SOME EFFECTS OF HYDROXYL COMPOUNDS ON PROSTATIC ACID PHOSPHATASE

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Three interactions between hydroxyl compounds and prostatic acid phosphatase have been found. First is the irreversible inhibition of the enzyme by alcohols, noted by HERBERT[1], who used ethanol inhibition to distinguish the prostatic enzyme from the normal acid phosphatase of blood serum, and later recorded by ABUL-FADL AND KING[2] for other alcohols.

Secondly, hydroxyl compounds may compete with water as acceptors of the phosphate radical from organic phosphates. The enzyme then acts as a phospho-transferase as well as a phosphatase. This effect has been found with many phosphatases since AXELROD reported phosphate transfer by a number of acid phosphatases from *p*-nitrophenyl-, phenyl- and phenolphthalein phosphates to a wide range of primary and secondary alcohols[3]. The same author showed, with labelled phosphate, that the contribution of free inorganic phosphate to the transferase effect was negligible, so that direct transfer from the substrate must be assumed[4].

Phosphate transfer has been shown with prostatic acid phosphatase by GREEN AND MEYERHOF, using acetyl phosphate, phosphocreatine and *p*-nitrophenyl phosphate as substrates, and glycerol and monosaccharides as acceptors[5]; and by BRAVERMANN AND CHARGAFF with phenyl phosphate as substrate and nucleosides as acceptors[6,7]. Transfer by other phosphatases has been reported by MEYERHOF AND GREEN[8], TSUBOI AND HUDSON[9], and MORTON[10].

There is general agreement, borne out in the present investigations, that simple hydrolysis decreases and phosphate transfer increases with increasing concentrations of the acceptor, though not necessarily to the same degree. Liberation of the organic radical may fall or remain steady, and in some instances in the present series it was found to rise, so that addition of hydroxyl compounds might give apparent activation if enzyme activity were measured by liberation of the organic radical, though phosphate liberation was decreased.

A third effect of long-chain hydroxyl compounds which was found in these investigations is protection of the enzyme from surface denaturation. This seems to be analogous to the protection previously noted with amino compounds[11,12], but much higher concentrations of hydroxyl compounds are required to give similar protection. It was found that denaturation of the enzyme is greatly assisted by traces of anionic detergents remaining in the tubes after washing, even after rinsing in tap and distilled water, but that this denaturation is truly a surface phenomenon is demonstrated by the maintenance of almost complete activity, even in such tubes, if the incubation mixture is covered with a layer of petroleum ether, and the diluted enzyme preparation introduced beneath this layer.

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MATERIALS AND METHODS

Specimens of seminal fluid, submitted for the investigation of fertility, were obtained from the pathological laboratory of the Bristol General Hospital, and stored in the refrigerator until required. Dilutions, ranging from 1/2,000 to 1/20,000 were made into physiological saline (0.9% NaCl w/v), using polythene vessels and pipettes to minimize surface denaturation at this stage. Specimens were selected which showed high acid phosphatase activity in the presence of gelatine 0.05% (w/v), and virtually none in its absence when 0.1 ml was incubated in 1 ml substrate mixture in open glass tubes which had been washed in Teepol and rinsed with tap and distilled water.

