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THE EFFECT OF CERTAIN CATIONS AND ANIONS ON THE ALKALINE PHOSPHOMONOESTERASE ACTIVITIES IN HUMAN DENTAL PLAQUE

by

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INTRODUCTION

The existence of certain factors in commercially available enzymes, human saliva, and some bacterial enzyme preparations having a phosphate liberating activity (*Paunio et al.*, 1968; *Mäkinen & Paunio*, 1969) has led the author to investigate phosphorolytic activity in human dental plaque material. This study was further stimulated by the knowledge that some of these phosphate liberating factors revealed high rates of phosphorolytic activity (data to be published). This particular study deals with the effect of certain metal ions and some related compounds on the rate of enzymic hydrolysis of p-nitrophenyl phosphate by enzyme preparations obtained through fractionation of human dental plaque material.

MATERIALS AND METHODS

Chemicals. All chemicals used in this study were supplied by E. Merck AG (Darmstadt, Germany) if not otherwise stated.

Enzyme preparations. Six different enzyme preparations were used in this study. The enzyme preparations were derived from human dental plaque by

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fractionation according to a method developed earlier. Briefly, the method was the following: Human dental plaque material was collected into iced 0.165 M NaCl solution, the mixture was centrifuged and the supernatant was collected and the solid discarded. The crude enzyme preparations obtained from different persons were pooled and then fractionated through Sephadex G-200 columns; further fractionation was performed using DEAE-cellulose ion exchange chromatography. Thus six different enzyme preparations were obtained, possessing p-nitrophenyl phosphate hydrolysing activity, which were then subjected to affector studies (for details of the fractionation, see *Paunio*, 1969). These enzyme preparations were numbered from E I to E VI.

Substrate. p-Nitrophenyl phosphate (Mann Research Laboratories, Inc., New York, N.Y., U.S.A.) was used in this study as the working substrate.

Determination of enzyme activity. The method was based on measurement of color intensity produced by the enzymatically liberated yellow p-nitrophenyl anion at 410 mμ (read on a Hitachi-Perkin Elmer UV-VIS Spectrophotometer). The procedure used was the following: From 10^{-3} or 5×10^{-3} M substrate stock solutions 0.1 ml was taken into the reaction mixture; the reaction mixture contained, in addition, 0.3 ml buffer (0.02 M boric acid borax buffer, pH 8.8), 0.1 ml water or other water soluble compounds to be tested, and finally 0.1 ml enzyme solution; the test tubes with the reaction mixtures were then incubated at 37 C for 22 hours. The use of prolonged incubation time in this study was determined by the evidently low enzyme activity in the enzyme preparations after fractionation. In test experiments concerning the effect of time on the rate of enzymic hydrolysis of p-nitrophenyl phosphate, the line drawn was still linear after 25 hours, when the rate of hydrolysis was plotted against the incubation time, thus ruling out possible autohydrolysis of the tested enzymes. After the incubation the reaction mixtures were placed in an iced water bath, followed by an addition of 1 ml 0.2 M NaOH.

In determining the effect of metal ions on the enzymic hydrolysis of the substrate, all experimental values were determined from spectrophotometric readings of both experiments and controls. The control experiments contained the tested metal ions at the same concentrations as in the experiments, though it should be remembered, that some metal ions catalyse a spontaneous hydrolysis of certain phosphate ester bonds (*Kroll*, 1952).

Affectors. The following ions and compounds were tested as chlorides if not otherwise stated: Al^{3+}, Cr^{3-}, $Na^{+}F^{-}$, $Sn^{2+}F^{-}$, Sn^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Sr^{2+}, Zn^{2+}, and $(NH_4)_6Mo_7O_{24}H_2O$. These ions or compounds were tested at different concentrations in the raction mixture, presented in more detail in the Results.

Expression of data. The rate of the enzymic hydrolysis of p-nitrophenyl phosphate was presented as v, which expresses the molar change of the liberated p-nitrophenyl anion in the reaction mixture per minute. One plotting method was used in presenting the results: the rate of hydrolysis (v) was plotted against the affector concentration.

