promotion of extracellular pectate lyase synthesis (basal levels of this enzyme are always present), together with induction of pectinolytic enzymes by the unsaturated disaccharide products of this enzyme. Collmer and Bateman have attributed induction in *Erwinia chrysanthemi* to the ring opened unsaturated monosaccharide product and its tautomer (*62,63*). In addition, it has been suggested that pectate

hydrolases are coordinately regulated with pectate lyase production in *E. chrysanthemi*, and that the action patterns of these two enzymes are complementary (*40*). The effect of water activity (a_w) on pectate lyase synthesis by *E. chrysanthemi* has been studied, and it was found that reduced water activity strongly repressed the production of extracellular pectate lyase (*64*). The molecular cloning of pectate lyase genes from *E. chrysanthemi* has resulted in their expression in *Escherichia coli*. Two pectate lyases, p*I* 8.8 and 9.8, were produced constituitively by *E. coli* and isolated from the periplasm and culture fluid. Catabolite repression of pectate lyase synthesis was observed in these *E. coli* clones (*65*). Insertion mutants of *E. chrysanthemi* have also been used to study pectate lyase induction (*66*).

Pectate lyases are primarily extracellular enzymes (Table 2) greatly facilitating their purification. This property and their scientific and commercial importance has led to the purification of more than two dozen pectate lyases. After recovery of the extracellular protein from the fermentation broth by precipitation, these enzymes are purified through anion and cation ion-exchange, gel-permeation chromatography, and isoelectric focusing. Affinity chromatography on Sepharose–polygalacturonamide has also been effectively used to purify pectate lyase (*54*). Cell-associated pectate lyase from *Erwinia* has been released by osmotic shock and then purified (*67*).

There are three distinct classes of pectate lyases (Table 1). Two act on full-length pectate polymers and are distinguished by their endo- and exolytic action patterns [**3**]. A third, oligogalacturonate lyase, acts exolytically on oligosaccharides formed by other pectinolytic enzymes. All three of these lyases can be distinguished from pectate hydrolases by assays specific for the unsaturated sugar formed by the action of a lyase (*68*). A second criterion useful in distinguishing hydrolytic enzymes from lyases is that the former show pH optima around 4.5, whereas the latter have optima closer to pH 8 (*40*).

A number of bacterial endopectate lyases have been well characterized (Table 2). Most are cationic proteins having mol wt of 20,000–42,500, with pH optima ranging from 7.5 to 10, and displaying a requirement for calcium (other divalent cations can often relieve this requirement). Unusual enzymes in this group include a thermally stable enzyme from *Bacillus stearothermophilus* (*54*), a high mol wt (105,000) lyase from *Clostritium felsineum* (*69*), and two low p*I* lyases from *E. chrysanthemi* (*70*) and *S. nitrosporeus* (*71*). Each of the four *Erwinia* species studied have isoenzymes that principally differ by p*I* (Table 2).

Two exopectate lyases from *Streptomyces massasporeus* (*72*) and *S. nitrosporeus* (*73,74*) have been well characterized. Both are anionic proteins of mol wt 35,000–50,000, having pH optima of 9.5. The *S. nitrosporeus* lyase is a glycoprotein (*74*). Exopectate lyases produced by a *Clostridia* (*75*) and an *Erwinia* (*63*) species are only partially characterized, but have the unusual feature of showing no calcium requirement.

All the oligogalacturonide lyases have exolytic action patterns, and two enzymes from *Erwinia* (mol wt 31,000) have been partially characterized (*46,63*).

LYASES ACTING ON ANIMAL POLYSACCHARIDES

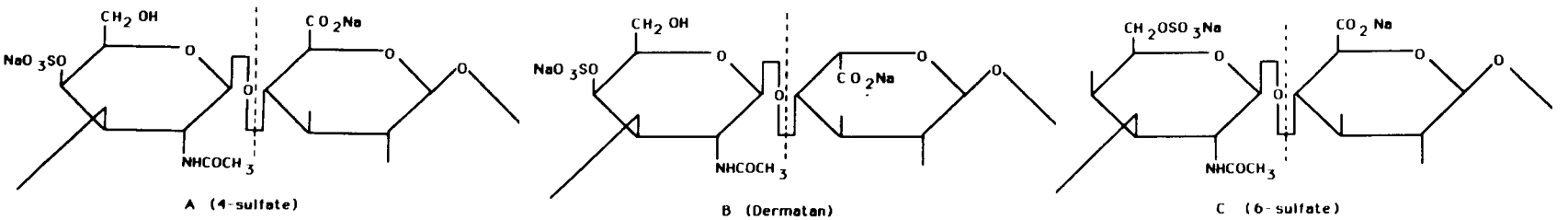
Acidic polysaccharides from animal (primarily mammalian) sources are linear copolymers comprised of alternating hexosamine and uronic acid residues. These polysaccharides are called glycosaminoglycans (*4,7*) and are (except in the case of hyaluronic acid) found covalently linked to a core protein in a very high molecular weight molecule called a proteoglycan. Hyaluronic acid and chondroitin are simple copolymers with a repeating disaccharide unit. The chondroitin sulfates show a slight variation in their repeating disaccharide units, whereas hyaluronic acid does not. A number of lyases act on both hyaluronic acid and the chondroitin sulfates. These enzymes are typically classified as either hyaluronidases or chondroitinases on the basis of which substrate yields the highest rate of reaction and most complete level of depolymerization (*76*). Heparin and heparan monosulfate are the most complex of the glycosaminoglycans, showing substantial variability in their repeating disaccharide sequence (*17*). The lyases acting on these acidic polysaccharides show specificity for the linkage type, the substitution on the sugar units comprising the linkage and, to a limited degree, the size of the substrate.

