THE ACTION OF MUCOPOLYSACCHARIDES ON PROSTATIC ACID PHOSPHATASE

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The action of mucopolysaccharides upon prostatic acid phosphatase was thought to be of interest in relation to the effect which chondroitin sulphate might have on phosphatase activity of metastases in bone from carcinoma of prostate. Chondroitin sulphate was expected to inhibit the enzyme, since it is a long-chain polymer with many acidic groups. KENT AND WHITEHOUSE[1] suggest a repeating disaccharide unit of one residue of glucuronic acid combined by 1–4 linkages with one residue of acetyl galactosamine, one hydroxyl of which is esterified with sulphuric acid, and quote a molecular weight of 260,000 for the undegraded substance, which would correspond to a chain-length of something over 1,000 residues. Denaturation of the enzyme by the sulphuric and carboxyl groups was expected, similar to that found with anionic detergents for other enzymes by WILLS[2], and with sodium dodecyl sulphate (SDS) for prostatic phosphatase in experiments reported in this paper.

In fact, chondroitin sulphate was found to inhibit the enzyme only slightly; and in the absence of other protective substances it also gave considerable protection against surface denaturation. Sodium alginate, which is a straight-chain polymer of the salt of β-D-mannuronic acid[3], gave similar results, though with greater inhibition and correspondingly less protection of the enzyme. Investigations with hydrolysates of these substances, and with simpler organic acids, appear to throw some light on the mechanisms involved.

MATERIALS AND METHODS

Specimens of human seminal fluid, obtained from the pathological laboratory of the Bristol General Hospital, were used as the source of prostatic acid phosphatase. These were stored in the refrigerator until required, when dilutions in physiological saline of the order of 1/2,000 to 1/20,000 were prepared, using polythene vessels and pipettes in order to minimise surface denaturation of the enzyme. 0.1 ml of diluted enzyme was added to 1 ml of 0.2% (w/v) solution of *para*-nitrophenyl phosphate in citrate buffer at pH 4.9 and 37° C in open glass tubes, which had been washed with Teepol and rinsed with tap-water, followed by distilled water. Specimens were selected which showed high acid phosphatase activity when 0.05% (w/v) gelatine was incorporated in the incubation mixture, but virtually none in its absence. Substances under investigation were added to the incubation mixture in known concentration. Duplicate estimations were carried out, and control tubes were incubated in which the addition of enzyme was omitted. Incubations were generally for 30 minutes. When *p*-nitrophenol estimations only were required, enzyme activity was stopped by the addition of 1 ml of the glycine-pyrophosphate buffer of HUGGINS AND TALALAY[4] at pH 11.2, followed by 2 ml of water. When *p*-nitrophenol and phosphate estimations were required on the same specimen, activity was stopped by the addition of 1 ml of 50% (w/v) trichloracetic acid. Tubes in which gelatine or other substances were precipitated were then centrifuged 10–30 minutes, after which 1.6 ml of the supernatant fluid was withdrawn and mixed with 2.4 ml distilled water. 1 ml of this mixture was treated with 3 ml *N*/2 NaOH for the estimation of *p*-

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nitrophenol, and the other 3 ml used for the estimation of phosphate by the method of FISKE AND SUBBAROW as given by KOCH AND HANKE[5].

Readings were made on a Spekker Absorptiometer, using monochromatic light from a filtered mercury-vapour lamp. For the estimation of *p*-nitrophenol, the mercury violet band at 4,360 A was isolated by Ilford Spectrum Violet filter No. 601 (Transmission 3850–4750 A) with the supplementary filter No. 809, cutting out light between 3,750 and 4,150 A. For phosphate estimations, the mercury yellow lines at 5,770 and 5,791 A were isolated by Ilford Spectrum Yellow filter No. 606 (Transmission 5,600 to 6,100 A). Enzyme activity was compared with that found in an incubation mixture containing 0.05 % (w/v) gelatine as its only addition. When *p*-nitrophenol and phosphate estimations were made on the same specimens, a *p*-nitrophenyl phosphate hydrolysate of known molarity was used as a standard of comparison, and the mean value in micromoles of phosphate and *p*-nitrophenol liberation in the tubes containing gelatine was taken as 100% activity.

Hydrolysates of chondroitin sulphate and sodium alginate were prepared by incubation in a boiling-water bath with hydrochloric acid in concentrations ranging from normal to 5 *N*. 2.5 *N* acid was found the most effective for the production of short-chain hydrolysates, as too much charring was obtained with more concentrated acid.