RESULTS

The presence of Mg^{2+} ions in the raction mixture did increase the rate of hydrolysis of p-nitrophenyl phosphate by all the tested enzyme preparations. Figure 1 shows typical results obtained in such experiments with the different enzyme preparations. Corresponding increases in the hydrolysis rate could also be obtained with a higher substrate concentrations (0.8×10^{-3} M).

The following experiments were performed using a 0.166×10^{-3} M Mg^{2+} ion concentration in the reaction mixture.

When metal ions such as Cr^{3+}, Ni^{2+}, Al^{3+}, Mn^{2+}, and $(NH_4)_6Mo_7O_{24}$ $4H_2O$ were tested, a marked decrease in the enzymic hydrolysis rate of the substrate could be notes. This was the case when all the enzyme preparations were tested, regardless of the substrate concentration used (Tables I—V).

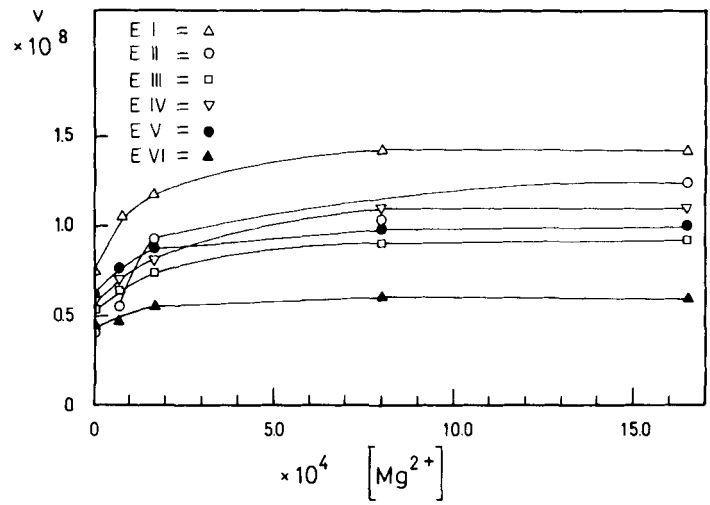

Fig. 1. The effect of Mg^{2+} ion (as $MgCl_2$) on the rate of hydrolysis of p-nitrophenyl phosphate by fractionated human dental plaque enzymes. Tested at 0.166 x 10^{-3}M substrate concentration. E I, E II etc. refer to the various enzyme preparations used.

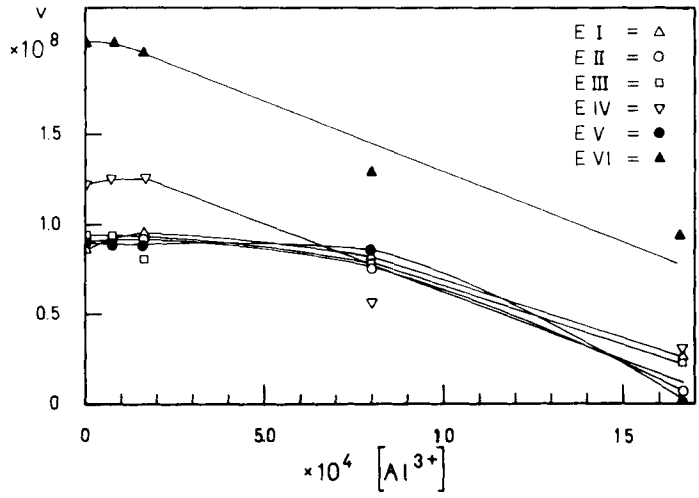

Fig. 2. The effect of Al^{3+} ion (as $AlCl_3$) on the rate of hydrolysis of p-nitrophenyl phosphate by fractionated human dental plaque enzymes. Tested at 0.8 x 10^{-3} M substrate concentration and in presence of 0.166 x 10^{-3} M $MgCl_2$.

Table VI presents the results obtained with Zn^{2+} ions, showing that Zn^{2+} ions did not exert any effect on the enzymic hydrolysis reaction.

