Studies on the Induction of Heparin-Degrading Enzymes in *Flavobacterium heparinum**

Carl P. Dietrich

ABSTRACT: Five enzymes from *Flavobacterium heparinum* acting in concert are able to degrade heparin to monosaccharides.

These enzymes have been identified as: a glucosaminidase able to degrade heparin to sulfated disaccharides and oligosaccharides; a glycuronidase which hydrolyzes the disaccharides to glucosamine 2,6-disulfate; a sulfamidase and a sulfoesterase able to remove sulfates from the N and O positions of glucosamine 2,6-disulfate and another sulfoesterase able to partially desulfate one of the disaccharides. The glucosaminidase, glycuronidase, and sulfamidase were induced when the bacteria were grown in the presence of heparin. Low activities of these enzymes could also be detected when the bacteria were grown in the absence of heparin. All three enzymes were also induced in the bacteria with the sulfated di-, tetra-, and hexasaccharides derived from heparin. Glucosamine *N*-sulfate and glucosamine 2,6-disulfate on the other hand were very poor inducers of these enzymes. Time curve studies on the induction of this enzymic system suggested that the sulfated disaccharides were the actual inducers. The induction by the tetrasaccharides, hexasaccharides, and heparin showed a lag period of about 1 hr, suggesting that these compounds were first hydrolyzed to disaccharides by enzymes present in low levels of activity in noninduced *Flavobacterium*. Actinomycin D, mitomycin C, and chloramphenicol were potent inhibitors of this induction process.

A *Flavobacterium* capable of degrading heparin was isolated from soil by Korn and Payza (1956). These authors observed that the bacteria had to grow in a medium containing heparin in order to induce the heparin-degrading system. It was soon demonstrated that this *Flavobacterium* contained also constitutional enzymes able to degrade other mucopolysaccharides such as chondroitin sulfate and hyaluronic acid without previous adaptation to these mucopolysaccharides (Hoffman *et al.*, 1957). These observations led Korn to hypothesize that heparin would induce a sulfamidase which would be responsible for the removal of sulfates from *N*-sulfate groups of heparin rendering the molecule susceptible to the action of the other mucopolysaccharide-degrading enzymes normally present in the bacteria (Korn, 1957). The isolation and characterization of several degradation products of heparin by degradation with adapted cells of *Flavobacterium heparinum* by Dietrich (1968a) suggested a sequential breakdown of heparin in which several enzymes were involved. Using the degradation products as substrates it was possible to demonstrate the presence of five enzymes in the *Flavobacterium* extracts which degraded heparin to monosaccharides. These enzymes were characterized as follows: a glucosaminidase which degrades heparin to sulfated oligosaccharides and disaccharides; a glycuronidase able to degrade the sulfated dissacharides to glucosamine 2,6-disulfate[1] and uronic acid; a sulfamidase and a sulfoesterase which desulfate glucosamine 2,6-disulfate to glucosamine. A second sulfoesterase able to desulfate partially the trisulfated disaccharide was also identified (Dietrich, 1968b, 1969a,c). The isolated sulfamidase was highly specific for glucosamine 2,6-disulfate with little or no activity upon heparin and other oligosaccharides. These results indicate that the induction of only a sulfamidase would not promote the degradation of heparin (as suggested by Korn) to the products obtained by Dietrich. Since the sulfamidase is specific for glucosamine 2,6-disulfate and since the constitutive enzymes of *F. heparinum* do not hydrolyze the native heparin molecule, one must suppose that the enzymes responsible for the breakdown of heparin to glucosamine 2,6-disulfate are also inducible.

The present paper describes the induction of an enzymic system in *F. heparinum* for the degradation of heparin. A study of the mechanism of induction of this enzymic system as well as the characterization of the inducer will be presented. A preliminary account of this work has been presented (Dietrich, 1969b).

Materials and Methods

F. heparinum (ATCC 13, 125) was grown in trypticase soy broth without dextrose media (27 g/l.) containing heparin (150 mg/l.). After growth for 36 hr at room temperature the cells were harvested and washed twice with 0.02 M potassium phosphate buffer (pH 7.0) and lyophilized. These will be referred to as induced cells. For the preparation of noninduced cells the bacteria were grown in the same media in which heparin was substituted by 0.25% glucose. The cells obtained after harvesting were lyophilized. In some instances the cells grown in glucose were used immediately after washing for induction studies.

