Carbohydrate Research, 37 (1974) 223–238

EFFECTS OF POLYANIONS AND POLYCATIONS ON THE TREHALOSE PHOSPHATE SYNTHETASE OF *Mycobacterium smegmatis**

ALAN D. ELBEIN AND MIKE MITCHELL

Department of Biochemistry, The University of Texas Health Science Center, San Antonio, Texas 78284 (U. S. A.)

(Received June 21st, 1974; accepted July 12th, 1974)

ABSTRACT

When tested as activators on the trehalose phosphate synthetase [UDP-D-glucose:D-glucose 6-phosphate α-D-glucosyltransferase, EC 2.4.1.15 (46)] from *Mycobacterium smegmatis*, heparin was the best, various other sulfated polysaccharides (especially chondroitin 4- and 6-sulfates, dermatan sulfate, heparan sulfate, and γ-carrageenan) and polynucleotides were good, but hyaluronic acid, D-galacturonan, dextran sulfate, and keratan sulfate, were poor. Digestion of chondroitin sulfate with hyaluronidase destroyed the activating ability, but separation of the digestion products on Sephadex G-100 resin gave large-molecular-weight components that still showed activating ability. A sulfated tetra- or octa-saccharide isolated from chondroitin sulfate did not activate the enzyme, nor did they prevent the activation by chondroitin sulfate, suggesting that these small polyanions do not bind to the enzyme. Among polycations, poly-DL-ornithine (mol. wt. 15,600 daltons) was the best inhibitor of the enzyme followed by poly-L-lysine (mol. wt. 4,000 daltons), poly-D-lysine (mol. wt. 70,000 daltons), poly-D,L-lysine (mol. wt. 35,000 daltons), and then poly-L-ornithine (mol. wt. 120,000 daltons); polyglycine, polyleucine, and polyhistidine showed no effect. In all cases, more polycation was required to inhibit the enzyme when heparin was used as the activator than when chondroitin sulfate was used. The order of mixing of various reaction components was important for the extent of inhibition, the greatest inhibition being observed when polyanion and polycation were mixed before the addition of enzyme, and the smallest when polyanion and enzyme were mixed before the addition of polycation.

INTRODUCTION

The trehalose phosphate synthetase [UDP-D-glucose:D-glucose 6-phosphate α-D-glucosyltransferase, EC 2.4.1.15 (46)] of *Mycobacterium smegmatis* is an unusual glycosyl transferase in that it is able to utilize five different D-glucose nucleotides

*Dedicated to the memory of Professor W. Z. Hassid.

(ADP-Glc, CDP-Glc, GDP-Glc, TDP-Glc, and UDP-Glc) as D-glucosyl donors for the synthesis of trehalose phosphate. However, in order for the enzyme to be active with UDP-Glc (and other pyrimidine sugar nucleotides), it requires the presence of a high-molecular-weight polyanion, such as ribonucleic acid, heparin, or chondroitin sulfate[1,2].

The present report describes the effect of various polyanions and polycations on the activity of this enzyme with both UDP-D-glucose and GDP-D-glucose as glucosyl donors. The best polyanion activators of the UDP-D-glucose:D-glucose 6-phosphate α-D-glucosyltransferase were those having charges due to sulfate or phosphate groups, with heparin showing maximum activation. However, some sulfated polymers, such as dextran sulfate, were poor activators, which indicates that additional factors are involved in activation. Although the optimum size for activation is not known with certainty, small oligonucleotides (12 bases or less) or sulfated oligosaccharides (octasaccharides or smaller) are not able to activate the enzyme. Further, when chondroitin sulfate was digested with hyaluronidase, only the high-molecular-weight components obtained from a separation on Sephadex G-100 resin were good activators. Various polycations, particularly poly-D,L-ornithine, inhibit the synthesis of trehalose phosphate, apparently by competing with the enzyme for the polyanion.

EXPERIMENTAL

Materials. — Various polyanions were kindly donated: chondroitin 4-sulfate and dermatan sulfate (sample A) by Dr. A. Davidson; hyaluronic acid, γ-carrageenan, alginic acid, and dextran sulfate by Dr. A. Stone; chondroitin-4 and 6-sulfates, oversulfated chondroitin sulfates, dermatan sulfate (sample B), keratan sulfates, and heparan sulfate by Dr. J. Cifonelli. Sulfated tetra- and octa-saccharide prepared from chondroitin sulfate were also kindly supplied by Dr. J. Cifonelli. Whale and shark chondroitin sulfate, heparin, poly-L-lysine (mol. wt. 4,000 daltons), poly-D-lysine (mol. wt. 70,000 daltons), poly-D,L-lysine (mol. wt. 35,000 daltons), poly-L-ornithine (mol. wt. 120,000 daltons), poly-D,L-ornithine (mol. wt. 15,000 daltons), poly-L-histidine (mol. wt. 15,000 daltons), polyglycine (mol. wt. 5–10,000 daltons) and high-molecular-weight polynucleotides were obtained from Sigma Chemical Co. (St. Louis, Ma. 63178). Sugar nucleotides (UDP-Glc and GDP-Glc) and other nucleotides were obtained from Calbiochem (La Jolla, Calif. 92037) or Sigma Chemical Co., oligo(thymidylic acid) (10 or 12–18 bases) and oligo(uridylic acid) (mol. wt. 7,500 daltons) from Miles Labs., Inc. (Kankakee, Ill. 60901).

Analytical methods. — Hexose content was determined by the anthrone method with D-glucose or trehalose as the standard[3]; protein by the Lowry procedure[4], uronic acids by the carbazole method[5], and nucleotides by their u.v. absorption.

Paper chromatography. — Descending paper chromatography was performed on Whatman 3 MM paper in: (A) 7:3 (v/v) ethanol–M ammonium acetate, pH 7.4; (B) 57:4:39 (v/v) 2-methylpropanoic acid–ammonium hydroxide–water; (C) 15:3:10:12

(v/v) 1-butanol–acetic acid–pyridine–water; (D) 5:3:2 (v/v) 1-butanol–pyridine–0.1M hydrochloric acid; and (E) 7:1:3 (v/v) 1-propanol–ethyl acetate–water. Sugars and oligosaccharides were detected with the silver nitrate[6] or acid aniline phthalate[7] reagents and nucleotides by their u.v. absorption.