This was especially the case in many experiments performed with metal ions when the experiments were performed using higher concentrations of substrate, i.e. the drop of the enzymic hydrolysis rate was not linear when the rate was plotted as a function of inhibitor concentration [I]. Figure 2 shows the results obtained in such experiments: no, or a very slight, decrease in the hydrolysis rate of the substrate in presence of low inhibitor concentrations, followed then by a rapid reduction in the hydrolysis rate in presence of higher inhibitor concentrations.

Sr^{2+} ions generally produced a slight activating affect on the hydrolysis rate of p-nitrophenyl phosphate by the different enzyme preparations. Table VII presents the results obtained in these experiments. The enzymes of Pool III and VI were unaffected by the Sr^{2+} ions. The other four enzyme preparations were activated by these ions.

F^- ions alone in the reaction mixture did not affect the enzymic hydrolysis by all the tested enzyme preparations (Table VIII). A marked decrease in the hydrolysis rate could, however, be achieved, when F^- ions were tested together with Sn^{2+} ions (Table IX). When Sn^{2+} ions were present alone a corresponding reduction in the rate of hydrolysis could be obtained (Table X).

Table I

The effect of Al^{3+} ions on the rate of hydrolysis of p-nitrophenyl phosphate by fractionated human dental plaque enzyme preparations performed at two different substrate concentration levels. The results are given as v, expressing the molar change of the liberated p-nitrophenol in the reaction mixture per min. ($x10^8$). The incubation was performed in 0.05 M ββ-glutaric acid buffer, pH 8.8 and in presence of 0.166×10^{-3} M Mg^{2+} ion conantration. The incubation time and temperature was 22 hours and 37°C respectively

Ion concentration (M)	Enzyme preparation											
	I		II		III		IV		V		VI	
	Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)	
	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}
Control	0.9	0.8	0.9	0.8	0.9	0.7	1.2	1.2	0.8	0.9	2.1	1.6
0.8×10^{-4}	0.8	0.7	0.9	0.9	0.8	0.7	1.3	1.2	0.7	0.8	2.0	1.6
0.166×10^{-3}	0.8	0.6	0.8	0.8	0.7	0.7	1.3	1.2	0.7	0.7	1.9	1.5
0.8×10^{-3}	0.7	0.6	0.7	0.7	0.7	0.6	0.6	0.7	0.7	0.4	1.3	1.6
0.166×10^{-2}	0.3	0	0.1	0	0.3	0	0.3	0	0	0.1	0.9	0.3

Table II

The effect of Ni^{2+} ions on the rate of hydrolysis of p-nitrophenyl phosphate by fractionated human dental plaque enzyme preparations performed at two different substrate concentration levels. The results are given as v, expressing the molar change of the liberated p-nitrophenol in the reaction mixture per min. ($x10^8$). The incubation was performed in 0.05 M ββ-glutaric acid buffer, pH 8.8 and in presence of 0.166×10^{-3} M Mg^{2+} ion concentration. The incubation time and temperature was 22 hours and 37°C respectively

Ion concentration (M)	Enzyme preparation											
	I		II		III		IV		V		VI	
	Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)	
	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8x10-3	0.166×10^{-3}	0.8×10^{-3}
Control	0.6	0.7	0.9	0.9	0.6	0.7	1.3	1.1	0.8	1.0	1.9	1.8
0.8×10^{-4}	0.5	0.6	0.8	0.6	0.5	0.4	1.0	0.9	0.2	0.3	1.8	1.7
0.166×10^{-2}	0.5	0.4	0.7	0.5	0.5	0.3	0.9	0.8	0.1	0	1.9	1.6
0.8×10^{-3}	0.1	0.07	0	0.1	0.3	0.1	0.1	0.6	0	0	1.7	1.3
0.166×10^{-2}	0.1	0	0	0	0.3	0	0	0.1	0	0	1.0	1.3