Induction of Enzymes in F. heparinum. For small-scale experiments in the induction of heparin-degrading enzymes the

* From the Department of Physiology and Pharmacology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada. *Received March 7, 1969.* Financial assistance was provided by grants to the author and to Professor L. B. Jaques from the Medical Research Council of Canada.

[1] The abbreviations used are: glucosamine *N*-sulfate, 2-deoxy-2-sulfoamino-D-glucose; glucosamine 2,6-disulfate, 2-deoxy-2-sulfoamino-D-glucose 6-*O*-sulfate; disulfated disaccharide, uronyl-2-deoxy-2-sulfoamino-D-glucose 6-*O*-sulfate; trisulfated disaccharide, 3-sulfouronyl-2-deoxy-2-sulfoamino-D-glucose 6-*O*-sulfate.

TABLE I: Glucosaminidase and Glycuronidase ("Heparinases") Activities in the Supernatant of 100,000*g* from Induced and Noninduced Cells.[a]

Source of Enzymes	Substrate	Products Formed (cpm)	
		Disaccharide	Glucosamine 2,6-Disulfate
Induced cells	Disulfated disaccharide		1906
	Trisulfated disaccharide		4165
	Tetrasaccharide	652	1853
	Heparin	1771	3729
Noninduced cells	Disulfated disaccharide		255
	Trisulfated disaccharide		221
	Tetrasaccharide	33	194
	Heparin	110	40

[a] Supernatants (50 μg) of 100,000*g* centrifugation prepared from induced and noninduced cells were incubated with about 50,000 cpm of *N*[$^{35}SO_4$]heparin, di- and tetrasaccharides in 1 mM $MgCl_2$–0.02 M phosphate buffer (pH 7.0) for 3 hr at 25° in a final volume of 20 μl. The reactions were stopped by heating, spotted on Whatman No. 1 paper, and chromatographed in isobutyric acid–1 M NH_3 solvent (5:3, v/v) for 36 hr. The products formed were localized by radioautography and counted in a liquid-scintillation spectrometer. The formation of disaccharides from the tetrasaccharide and heparin is due to the action of the glucosaminidase and the formation of glucosamine 2,6-disulfate from the disaccharides is due to the action of the glycuronidase.

following method was used. The cells were grown for 36 hr in dextrose as described above, washed three times with fresh trypticase soy broth without dextrose, and resuspended in a media containing 10 mg of trypticase soy broth minus dextrose, 10 mg of casein hydrolysate, and 5 mg of yeast extract in 10 ml of 0.02 M phosphate buffer (pH 7.0). A suspension of about 20 mg (wet weight) of cells/ml was prepared; 1-ml aliquots were then immediately incubated with heparin or other substances at room temperature for different periods to induce the heparin-degrading enzymes. After incubation the cell suspensions were diluted to 10 ml with 0.02 M phosphate buffer (pH 7.0) and harvested. After three washes the cells were lyophilized, resuspended in 100 μl of 0.02 M potassium acetate buffer (pH 7.0), and incubated with the substrates as described below.

Preparation and Assay of Enzymes. Lyophilized cells (100 mg) were resuspended in 10 ml of 0.02 M sodium acetate buffer (pH 7.0) and treated ultrasonically for 5 min in a Bronwill Sonicator with cooling. The suspension was then diluted to 30 ml with the same buffer and centrifuged at 10,000*g* for 15 min. The supernatant obtained was again centrifuged to 100,000*g* for 1 hr. The pellet formed was washed twice with potassium acetate buffer (0.02 M) and resuspended in 2 ml of the same buffer. The supernatant of 100,000*g* was lyophilized. All the operations were carried out at 3°. In some instances lyophilized cells were used without sonication. These preparations are able to degrade heparin to the same extent as the ones obtained after sonication (Dietrich, 1968a). The incubation mixtures containing the enzymes and heparin or other substrates were analyzed by chromatography in isobutyric acid–NH_3 solvent and electrophoresis as described previously (Dietrich, 1969a,c).