p-Nitrophenyl phosphate was used as substrate throughout the series, in the concentration given by BESSEY, LOWRY AND BROCK[13]—0.2% (w/v) in the buffered mixture before the addition of the enzyme preparation—but the citrate buffer of ABUL-FADL AND KING[14] at pH 4.9 was used instead of alkaline buffer. Substances under investigation were incorporated in this mixture in varying concentrations, either with or without gelatine 0.05% (w/v), and phosphatase activity was compared to that obtained in the presence of this concentration of gelatine without other additions. Open glass tubes were used, containing 1 ml of the substrate mixture, to which was added 0.1 ml of the enzyme dilution. Incubation was generally for 30 min at 37°. In preliminary investigations where *p*-nitrophenol estimations only were made, activity was stopped by the addition of 1 ml of the glycine-pyrophosphate buffer of HUGGINS AND TALALAY[15], followed by 3 ml water. When liberation of *p*-nitrophenol and phosphate were to be estimated on the same specimen, activity was stopped by the addition of 1 ml 50% (w/v) trichloroacetic acid; this strength being found necessary to precipitate the gelatine in the standard tubes. Tubes containing gelatine or other precipitate were then centrifuged; 20–30 minutes being required to remove the gelatine satisfactorily. 1.6 ml was pipetted from each tube into 2.4 ml of distilled water and mixed. 1 ml of this mixture was taken for the estimation of *p*-nitrophenol liberation; to this was added 3 ml of $N/2$ NaOH, and the colour density compared with that of a *p*-nitrophenol standard. The remaining 3 ml was used for the estimation of liberated phosphate by the method of Fiske and Subbarow as given by KOCH AND HANKE[16], using a phosphate standard of like molarity to the *p*-nitrophenol standard. Tubes were incubated in duplicate, with controls treated similarly except for the addition of the enzyme preparation. The mean value of *p*-nitrophenol and phosphate liberation in the presence of gelatine 0.05% was taken as the standard to which all other values were compared. In the presence of polyhydric alcohols and some sugars, the development of the blue colour of reduced phosphomolybdate is extremely slow, and it was necessary to put up standard control tubes, with the addition of the test substance, to ensure that full colour production had occurred before readings were taken.

Readings were made on a Spekker Absorptiometer, using a mercury-vapour lamp. For phosphate estimations, the yellow mercury lines at 5770 and 5791 A were isolated by the used of Ilford Spectrum Yellow Filter, No. 606 (transmission between 5600 and 6100 A), and for the estimation of *p*-nitrophenol the mercury violet band at 4360 A was isolated by Ilford Spectrum Violet Filter, No. 601 (transmission 3850–4750 A) with the supplementary filter No. 809, which cuts out light between 3750 and 4150 A.

The average chain-lengths of hydrolysates of soluble starch were calculated from estimations of reducing sugar in suitable dilutions by the method of KING AND GARNER[17] as quoted by DELORY[18]. The known concentration of starch hydrolysate, divided by the estimated concentration of reducing sugar, was taken as the mean chain-length; when this figure was under 2, it was multiplied by 1.1 to take into account the increase of weight on hydrolysis; with longer chain-lengths this was treated as negligible.

RESULTS

Monohydric alcohols

Fig. 1 shows the activity of prostatic acid phosphatase in the presence of different concentrations of *n*-propanol, and indicates the reference points which are used for comparison in the tables; the concentration at which phosphate liberation is halved; that at which *p*-nitrophenol liberation in millimoles is 110% phosphate liberation, so that phosphate transfer is 10%; and the concentration at which *p*-nitrophenol liberation drops to the original value, obtained in the presence of gelatine only.

Table I gives comparative figures for different alcohols. Both inhibition and phos-

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phate transfer increase with the chain-length of primary alcohols. *iso*-Butanol shows inhibition similar to that of *n*-butanol, but less transfer. *sec.*-Butanol shows inhibition between those of *n*-butanol and *n*-propanol, but a transfer ratio near that of ethanol; with *tert.*-butanol neither inhibition nor transfer was found. These results agree with those of ABUL-FADL AND KING, who found that inhibition of prostatic phosphatase by alcohols increased in going up the series[2]; and of AXELROD, who showed with other acid phosphatases that primary alcohols were more effective acceptors than secondary, and that tertiary alcohols would not act as phosphate acceptors[3].