Table III

The effect of Mn^{2+} ions on the rate of hydrolysis of p-nitrophenyl phosphate by fractionated human dental plaque enzyme preparations performed at two different substrate concentration levels. The results are given as v, expressing the molar change of the liberated p-nitrophenol in the reaction mixture per min. ($x10^8$). The incubation was performed in 0.05 M ββ-glutaric acid buffer, pH 8.8 and in presence of 0.166×10^{-3} M Mg^{2+} ion concentration. The incubation time and temperature was 22 hours and 37°C respectively

Ion concentration (M)	Enzyme preparation											
	I		II		III		IV		V		VI	
	Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)	
	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.66×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}
Control	0.8	0.9	1.3	1.4	1.3	0.9	2.2	1.3	1.7	1.2	2.8	2.7
0.8×10^{-4}	0.6	0.7	1.7	1.7	1.4	0.9	2.3	1.2	1.8	1.1	2.7	2.7
0.166×10^{-3}	0.3	0.5	0.1	0.5	0.1	0.4	0.8	1.1	0.4	0.6	1.2	1.5
0.8×10^{-3}	0.1	0.07	0.1	0.4	0	0.07	0	0.8	0	0.2	0.7	1.1
0.166×10^{-2}	0	0	0	0	0	0	0	0.4	0	0	0.3	0.1

Table IV

The effect of molybdate on the rate of hydrolysis of p-nitrophenyl phosphate by fractionated human dental plaque encyme preparations performed at two different substrate concentration levels. The results are given as v, expressing the molar change of the liberated p-nitrophenol in the reaction mixture per min. ($\times 10^8$). The incubation was performed in 0.05 M ββ-glutaric acid buffer, pH 8.8 and in presence of 0.166×10^{-3} M Mg^{2+} ion concentration. The incubation time and temperature was 22 hours and 37°C respectively

Ion concentration (M)	Enzyme preparation											
	I		II		III		IV		V		VI	
	Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)	
	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-2}	0.8×10^{-3}
Control	0.9	0.8	0.8	0.5	0.7	0.3	1.3	0.9	0.9	0.7	2.1	1.8
0.8×10^{-4}	0.7	0.3	0.7	0.4	0.6	0.3	1.1	0.8	0.8	0.5	1.9	1.4
0.166×10^{-3}	0.7	0.07	0.6	0.3	0.5	0.03	1.0	0.6	0.7	0.5	1.6	1.2
0.8×10^{-3}	0	0	0	0	0	0	0	0	0	0	0	0
0.166×10^{-2}	1.2	0	1.3	0	1.3	0	0.9	0	0.8	0	1.5	0

Table V

The effect of Cr^{3+} ions on the rate of hydrolysis of p-nitrophenyl phosphate by fractionated human dental plaque enzyme preparations performed at two different substrate concentration levels. The results are given as v, expressing the molar change of the liberated p-nitrophenol in the reaction mixture per min. ($\times 10^8$). The incubation was performed in 0.05 M ββ-glutaric acid buffer, pH 8.8 and in presence of 0.166×10^{-3} M Mg^{2+} ion concentration. The incubation time and temperature was 22 hours and 37°C respectively

Ion concentration (M)	Enzyme preparation											
	I		II		III		IV		V		VI	
	Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)	
	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}
Control	0.8	0.7	0.6	0.5	0.7	0.6	0.9	0.8	0.6	0.5	1.6	1.4
0.8×10^{-4}	0.6	0.5	0.5	0.4	0.7	0.7	0.9	0.8	0.6	0.5	1.5	1.3
0.166×10^{-3}	0.01	0.4	0.5	0.4	0.7	0.7	0.9	0.9	0.7	0.5	1.4	1.2
0.8×10^{-3}	0.07	0.2	0.6	0.7	0.5	0.2	0.9	0.4	0.3	0.4	1.1	1.1
0.166×10^{-2}	0	0	0	0	0	0	0	0	0	0	0.2	0

Table VI

The effect of Zn^{2+} ions on the rate of hydrolysis of p-nitrophenyl phosphate by fractionated human dental plaque enzyme preparations performed at two different substrate concentration levels. The results are given as v, expressing the molar change of the liberated p-nitrophenol in the reaction mixture per min. ($x10^8$). The incubation was performed in 0.05 M ββ-glutaric acid buffer, pH 8.8 and in presence of 0.166×10^{-3} M Mg^{2+} ion concentration. The incubation time and temperature was 22 hours and 37°C respectively

