The Effect of Alcohols on the Hydrolysis of Sodium Phenolphthalein Diphosphate by Prostatic Extracts

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King (1943) synthesized several phosphoric esters which liberate chromogens after phosphatase hydrolysis. Among these were the calcium, barium and lead salts of phenolphthalein phosphate. He found, however, that these compounds were not suitable as a substrate for plasma phosphatase determinations owing to the slow rate of hydrolysis. Huggins & Talalay (1945) synthesized the sodium salt of phenolphthalein phosphate and considered it to be sufficiently rapidly hydrolyzed by phosphatase to constitute a suitable substrate for measuring phosphatase activity in plasma and other biological fluids. They described a method of measuring acid phosphatase activity using this substrate buffered at pH 5.4 and measuring colorimetrically the free phenolphthalein liberated. Whilst comparing this method with that of Gutman, Gutman & Robinson (1939), it was noticed that the addition of ethanol to the digest greatly increased the free phenolphthalein liberation.

EXPERIMENTAL

Preparation of enzyme. Human prostate was used as enzyme source. Partial purification was effected by the method of Kutscher & Worner (1936) slightly modified. The prostate was finely chopped, suspended in three times its own weight of distilled water, and autolyzed under toluene for 3 or 4 days. After centrifuging, the supernatant fluid was dialyzed against distilled water, and the protein precipitate filtered off. The preparation was freeze-dried and stored in the refrigerator. For use 6 mg. were dissolved in 50 ml. of distilled water.

Pentasodium phenolphthalein diphosphate was synthesized and acid-phosphatase activity estimated by the method of Huggins & Talalay (1945). Chloroform, however, was omitted from the substrate solution because irregular results were found in its presence.

Procedure. The phosphate liberated was measured as in the Bodansky (1933) method for the estimation of alkaline phosphatase. Simultaneous estimations of phenolphthalein and phosphate were carried out after digestion at 37° for 1 hr., both without any alcohol, and in the presence of ethanol, methanol, *n*-propyl and isopropyl alcohols and ethylene glycol.

The following buffered substrate was used: 1.216 g. sodium phenolphthalein phosphate, 11.7 g. sodium acetate (hydrated) and 0.79 ml. glacial acetic acid, dissolved in water and made up to 1 l. Each estimation was carried out in duplicate. Colorimeter tubes were set up in the water bath at 37° containing:

Tube (1) 2.5 ml. buffered substrate; 2.5 ml. distilled water; 0.5 ml. enzyme solution.

Tube (2) 2.5 ml. buffered substrate; 2.5 ml. diluted alcohol; 0.5 ml. enzyme solution.

1 ml. was immediately removed and discarded from (1), and 1 ml. from (2) was removed and run into a tube containing 5 ml. 12% (w/v) trichloroacetic acid and 4 ml. distilled water. This was centrifuged, and the initial phosphate determined by the amount of colour developed from 5 ml. of supernatant fluid. The tubes were then incubated for 1 hr., after which sufficient pure alcohol was added to (1) to give it the same concentration of alcohol as (2). The volume of (2) was then adjusted to that of (1) by adding alcohol of the appropriate strength. This dilution of the digest was allowed for in calculating the results. 1 ml. was then removed from (1) and (2) into tubes containing 5 ml. 12% (w/v) trichloroacetic acid and 4 ml. distilled water, centrifuged, and the supernatant fluid used to obtain the amount of free phosphate after digestion. To the remainder of the solution in the colorimeter tubes, glycine buffer and water were added, and the amount of free phenolphthalein liberated determined.

RESULTS

The effect of different concentrations of ethanol on the phenolphthalein liberated by the enzyme was determined. Approximately 15% (v/v) was found to show the greatest effect (Fig. 1). For the other alcohols, a concentration giving a marked increase in the amount of phenolphthalein liberated was chosen. The differences between the amounts of phenolphthalein and phosphate liberated with and without alcohol were measured, and the percentage increase or decrease calculated (Table 1). For each alcohol there is a large increase in the amount of free phenolphthalein liberated. As regards phosphate liberation it appears that methyl, ethyl and *n*-propyl alcohols, at the concentrations used, cause no increase in phosphate liberation, whereas isopropyl alcohol produces a marked increase in the amount of phosphate liberated, and ethylene glycol a marked decrease.

DISCUSSION

Axelrod (1947) using an acid-optimum phosphatase prepared from orange juice, and monophenyl phosphate, *p*-nitrophenyl phosphate or sodium phenolphthalein phosphate as substrate, found that in the presence of certain alcohols, transference of phosphate from the substrate to the alcohol is catalyzed

by a newly described enzyme. Such a reaction would account for the results described in the present

a transphosphorylase which catalyze the following reactions:

ϕ = phenolphthalein; A = alcohol.

Thus, in the presence of an alcohol, there is an increase in the liberation of free phenolphthalein, but no such effect on phosphate liberation.

In the case of ethylene glycol, the transphosphorylase presumably competes more effectively than the phosphatase for the phenolphthalein phosphate, whereas the case of *isopropyl* alcohol suggests that the alcohol phosphate is split again. Fig. 1 indicates that the transphosphorylase is inactivated by 20 % (v/v) ethanol.

SUMMARY

1. The formation of free phenolphthalein from pentasodium phenolphthalein diphosphate by prostatic phosphatase preparations acting at pH 5.4 and 37° is increased by methyl, ethyl, *n*-propyl and *isopropyl* alcohols, and ethylene glycol.

2. The formation of free phosphate under the above conditions is apparently unaffected by methyl, ethyl, and *n*-propyl alcohols, increased by *isopropyl* alcohol and decreased by ethylene glycol.

3. An interpretation of these results in terms of transphosphorylation is suggested.

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Fig. 1. The effect of different concentrations of ethanol on the production of free phenolphthalein by the action of prostatic phosphatase extract on pentasodium phenolphthalein diphosphate.

communication, assuming that there are two enzymes present, an acid-optimum phosphatase and

Table 1. The effect of alcohols upon the amounts of phenolphthalein and phosphate liberated from pentasodium phenolphthalein diphosphate, by incubation with a prostatic extract

(ϕ = phenolphthalein. A = alcohol. Inorganic phosphate is expressed as mg. P/l.)

Alcohol (%, v/v)	ϕ liberated (mg./l.)		P liberated (mg./l.)		Increase (%)	
	With A	Without A	With A	Without A	ϕ	P
15% ethyl	75.8	45.0	18.6	19.4	+ 66	- 4
	94.5	58.5	16.6	15.6	+ 62	+ 6
	91.0	52.5	20.4	18.5	+ 74	+10
10% methyl	89.0	52.0	16.3	17.1	+ 71	- 5
	97.0	54.0	15.7	16.9	+ 79	- 7
	90.0	49.2	15.4	15.9	+ 83	- 3
5% <i>n</i> -propyl	86.5	56.0	20.9	19.7	+ 53	+ 6
	85.0	56.0	21.6	22.4	+ 52	- 7
	90.0	58.0	20.3	18.9	+ 55	+ 6
10% <i>isopropyl</i>	88.5	56.5	27.7	20.7	+ 57	+34
	83.0	51.5	26.5	17.7	+ 61	+50
	88.5	54.5	25.4	18.2	+ 62	+39
20% ethylene glycol	92.5	53.5	18.0	20.0	+ 73	-10
	89.5	41.2	11.8	19.4	+117	-39
	94.5	51.5	15.7	22.2	+ 84	-30

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