Chemicals. *N*[$^{35}SO_4$]Heparin was obtained from Calbiochem (Los Angles, Calif.) with a specific activity of 0.68 mCi/g. *N*-$^{35}SO_4$-Labeled glucosamine 2,6-disulfate, sulfated disaccharides, tetrasaccharides, and hexasaccharides were prepared from *N*-$^{35}SO_4$-labeled heparin by degradation with crude enzymes of *F. heparinum* as previously described for nonradioactive products (Dietrich, 1968a). The radioactive measurements were corrected for the normal decay of ^{35}S (half-life 88 days). Sulfated heparin, chondroitin sulfate A/C, starch sulfate, and sodium mannuronate were materials supplied by the late Dr. A. Winterstein, Hoffman-LaRoche AG, Basel, Switzerland. Dermatan sulfate was a preparation purified by Dr. R. Pearce, University of British Columbia, Vancouver, B. C., from material supplied by Dr. Winterstein. Heparitin sulfate was a preparation purified by Dr. R. Pearce from material supplied to us by Dr. J. T. Correll, Upjohn Co., Kalamazoo, Mich. Rhamnose was given by Dr. D. E. Eveleigh, Prairie Regional Laboratory, Saskatoon, dextran sulfate by Meito-Sangyo Co., Japan, and oxidized cellulose by Tennessee Eastman Corp.

Other Methods. Protein was measured by the method of Lowry *et al.* (1951). Reducing sugars were detected on thin-layer and paper chromatography by $AgNO_3$ reagent and oligosaccharides and heparin by the toluidine blue reagent as previously described (Dietrich, 1968a).

Results

Glucosaminidase and Glycuronidase ("Heparinases") Activities in Induced and Noninduced Cell Extracts. The radioactive disaccharides, tetrasaccharides, and heparin were incubated with the supernatant of 100,000*g* centrifugation obtained from *F. heparinum* grown in the presence and absence of heparin. The results obtained are shown in Table I. The glycuronidase which hydrolyses tri- and disulfated disaccharides to glucosamine 2,6-disulfate and uronic acid is present in hign amounts only in the supernatant of 100,000*g* prepared from induced cells. Low levels of activity were detected in the noninduced supernatant. Table I shows that heparin is extensively degraded

TABLE II: Sulfamidase and Sulfoesterase Activities from Precipitate and Supernatant of 100,000*g* from Induced and Noninduced Cells.[a]

Source of Enzymes	Products Formed from Glucosamine 2,6-Disulfate (cpm)	
	Glucosamine *N*-Sulfate	Inorganic Sulfate
Supernatant from induced cells	2610	2710
Pellet from induced cells	3700	5340
Supernatant from noninduced cells	1765	80
Pellet from noninduced cells	330	184

[a] Supernatant (50 μg) and pellet from 100,000*g* centrifugation from induced and noninduced cells were incubated with 40,000 cpm of *N*[$^{35}SO_4$]glucosamine 2,6-disulfate in 1 mM $MgCl_2$–0.04 M potassium acetate buffer (pH 7.0) for 1 hr at 25° in a final volume of 20 μl. The reaction mixtures were heated after incubation for 1 min at 100°, spotted on Whatman No. 3MM paper, and subjected to electrophoresis in 0.3 M pyridine–acetate buffer (pH 4.5) at a potential gradient of 10 V/cm for 3 hr. The products formed were localized by radioautography and counted. The formation of radioactive glucosamine *N*-sulfate is the result of the sulfoesterase activity and the formation of radioactive inorganic sulfate is due to the activity of the sulfamidase.

TABLE III: Recombination of Enzyme Fractions from Induced and Noninduced Cells.[a]

Conditions	Inorganic Sulfate Produced from (cpm)	
	Heparin	Glucosamine 2,6-Disulfate
Pellet from induced cells	429	5340
Pellet from induced cells + supernatant of noninduced cells	870	4815
Supernatant of noninduced cells	275	233
Pellet from induced cells + supernatant of induced cells	5650	6800
Supernatant of induced cells	1210	1150

[a] This experiment was performed as described in Table II with 50 μg of each protein fraction and 50,00 cpm of each substrate in a final volume of 30 μl. The radioactive inorganic sulfate formed was isolated by electrophoresis.

to disaccharides only in the presence of the enzymes prepared from induced cells. The enzyme responsible for the degradation of heparin to these disaccharides is a glucosaminidase (Dietrich, 1968b, 1969c). The glucosamine 2,6-disulfate produced in this experiment reflects the activity of both glucosaminidase and glycuronidase. The results thus indicate that the glucosaminidase and the glycuronidase are induced enzymes. Noninduced cells have a relatively low level of activity of these two enzymes and induction by growing these cells in the presence of heparin promotes about 20-fold increase in these activities.