Isolation and assay of the trehalose phosphate synthetase. — The enzyme was isolated from *Mycobacterium smegmatis* and partially purified by chromatography on DEAE-cellulose as previously described[2]. Reaction mixtures for the determination of enzymic activity contained the following components in a final volume of 0.2 ml (in μmoles): UDP-Glc or GDP-Glc, 0.5; D-glucose 6-phosphate, 1; magnesium chloride, 0.75; tris(hydroxymethyl)aminomethane (Tris) buffer, pH 7.0, 10; and an appropriate amount of enzyme. Polyanions and polycations were added to incubation mixtures in various amounts, as indicated in the legends of the figures. The optimum concentration of polyanion was determined for each enzyme preparation. Incubations were usually done at 37° for 15 min. Trehalose phosphate formation was then determined by a colorimetric procedure using the anthrone reagent[2]; this method is based on the stability of trehalose phosphate (and trehalose) to both acid and alkali. Thus, after incubation the reaction was stopped by the addition of M hydrochloric acid (50 μl), and the tubes were heated for 15 min in a boiling water-bath to hydrolyze any remaining sugar nucleotide; 2% sodium hydroxide (0.3 ml) was added, and the tubes were heated for an additional 15 min to destroy any sugars having a free carbonyl group. An aliquot of the incubation mixture was removed and the content of trehalose phosphate was determined by the anthrone method.

Preparation of sulfated oligosaccharides. — Chondroitin sulfate (Sigma Chemical Co.) was treated with hyaluronidase (EC 3.2.1.35, Worthington Biochemical Corp., Freehold, N. J. 07728), as described by Gregory *et al.*[8]. Aliquots of the reaction mixture were removed at various times and heated for 15 min to destroy the hyaluronidase. The precipitate was removed by filtration and the supernatant was dialyzed overnight to remove the salts. The oligosaccharides were precipitated by the addition of ethanol (3 vol.), after addition of one drop of a saturated solution of sodium acetate. After being kept overnight at −20°, the precipitate was isolated by centrifugation, washed with 80% ethanol, acetone and ether, and air-dried. Solutions of the oligosaccharides, containing a known amount based on dry weight and carbazole reaction, were tested as activators of the synthetase. The molecular weight of the fragments in these hydrolyzates was determined by paper chromatography in Solvents C, D, and E, and also by gel filtration on Sephadex G-50 or G-100 resins. Gel filtration on columns of Sephadex resin was performed in 0.05M acetic acid containing 0.4M sodiumchloride; fractions were tested with the carbazole reaction and assayed for their activating ability.

Preparation of oligonucleotides. — Poly(uridylic acid) (mol. wt. 100,000 daltons) and oligo(uridylic acid) were partially degraded by potassium hydroxide hydrolysis. The nucleotides (10 mg) were dissolved in water (2 ml) and M potassium hydroxide (1 ml) was added. The mixtures were incubated at 37° and 0.3-ml aliquots were removed at various times, neutralized with perchloric acid, and cooled at 0° for

30 min. The precipitate was removed by centrifugation, and the supernatant was tested for its ability to activate the trehalose-phosphate synthetase. The concentration of nucleotide in each case was determined by u.v. absorption at 260 nm. Each aliquot was streaked on a sheet of SS 589 Blue Ribbon paper and chromatographed for 48–96 h in Solvents A or B. Various nucleotide bands were eluted from the paper and assayed for activating ability.

RESULTS

Effect of polyanions. — Activation of the trehalose phosphate synthetase by high-molecular-weight polyanions. A number of high-molecular-weight polyanions were able to activate the trehalose phosphate synthetase to use UDP-Glc as a D-glucosyl donor. As shown in Fig. 1 and Table I, the various polyanions had considerably different ability to stimulate the enzyme. The best activator appears to be heparin, which contains both *N*- and *O*-sulfate groups as well as uronic acid residues and, apparently, has the highest sulfate content (and charge density) of the various glycosaminoglycans. Chondroitin 4-sulfate (98% of 4-sulfate groups), and 6-sulfates (80%

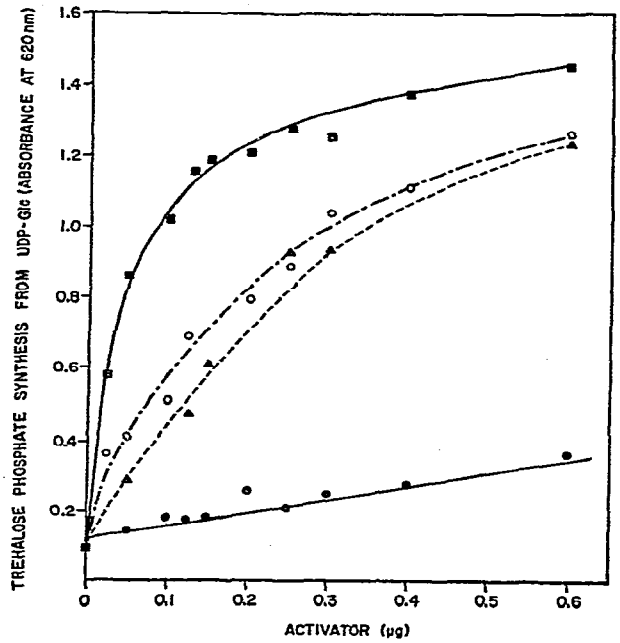

Fig. 1. The effect of various polyanions on the activity of the trehalose phosphate synthetase with UDP-Glc as substrate. The reaction mixtures were as described in the Experimental section, but the amount of polyanion was varied as indicated. Each incubation mixture contained 130 μg of the enzyme (purified by chromatography on DEAE-cellulose). Heparin (–■–■–■–); dermatan sulfate (sample B) (·–○–·–○–·); chondroitin 6-sulfate (––▲–––▲–––▲––); hyaluronic acid and D-galacturonan (–●–●–●–).