Number-average chain-lengths were calculated from the estimation of reducing-sugar by the method of KING AND GARNER[6,7]. The concentration of solution used, divided by the estimated concentration of reducing end-groups, was taken as the mean chain-length.

RESULTS

Sodium dodecyl sulphate (SDS)

SDS causes marked inhibition of prostatic acid phosphatase, which is slightly increased by reduction in the concentration of substrate, but much more markedly by reduction in the concentration of gelatine, as shown in Table I. It was not found possible to reverse inhibition by the addition of gelatine after incubation with SDS was begun. If incubation was carried out in the absence of gelatine, but under a layer of petroleum ether, which gives a large measure of protection to the enzyme from surface denaturation, complete and irreversible inhibition could be obtained with a concentration of 0.0001 *M* SDS; a concentration less than that at which WILLS[2] found complete reversibility of the inactivation of urease by SDS.

TABLE I

INHIBITION OF PROSTATIC ACID PHOSPHATASE BY SODIUM DOCEDYL SULPHATE (SDS) with different concentrations of substrate (*p*-nitrophenyl phosphate) and of gelatine

Concentration % (w/v) of substrate	*Concentration % (w/v) of gelatine*	*Millimoles of SDS per litre for 50% inhibition*
0.2	0.05	0.35
0.02	0.05	0.25
0.2	0.0005	0.02

Chondroitin sulphate

Fig. 1 shows the activity of prostatic acid phosphatase in the presence of gelatine 0.05% (w/v) and of different concentrations of chondroitin sulphate preparations of estimated chain-lengths 660 residues (Evans) and 197 residues (Light's), and of a hydrolysate of the latter of 2.6 residues. All inhibit the enzyme to some extent, but the hydrolysate much more markedly than either of the original preparations, which show only slight inhibition at similar levels.

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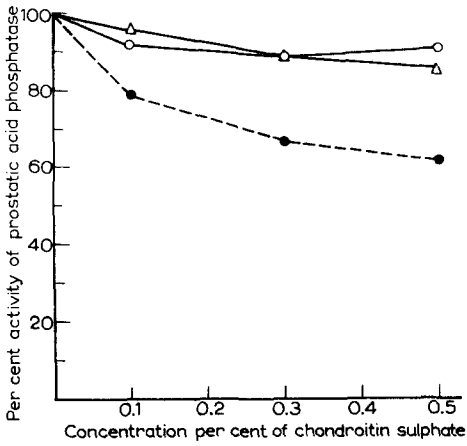

Fig. 1. Inhibition of prostatic acid phosphatase by chondroitin sulphate. △—△ Activity with chondroitin sulphate, chain-length 660 residues O—O Activity with chondroitin sulphate, chain-lenght 197 residues; ●---● Activity with hydrolysate, chain-length 2.6 residues. Gelatine 0.05% (w/v) throughout.

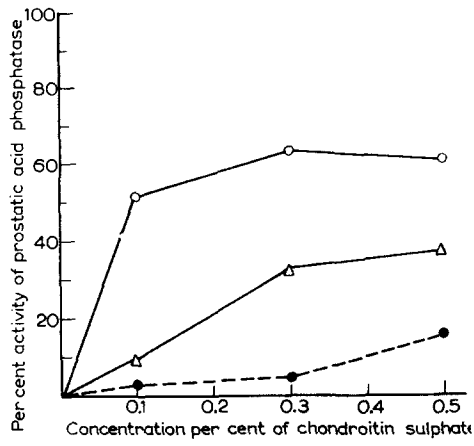

Fig. 2. Protection of prostatic acid phosphatase by chondroitin sulphate. △—△ Activity with chondroitin sulphate, chain-length 660 residues; O—O Activity with chondroitin sulphate, chain-length 197 residues; ●---● Activity with hydrolysate, chain-length 2.6 residues. 100% = activity with gelatine 0.05% (w/v).

Fig. 2 shows the effect of the three in the absence of gelatine. All give some protection, but at widely different levels; the greatest protection being given by the sample of intermediate chain-length, and the least by the short-chain hydrolysate. Table II shows the effect on inhibition by a hydrolysate of mean chain-length 3.8 residues of varying substrate concentration, and the concentration of gelatine. Inhibition is increased if either is reduced, suggesting that the effect is partly due to denaturation, and partly to competition with the substrate.

No transfer of phosphate from the substrate to chondroitin sulphate or its hydrolysates was found with concentrations up to 1%.

TABLE II

INHIBITION OF PROSTATIC ACID PHOSPHATASE BY CHONDROITIN SULPHATE HYDROLYSATE (CHAIN-LENGTH 3.8 RESIDUES)

with different concentrations of substrate (*p*-nitrophenyl phosphate) and of gelatine.