Chondroitin Sulfate Lyases

Chondroitin is a glycosaminoglycan commonly associated with the extracellular matrix in mammals and one of the major components of the cartilage (*7*). The three major types of chondroitin are chondroitin sulfate A, B (dermatan sulfate), and C [**4**]. There are other rare types of chondroitin, such as chondroitin sulfate D and E (*7*). Chondroitins often contain common sequences, making this nomenclature confusing and increasingly outdated (*17*). The easiest way to differentiate among the chondroitins is to examine the redundancy of a few specific sequences within the polymer. In addition to these common sequences, there are regions of higher sulfation, two or more sulfates/disaccharide, and regions of low sulfation, unsulfated chondroitin sulfate sometimes being called chondroitin. Chondroitins are isolated from proteoglycans and chondroitin A, B, and C are often found bound to different core proteins (*2*). Although the molecular weight of chondroitins vary (depending largely on the source), typical average molecular weights of chondroitin sulfate A, B, and C have been reported as 15,000, 45,000, and 29,000, respectively (*77*).

Chondroitin lyases (chondroitinases) are most commonly obtained from *Proteus vulgaris, Arthrobacter aurescens, Bacteroides thetaiotamicron,* and *Flavobacterium heparinum. Pseudomonas fluorescens* and *P. aeruginosa*

CHONDROITIN SULFATE LYASES



Scheme 4. Chondroitin sulfates A, B, and C sodium salts.

also show the capacity to degrade chondroitin sulfates (*78*). Chondroitinase ABC from *P. vulgaris*, first prepared by Dodgson and Lloyd (*79*), is produced when induced by chondroitin sulfate or *N*-acetylgalactosamine. The production of this enzyme appears to be under catabolite repression by glucose and several other carbon sources (*80*). *Arthrobacter aurescens* is a soil isolate that secretes an extracelluar chondroitinase AC (*81*). *Bacteroides thetaiotaomicron*, isolated from the human colon, is a Gram-negative anaerobe that produces two different chondroitinase ABCs (*82*). Although present constituitively at low levels, the production of these enzymes can be stimulated by addition of chondroitin sulfate or a chondroitin sulfate derived oligomer of octasaccharide size or larger. These enzymes are cell associated, probably residing in the periplasmic space. *Flavobacterium heparinum*, a Gram-negative facultative anaerobe, produces constituitive chondroitinases C (*83*) and AC (*84*) and chondroitinase B, which is only present at very low levels in noninduced cultures (*85,86*). These chondroitinases are cell associated. Chondroitinase B is slowly induced by either chondroitin sulfate A, B, or C, whereas the unsulfated, the 4-, or the 6-sulfated chondroitinase derived disaccharide gives almost immediate induction (*86*). Other sources of chondroitinases include oral endodontal isolates (*87*), pathogenic *Bacteroides* (*88,89*), and 253 chondroitinase producing bacteria obtained from land and aquatic environments (*90*). The majority of these isolates are members of one of seven genera: *Aeromonas, Vibrio* or *Beneckea, Flavobacterium, Proteus, Micrococcus,* or *Arthrobacter.* Of special interest is an *Aeromonas* species (probably *A. liquefaciens*) that produces large quantities of extracellular chondroitinase AC (*90*).

The bacteria discussed above consist of Gram-negative and -positive organisms and range from strict aerobes to strict anaerobes (Table 2). These chondroitin lyase producers are isolated from decomposing animal protein (*P. vulgaris* and *Micrococcus varians*), the soil, or the gut or rumen of various animals and are generally nonpathogenic (except, possibly, in the case of *Vibrio sp.*).

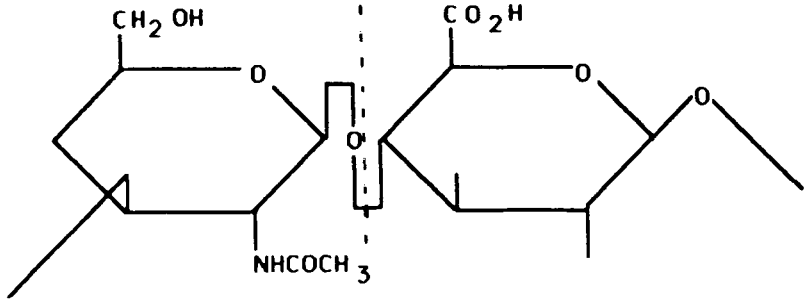
Chondroitinase ABC from *P. vulagaris* has been purified by ammonium sulfate precipitation, followed by DEAE–cellulose and phosphocellulose chromatography, giving a single band on PAGE (*84*). Two chondroitinase ABCs from *B. thetaiotaomicron* were purified to homogeneity by DEAE–cellulose, heparin–agarose, phosphocellulose, and gel-permeation chromatography (*82*). Chondroitinase AC from *F. heparinum* was released by freeze-drying and purified by agarose gel-electrophoresis (*86*). Chondroitinase AC from *A. aurescens* has also been purified to homogeneity (*91*). Flavorbacterial chondroitinases B and C have been purified either in the same manner as chondroitinase AC (*86*) or by hydroxylapatite followed by affinity chromatography on chondroitin sulfate B-treated heparin–agarose (for chondroitinase B) and chondroitin sulfate B treated chondroitin sulfate B-agarose (*92*).