Ion concentration (M)	Enzyme preparation											
	I		II		III		IV		V		VI	
	Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)	
	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}
Control	0.7	0.3	0.9	0.7	0.5	0.3	1.3	0.9	1.3	2.2	1.9	1.1
$0.8x10^{-4}$	0.6	0.3	0.8	0.8	0.4	0.3	1.2	0.8	1.2	2.2	1.8	1.3
0.166×10^{-3}	0.6	0.3	0.6	0.9	0.5	0.4	1.3	0.9	1.2	2.1	1.8	1.2
0.8×10^{-3}	0.6	0.3	1.0	0.8	0.5	0.3	1.3	0.9	1.3	2.3	1.8	1.3
0.166×10^{-2}	0.7	0.3	0.9	0.6	0.5	0.3	1.2	0.8	1.2	2.2	1.7	1.2

Table VII

The effect of Sr^{2+} ions on the rate of hydrolysis of p-nitrophenyl phosphate by fractionated human dental plaque enzyme preparations performed at two different substrate concentration levels. The results are given as v, expressing the molar change of the liberated p-nitrophenol in the reaction mixture per min. ($\times 10^8$). The incubation was performed in 0.05 M ββ-glutaric acid buffer, pH 8.8 and in presence of 0.166×10^{-3} M $Mg^{2}\times$ ion concentration. The incubation time and temperature was 22 hours and 37°C respectively

Ion concentration (M)	Enzyme preparation											
	I		II		III		IV		V		VI	
	Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)	
	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	$0.166\times10t^{3}$	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}
Control	0.9	0.6	0.9	0.9	0.4	0.5	0.8	1.3	1.4	1.3	1.1	0.9
0.8×10^{-4}	0.8	0.6	0.9	1.0	0.4	0.4	0.8	1.4	1.5	1.3	1.0	0.9
0.166×10^{-3}	0.9	0.6	0.9	0.9	0.4	0.5	0.9	1.5	1.6	1.4	1.0	0.9
0.8×10^{-3}	1.0	0.7	1.0	1.4	0.5	0.4	1.3	1.7	1.8	1.5	1.0	0.9
0.166×10^{-2}	1.0	0.7	1.0	1.5	0.4	0.5	1.5	1.9	1.9	1.8	1.0	1.0

Table VIII

The effect of Na^+F^- ions on the rate of hydrolysis of p-nitrophenyl phosphate by fractionated human dental plaque enzyme preparations performed at two different substrate concentration levels. The results are given as v, expressing the molar change of the liberated p-nitrophenol in the reaction mixture per min. ($\times 10^8$). The incubation was performed in 0.05 M ββ-glutaric acid buffer, pH 8.8 and in presence of 0.166×10^{-3} M $Mg^{2}\times$ ion concentration. The incubation time and temperature was 22 hours and 37°C respectively

Ion concentration (M)	Enzyme preparation											
	I		II		III		IV		V		VI	
	Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)	
	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}
Control	0.8	0.7	0.9	0.7	0.6	0.3	1.3	1.0	1.2	1.4	1.3	1.7
0.8×10^{-4}	0.8	0.7	0.9	0.6	0.6	0.3	1.3	0.9	1.2	1.4	1.4	1.8
0.166×10^{-3}	0.7	0.6	0.9	0.7	0.5	0.4	1.3	1.0	1.2	1.3	1.3	1.7
0.8×10^{-3}	0.8	0.7	0.9	0.7	0.6	0.5	1.4	1.0	1.3	1.4	1.4	1.8
0.166×10^{-2}	0.8	0.7	0.9	0.8	0.6	0.4	1.3	1.1	1.2	1.4	1.4	1.8