Concentration % (w/v) of substrate	*Concentration % (w/v) of gelatine*	*% Activity*	
		0.5% (w/v) Hydrolysate	*1.0% (w/v) Hydrolysate*
0.2	0.05	78	61.5
0.02	0.05	62.5	39
0.2	0.0005	68.5	42

Sodium alginate (B.D.H.)

Fig. 3 shows the effect of sodium alginate, of estimated mean chain-length 556 residues, on the activity of prostatic acid phospatase, in the presence and absence of gelatine 0.05% (w/v). Inhibition is considerably greater than with either sample of

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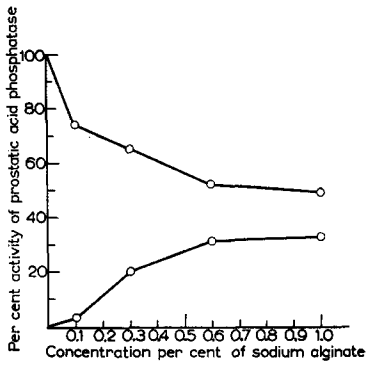


Fig. 3. Prostatic acid phosphatase activity with sodium alginate. Upper line: activity in the presence of gelatine 0.05% (w/v). Lower line: activity with no gelatine.

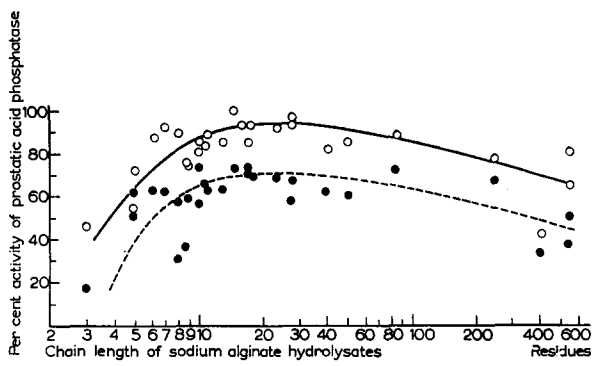
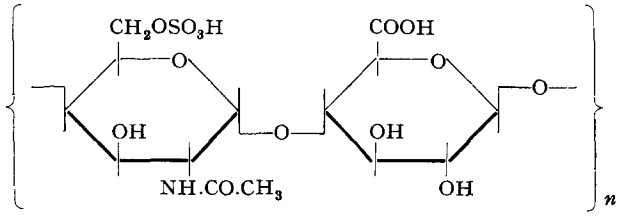


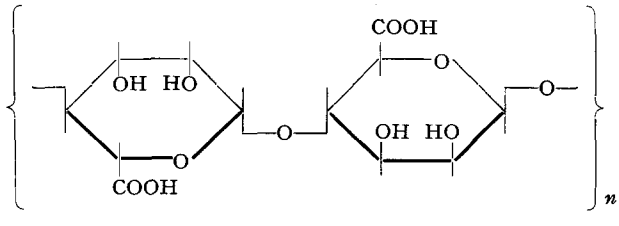
Fig. 4. Prostatic acid phosphatase activity with sodium alginate hydrolysates. O—O Activity with 0.8% hydrolysate + 0.05% gelatine. ●---● Activity with 1.0% hydrolysate only.

chondroitin sulphate, but protection only slightly less than that shown by the sample of comparable chain-length. Fig. 4 shows curves derived from a number of experiments showing the variation with mean chain-length of protection and inhibition by solutions of hydrolysates. With chains of 10 to 100 residues there is greater protection and less inhibition than with the undegraded substance, but with the short-chain oligosaccharides inhibition rapidly increases at the expense of protection. There would seem to be little difference, except the somewhat greater inhibition by alginate, between the effects of alginate and chondroitin sulphate hydrolysates of like chain-length; though chondroitin sulphate has both carboxyl and sulphuric acid groups and alginate carboxyl only, as shown in the following approximate formulae[1,3].

Chondroitin sulphate



Alginate



It was not expected that the amino groups of chondroitin sulphate would show any protective effect, since it was found that acetylation destroys the protective effect of amino compounds[8,9].

As with chondroitin sulphate, inhibition from sodium alginate increases with a decrease in either gelatine or substrate concentration, as shown in Table III, and there is a partial reversal of inhibition if gelatine is added after the start of incubation. No

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transfer of the phosphate radical from the substrate to alginate or its hydrolysates was found.

