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THE PHOSPHATE-LIBERATING ACTIVITY OF A MOLD NEUTRAL DEXTRANASE PREPARATION

by

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INTRODUCTION

During recent years several papers have been published on possible enzymic liberation of phosphate from human dental enamel and dentine (*Paunio et al.*, 1968; *Mäkinen & Paunio*, 1969; *Kreitzman et al.*, 1969 and *Mäkinen & Paunio*, 1970). It is reasonable to assume that the near proximity of the plaque enzymes and substrate, e.g. the phosphoprotein matrix of dental enamel, or the phosphours containing peptides like those described by *Seyer* and *Glimcher* (1969), almost certainly will result in an enzymic degradation of the organic matrix of the tooth resulting, for instance, in a measurable amount of liberated phosphate.

The occurrence of an enzymic hydrolysis reaction like this may, however, for several reasons be hard to demonstrate. The most important reason may be that the reactions involved are extremely slow. On the other hand, when commercially available enzymes have been used in earlier studies, a clear liberation of phosphate has been demonstrated. In some instances the increase in the liberation of phosphate has among others been ascribed to ammonium sulphate present in the enzyme preparations, this leading to difficulties in interpreting experimental data (*Mäkinen & Paunio*, 1969; *Mäkinen & Paunio*, 1970) as ammonium sulphate has been found to be one of the phosphate-liberating factors in the tested enzyme preparations. Despite of this,

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the possibility of an enzymic reaction in the liberation of phosphate from human dental enamel cannot be ruled out, although the removal of ammonium sulphate from a commercial preparation of *E. coli* alkaline phosphatase decreased the rate of liberation of phosphorus. However, in spite of the removal of ammonium sulphate, a phosphate-liberating peak constantly appeared in chromatograms obtained from CM-cellulose chromatography, although the fractions involved did not contain any measurable amounts of phosphorus. This suggests that certain specific factors still might be involved in the liberation of phosphate. These findings will be discussed in forthcoming papers.

The purpose of the present communication is to show to which extent ammonium sulphate present in commercial mold dextranase preparation accomplishes the liberation of phosphate from human dental enamel and dentine.

MATERIALS AND METHODS

The materials and methods were the same as described in earlier papers (*Paunio et al.*, 1968; *Mäkinen & Paunio*, 1970). The dextranase, Dextranase N (from Penicillum lilacinum NRRL 896), was obtained from Swiss Ferment Company Ltd., Basle.

RESULTS

Table I shows how the liberation of phosphate increases with increasing concentration of the enzyme preparation in the reaction mixture. Removal of small molecules by dialysis or by molecular exclusion by the aid of Sephadex G-25 caused a practically total disappearance of the phosphate-liberating activity.

Table I.

Effect of enzyme concentration on the liberation of phosphorus from human dental enamel and dentine powder, tested in 0.1 M tris-HCl buffer, pH 7.0, for 1 hour at 37° C.

Amount of enzyme preparation (mg)	Phosphorus (μg per ml)	
	Enamel	Dentine
1	0.6	0.2
2	1.5	2.2
3	3.0	2.7
4	3.0	3.9
5	3.1	5.1
10	4.2	8.7
15	6.0	