TABLE I

ACTIVATION OF TREHALOSE PHOSPHATE SYNTHETASE BY VARIOUS POLYANIONS

Polyanion	*Trehalose phosphate formed after addition of activator (μg)*[a]				
	0.02	*0.05*	*0.1*	*0.2*	*0.4*
Chondroitin 4-sulfate	0.29	0.32	0.38	0.44	0.50
Chondroitin 6-sulfate	0.30	0.32	0.35	0.45	0.58
Oversulfated chondroitin sulfate	0.31	0.39	0.45	0.63	0.83
Dermatan sulfate (sample A)	0.40	0.46	0.61	0.81	0.95
Dermatan sulfate (sample B)	0.36	0.51	0.72	0.86	1.02
Heparin	0.37	0.54	0.79	0.89	1.09
Heparan sulfate	0.29	0.38	0.58	0.67	0.82
Keratan sulfate (sample A)	0.28	0.23	0.28	0.36	0.53
Keratan sulfate (sample B)	0.24	0.23	0.2	0.2	0.18
Dextran sulfate	0.19	0.21	0.23	0.22	0.23
Hyaluronic acid	0.21	0.22	0.21	0.21	0.15
γ-Carrageenan	0.28	0.37	0.55	0.78	0.97
Ribonucleic acid (Peak-2)	0.39	0.48	0.65	0.74	0.94
None	0.21				

[a]Absorbance at 620 nm.

of 6-sulfate groups) and dermatan sulfate (90% of 4-sulfate groups), all containing uronic acid residues and *O*-sulfate groups and about one half of the sulfur content of heparin, were all fairly good activators of the enzyme, although less effective than heparin. Heparan sulfate, which has *N*- and *O*-sulfate groups and the same amount of sulfate groups as chondroitin sulfate, showed approximately the same amount of activity as did chondroitin sulfate. γ-Carrageenan, a sulfated polysaccharide, was also a fairly good activator, but keratan sulfate was a poor activator and dextran sulfate was inactive. Likewise, polyanions, such as hyaluronic acid and D-galacturonan, which have charges due to carboxyl groups, were not activators of the synthetase. With each enzyme preparation, the optimum concentration of polyanion activator had to be determined, since the amount of activator necessary for maximum activity did vary from one enzyme preparation to another. However, the relative effectiveness of the various polyanions remained very much the same, as shown in Fig. 1 and Table I. It should be pointed out that the assay used to determine enzymic activity is not sensitive enough to differentiate between small variations in activating capacity. Thus, dermatan sulfate, heparan sulfate, γ-carrageenan, and chondroitin 6-sulfate may all be equally effective as activators.

Various polynucleotides are also effective as activators of the trehalose phosphate synthetase, as shown in Fig. 2. High-molecular-weight poly(cytidylic acid) and poly(uridylic acid) were fairly good activators of the enzyme, although they appeared to be somewhat less effective than chondroitin sulfate. On the other hand, high-molecular-weight poly(adenylic acid) and poly(guanylic acid) were poor activators of the enzyme. Although oligo(uridylic acid) had previously been found[2] to be an

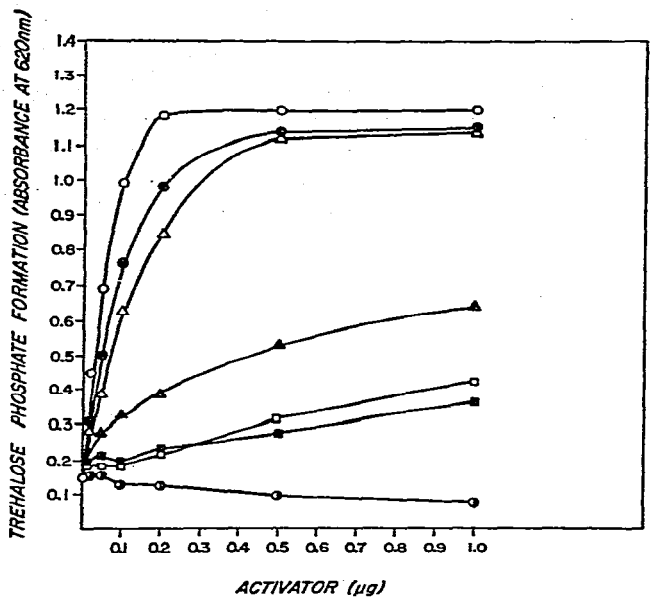

Fig. 2. The effect of various polynucleotides on the activity of the trehalose phosphate synthetase with UDP-Glc as substrate. Reaction mixtures were as described in the Experimental section, but the amount of polynucleotide was varied as indicated. Each incubation mixture contained 130 μg of enzyme (purified by chromatography on DEAE-cellulose). Dermatan sulfate (–○–○–○–); poly(C) (–●–●–●–); poly(U) (mol. wt. >100,000) (–△–△–△–); poly(A) (mol. wt. ~100,0) (–▲–▲–▲–); poly(G) (mol. wt. >100,000) (–■–■–■–); oligo(U) (mol. wt. >500)00 (–□–□–□–); and oligo(dT) (–◑–◑–◑–).

activator at low concentrations (0.1–0.5 μg/0.2 ml), in these experiments it was found to be a poor activator unless the concentrations were increased to 1–5 μg/0.2 ml. One possible explanation for this different behavior is that the oligo(uridylic acid) sample used in these studies is different from that used previously, which might have been contaminated by some high-molecular-weight material. It is also possible that the present preparation had inadvertantly been exposed to ribonuclease; no attempt was made at this time to verify its molecular size. Oligo(deoxythymidylic acid), containing 12–18 bases, was also a poor activator of the enzyme .The high-molecular weight poly(uridylic and cytidylic) acids were treated with 0.3M potassium hydroxide for various periods of time, and the degradation products were tested as activators of the enzyme. Both preparations lost their activating ability within the first 30 min of incubation. When these reaction mixtures were fractionated on paper chromatograms, a variety of bands that ranged from material remaining at the origin through tetra-, tri-, di-, and mono-nucleotides were observed. None of these compounds, including the bands eluted from the origin, showed any activating ability. On the basis of these experiments and the ones described further, it seems likely that a high-molecular-weight (at least 10,000 daltons), highly charged polyanion is necessary for activation of the enzyme.