Chondroitinase ABC from *P. vulgaris* has a mol wt slightly lower than 150,000, with pH optima of 8.0, 6.8, and 6.2 on chondroitin sulfates, chondroitin, and hyaluronic acid, respectively (*84*). The two chondroitinases produced by *B. thetaiotaomicron* have mol wt and pIs of 104,000 and 7.9 and 108,000 and 8.0, respectively (*82*). Neither showed subunits and both were inhibited by heparin. These chondroitinases had similar K_m values of 40–70 μg/mL on chondroitin sulfate and 300–400 μg/mL on chondroitin. Finally, the pH optima on chondroitin sulfate are 7.2 and 7.6 and on chondroitin, 7.0 and 6.8, respectively. Partial chemical fragmentation showed no major peptides common to both enzymes (*82*). *Arthrobacter aurescens* chondroitinase AC is a monomeric glycoprotein with a mol wt of 76,000, stable below 45°C and between pH 4.9 and 7.4, with activity optima at 50°C and pH 6.0 (*81*). Amino acid analysis shows 40% hydrophobic amino acids (*81*), in contrast to the 4.6% found in *Staphylococcus* hyaluronidase (*93*). Chondroitinase AC from *F. heparinum* has a mol wt of 70,000–80,000 (*94*) and an activity optima at 40°C, pH 8 (*86*), and at an ionic strength of 0.05*M* (*86,95*). Calcium has been reported to both enhance (*84,95*) and decrease the rate of enzyme catalysis (*86*). Chondroitin sulfate B competitively inhibits (*84*), and heparin may (*84*) or may not (*95*) competitively inhibit, chondroitinase AC. Chondroitinase AC from both *F. heparinum* and *A. aurescens* acts endolytically on chondroitin sulfate substrate, the former by a random attack, the latter by a stepwise attack (*96*). In addition, both chondroitinase ACs act at higher rates on chondroitin and hyaluronic acid than on chondroitin sulfate A and C. Chondroitinase B has activity optima at 20°C, pH 8, and an ionic strength of 0.02*M*. Calcium has no effect on enzyme activity. The flavobacterial chondroitinases are all cationic proteins, as judged from their ability to migrate toward the negative pole when subjected to electrophoresis on a pH 8.5 agarose gel (*86*).

Hyaluronate Lyases

Hyaluronic acid is an unsulfated glycosaminoglycan with a mol wt of 200,000 (*77*) or higher and is a major component of connective tissue, such as cartilage (*3*). Unlike the other glycosaminoglycans, hyaluronic acid is not found covalently attached to a core protein (*7*). A copolymer of D-glucuronic acid and 2-acetamido-2-deoxy-D-glucose **[5]**, hyaluronic acid is structurally similar to chondroitin sulfates A and C (*2*).

Karl Meyer and coworkers (*1,97*) first showed that the cleavage of hyaluronic acid and chondroitin sulfates A and C by bacterial enzymes proceeded through β-elimination. Studies on the mechanism of action of hyaluronate lyase showed that the treatment of hyaluronic acid labeled at the 5-position of the uronic acid residue resulted in the loss of tritium label (*10*). In addition, the eliminated hemiacetal failed to incorporate ^{18}O when the reaction was carried out in $H_2{}^{18}O$ (*11*). These results suggest a



Scheme 5. Sodium hyaluronate.

mechanism involving the direct elimination of hemiacetal from uronic acid residue (*14*).

Hyaluronate lyase is produced almost exclusively by bacteria (Table 2). These include both Gram-negative and -positive bacteria and range from aerobes to obligate anaerobes. These bacteria are often pathogenic and are isolated from dead or dying animal tissue, pus, and wounds. Soil, gut, and rumen isolates that produce hyaluronate lyases are generally nonpathogenic. Cutaneous *Propionibacterium acnes, P. avidum,* and *P. granulosum* all produce extracellular hyaluronate lyase (*98–100*). Hyaluronidase is a product of *Streptomyces hyalurolyticus* (*101*), a *Bacteroides* species (*89,102*), *Staphylococcus aureus* (*93*), *Clostridium welchii,* and type II *Pneumococcus* (*1,97*). *Streptococcus mitis* (*103*) and *Peptostreptococci* (*104*) isolated from the oral cavity, *Streptococcus equisilimilis* (*105*), *S. pyrogenes* (*106*), *Streptococci group C* (*24*), and *Streptococci group A* (*15*) all produce extracellular hyaluronidases. A *Streptococcal* bactriophage borne hyaluronidase has also been reported (*106*). Hyaluronidase is produced by both virulent and temperate forms of this phage (*107*). Recently, hyaluronidases isolated from nonmicrobial sources, such as the venom of the honey bee *Apis mallifera* (*108*) and by inference, an immunologically related enzyme from yellow jacket *Vespula maculifrons* (*109*), have been classified as lyases. It is unclear from the assay (*110*) used to measure the activity of these enzymes whether they are lyases or hydrolases. In the past, these enzymes were classified as hydrolases (*111*) and were thought to be similar to the mammalian testicular hyaluronidases.