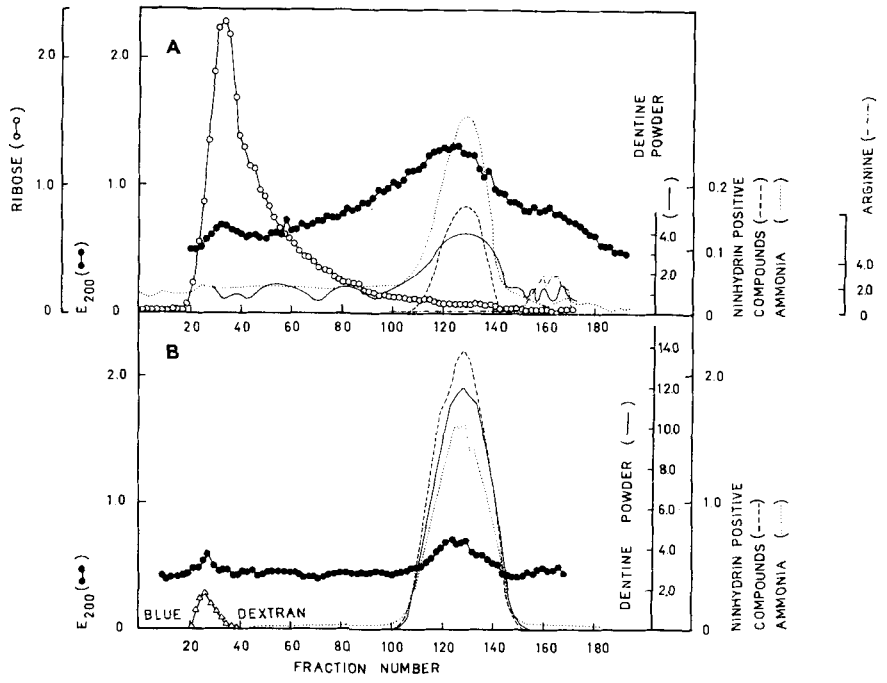

Fig. 1. Molecular exclusion chromatography of neutral dextranase preparation (A) and pure ammonium sulphate solution (B). Column: Bio-Gel P-300 (59.0 cm x 2.5 cm); elution buffer: 0.01 M tris-HCl buffer, pH 7.0; sample: 100 mg enzyme preparation dissolved in 2.0 ml of cold (+ 4° C) water. (In B, 1.4 mg ammonium sulphate dissolved in 2.0 ml cold (+ 4° C) water, was applied to the column). Flow rate: 6 ml per hour; hydrostatic pressure: 15 cm; temperature: + 2° C; fraction volume: 3 ml. o—o, determination of ribose (in μg per ml); •—•, E_{220}; . . ., ammonium sulphate (in E_{540}); — — —, ninhydrine-positive compounds (in E_{540}), ————, liberation of phosphate from human dentine powder (in μg phosphorus per ml); —.—.—, Sagaguchi reaction. For the determination of various chemical compounds and groups, see Mäkinen & Paunio 1969, 1970.

Fig. 1 shows the fractionation of the neutral dextranase preparation and pure ammonium sulphate on Bio-Gel P-300 columns. The peaks representing ammonium sulphate and the liberation of phosphate coincided in all the experiments. Removal of ammonium sulphate and subsequent molecular exclusion chromatography with the desalted enzyme preparation yielded no phosphate-liberating peaks. The phosphate-liberating effect shown in Fig. 1 was obtained with dentine powder as »substrate». Corresponding results were constantly obtained in experiments with enamel powder.

Table II shows the effect of some ammonium and sodium salts on the dissolution rate of human dental enamel presented as liberated phosphate during 1 hour incubation at 37° C, showing that increasing salt concentration results in an increasing dissolution rate of dental enamel and dentine.

Table II.

The effect of certain salts on the liberation of phosphate from human dental enamel powder (expressed as μg P per ml), tested in 0.1 M tris-HCl buffer, pH 7.0, for 1 hour at 37°C.

Salt	Salt concentration (%)									
	0	1	5	10	15	20	25	30	35	45
$(NH_4)_2SO_4$	7.2		16.8	16.8	12.2		14.3		13.0	13.0
NH_4Cl*)	7.2	9.1	12.2	27.6	41.6	41.6	46.9	64.0		
Na_2SO_4	7.2	26.2	51.1	63.1	63.6	63.6	62.5	59.1		
NaCl*)	7.2	7.7	10.1	12.4	24.0	20.0	21.0	26.0		

*) The values shown are approximations, calculated from extrapolated standard curves. (The absolute value of phosphorus is not known due to the interference of NaCl and NH_4Cl with the phosphorus assay).

DISCUSSION

The aim of this communication was to show that enzyme preparations may contain chemical compounds such as ammonium sulphate, which may interfere with certain experiments where enzymes and enamel or dentine powder were incubated in the same reaction mixture. The effect of neutral dextranase on the liberation of phosphate from human dental enamel and dentine could almost entirely be ascribed to ammonium sulphate in the dextranase preparation. The ammonium sulphate present in the neutral dextranase preparation could easily by removed without any apparent loss in the enzyme activity. In this respect it was also evident that the desalted dextranase preparation studied here can be used without apparent danger in preparations for enhancing the elimination of dental plaque.

The liberation of phosphate caused by ammonium sulphate may alter some of the factors important for the solubility of dental enamel by changing for instance, the ionic strength of the solution. Secondly, the existence of different ions in the solution may alter the solubility of enamel and dentine resulting in this case in a elevated solubility in a way described by *La Mer* (1962).

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SUMMARY

The results show that a mold neutral dextranase (Dextranase N) preparation displayed a phosphate-liberating activity towards human dental enamel and

dentine. Dialyses and molecular exclusion chromatography revealed that the phosphate-liberating activity was evidently due to ammonium sulphate present in the dextranase preparation. The removal of ammonium sulphate salt resulted in a loss of the phosphate liberating activity towards human dental enamel and dentine.

RÉSUMÉ

LIBÉRATION DE PHOSPHATE PAR UNE PRÉPARATION DE DEXTRANASE NEUTRE PROVENANT DE MOISISSURES

Les résultats de la présente étude ont montré qu'une préparation de dextranase neutre (Dextranase N) provenant de moisissures présentait lorsqu'on la faisait agir sur la dentine et l'émail dentaire humains une activité libérant le phosphate. Par dialyses et chromatographie par exclusion moléculaire, il a été mis en évidence que l'activité libérant le phosphate était sans aucun doute due au sulfate d'ammonium présent dans la préparation de dextranase. En enlevant le sulfate d'ammonium, on obtenait la disparition de cette activité envers la dentine et l'émail dentaire humains.

ZUSAMMENFASSUNG

ÜBER DIE PHOSPHAT-LIBERIERENDE AKTIVITÄT EINES NEUTRALEN DEXTRANASEPRÄPARATES VOM SCHIMMELPILTZ

Die Ergebnisse zeigten, dass neutrales Dextranasepräparat (Dextranase N) vom Schimmelpilz, Phosphat im menschlichen Zahnschmelz und Dentin freisetzt. Dialyse und molekulare Siebung erwiesen, dass die Phosphat-freisetzende Wirkung offensichtlich auf der Anwesenheit von Ammoniumsulfat in dem Dextranasepräparat beruht. Der Entzug von Ammoniumsulfatsalz bewirkte den Verlust der Phosphat-liberierenden Aktivität gegenüber menschlichem Zahnschmelz und Dentin.

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