Determination of optimum size of the activator. — In order to determine the lowest-molecular-weight polyanion that would still activate the synthetase, a sulfated tetrasaccharide and a sulfated octasaccharide, obtained by hyaluronidase digestion of chondroitin sulfate and kindly donated by Dr. J. Cifonelli, were tested as activators of the enzyme. As shown in Fig. 3, neither of these compounds had any effect on the enzyme, even when they were tested at high concentrations. In fact, not only were

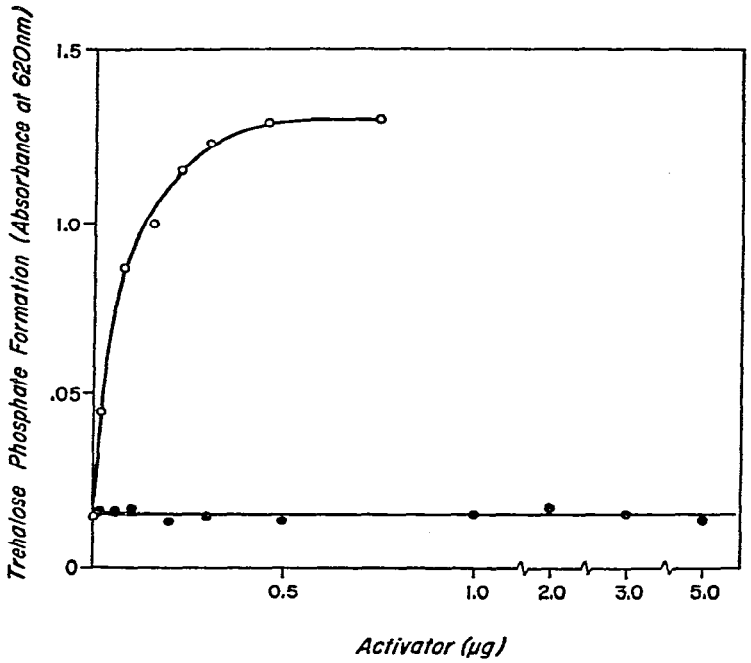

Fig. 3. Comparison of the activating ability of a sulfated tetra- or octa-saccharide (–●–●–●–) to that of dermatan sulfate (sample B) (–O–O–O–) on the trehalose phosphate synthetase. Reaction mixtures were as described in the Experimental section, except that the concentration of activator was varied as indicated. Each incubation contained 150 μg of enzyme.

they unable to activate, but they did not compete with chondroitin sulfate and prevent its activation of the enzyme. In this experiment, the octasaccharide (5, 10, or 20 μg) was added to reaction mixtures containing chondroitin sulfate as the activator, and trehalose phosphate formation was determined as usual. If the octasaccharide was bound to the enzyme, it should have competed with chondroitin sulfate and prevented activation. However, no such inhibition was observed, which indicates that the octasaccharide either was not bound or was bound very weakly to the enzyme.

Larger-molecular-weight oligosaccharides were obtained by digestion of chondroitin sulfate with hyaluronidase as described in the Experimental action. The various hydrolyzates were tested as activators of the synthetase. As shown in Fig. 4, after an hydrolysis of 4 h, the sample still had fairly good activating ability,

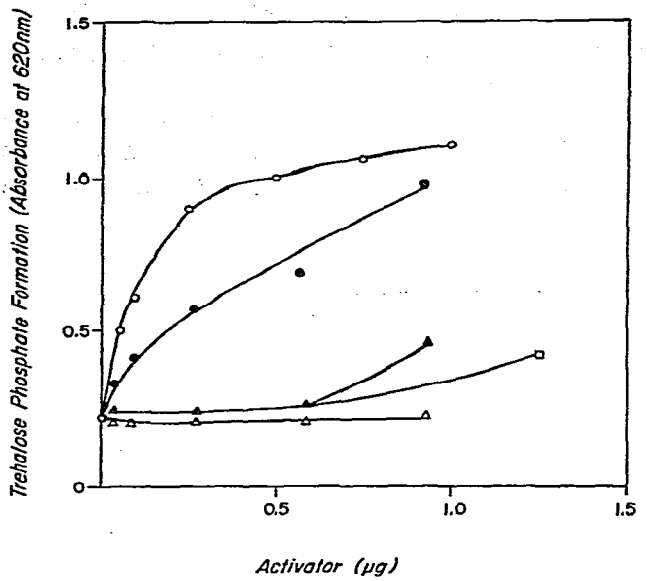

Fig. 4. The effect of hyaluronidase treatment on the activating ability of chondroitin sulfate (Sigma). Chondroitin sulfate was treated with hyaluronidase for varying times and the degradation products were precipitated with alcohol and then tested as activators. Reaction mixtures were as described in the Experimental section and contained 120 μg of enzyme. Varying amounts of each digestion product were added as indicated. Untreated chondroitin sulfate (–O–O–O–); 4-h (–●–●–●–), 22-h (–▲–▲–▲–), 96-h (–□–), and 120-h hydrolyzate (–△–△–△–).

but the 22-hour hydrolyzate had much lower activity, and longer times of hydrolysis essentially abolished the activity. The 4- and 22-hour hydrolyzates were fractionated on a column of Sephadex G-100 resin as shown in Fig. 5. Fractions were pooled as shown by the brackets and each was tested for its activating ability. Several standard compounds were also filtered through this column, as shown in Fig. 6: Chondroitin 4-sulfate was resolved into 2 peaks, which were pooled as indicated by the brackets. The sulfated octasaccharide standard emerged as a single symmetrical peak well after undegraded chondroitin sulfate. Fig. 7 shows a comparison of the relative activating ability of the various fractions eluted from the column of Sephadex G-100 resin. In each case, the fastest moving component (*i.e.*, the highest-molecular-weight species) showed the best activating ability. Thus, fractions 4_1, 22_1, and C-4-S_1 were fairly good activators, whereas other fractions were less active (4_2) or were inactive (22_2 and 22_3). The reason for the poor activating ability of peak 22_2 is not understood at this time. Fraction C-4-S_2, the slower moving component of chondroitin 4-sulfate was also found to be less effective than C-4-S_1. It should be pointed out that the reference compound used in these various experiments is dermatan sulfate and that unfractionated chondroitin 4-sulfate showed an activity much lower than that of dermatan sulfate (see Table I). This may be due to the presence of two components and because the latter-eluted component (lower molecular weight) is a

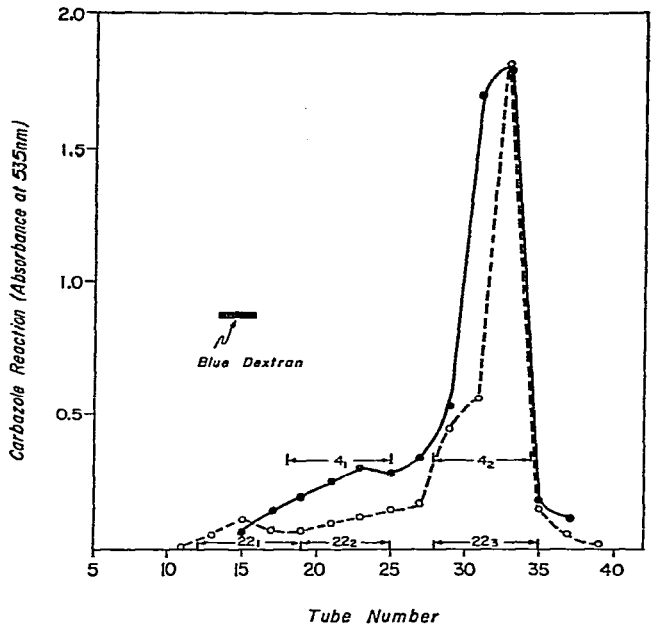

Fig. 5. Chromatography on a column of Sephadex G-100 resin of the 4- (—●—●—●—) and 22-h (———O——O——O———) hydrolyzate of chondroitin sulfate by hyaluronidase. The material was placed on the column in 0.05M acetic acid and eluted with the same solvent. Fractions were pooled as shown by the brackets: Fractions 4_1 and 4_2 for the 4-h hydrolyzate and Fractions 22_1, 22_2, and 22_3 for the 22-h hydrolyzate.