The effects of pH, oxygen, and glucose on the production of extracellular hyaluronidase from *Propionibacterium sp.* has been studied extensively (*98–100*), using continuous culture. Growth and enzyme production on glucose in the absence of hyaluronate took place at pH values ranging from 5 to 8, with both optima at pH 6. All three *Propionibacteria sp.* are capable of growth in 0–100% of air saturation, but the presence of oxygen inhibited both growth and hyaluronidase production. *Propionibacterium avidum* was the species best adapted for anerobic growth. The organisms showed improved growth with the addition of glucose, and the production of hyaluronidase increased with the increasing glucose, up to 0.2 w/v%, then decreased with the addition of more glucose. The

production of extracellular hyaluronidases from *S. aureus* (*112*) showed that growth could occur at pH 5.5–9.5, with optimum production of hyaluronidase at pH 7–7.5. Aeration, as well as the addition of yeast extract, was required, and in continuous culture the level of extracellular hyaluronidase decreased throughout the entire fermentation. Several *Peptostreptococci* were examined in liquid culture (*104*) and found to produce extracellular hyaluronidase anerobically at 37°C.

Several hyaluronidases have been purified to homogeneity without excessive difficulty because of their extracellular nature. *Staphylococcal* hyaluronidase isolated from the culture supernatant has been purified by salt and acetone precipitation, followed by gel-permeation chromatography (*113*) and by isoelectric focusing and gel-permeation chromatography (*114*). *Streptococcal* hyaluronidases have been purified, using salt precipitation, ion-exchange, and gel-permeation chromatography (*115,116*). A hyaluronidase from *S. hyalurolyticus* has been purified, using hydrophobic–ionic chromatography on an amberlite ion-exchange resin (*101*). A hyaluronidase from *P. acnes* has also been purified to homogeneity (*117*). Recently, a hyaluronate lyase from *Peptostreptococcus,* with a very high mol wt of 160,000, was purified to homogeneity by gel-permeation chromatography (*118*). This enzyme acts well on hyaluronic acid and chondroitin sulfates A and C. Bacteriophage hyaluronidase has also been purified (*119*). Finally, bee venom hyaluronidase (this may be a hydrolase) was purified by gel-permeation and ion-exchange and immuno- and heparin–Sepharose affinity chromatography (*108*).

Staphylococcus aureus produces two hyaluronidases (*114*), both having identical mol wt of 84,000 (*93*), but with slightly different isoelectric points (p*I* 7.4 and 7.9). The p*I* 7.4 isoenzyme was slowly converted to the p*I* 7.9 species during incubation in phosphate buffer (*114*). At least one of these isoenzymes is a glycoprotein that has been crystallized. An amino acid analysis shows this protein to be rich (82%) in basic amino acids and poor (4.6%) in hydrophobic amino acids (*93*). These isoenzymes are insensitive to mild reducing agents and do not require a divalent metal cofactor (*114*). Several *Streptococcal* hyaluronidases have been purified and are anionic proteins with mol wt ranging from 50,000 to 100,000. Again, the presence of isoenzymes has been suggested here, as well (*105*), and the enzyme from *Streptococci group A* is a glycoprotein (*116*). *Propionibacterium acnes* hyaluronidase has a mol wt of 80,000 (*117*), quite similar to the other microbial hyaluronate lyases. This enzyme has the unusual property of being extremely nonantigenic, a very uncharacteristic trait for a pathogenic microbial enzyme (*120*). The hyaluronidases from *P. acnes* and *P. granulosum* are immunologically related, but not identical (*120*). A bacteriophage hyaluronidase, isolated from a temperate form, has been characterized (*119*). The bacteriophage hyaluronidases from virulent and temperate forms were immunologically distinct. Bee venom hyaluronidase has a considerably lower mol wt, 41,000, and a higher isoelectric point, p*I* 9, than observed in microbial

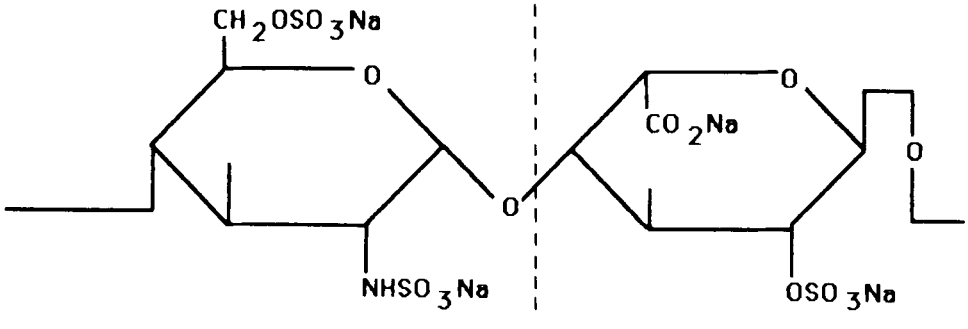
hyaluronidases (*108*). Like some microbial enzymes, bee venom hyaluronidase is a glycoprotein, but, surprisingly, its rich level of acidic amino acids makes this enzyme similar to anionic and dissimilar to cationic microbial hyaluronidases. Another interesting feature of this enzyme is its blocked *N*-terminus.

Heparin Lyases

Heparin [6] is a polysaccharide anticoagulant prepared from bovine lung and porcine mucosa (*7*). It is the most acidic natural product, having, on the average, four negative charges/saccharide residue (three sulfates and one carboxylate group). Heparin is both polydisperse (mol wt 5000–40,000; average mol wt 14,000) and microheterogeneous, having a great degree of structural variation (*17*).