Sulfamidase and Sulfoesterase Activities in the Supernatant and Precipitate of 100,000g Induced and Noninduced Cells of F. heparinum. Table II shows the amount of glucosamine *N*-sulfate and inorganic sulfate formed from *N*[$^{35}SO_4$]glucosamine 2,6-disulfate by the action of the sulfatases. The formation of radioactive inorganic sulfate is due to the action of the sulfamidase. This activity is practically absent from the supernatant as well as the precipitate of 100,000*g* noninduced cells. The sulfoesterase activity on the other hand is present in the 100,000*g* supernatant of both adapted and nonadapted cell extracts. An interesting observation is that the sulfoesterase is almost absent from the pellet of nonadapted cell extracts. The significance of this finding has not yet been established.

Recombination of Enzymes from Induced and Noninduced Cells from F. heparinum. The pellet prepared from induced cells containing the sulfamidase degrades heparin to inorganic sulfate only to a small extent when compared with the action of this enzyme upon glucosamine 2,6-disulfate (Table III). Korn (1957) has suggested that partial N desulfation of the heparin molecule could render it susceptible to the action of the constitutional mucopolysaccharidases. In order to verify whether the sulfamidase stimulates the breakdown of heparin by these constitutional enzymes from noninduced cells this enzyme was combined with extracts of these cells. Table III shows that no increase in the breakdown occurred by this recombination. The controls (nonadapted supernatant and adapted precipitate alone) show that the slight increase observed is only due to additive effects of the two enzyme systems brought together. When the reconstitution is made with both supernatant and precipitate of adapted enzymes the release of inorganic sulfate is comparable with the release of inorganic sulfate from glucosamine 2,6-disulfate. Oligo- and disaccharides are formed in the sequential degradation of heparin to glucosamine 2,6-disulfate only when induced supernatant alone or in combination with the pellet of induced cells is used. Only a small amount of these compounds could be detected when the supernatant of noninduced cells was incubated with heparin. These results rule out the possibility of breakdown of partially N-desulfated heparin (if it is formed by the action of the sulfamidase) by constitutional enzymes.

Induction of Heparin-Degrading Enzymes by Heparin and Structurally Related Compounds. In order to verify what parts of the heparin molecule are essential to promote the induction of heparin-degrading enzymes in *F. heparinum*, compounds structurally related to heparin were tested. The results are summarized in Tables IV and V. Among the mucopolysaccharides tested only heparitin sulfate, sulfurated heparin, and heparin are capable of promoting significant induction of the heparin-degrading enzymes. The compounds presented in Table V are those obtained by degradation of heparin by induced enzymes from *F. heparinum*. The di-, tetra-, and hexasaccharides have induction properties similar to heparin while the sulfated monosaccharides are very poor inducers. A very important observation derived from these experiments is that substrates of the sulfamidase, *i.e.*, glucosamine *N*-sulfate and glucosamine 2,6-disulfate, do not promote any considerable induction of this

TABLE IV: Induction of "Heparinases" in *F. heparinum* by Several Mucopolysaccharides and Disaccharides.[a]

Inducer	"Heparinases" Act. (cpm/mg of protein per hr)
Heparin	1040
Heparitin ulfate	1258
Sulfurate heparin	1473
Dermata . sulfate	276
Chondro.tin sulfate A/C	341
Hyaluronic acid	324
Dextran sulfate	276
Starch sulfate	411
Sodium mannuronate	566
Oxidized cellulose	415
Lactose	300
Maltose	280
Melizitose	295
Rhamnose	310
Glucose	270