poorer activator, whereas the activity of C-4-S_1 is more similar to that of dermatan sulfate. These experiments, suggest that the molecular weight of the activator must be in the order of 10–12,000 daltons or higher.

Various other glycosaminoglycans were also treated with hyaluronidase to determine the effect that this enzyme would have on their activating ability. These glycosaminoglycans included chondroitin 4- and 6-sulfates, oversulfated chondroitin sulfate, dermatan sulfate, and heparin. After 24 h of enzymic digestion, chondroitin 4- and 6-sulfates and oversulfated chondroitin sulfate had lost their activating ability, whereas dermatan sulfate and heparin were still effective activators. These results are explained by the resistance of heparin and dermatan sulfate to hyaluronidase, whereas the chondroitin sulfates are hydrolyzed by this enzyme.

*Effect of polycations. — Inhibition of the trehalose phosphate synthetase by poly-*D,L*-ornithine.* The polycation, poly-D,L-ornithine, when added to incubation mixtures at low concentrations, was found to inhibit the activity of the synthetase when UDP-glucose was used as the substrate. Fig. 8 shows the effect of adding increasing amounts of poly-D,L-ornithine to incubation mixtures in which either heparin or chondroitin sulfate was used as an activator of the enzyme. It should be noted that considerably larger amounts of poly-D,L-ornithine were necessary to inhibit the enzyme when heparin was used as the activator than when chondroitin sulfate was used. These

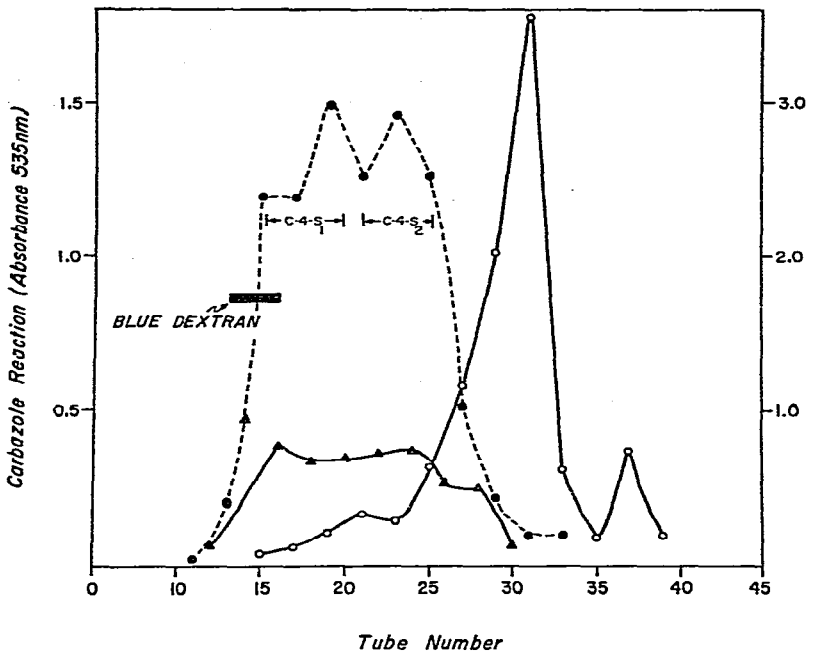

Fig. 6. Chromatography on a column of Sephadex G-100 resin of chondroitin sulfate (Sigma) (–▲–▲–▲–), chondroitin 4-sulfate (– – –●– –●– –●– – –), and sulfated octasaccharide (–O–O–O–). For details see legend of Fig. 5. Fractions were pooled as shown by the brackets: Fractions C-4-S_1 and C-4-S_2 for chondroitin 4-sulfate.

data support our earlier observation that heparin is a better activator of the enzyme than is chondroitin sulfate and suggest that heparin binds more tightly to the enzyme than does chondroitin sulfate. It is interesting to note that poly-D,L-ornithine did not significantly inhibit the activity when GDP-glucose was used as the substrate. In this case no activator was added, since polyanion is not required for activity with GDP-Glc. The reason for the increase of enzymic activity at low poly-D,L-ornithine concentrations is not known at this time. The converse of the above experiment was also performed, as shown in Fig. 9. Poly-L-ornithine and poly-L-lysine, at 0.02 μg per incubation mixture, inhibited enzymic activity with UDP-glucose, but this inhibition could be overcome by addition of increasing amounts of chondroitin sulfate. Poly-L-ornithine was a better inhibitor than poly-L-lysine, since it took a much greater amount of chondroitin sulfate to overcome the inhibition of the enzyme by the first-named polycation.

Effect of various polycations on enzymic activity. Other polycations were tested as inhibitors of the enzyme, as shown in Fig. 10. In all of these cases, chondroitin sulfate was used as the activator. Poly-D,L-ornithine (mol. wt. 15,600) was the most effective inhibitor, whereas poly-L-ornithine, which has a higher molecular weight (120,000), was much less effective as an inhibitor. Low-molecular-weight