Flavobacterium heparinum is a soil isolate (*121*) found to be capable of utilizing heparin as a sole carbon and nitrogen source by producing a heparin lyase. *Flavobacterium heparinum* is a small (approximately 1×0.3 μm rod), yellow, nonmotile, nonsporeforming, Gram-negative, facultative anerobe (*122*). Heparinase activity has also been observed in several *Bacteroides sp.* (rumen isolates), including *B. melaninogenicus, B. oralis,* (*123*), *B. ovatus, B. thetaiotaomicron* (*89,124*), and others (*89*), although this activity has not been measured by assays capable of distinguishing between lyases and hydrolases. Although it is likely that all these heparinases are eliminases, *F. heparinum* is the only organism established as producing an eliminase acting on heparin.

Heparin lyase (EC 4.2.2.7) is an inducible cell associated enzyme produced by *F. heparinum.* The kinetics of its induction, as well as the regulation of its synthesis, has been extensively studied (*122,125*). Although heparinase is induced by heparin (or a heparinase derived heparin oligosaccharide or metabolite), it is produced constituitively at low levels and can be derepressed by the removal of sulfate from the culture medium (*125*). The synthesis of this enzyme shows only weak carbon and nitrogen catabolite repression (*125*).



Scheme 6. Heparin (sodium salt).

Heparinase is a cell associated enzyme, probably residing in the periplasmic space (*122,125*). Disruption of the cells obtained from the fermentation broth is usually accomplished by sonication, homogenization, or repeated freeze–thawing, and the contaminating nucleic acids are precipitated with protamine. Hydroxylapatite, followed by cellulose-phosphate chromatography, separates heparinase from the other flavorbacterial eliminases (*126–128*). Affinity chromatography using heparin–Sepharose is ineffective (*129*); however, dermatan sulfate-coated dermatan sulfate–Sepharose has been successfully used to purify heparinase (*128*). Heparinase has been recently purified to homogeneity by hydroxylapatite, chromatofocusing, and gel-permeation chromatography (*130*). Polyclonal antibodies prepared against this homogeneous enzyme have also been used in its affinity purification (*131*).

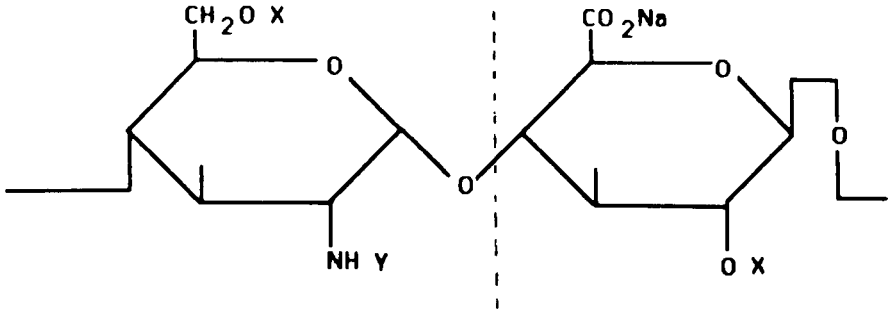
The properties of purified heparinase have been well characterized (*130*). Heparinase is a monomeric, cationic (pI = 8.5) protein of mol wt 42,800, containing a large number of lysine residues (10%). Heparinase is most stable at <30°C, pH 6.5, and an ionic strength of 0.3M. It is sensitive to mercaptoethanol and mercury (II) salts, yet it contains only four cysteine residues. Heparinase displays a maximum activity at 37°C, pH 6.5, and an ionic strength of 0.08M. Although calcium and other divalent cations have been reported to activate heparinase (*126*) and are generally included in assay buffers, others fail to detect this activation (*132*) and have even used EDTA buffer to assay heparinase activity (*127*). Heparinase has an activation energy of 4.5 kcal/mol, a K_m of $8 \times 10^{-6}M$, and a V_{max} of $4 \times 10^{-7}M$/min (*130*). Several synthetic polyanions, including polyvinyl sulfate ($K_i = 3 \times 10^{-8}M$), act as heparinase inhibitors; no other glycosaminoglycans or heparin fragments inhibit heparinase (*129*). When its polyclonal antibodies are added to heparinase, it is activated, rather than inhibited (*131*). Heparinase cleaves specifically at heparin's most frequently occurring linkage: →4)-2-deoxy-2-sulfamino-α- D-glucopyranose-6-sulfate-(1→4)-α-L-idopyranosyluronic acid-2-sulfate-(1 → [**6**] (*133,134*). A tetrasaccharide possessing this linkage is heparinase resistant (*135*), but not a heparinase inhibitor, suggesting a minimum substrate size is required for binding (*134*). This linkage is also present in heparan monosulfate, to about 25% (*136*) of that occurring in heparin, thus making this polysaccharide also a substrate.

Heparan Lyases

Heparan is usually isolated as a byproduct in the manufacture of the anticoagulant drug heparin (*137*). Its structure [**7**] is similar to heparin's [**6**] (*138*). Heparin and heparan glycosaminoglycans are each derived from proteoglycans having core proteins with different sequences (*4*). Heparan is a major component of the extracellular matrix (*4*) and is polydisperse, having an average mol wt of 50,000 when obtained from bovine aorta (*77*).

HEPARAN MONOSULFATE

(Sodium Salt)



Scheme 7. X = SO Na or H; Y = $COCH_3$, SO_3 Na or H.