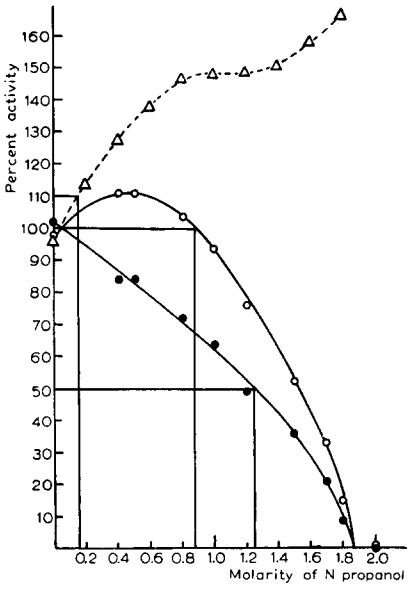

Fig. 1. Activity of prostatic acid phosphatase in the presence of *n*-propanol. Incubations with *p*-nitrophenyl phosphate 0.2 % in citrate buffer at pH 4.9, in the presence of gelatine 0.05 %. ○ Liberation of *p*-nitrophenol; ● Liberation of phosphate; △ Liberation of *p*-nitrophenol expressed as percentage of phosphate liberation. Reference points indicated. 10 % transfer of phosphate radical (*p*-nitrophenol liberation = 110 % phosphate liberation); 50 % inhibition of phosphate liberation; 100 % *p*-nitrophenol liberation.

p-Nitrophenol liberation is increased at initial concentrations of alcohols, except with *iso*butanol and *tert.*-butanol. In the absence of gelatine, slight protection from surface denaturation was sometimes found; this was irregular in its occurrence, but the effect appeared to increase with chain-length.

TABLE I

EFFECTS OF MONOHYDRIC ALCOHOLS ON PROSTATIC ACID PHOSPHATASE ACTIVITY

Alcohol	*50% Phosphate inhibition*	*10% Transfer*	*100% p-Nitrophenol*	*Protective activity*
Methanol	4.3 *M*	0.7 *M*	3 *M*	± at 5 *M*
Ethanol	2.4 *M*	0.4 *M*	0.8 *M*	Slight at 2–3 *M*
n-Propanol	1.25 *M*	0.16 *M*	0.9 *M*	Slight at 1.5 *M*
n-Butanol	0.5 *M*	0.02 *M*	0.5 *M*	± 0.2–0.4 *M*
iso-Butanol	0.4 *M*	0.05 *M*	0	None to 1.2 *M*
Secondary butanol	0.8 *M*	0.5 *M*	0.4 *M*	None to 0.75 *M*
Tertiary butanol	No inhibition	No transfer	0	None to 0.6 *M*

Glycols (Table II)

These form a similar series to the primary alcohols, but with slightly less inhibition and greater transfer with the same chain-length. Increased liberation of *p*-nitrophenol was found at initial concentrations of trimethylene and hexamethylene glycols, but not with ethylene glycol. Protection from surface denaturation was more definite, and again increased with chain-length.

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TABLE II

EFFECTS OF GLYCOLS ON PROSTATIC ACID PHOSPHATASE ACTIVITY

Glycol	*50% Phosphate inhibition*	*10% Transfer*	*100% p-Nitrophenol*	*Protective activity*
Ethylene glycol	3 *M*	0.4 *M*	0	None to 6 *M*
Trimethylene glycol	1.6 *M*	0.1 *M*	1.1 *M*	from 0.4 *M*
Hexamethylene glycol	0.4 *M*	0.01 *M*	1.0 *M*	from 0.2 *M*

Polyhydric alcohols (Table III)

These are not such effective phosphate acceptors as the glycols, and chain-length has little effect on the extent of phosphate transfer, which is constant for glycerol, erythritol, mannitol and sorbitol, though greater for dulcitol. Inhibition increases with chain-length, and, in the hexahydric alcohols, appears to be increased by the trans-configuration of secondary hydroxyl groups. Increased liberation of *p*-nitrophenol was found with glycerol and erythritol, but with none of the hexitols. No protective activity was found.