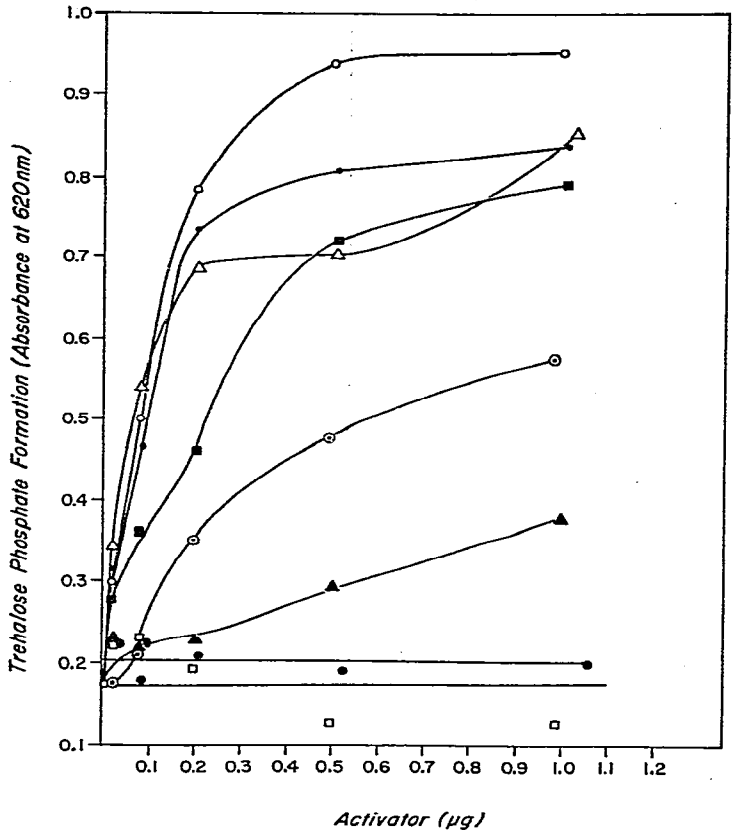

Fig. 7. Activating ability of the various fractions obtained from the column of Sephadex G-100 resin (Figs. 4 and 5). The reaction mixtures were as described in the Experimental section with UDP-Glc as substrate and contained 120 μg of enzyme. Each fraction was isolated from the column and tested at the concentrations shown in the figure: undegraded chondroitin sulfate (-○-○-○-), Fractions C-4-S_1 (-◆-◆-◆-), 4_1 (-△-△-△-), 22_1 (-■-■-■-), C-4-S_2 (-⊙-⊙-⊙-), 4_2 (-▲-▲-▲-), 22_2 (-●-●-●-), and 22_3 (-□-□-□-).

poly-L-lysine (mol. wt. 4000) was a better inhibitor than the high-molecular-weight poly-D-lysine (mol. wt. 70,000) and poly-D,L-lysine (mol. wt. 35,000). Thus, a molecular weight of 4000–16,000 appears to be optimum for inhibition. Poly-L-histidine, polyglycine, and poly-L-leucine (not shown in Fig. 10), which are presumably uncharged at pH 7.5, did not show any inhibition, even at high concentrations. Thus, in addition to an optimum molecular size, the polyamino acid must have a positive charge at pH 7.0.

Order of mixing. As might be expected, the order of mixing the polycation, polyanion, and synthetase was important (see Fig. 11). The greatest inhibition of the UDP-Glc:D-glucose 6-phosphate α-D-glucosyltransferase activity was observed when polyanion (chondroitin sulfate) and polycation (poly-D,L-ornithine) were mixed before the addition of enzyme. On the other hand, the least inhibition was seen

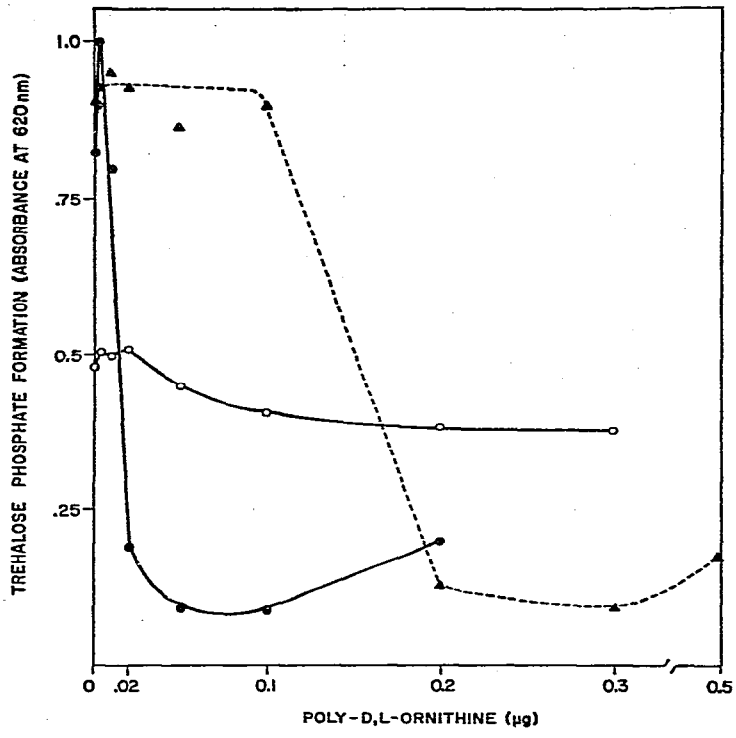

Fig. 8. The effect of poly-D,L-ornithine on the activity of the trehalose phosphate synthetase. The reaction mixtures were as described in the Experimental section with either UDP-Glc or GDP-Glc as D-glucosyl donor. When UDP-Glc was used as substrate, either chondroitin sulfate (0.2 μg, –●–●–●–) or heparin (0.2 μg, ---▲---▲---) was used as the activator. Neither were required when GDP-Glc (–O–O–O–) was used. In this experiment, all the components of the reaction mixture including polyanion and polycation were mixed before the addition of enzyme (120 μg).

when polyanion and enzyme were mixed before the addition of polycation. Thus, the enzyme and the polycation apparently compete for the polyanion.

DISCUSSION

The trehalose phosphate synthetase represents an interesting example of a protein:polyelectrolyte interaction in which a polyanion apparently binds to the enzyme and somehow alters its conformation to enable it to utilize a given substrate (in this case, UDP-glucose). Although the nature of the change in the protein structure that is induced by the polyanion is not known at this time, it is apparent that a variety of different polyanions are capable of causing this change. Two general requirements appear to be necessary in order for these polyanions to function as activators of the trehalose phosphate synthetase: (a) the macromolecular nature of the polyanion, and (b) the strong-negatively charged nature of the polymer. Thus, heparin, which has both *N*- and *O*-sulfate groups and the greatest charge density of