The only organisms reported to produce heparan lyases or heparitinases is *F. heparinum* (*126,136*). Two heparitinases, with differing specificities, described as heparitinase 1 and 2, have been reported (*139,140*). Heparitinase production is induced by heparin or heparan (*126,139*). Little is known about the regulation of heparitinase production, but it may be under the same control as heparinase (*125*).

Heparitinases are cell-associated enzymes and require cell disruption before purification (*126*). Following sonication and acetone precipitation, purification by electrophoresis (*136,140*) has afforded two cleanly resolved heparitinases; heparitinase 1 and 2 (*140*). Purifications, using hydroxylapatite chromatography, followed by chromatography a second time on hydroxylapatite or phosphocellulose, result in the isolation of only heparitinase 1 (*126,128*). Further purification by affinity chromatography on heparin-coated heparin–Sepharose yields a highly purified preparation of heparitinase 1 (*128*). It appears that neither of these heparitinase preparations contain any heparitinase 2 (*126,128*).

No molecular weight has been reported for either heparitinase 1 or 2. By examining the electrophoretic separation of the heparitinases and heparinase on agarose at pH 7 (*140*), both heparitinases appear to be basic proteins migrating with flavobacterial heparinase (p*I* = 8.5) (*130*). Heparitinases 1 and 2 have temperature and pH activity optima of 45°C and pH 6–7 and 8–8.5, respectively (*140*). Both heparitinases are almost completely inhibited at an ionic strength of 0.2*M*. Divalent cations, such as calcium, do not enhance the activity of either heparitinase, whereas iron (II) inhibits both and zinc (II) inhibits only heparitinase 2 (*140*). The major distinction between these two enzymes appears to lie in their linkage specificity. Heparitinase 1 cleaves heparan [7] at the →4)-2-deoxy-2-acetamindo-α-D-glucopyranose-6-(sulfate or hydroxyl)-(1→4)-β-D-

glucopyranosyluronic acid-(1→ linkage (*128,133,140*). Heparitinase 2 reportedly cleaves heparan [7] at the →4)-2-deoxy-2-sulfamino-α-D-glucopyranosluronic acid-(1→ linkage (*140*). Heparitinase is reported to show no activity against heparin (*126,136*), even though heparin contains linkages corresponding to those defining heparitinase specificity, this may be because of the inhibition of heparitinase by heparin.

LYASES ACTING ON MICROBIAL POLYSACCHARIDES

The production of microbial acidic polysaccharides and their metabolism is not well characterized. Microbial alginate and its depolymerization by microbial alginase is discussed above. A number of other acidic polysaccharides of microbial origin are known, but because of their diversity and complex structure, research directed at their enzymatic depolymerization is only in its infancy. One such microbial polysaccharide, emulsan [8], is typical of complex acidic microbial polysaccharides, in that it performs a specialized function as a bioemulsifier. Emulsan and a second acidic polysaccharide, as well as two lyases that act on these polymers, are discussed below in detail.

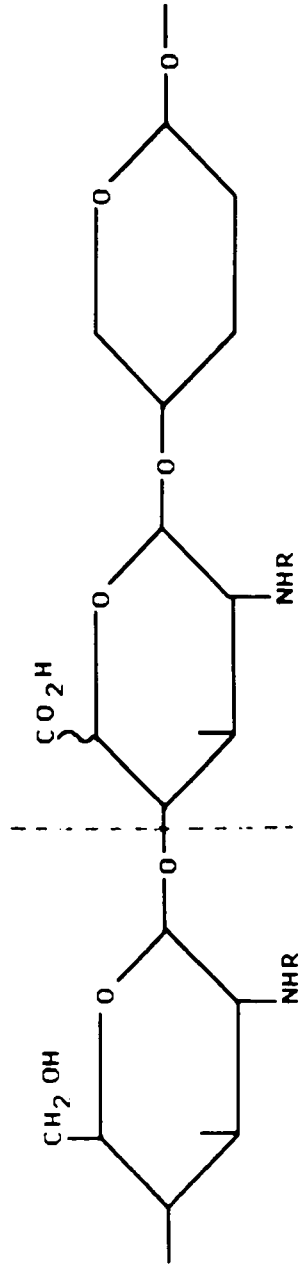
Emulsan Lyase

Emulsan [8] is a bioemulsifier produced by *Acinetobacter calcoaceticus* while growing on hydrocarbons (*8,141*). Emulsan consists of a linear heteropolysaccharide backbone comprised of D-galactosamine, an aminouronic acid, and a third unidentified sugar (*141*). Fatty-acid side chains are joined to this backbone through *O*-acetyl and *N*-acyl linkages; in addition there is also some associated protein.

Emulsanase activity has been obtained in a soil isolate tentatively classified as a *Zoogloea* species (*142*). This extracellular lyase is induced by emulsan. After recovery from the fermentation supernatant, it was partially purified by ammonium sulfate precipitation and its mol wt estimated at 89,000 by gel-permeation chromatography. This enzyme is an eliminase, cleaving emulsan (mol wt 330,000) into unsaturated oligosaccharides (average mol wt 3000) in an endolytic fashion. Emulsanase may have a minimum substrate size requirement (*142*).