[a] Each compound (100 μg) was incubated with 20 mg (wet weight) of *F. heparinum* cells (previously grown in glucose) in 1 ml of tripticase soy broth–casein–yeast extract media for 20 hr at room temperature. The cells were washed three times with 0.02 M phosphate buffer (pH 7.0) by centrifugation and resuspended in 100 μl of 0.02 M phosphate buffer; 10-μl aliquots were incubated with 60,000 cpm of $N[^{35}SO_4]$heparin in 1 mM $MgCl_2$–0.03 M phosphate buffer in a final volume of 20 μl. After 6-hr incubation at 25° 20 μl of phenol 80% was added and after 30-min periodical agitation, the water phase was separated by centrifugation and 10 μl applied to Whatman No. 1 paper. The radioactive glucosamine 2,6-disulfate formed was analyzed by chromatography as described in Table I. This compound was considered to be the end product of the combined activities of the glucosaminidase and the glycuronidase ("heparinases").

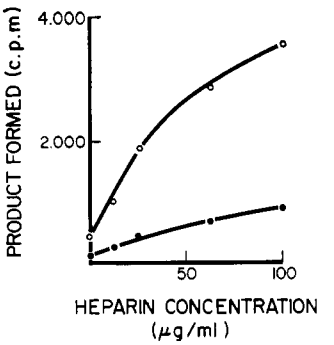

FIGURE 1: Effect of heparin concentration in the induction of the "heparinases" and the sulfamidase in *F. heparinum* cells. This experiment was performed as described in Table IV except that the cells were induced with increasing concentrations of heparin as indicated. After induction and lyophilization the cells were incubated with $N[^{35}SO_4]$heparin and $N[^{35}SO_4]$glucosamine 2,6-disulfate. The $N[^{35}SO_4]$disaccharides and glucosamine 2,6-disulfate formed from heparin ("heparinases" activities; O—O) and inorganic $^{35}SO_4$ formed from glucosamine 2,6-disulfate (sulfamidase activity; ●—●) were analyzed as described in Tables I and II.

enzyme. Conversely the disaccharides are potent inducers of both heparinases although not a substrate for one of these enzymes, *i.e.*, the glucosaminidase. Table IV also shows that disaccharides with α or β linkages are unable to induce any of these enzymes.

Kinetics of Induction of the "Heparinases" and the Sulfamidase in F. heparinum by Heparin, Oligosaccharides, and Disaccharides. The induction of the "heparinases" and the sulfamidase is dependent upon the concentration of heparin (Figure 1). The "heparinases" seem to be three to four times more active than the sulfamidase. The time course of induction of these enzymes is shown in Figure 2. When heparin is used as inducer there is a lag period of about 1 hr for the onset of induction of the "heparinases." During this period only low activities of the glucosaminidase, glycuronidase, and sulfamidase could be detected. In contrast when the disaccharide is used as inducer no lag period could be detected and all the three enzymes were induced by both disaccharides (see Figure 3). The sulfated monosaccharides, *e.g.*, glucosamine *N*-sulfate and glucosamine 2,6-disulfate, are very poor inducers for the three enzymes, while glucose has no inducing effect during the period used in this experiment. Figure 3 shows the kinetics of a long-term induction using all known degradation products derived from heparin as well as heparin. The lag period of induction of the heparinases by the hexasaccharide is more pronounced than the lag observed when heparin was used as inducer. The formation of induced enzymes levels off after about 4 hr of incubation with the inducers and then remains almost unchanged until 18 hr. Only in the presence of disulfated disaccharide the concentration of the induced enzymes drops after the 4th hr of incubation. This drop of activity could be due to the disappearance of the inducer from the medium. Another experiment performed with radioactive degradation

TABLE V: Induction of the "Heparinases" and Sulfamidase in *F. heparinum* by Heparin and Its Degradation Products.[a]

Inducer	Enzymic Act. (cpm/mg of protein hr)	
	"Heparinases"	Sulfamidase
Glucosamine 2,6-disulfate	300	66
Glucosamine *N*-sulfate	320	60
Disulfated disaccharide	1050	380
Trisulfated disaccharide	1905	690
Tetrasaccharide	1880	905
Hexasaccharide	2232	670
Heparin	1260	390
Glucosamine	225	64
Glucuronic acid	170	66
Glucose	214	84

[a] This experiment was performed as described in Table IV. Also 10,000 cpm of $N[^{35}SO_4]$glucosamine 2,6-disulfate was incubated with the induced cells for the test of the sulfamidase activity. The radioactive inorganic SO_4 formed was analyzed by electrophoresis as described in Table I.