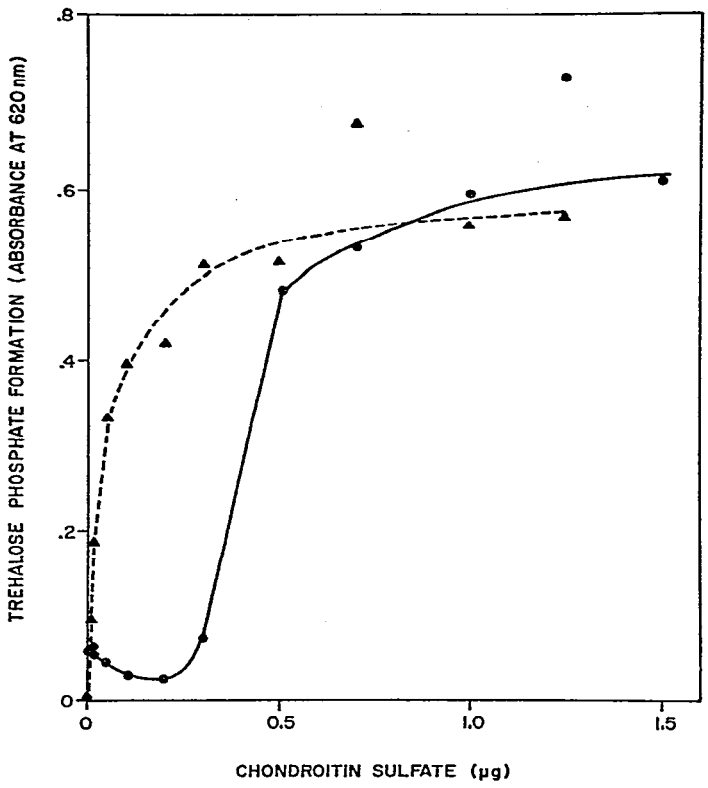

Fig. 9. The effect of increasing concentrations of chondroitin sulfate on the inhibition of trehalose phosphate synthetase by poly-L-ornithine (–●–●–●–) and poly-L-lysine (---▲---▲---). The reaction mixtures were as described in the Experimental section and contained UDP-Glc as substrate, the enzyme (120 μg), poly-L-ornithine (0.02 μg), or poly-L-lysine (0.02 μg). The reaction was initiated by the addition of enzyme.

the various glycosaminoglycans, is a better activator than are chondroitin sulfate and the polynucleotides, whereas hyaluronic acid (or other polyanions having carboxyl groups as the only negatively charged groups) is a poor activator.

A molecular weight of 11,000 daltons or above appears to give maximum stimulation to the enzyme. Thus, heparin is reported to have a molecular weight of about 11,000 whereas that of chondroitin 4-sulfate is ~12,000 and that of dermatan sulfate ~27,000. Degradation of chondroitin sulfate with hyaluronidase led to the rapid disappearance of the activating ability within the first 22 h. When the 4- and 22-h hydrolyzates were fractionated on a column of Sephadex G-100 resin, it was found that only the high-molecular-weight components, which emerged in the same location as intact chondroitin sulfate, had good activating ability. On the other hand, the low-molecular-weight components obtained from the Sephadex resin column, as well as a sulfated tetrasaccharide and a sulfated octasaccharide (isolated from chondroitin sulfate by Dr. J. Cifonelli) were ineffective in activating the enzyme.

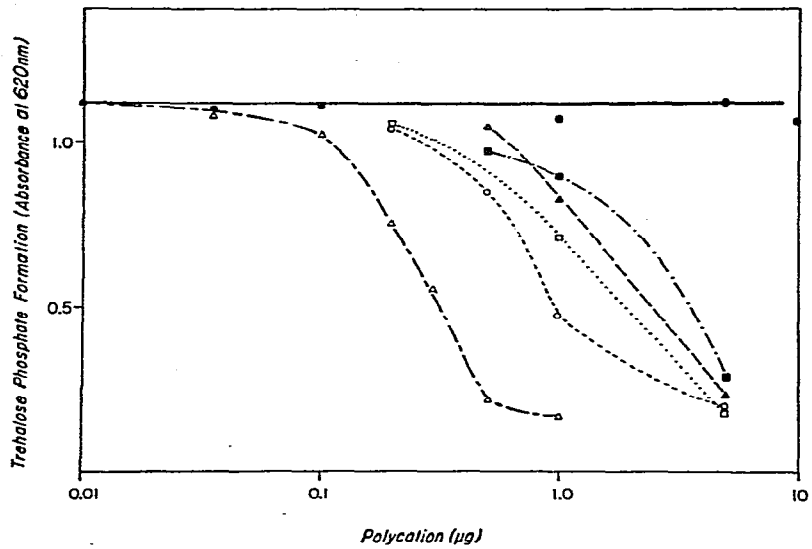

Fig. 10. The effect of various polyamino acids on the activity of the trehalose phosphate synthetase, with UDP-Glc as substrate. The reaction mixtures were as described in the Experimental section and contained enzyme (150 μg) and chondroitin sulfate (0.2 μg) as the activator. The concentration of polycation was varied as indicated in the figure. In each case, the reaction was initiated by the addition of enzyme. Poly-L-histidine (mol. wt. 15,000) and polyglycine (mol. wt. 6,000) (–●–●–●–); poly-L-ornithine (mol, wt. 120,000) (.–■–.–■–.–); poly-D,L-lysine (––▲––▲––); poly-D-lysine (mol. wt. 70,000) (···□···□···); poly-L-lysine (mol. wt. 4000) (–––○–––○–––); and poly-D,L-ornithine (mol. wt. 15,600) (––△–––△––).

A number of other enzymes have been described that are activated by various high-molecular-weight polyelectrolytes. Probably the most widely studied is lipoprotein lipase and its interaction with heparin[10–15]. However, various other enzymes, such as β-glucuronidase, hyaluronidase, aldolase, polynucleotide phosphorylase, etc., also show an interaction with either polyanions or polycations. Several reviews have been published on the interactions of various proteins with polyelectrolytes[16–18].