TABLE III

EFFECTS OF POLYHYDRIC ALCOHOLS ON PROSTATIC ACID PHOSPHATASE ACTIVITY

	Glycerol	*Erythritol*	D-*Mannitol*	D-*Sorbitol*	*Dulcitol*
	CH_2OH HCOH CH_2OH	CH_2OH HCOH HCOH CH_2OH	CH_2OH HOCH HOCH HCOH HCOH CH_2OH	CH_2OH HCOH HOCH HCOH HCOH CH_2OH	CH_2OH HCOH HOCH HOCH HCOH CH_2OH
50% Phosphate inhibition	3.3 *M*	1.5 *M*	1.4 *M*	0.9 *M*	0.35 *M*
10% Transfer	0.2 *M*	0.2 *M*	0.2 *M*	0.2 *M*	0.1 *M*
100% *p*-nitrophenol	4.2 *M*	1.2 *M*	0	0	0

Monosaccharides (Table IV)

Trans-configuration appears to increase both inhibition and transfer to some extent. Inhibition is slightly greater with ketoses than with the corresponding aldoses, but phosphate transfer is approximately the same. There was no increase in *p*-nitrophenol liberation, and no protective activity was found.

Oligosaccharides (Table V)

Phosphate transfer to sucrose and maltose is approximately the same as to fructose and glucose respectively, but inhibition is more rapid with the disaccharides. *p*-Nitrophenol liberation is not increased. Slight variable protection was found with high concentrations of sucrose. Investigations were limited by the comparatively low solubility of many oligosaccharides.

Starch and starch hydrolysates (Table VI)

"Soluble Starch" (Ferris, Ltd.) was used for these investigations, as this should be composed mainly of straight-chain molecules, and so be more directly comparable with

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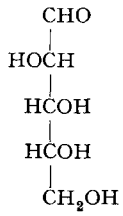
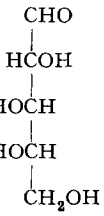
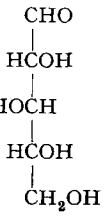
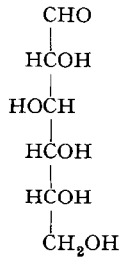
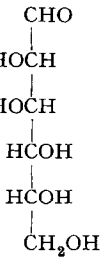
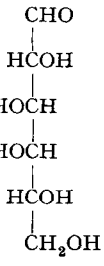
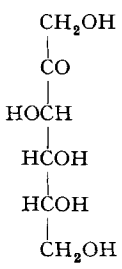
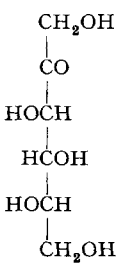
TABLE IV

EFFECTS OF MONOSACCHARIDES ON PROSTATIC ACID PHOSPHATASE ACTIVITY

Aldo-pentoses	D (—) *Arabinose*	L (+) *Arabinose*	D (+) *Xylose*
			
50 % Phosphate inhibition	1.8 *M*	1.6 *M*	1.6 *M*
10 % Transfer	0.8 *M*	0.4 *M*	0.6 *M*

Aldo-hexoses	D (+) *Glucose*	D (+) *Mannose*	D (+) *Galactose*
			
50 % Phosphate inhibition	2.6 *M*	2.3 *M*	1.9 *M*
10 % Transfer	0.7 *M*	1.2 *M*	0.4 *M*

Keto-hexoses	D (—) *Fructose*	L (—) *Sorbose*
		
50 % Phosphate inhibition	2.0 *M*	1.6 *M*
10 % Transfer	1.0 *M*	0.4 *M*

the oligosaccharides. With short-chain hydrolysates, transfer is similar and inhibition somewhat greater than with the same absolute concentration of glucose; but both show an increase with increasing chain-length at like molar concentrations. There is no increase in *p*-nitrophenol liberation. Investigations with starch and long-chain hydrolysates were limited by the high viscosity of concentrated solutions.