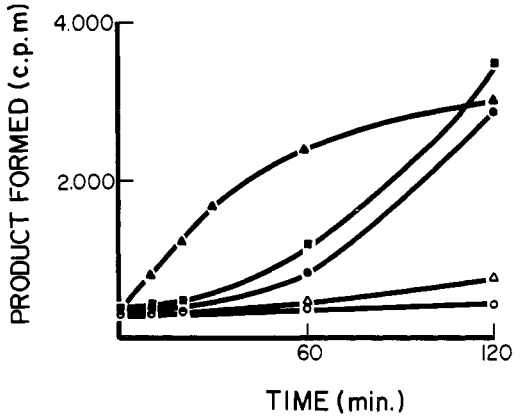

FIGURE 2: Short-term induction of the "heparinases" in *F. heparinum* by heparin and heparin degradation products. *F. heparinum* cells (30 mg wet weight) grown in glucose were incubated with 100 μg of heparin and degradation products in trypticase soy broth–casein-yeast extract media in a final volume of 1 ml; 100-μl aliquots were taken at the times indicated, diluted with 5 ml of cold 0.02 M phosphate buffer (pH 7.0), and harvested. After the third wash the cells were lyophilized. After lyophilization the cells were incubated with N[$^{35}SO_4$]heparin and the products formed analyzed as described in Table IV. The radioactive disaccharide and glucosamine 2,6-disulfate formed from cells induced with disaccharides (▲—▲), hexasaccharide (●—●), heparin (■—■), glucosamine 2,6-disulfate (△—△), and glucose (○—○) were analyzed by chromatography.

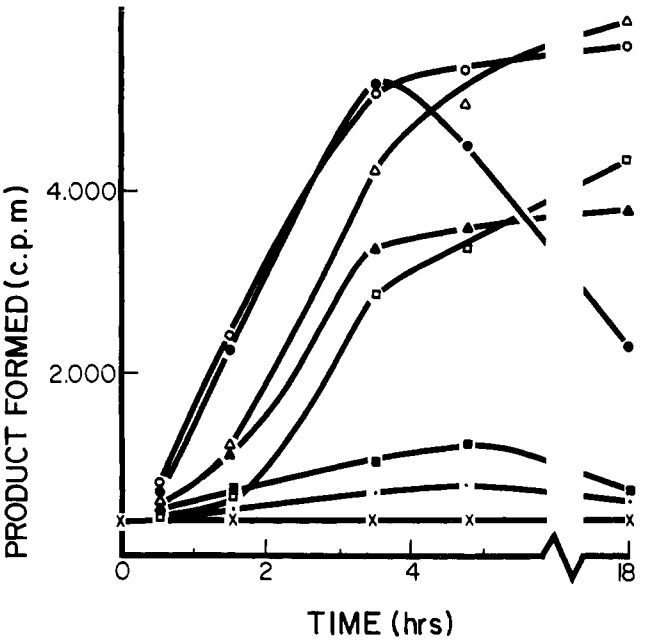

FIGURE 3: Long-term induction of the "heparinases" in *F. heparinum* by heparin and heparin degradation products. The conditions of this experiment were the same as the ones described in Figure 2 except that longer periods of induction and different degradation products were used as inducers. The following compounds were used as inducers: (○—○) trisulfated disaccharide, (●—●) disulfated disaccharide, (△—△) tetrasaccharide, (□—□) hexasaccharide, (▲—▲) heparin, (■—■) glucosamine 2,6-disulfate, (·—·) glucosamine N-sulfate, and (×—×) glucose.

products indicated that after 18-hr induction the disulfated disaccharide was completely degraded whereas about 20% of the other inducers was still in the media.