A large number of enzymes have also been shown to be inhibited by either polycations or polyanions[16,18,19]. As might be expected, enzymes which are activated by polyanions are usually inhibited by polycations (or at least activation is prevented) and *vice versa*. A similar phenomenon has been observed with the trehalose phosphate synthetase, in which activity with UDP-glucose is inhibited by a number of polycations. This is probably not an inhibition of enzymic activity *per se*, but instead an inhibition (or prevention) of the activation phenomenon shown by various polyanions. Interestingly enough, there are profound differences in the inhibitory effect of the various polyamino acids, depending on their charge and probably also on their molecular weight. Thus, poly-D,L-ornithine, which has a molecular weight of 15,600 daltons, was the best inhibitor, but high-molecular-weight poly-L-ornithine (mol. wt. 120,000 daltons) was much less effective. Likewise, low-molecular-weight poly-L-lysine (mol. wt. 4000 daltons) was a better inhibitor than were the high-molecular-

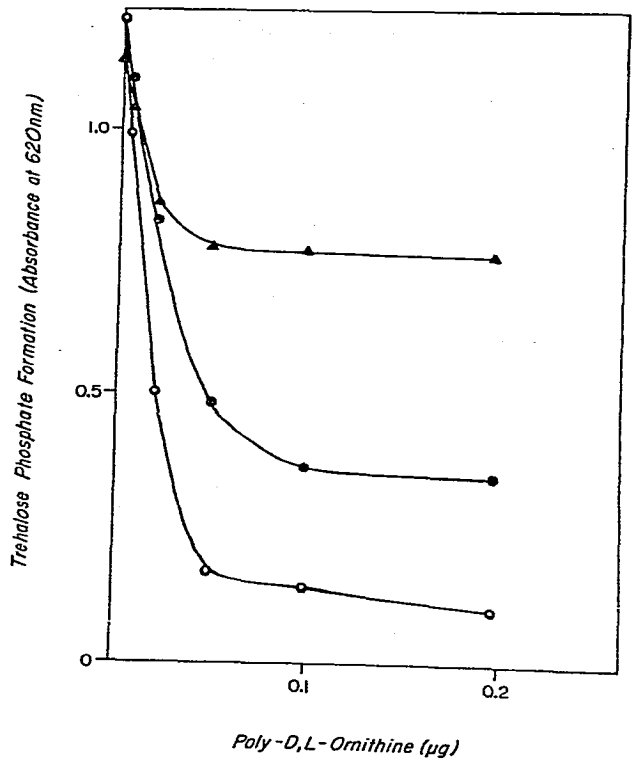

Fig. 11. The effect of order of mixing of the reaction components on the activity of the trehalose phosphate synthetase. In a 5-min preincubation, the following components were mixed: chondroitin sulfate (0.2 μg) and enzyme (–▲–▲–), poly-D,L-ornithine (concentration as shown) and enzyme (–●–●–), and chondroitin sulfate (0.2 μg) and poly-D,L-ornithine (concentration as shown) (–O–O–). At the end of the preincubation, the final component was added (poly-D,L-ornithine, or chondroitin sulfate, or enzyme) as well as the substrates, and the mixture was incubated for an additional 15 min. Trehalose phosphate formation was determined as indicated in the Experimental section and 150 μg of enzyme was used.

weight poly-D- and D,L-lysines, but all these compounds required higher concentrations for inhibition than did poly-D,L-ornithine. The polycations apparently compete with the enzyme for the polyanion, since the order of mixing the various components is important in the inhibitory process. Thus, if enzyme and polyanion are mixed before the polycation is added, much less inhibition occurs, presumably because the polyanion is bound to the enzyme and is more difficult to remove. This conclusion is supported by the observation that it takes a much greater amount of polycation to inhibit the enzyme when heparin is used as the activator than when chondroitin sulfate is used, probably heparin, which has a higher charge density and is a better activator, binds more tightly to the protein. Other polyamino acids, such as polyglycine, poly-L-histidine, and poly-L-leucine, which have little or no charge at pH 7.5, showed no effect on enzymic activity, even at high concentrations.

Gelman *et al.*[20] studied the ionic interactions between chondroitin 6-sulfate and poly-L-lysine by circular dichroism. The circular dichroism spectrum of a mixtures

of polyanion and polycation was quite different from that predicted from data for the individual components. The authors suggest that the interaction forces the polypeptide to adopt the α-helical configuration rather than the charged coil form expected at neutral pH in the absence of the polysaccharide.

ACKNOWLEDGMENT

This work was supported by a grant (AI-09402) from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U.S. Public Health Service, and a grant from the Robert A. Welch Foundation.

REFERENCES

1 C. LIU, B. W. PATTERSON, D. LAPP, AND A. D. ELBEIN, *J. Biol. Chem.*, 244 (1969) 3728–3731.
2 D. LAPP, B. W. PATTERSON, AND A. D. ELBEIN, *J. Biol. Chem.*, 246 (1971) 4567–4579.
3 F. A. LOEWUS, *Anal. Chem.*, 24 (1952) 219.
4 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265–275.
5 Z. DISCHE, *Methods Carbohyd. Chem.*, 1 (1962) 488.
6 W. E. TREVELYAN, D. P. PROCTER, AND J. S. HARRISON, *Nature*, 116 (1950) 444–445.
7 S. ARONOFF, *Techniques of Radiobiochemistry*, Iowa State College Press, Ames, Iowa, 1956, pp. 00.
8 J. D. GREGORY, T. C. LAURENT, AND L. RODÉN, *J. Biol. Chem.*, 239 (1964) 3312–3320.
9 R.-H. IVERIUS, *Biochem. J.*, 124 (1971) 677–683.
10 E. D. KORN, *J. Biol. Chem.*, 215 (1955) 1–14.
11 E. D. KORN, *J. Biol. Chem.*, 215 (1955) 15–26.
12 P. BERNFELD AND T. F. KELLEY, *J. Biol. Chem.*, 238 (1963) 1236–1241.
13 P.-H. IVERIUS, U. LINDAHL, T. EGELRUD, AND T. OLIVECRONA, *J. Biol. Chem.*, 247 (1972) 6610–6616.
14 D. S. ROBINSON, *Quart. J. Exp. Physiol.*, 41 (1956) 195–204.
15 A. E. HORNER, *Proc. Nat. Acad. Sci. U.S.*, 69 (1972) 3469–3473.
16 A. D. ELBEIN, *Advan Enzymol.*, 40 (1974) 29–64.
17 P. BERNFELD, in E. A. BALAZS AND R. W. JEANLOZ (Eds.), *The Amino Sugars*, Vol. IIB, Academic Press, New York, 1966, pp. 213–228.
18 P. BERNFELD, in R. M. HOCHSTER AND J. H. QUASTEL, (Eds.), *Metabolic Inhibitors*, Vol. II, Academic Press, New York, 1963, pp. 437–000.
19 M. SELA AND E. KATCHALSKI, *Advan Protein Chem.*, 14 (1959) 391–478.
20 R. A. GELMAN, W. B. RIPPON, AND J. BLACKWELL, *Biochem. Biophys. Res. Commun.*, 48 (1972) 708–711.