TABLE III

INHIBITION OF PROSTATIC ACID PHOSPATASE BY SODIUM ALGINATE

with different concentrations of substrate (*p*-nitrophenyl phosphate) and of gelatine

Concentration % (w/v) of substrate	*Concentration % (w/v) of gelatine*	*Concentration % (w/v) of alginate for 50% inhibition*
0.2	0.05	0.6
0.02	0.05	0.12
0.2	0.0005	0.1

Approximate estimations of Michaelis constants of inhibitors and the derived constants for *p*-nitrophenyl phosphate, using the graphic method of HUNTER AND DOWNS[10,11], are shown in Table V. Figures for alginate and chondroitin sulphate are derived from the inhibitions found with a gelatine concentration of 0.05% (w/v). The estimated constants for the substrate are widely divergent both from each other and from those found using the simpler acids showing purely competitive inhibition, which are in fair agreement.

Saccharate, gluconate, oxalate and tartrate

ABUL-FADL AND KING[12] noted the specific inhibition of prostatic acid phosphatase by *l*-tartrate. TSUBOI AND HUDSON[13] observed that this inhibition is competitive, and that oxalate shows weak competitive inhibition of the enzyme. Gluconate and saccharate are found to show competitive inhibition of an order similar to that for oxalate. The amounts of these substances required to give 50% inhibition under standard conditions are listed in Table IV; their Michaelis constants, and the derived constants for *p*-nitrophenyl phosphate[10,11] are shown in Table V. Inhibition remains the same when greater dilutions of gelatine are used, so would seem to be simply competitive, with no effect due to denaturation. In the absence of gelatine, there is a certain amount of protection from denaturation with saccharate and gluconate, but this extends over a small range of concentrations only, being quickly masked by the greater inhibition.

TABLE IV

INHIBITORS OF PROSTATIC ACID PHOSPHATASE

Inhibitor	*Concentration required to give 50% inhibition using p-nitrophenyl phosphate 0.2% (0.0076 M) at pH 4.9 in the presence of 0.05 gelatine*
SDS	0.00035 *M*
Saccharate	0.35 *M*
Gluconate	0.1 *M*
Oxalate	0.064 *M*
Tartrate	0.002 *M*
Glucose	3.6 *M* (*p*-nitrophenol liberation) 2.6 *M* (Phosphate liberation)
Chondroitin sulphate	(10% inhibition at 0.2% or approximately 0.00006 *M*)
Alginate	0.6% or approximately 0.000065 *M*

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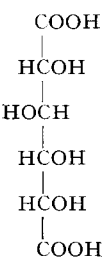
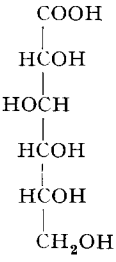
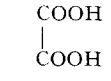
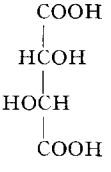
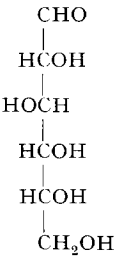
TABLE V

INHIBITORS OF PROSTATIC ACID PHOSPHATASE

Estimations of Michaelis constants of inhibitors and of the substrate (*p*-nitrophenyl phosphate).

Inhibitor		*kI*	*kI/kS*	*kS*
Saccharate		0.01 *M*	52	0.00019 *M*
Gluconate		0.003 *M*	13	0.00023 *M*
Oxalate		0.002 *M*	8.1	0.00025 *M*
Tartrate		0.00006 *M*	0.26	0.00023 *M*
Glucose		3.6 *M* (*p*-nitrophenol liberation) 2.6 *M* (Phosphate liberation)		
Chondroitin sulphate hydrolysate Chain-length 3.8 residues Molecular weight approx. 800		0.008 *M*	:	0.008 *M*
Sodium alginate Chain-length 556 residues Molecular weight approx. 92,000		0.0000054 *M*	0.007	0.0008 *M*

Glucose and starch

These substances have been more fully dealt with in a previous paper[14], but some mention of them here is appropriate, in view of the structural relationship between glucose and gluconate; starch and the acid mucopolysaccharides. Glucose shows extremely weak inhibition of prostatic acid phosphatase, accompanied by some phosphate transfer from the substrate to the glucose radical. There is no competition with the substrate, and no opposition to gelatine, nor was any protective effect found. Starch and its hydrolysates, however, give protection from surface denaturation comparable to that shown by chondroitin sulphate and sodium alginate. Some inhibition of the enzyme is shown by short-chain hydrolysates, but with starch itself, and hydrolysates of chain-length over 10 residues, this could not be detected.

DISCUSSION

Three kinds of inhibition of prostatic acid phosphatase have been met with in this investigation.

(i) SDS appears to inhibit by denaturation of the enzyme, in opposition to such protective substances as gelatine, other proteins and the long-chain amino-compounds[8,9].