Acidic Heteropolysaccharide Lyase

Capsular and excreted extracellular acidic polysaccharides of *Rhizobium trifolii* are degraded by bacteriophage-induced acidic heteropolysaccharide lyases (*143*). The acidic oligosacchairde products obtained were comprised of glucose, galactose, glucuronic acid, and 4-deoxy-L-*threo*-hex-4-enopyranosyluronic acid (this could be derived from either glucuronic or iduronic acid) in a ratio of 5:1:1:1. The two lyases isolated gave very high molecular weights by gel-permeation chromatography



Scheme 8. Emulsan; R = fatty acid side chain.

under nondenaturing conditions (Table 2). One of these lyases acts endolytically and the other exolytically, and both are deactivated by treatment with EDTA and reactivated by the addition of calcium.

GENERAL CHARACTERISTICS OF LYASES AND THEIR PRODUCTION

General Lyase Properties

Lyases (Table 2) are enzymes of low to intermediate molecular weight (20,000–110,000) and are characteristically monomeric, giving the same molecular weight when measured under reducing and nonreducing conditions. Amino acid analysis has been performed on chondroitinases, heparinases, hyaluronidases, and pectate lyases; several of these enzymes have been found to be glycoproteins. Most lyases are cationic, with the notable exceptions of *Strepcoccocal* hyaluronidases (*105*) and a pectate lyase from *E. chrysanthemi* (*46,70*) and one from *S. nitrosporeus* (*71*). Most polysaccharide lyases have activity optima above pH 6, with the hyaluronidases generally having the lowest, and the pectate lyases the highest, pH optima. Most lyases act endolytically and have no minimum size requirement for substrate. The exceptions to this rule are the pectate lyases. Oligogalacturonate lyase acts exolytically on small substrate molecules and exopectate lyase on larger substrate chains. An absolute calcium requirement is present only in the pectate lyases, although other lyases all seem effected to a lesser degree by the presence and concentration of divalent cation, some being inhibited and others stimulated. These observations can be rationalized by proposing two distinct effects: divalent calcium acting as a cofactor for the enzyme and divalent metal binding to the acidic polysaccharide substrate, either enhancing or reducing its susceptability to enzymatic attack.

As discussed earlier, lyases can act by two eliminative mechanisms (Fig. 2). A concerted (E2) elimination is possible only when the leaving groups are in an anti (diaxial) relationship. Alternatively, a stepwise elimination, possibly involving a carbanion intermediate, might occur when the leaving groups are in an axial–equatorial relationship. Because of the very different nature of these mechanisms, it would be unexpected to see an enzyme that acted on substrates having both diaxial and axial–equatorial orientations. The only lyases that act at both such linkages are the chondroitinase ABC enzymes. These enzymes are larger than most lyases, having mol wt $> 100{,}000$. Although they are monomeric, their larger size suggests the presence of multiple catalytic domains. Detailed kinetic studies on the action of these enzymes on chondroitin sulfate B (diaxial orientation) and on chondroitin sulfates A and C (axial–equatorial orientation) might shed some light on this question.

Organism Types Producing Lyases and Their Characteristics

Organisms that produce polysaccharide lyases would be expected to have access to their polysaccharide substrates or to other smaller molecules bearing the appropriate linkage region (*144*). As the examples above indicate, these organisms can be subdivided into pathogenic and nonpathogenic microbes, and certain properties are associated with each of these groups. The pathogenic organisms tend to produce constituitively and are generally not under catabolite regulation. These organisms often produce isoenzymes capable of acting in a concerted fashion (*145*) on the acidic polysaccharide substrate under a variety of pH conditions and are often accompanied by the corresponding hydrolases (*144*). Finally, enzymes from pathogenic organisms are often extracellular in nature. The nonpathogenic organisms (*146*) most often fall into three classes: (1), soil isolates, (2), isolates from infected plant and animal tissue, and (3), isolates from the gut or rumen. These locations all have one property in common, a large concentration of substrate. Hence, it appears that these organisms are using their enzymes primarily to release carbon sources for growth rather than to invade tissue. In addition, the nonpathogenic organisms generally grow better on their acidic polysaccharide substrate as a sole carbon source than do the pathogens (*19,56,58*). Nonpathogens (*146*) produce cell-associated enzymes (Table 2), often localized in the periplasmic space, permitting them to keep the oligosaccharide products to themselves rather than spilling them out to their environment. Many of these nonpathogenic organisms also produce multiple lyases, each acting on closely related acidic polysaccharides, whereas pathogens produce a large number of isoenzymes, all acting on the same substrate (Table 2). This widens the niche of these nonpathogenic organisms. Finally, nonpathogenic organisms generally do not produce polysaccharide hydrolases, as do their pathogenic counterparts (*144*).

Although polysaccharide lyases are predominantly a bacterial product (Table 2), fungal lyases have been found, and these are particularly responsible for the depolymerization of plant polysacchardies (*144*). Like their bacterial counterparts, these fungi include both pathogenic and nonpathogenic organisms, but unlike the broad range of bacteria, fungal lyase production is limited (*144*) to only two classes of higher fungi. All of the fungal lyases are characteristically extracellular enzymes.

In addition to microbial sources, lyases of viral and animal origins have recently been reported. A not-unexpected viral source of lyase is a bacteriophage that infects a *Streptococci* species that produces a hyaluronidase (*119*). By contrast, bee (*108*) and wasp venom (*109*) hyaluronate lyases are entirely unexpected and as yet unconfirmed. The isolation of hepatopancreatic alginate lyases from shellfish, although originally ascribed to the presence of contaminating bacterial lyase production, has now apparently been confirmed (*29*). Further research will establish whether this and other nonmicrobial lyase production is widespread.