Effect of Antibiotics in the Induction of the "Heparinases" in F. heparinum with Heparin. Mitomycin C, actinomycin D, and chloramphenicol inhibit the induction of the glycuronidase and the glucosaminidase to the extent of about 65% at a concentration of 20 μg/ml as shown in Table VI. Puromycin and penicillin have only a slight effect on the induction of these enzymes, at this concentration. These results strongly indicate that protein synthesis and RNA synthesis are involved in the induction of these enzymes. The lack of effect of puromycin is not easily understood. It is possible that this compound might complex with heparin thus losing its activity as inhibitor of protein synthesis while the concentration of noncomplexed heparin is still enough to promote the induction.

TABLE VI: Effect of Antibiotics in the Induction of the Glucosaminidase and Glycuronidase by Heparin in *F. heparinum*.[a]

Antibiotic Added	Enzymic Activities	
	Glucosaminidase (%)	Glycuronidase (%)
None	100	100
Mitomycin C	31	34
Actinomycin D	29	26
Chloramphenicol	39	40
Puromycin	93	85
Penicillin	96	84

[a] This experiment was performed as described in Table IV except that 100 μg of heparin was used as an inducer in the presence of 20 μg of each of the antibiotics. The cells after induction were incubated with N[$^{35}SO_4$]heparin and N-[$^{35}SO_4$]-trisulfated disaccharide. The products formed were analyzed as described in Table I.

Discussion

The results presented in this paper clearly indicate that the induction of a single enzyme, *i.e.*, the sulfamidase, is not sufficient to promote the degradation of heparin to the degradation products obtained. Actually, three enzymes taking part in the degradation of heparin are concomitantly induced in *F. heparinum* when this bacterium is grown in the presence of heparin.

Some degradation products of heparin are better inducers for the three enzymes than heparin itself. The di- and trisulfated disaccharides are able to promote almost immediate induction of the heparin-degrading enzymes while a lag period of 1 hr precedes the induction by heparin and the oligosaccharides. Since a low level of activities of the degrading enzymes is present in noninduced cells one is inclined to suggest that the actual inducers are the disaccharides which can be formed from heparin by the action of these low level activities of degrading enzymes. The lag period observed would be accounted for by the slow formation of disaccharides in the noninduced cells. A corroborating evidence for this hypothesis is the fact that the lag period in the induction of the enzymes by the hexasaccharide is more pronounced than that of heparin. It had already been shown that heparin is a better substrate for

the glucosaminidase than the hexasaccharide (Dietrich, 1969c) thus producing disaccharides at a faster rate, and concomitantly a less pronounced lag period. Nevertheless the possibility that all these compounds can act as inducers and that the lag period is due to the difficulty of penetration is not yet discarded. Difficulty of penetration does not seem to be a convincing explanation since the hexasaccharide gives a larger lag period than the intact heparin molecule. Furthermore a pilot experiment performed with radioactive heparin and degradation products indicate that these compounds do not seem to penetrate in *F. heparinum* cells.

In contrast to the remarkable inducing effect of the di-, tetra-, and hexasaccharides and heparin, the sulfated monosaccharides, *i.e.*, glucosamine *N*-sulfate and glucosamine 2,6-disulfate, are very poor inducers of all the three enzymes. This observation is in disagreement with the recent findings of Lloyd *et al.* (1968) who claimed that glucosamine *N*-sulfate is as good an inducer of the sulfamidase as heparin in *F. heparinum* cells. It is possible that these authors were observing the induction during the lag period of induction of these enzymes by heparin, or after an exceedingly long period of induction in which all the inducer might have been degraded. These authors also pointed out that no heparin-degrading enzymes could be observed in noninduced cells. These results contrast with our observations as well as the ones made by Payza (1962) where he was able to identify a low activity heparin-degrading system in *F. heparinum* grown in the absence of heparin.

The pattern of induction of the heparin-degrading enzymes in *F. heparinum* here described is compatible with the Jacob and Monod's (1961) theory for the mechanism of enzyme induction. Firstly the inducer, *i.e.*, the disaccharide, promotes the induction of a series of enzymes in the pathway of heparin degradation. Secondly, the inducer is not necessarily the substrate for all the enzymes induced, and conversely the substrate for some of these enzymes do not promote induction. Thirdly, noninduced cells contain a low level of activity of the enzymes indicating that there is a genetic code already present in these cells, and that the function of the inducer is to enhance, by derepression, the production of these enzymes. Finally the action of antibiotics on the induction process strongly supports the participation of mRNA and protein synthesis in this process.

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