Table IX

The effect of $Sn^{2+}F^-$ ions on the rate of hydrolysis of p-nitrophenyl phosphate by fractionated human dental plaque enzyme preparations performed at two different substrate concentration levels. The results are given as v, expressing the molar change of the liberated p-nitrophenol in the reaction mixture per min. ($\times 10^8$). The incubation was performed in 0.05 M ββ-glutaric acid buffer, pH 8.8 and in presence of 0.166×10^{-3} M Mg^{2+} ion concentration. The incubation time and temperature was 22 hours and 37°C respectively

Ion concentration (M)	Enzyme preparation											
	I		II		III		IV		V		VI	
	Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)	
	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}
Control	0.8	0.7	0.8	0.6	0.7	0.8	1.2	1.0	1.0	0.8	1.1	1.3
0.8×10^{-4}	0.7	0.6	0.7	0.6	0.7	0.8	1.2	0.9	1.0	0.8	1.1	1.3
0.166×10^{-3}	0.7	0.5	0.7	0.6	0.7	0.8	1.1	0.9	0.9	0.7	1.0	1.3
0.8×10^{-3}	0.6	0.2	0.6	0.5	0.4	0.5	0.8	0.7	0.9	0.6	0.9	1.2
0.166×10^{-2}	0	0.07	0	0.2	0	0.3	0.3	0.6	0.3	0.4	0.3	0.1

Table X

The effect of Sn^{2+} Cl^- ions on the rate of hydrolysis of p-nitrophenyl phosphate by fractionated human dental plaque enzyme preparations performed at two different substrate concentration levels. The results are given as v, expressing the molar change of the liberated p-nitrophenol in the reaction mixture per min. ($\times 10^8$). The incubation was performed in 0.05 M ββ-glutaric acid buffer, pH 8.8 and in presence of 0.166×10^{-3} M Mg^{2-} ion concentration. The incubation time and temperature was 22 hours and 37°C respectively

Ion concentration (M)	Enzyme preparation											
	I		II		III		IV		V		VI	
	Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)		Substrate conc. (M)	
	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}	0.166×10^{-3}	0.8×10^{-3}
Control	0.7	0.9	0.6	0.6	0.8	0.8	1.0	1.2	1.2	1.3	1.0	1.3
0.8×10^{-4}	0.6	0.8	0.6	0.6	0.7	0.8	0.9	1.2	1.3	1.2	0.9	1.2
0.166×10^{-3}	0.5	0.8	0.3	0.6	0.6	0.8	0.8	1.2	1.0	1.2	0.7	1.1
0.8×10^{-3}	0.07	0.7	0	0.5	0.07	0.9	0.3	0.9	0.6	0.8	0.5	1.0
0.166×10^{-2}	0	0	0	0.4	0	0.5	0.2	0.8	0.5	0.5	0.4	0.8

DISCUSSION

Because Mg^{2+} ions clearly activated the enzymic reactions and because the evidently low enzyme activities existing in the enzyme preparations it was found necessary to performe all tests in presence of a constant $MgCl_2$ concentration.

Referring again to Mg^{2+} ion activation of all the tested enzyme preparations, the possibility aries of these enzymes being metal dependent. This hypothesis is further supported by other (unpublished) studies with Ca^{2+} ions, showing a corresponding effect on the enzymes tested in this study. The results obtained with both Zn^{2+} and Sr^{2+} ions, with no, or slight, activating effect on the enzymes, seem to suggest that this is not the case. This phenomenon could, however, be explained along the same lines: if all the experiments were performed in presence of Mg^{2+} ions, the active site would already be saturated with Mg^{2+} ions and so display perhaps the same enzyme activity towards p-nitrophenyl phosphate as when saturated with Zn^{2+} ions or Sr^{2+} ions. Fluoride ions were, not unexpectedly, completely inactive towards the enzymic hydrolysis reaction by all the tested enzymes. In other corresponding studies with other enzymes (data to be published) similar results were obtained. The results obtained in this study, as well as those other studies, suggest then that F^- ions exert an effect of the same nature as halogenas on many known enzymes. In practical terms, the caries inhibiting effect displayed by F^- ions may be primarily an effect on the inorganic phase of the tooth, rather than an activating or inhibiting effect on the enzymes existing in human dental plaque material.