Why do microorganisms cleave polysaccharides eliminatively rather than via hydrolysis, the preferred mechanism in higher animals? The reasons are not entirely clear, requiring some speculation. Unsaturated oligosaccharide residues, formed by eliminative polysaccharide cleavage, are Michael receptors and subject to nucleophilic attack (*147*). This reactivity may cause toxicity problems in higher animals. In addition, these unsaturated sugars would require additional enzymes (*63*) to obtain metabolites capable of entering the carbon pool. This added set of enzymes might have to be constituitive to prevent the buildup of potentially toxic unsaturated sugars, and maintaining these enzymes might prove costly to higher animals. From the microbial side, the use of eliminative cleavage in preference to hydrolysis might actually be favorable. Many lyase-producing organisms grow on acidic polysaccharides as a sole carbon source. The inability of higher animals to efficiently metabolize these unsaturated sugars (*148*) might reduce competition for this carbon source. Plants recognize these unsaturated sugars as foreign and respond by mounting a defense. These unsaturated sugars of microbial origin can act as elicitors that induce plant production of low molecular weight antimicrobial agents called phytoalexins (*55*). Bacteria producing hyaluronate lyase are frequently animal pathogens. Even though hyaluronate lyase is not very antigenic (*120*), antibodies have been prepared to the unsaturated carbohydrate products of this and other lyases (*149*). This may represent a possible defense mechanism against microbial invasion of extracellular matrix. The lyase mechanism might also provide advantages from the growth environment of microorganisms. Both chemical and enzymatic eliminative cleavage is typically base catalyzed, whereas hydrolysis occurs predominantly through acid catalysis. As a microorganism ferments acid polysaccharides, the culture pH increases (*59*). This increasingly basic environment favors eliminative cleavage. For example, pectate lyases have their pH optima around 8.5 and pectate hydrolases have theirs at pH 4.5 (Table 2). Most lyases have basic pH optima and most are extracellular or periplasmic, indicating that these enzymes have been adapted for their environment. Lastly, lyase cleavage of acidic polysaccharides might simply be the lower energy pathway. Within the active site of a hydrolytic enzyme, an enzyme must accommodate the acid polysaccharide and the water substrate molecule. The tight, highly ordered association between the acidic polysaccharide and water might interfere with its addition to the glycosidic linkage.

Potential New Sources of Polysaccharide Lyases

Other acid polysaccharides may potentially serve as substrates for as yet undiscovered lyases. A number of highly branched polysaccharides containing a mixture of acidic and neutral sugars are produced in abundance by plants (*2*). Gums, which provide protection to plants against microbial infection, include gum arabic, ghatti, and tragacanth; all con-

tain a substantial quantity of glucuronic acid. Additionally, plant and algal mucilages also contain glucuronic or galacturonic acids. Although these polymers are more highly branched than pectin and possess only a fraction of the acidic saccharide units present in either pectin, pectate, or alginate, they may still serve as suitable substrates for lyases. Lyases have been found that act on most known classes of acidic polysaccharides from animal sources, including all of the glycosaminoglycans, but there are regions within these polysaccharides that are resistant to the action of the known lyases. Such regions include the "over-sulfated" sequences in chondroitin sulfates (i.e., chondroitin sulfate D) (*7*) and certain sequences in heparin (*17,138*). It is on these specific sites that new polysaccharide lyases might be found to act. Polysaccharides produced by microorganisms (*6*) are by far the least well characterized, and it is against these substrates that the largest number of new types of polysaccharide lyases might be found. Extracellular alginic acid from *A. vinelandii*, comprised predominantly of partially acetylated mannuronic acid, has been shown to be a substrate for alginase (*19*). Similarly, other extracellular microbial polysaccharides, such as Xantham gums (branched polysaccharides containing glucuronic acid) (*2*), might also act as a substrate for a yet undiscovered polysaccharide lyase. Other sources are bacterial lipopolysaccharides, such as emulsan (*8*), discussed above, or those found in enteric bacteria that contain glucuronic and galacturonic acids (*2*). Finally, capsular polysaccharides (*6,144*) possess unusual aminouronic acid residues (*2*) that should be susceptable to eliminative cleavage.

In addition to searching for microbial lyase activity, in light of recent reports of a mollusk aliginate lyase (*29*), it might be wise to extend the search to other animal sources. Reports of a lobster-produced heparan monosulfate (*150*), as well as a clam heparin (*151*), suggest that these shellfish might have metabolic lyases similar to those found in the mollusk, which act on their own acidic polysaccharides. A reexamination of a reported, albeit unconfirmed, bee venom hyaluronate lyase (*108*) may also be warranted.

CONCLUSIONS

Lyases are a class of enzymes that act on a wide variety of naturally occurring acidic polysaccharides. These enzymes act eliminatively by either concerted or stepwise mechanisms to produce unsaturated oligosaccharides. Lyases are primarily produced by microbes and most commonly by bacteria. A wide variety of both pathogenic and nonpathogenic bacteria and fungi produce lyases. Pathogens produce lyases to assist their invasion of plant and animal tissues, whereas nonpathogenic microbes appear to use lyases to prepare a carbon source that they are capable of assimilating. New potential sources of lyases include microbes

growing on complex acidic polysaccharides, as well as from animal tissue. The mechanism, sources, production, and uses of this class of enzyme represent an area ripe for further investigation.

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