(ii) Saccharate, gluconate, oxalate and tartrate compete with the substrate for the active centre of the enzyme.

(iii) Inhibition by glucose is not modified either by substrate concentration or by the concentration of protective substances. It is associated, as is inhibition by other hydroxyl compounds, with phosphate transfer from the substrate to the inhibitor; and though there is no direct relationship between inhibition and the amount of transfer, it would seem likely that both are due to competition with the hydroxyl group of water, or interference with its access to the enzyme[14].

The acid polysaccharides, chondroitin sulphate and alginate, show a combination of two of these three types of inhibition; there is some competition with the substrate, and some opposition to protective substances. Besides this they have a third effect, found also with starch and certain other hydroxyl compounds[14], in that they themselves protect the enzyme from surface denaturation. Slight protection is also shown by gluconate and saccharate, but is rapidly masked by the more marked inhibition from these simpler acids.

Protection of prostatic phosphatase by alginate is greatest with hydrolysates of moderate chain-length, from about 10 to 100 residues, and less for the same absolute concentration with both the undegraded substance and short-chain hydrolysates. Chondroitin sulphate again shows greatest protection at intermediate chain-length, roughly paralleling the effect of alginate; though inhibition with chondroitin sulphate does not increase so markedly with increased chain-length.

The degree of polymerization of chondroitin sulphate in bone matrix is thus likely to affect the activity of acid phosphatase in metastases from carcinoma of prostate —partial degradation of the mucopolysaccharide possibly leading to greater activity of the enzyme than with full polymerization, and breakdown to short chain oligosaccharides or constituent units causing considerable inhibition. Both the metabolism of the tumour and its effect on its environment by the liberation of inorganic phosphate

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may thus depend to a considerable extent on the state of polymerization of the mucopolysaccharides of bone matrix.

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SUMMARY

1. Inhibition of prostatic acid phosphatase by denaturation is shown by SDS. This is opposed by protective substances such as gelatine.

2. Saccharate, gluconate, oxalate and tartrate inhibit the enzyme competitively.

3. Glucose shows weak inhibition which is neither competitive nor an effect of denaturation.

4. Chondroitin sulphate and sodium alginate show inhibition which appears to be a combination of denaturation and competition.

5. Saccharate, gluconate, chondroitin sulphate and sodium alginate, as well as starch, in the absence of other protective substances, give some protection from surface denaturation. This is more marked with the acid polysaccharides than with the simpler acids, and varies with the chain-length of polysaccharides and their hydrolysates; the protection from the same concentration by weight being greater for chains of moderate length (10 to 100 residues) than for either very long or very short chains.

6. It is suggested that the degree of polymerization of chondroitin sulphate in bone may affect the activity of prostatic acid phosphatase in metastases from carcinomas of prostate.

REFERENCES

[1] P. W. KENT AND M. W. WHITEHOUSE, *Biochemistry of the Aminosugars*, Butterworth's Sci. Publ., London, 1955, pp. 75, 79–82.
[2] E. D. WILLS, *Biochem. J.*, 57 (1954) 109.
[3] E. G. V. PERCIVAL, *Structural Carbohydrate Chemistry*, Frederick Muller, Ltd, London, 1950, p. 159.
[4] C. HUGGINS AND P. TALALAY, *J. Biol. Chem.*, 159 (1945) 399.
[5] F. C. KOCH AND M. E. HANKE, *Practical Methods in Biochemistry*, Baillère, Tindall & Cox, London, 1953, p. 227.
[6] E. J. KING AND R. J. GARNER, *J. Clin. Pathol.* 1 (1947) 30.
[7] G. E. DELORY, *Photoelectric Methods in Clinical Biochemistry*, Hilger & Watts, London, 1949, p. 70.
[8] G. M. JEFFREE, *Nature*, 175 (1955) 509.
[9] G. M. JEFFREE, *Biochim. Biophys. Acta*, 20 (1956) 503.
[10] A. HUNTER AND C. E. DOWNS, *J. Biol. Chem.* 157 (1945) 427.
[11] T. S. WORK AND E. WORK, *The Basis of Chemotherapy*, Oliver & Boyd, Edinburgh and London, 1948, pp. 166–170.
[12] M. A. M. ABUL-FADL AND E. J. KING, *Biochem J.*, 45 (1949) 51.
[13] K. K. TSUBOI AND P. B. HUDSON, *Arch. Biochem. Biophys.*, 55 (1955) 191.
[14] G. M. JEFFREE, *Biochim. Biophys. Acta*, 23 (1957) 155.

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