The hypothesis put forward in this study should be confirmed with thorough kinetic studies. This could, however, not be performed in this study while no valid K_i values could be obtained, due to the nonlinear curves obtained when for instance reciprocal of the rate of hydrolysis (v) was plotted as the function of the inhibitor concentration.

SUMMARY

All tested fractioned enzyme preparations were activated by Mg^{2+} ions. When the affector studies were performed in presence of Mg^{2+} ions the following metal ions were strong inhibitors of the enzymic hydrolysis of p-nitrophenyl phosphate: Cr^{3+}, Ni^{2+}, Al^{3+}, Mn^{2+}. Zn^{2+} ions showed no effect on the rate of hydrolysis. Two enzyme preparations were not affected, and four enzyme preparations were activated by Sr^{2+} ions. F^- ions were completely non-reacting to the enzymic hydrolysis of the substrate used. When

F^- ions were tested together with Sn^{2+} ions a reduction of the hydrolysis rate could be obtained. The effect of the tested elements on the enzymic hydrolysis reaction of the fractioned enzymes is discussed in terms of a possible metal-dependent character of these enzymes.

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RÉSUMÉ

ACTION DE CERTAINS CATIONS ET ANIONS SUR LES ACTIVITÉS PHOSPHOMONOESTÉRASIQUES ALCALINES DANS LA PLAQUE DENTAIRE HUMAINE.

Toutes les préparations enzymatiques fractionnées qui ont été testées ont été activées par les ions Mg^{2+}. Lorsque l'étude des effecteurs était faite en présence d'ions Mg^{2+}, les ions métalliques suivants agissaient comme inhibiteurs puissants de l'hydrolyse enzymatique du p-nitrophénylphosphate: Cr^{3+}, Ni^{2+}, Al^{3+}, Mn^{2+}. Les ions Zn^{2+} restaient sans effet sur le degré de l'hydrolyse. Les ions Sr^{2+} restaient sans effet sur deux des préparations enzymatiques et en activaient quatre. Les ions F^- restaient absolument sans réaction à l'égard de l'hydrolyse enzymatique du substrat utilisé. Quand les ions F^- étaient testés avec les ions Sn^{2+}, une réduction du degré de l'hydrolyse pouvait être obtenue. L'auteur discute l'action des éléments testés sur la réaction d'hydrolyse enzymatique des enzymes fractionnés en fonction d'un éventuel caractère de dépendance de ces enzymes à l'égard des métaux.

ZUSAMMENFASSUNG

DIE WIRKUNG BESTIMMTER METALLIONEN UND GEWISSER VERBINDUNGEN AUF DIE AKTIVITÄT DER ALKALISCHEN PHOSPHOMONOESTERASE DER MENSCHLICHEN ZAHNPLAQUE

Alle untersuchten fraktionierten Enzympräparate wurden durch Mg^{2+}-Ionen aktiviert. Bei Fortführung der Untersuchungen in Gegenwart von Mg^{2+}-Ionen erwiesen sich folgende Metallionen als starke Inhibitoren der enzymatischen Hydrolyse von p-Nitrophenylpgosphat: Cr^{3+}, Ni^{2+}, Al^{3+} und Mn^{2+}. Zn^{2+}-Ionen zeigten keine Wirkung auf die Hydrolysenrate. Zwei der Enzympräparate wurden durch Sr^{2+}-Ionen nicht beeinflusst, vier andere aber aktiviert. F^--Ionen ergaben überhaupt keine Reaktion gegenüber der enzymatischen Hydrolyse des verwendeten Substrats. Als F^--Ionen zu-

sammen mit Sn^{2+}-Ionen untersucht wurden, konnte eine Minderung der Hydrolysenrate erreicht werden. Die Wirkung der getesteten Elemente auf die enzymatisch-hydrolytische Reaktion der fraktionierten Enzyme wird in Hinblick eines möglicherweise metallabhängigen Charakters dieser Enzyme diskutiert.

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