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TABLE V

EFFECTS OF OLIGOSACCHARIDES ON PROSTATIC ACID PHOSPHATASE ACTIVITY

	50% *Phosphate inhibition*	10% *Transfer*	*Protective activity*
Sucrose α D-glucopyrano-1:2 β D-fructofuranoside	1.5 *M*	1.1 *M*	± 0.5 – 1.5 *M*
Maltose α D-glucopyrano-1:4 α D-glucopyranoside	1.4 *M*	0.5 *M*	None to 1.5 *M*
Lactose α D-glucopyrano-1:4 β D-galactopyranoside	(5% inhibition at 0.5 *M*)	(No transfer to 0.5 *M*)	None to 0.5 *M*
Raffinose α D-galactopyrano-1:6 α D-glucopyrano-1:4 β D-fructofuranoside	(No inhibition to 0.1 *M*)	(No transfer to 0.1 *M*)	None to 0.1 *M*

TABLE VI

EFFECTS OF STARCH HYDROLYSATES ON PROSTATIC ACID PHOSPHATASE ACTIVITY

	Glucose units	*Mol. wt.*	50% *Protection*		10% *Transfer*		10% *Inhibition*	
			%	*Molarity*	%	*Molarity*	%	*Molarity*
"Soluble starch"	223	36,000	1.2	0.0003				
Hydrolysate	30	4,860	0.9	0.002				
Hydrolysate	3.5	585	0.4	0.007	12	0.2	7	0.12
Hydrolysate	1.3	222	1.0	0.045	9	0.4	5.5	0.25
Glucose	1.0	180	No protection		12.6	0.7	13.5	0.75

Though glucose gives no protection from surface denaturation, this is found throughout the series of starch hydrolysates; and though the absolute effect is greatest with comparatively short-chain hydrolysates, the molar effect increases with chain-length throughout the series.

DISCUSSION

With starch hydrolysates and the glycols, the power to protect prostatic acid phosphatase from surface denaturation appears to depend on the chain-length. In the presence of secondary alcoholic groups, a much longer chain is required for a like effect than with the glycols. This protection appears similar to that previously obtained with amino-compounds[11,12], but the concentrations required are enormously greater; 0.4 *M* trimethylene glycol or 0.2 *M* hexamethylene glycol give protection approximately equivalent to that given by 11.0 m*M* trimethylene diamine or 0.2 m*M* hexamethylene diamine.

The ability to act as phosphate acceptors with prostatic phosphatase appears to depend on the spacing between hydroxyl groups, rather than upon molecular size; it increases rapidly with the chain-length of glycols, but with polyhydric alcohols depends more on the *cis*- or *trans*-configuration of the secondary hydroxyl groups. Its increase with the chain-length of monohydric alcohols may possibly depend on association of the alcohol molecules.

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The power to inhibit prostatic phosphatase increases with the molecular weight of comparable series, but appears to decrease with an increase in the number of hydroxyl groups. It is greater with monohydric alcohols than with glycols, and less again with polyhydric alcohols.

Increased liberation of *p*-nitrophenol occurs when phosphate transfer is greater than the inhibition of phosphatase activity, and in general is found with straight-chain compounds with widely-spaced hydroxyl groups. It was not seen with hexitols, sugars or starch hydrolysates.

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SUMMARY

1. Three effects of hydroxyl compounds on prostatic acid phosphatase activity have been investigated.

2. Protection from surface denaturation was found with glycols and starch hydrolysates, and increased with chain-length in each series.

3. Prostatic phosphatase was found to transfer phosphate from *p*-nitrophenyl phosphate to primary and secondary alcohols, glycols, polyhydric alcohols, sugars and starch hydrolysates. The effect increased with the spacing between hydroxyl groups in the molecule.

4. Inhibition of prostatic acid phosphatase activity was shown by the same series of compounds, but was found to increase with molecular weight and to decrease with an increase in the number of hydroxyl groups in the molecule.

5. When phosphate transfer exceeded inhibition, an increased liberation of *p*-nitrophenol was found.

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