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## ENZYMATIC RELEASE OF PHOSPHORUS FROM HUMAN DENTAL ENAMEL BY ENZYME PREPARATIONS OF E. COLI

A PRELIMINARY REPORT

by

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Biochemically it is evident that the continuous proximity of plaque enzymes and substrate (the phosphoprotein matrix of enamel and dentine) almost certainly results in the liberation of phosphorus. Because the corresponding enzyme activity of plaque may be too low to permit its identification, an attempt was made to clarify the problem by using commercial enzyme preparations. It was thus found that *Bacillus subtilis* subtilopectidase A (EC 3.4.4.16) preparations, or components present in it, are capable of liberating low amounts of phosphorus from powdered human dental enamel (Paunio, Mäkinen & Scheinin, 1968).

More recent investigations carried out in the same laboratory have revealed that preparations of *E. coli* alkaline phosphatase (EC 3.1.3.1), Type III-S (Sigma Chemical Company, St. Louis, Mo., USA) display even greater phosphorus-liberating activity than those of subtilopectidase A. Molecular exclusion chromatography on Sephadex® G-200 and Bio-Gel P-300 columns have shown that the liberation of phosphorus is not caused by the alkaline phosphatase preparation itself but by a component with lower molecular weight present in the commercial enzyme preparations of *E. coli* (Fig. 1). The phosphorus-liberating peak coincides with the peak representing nin-

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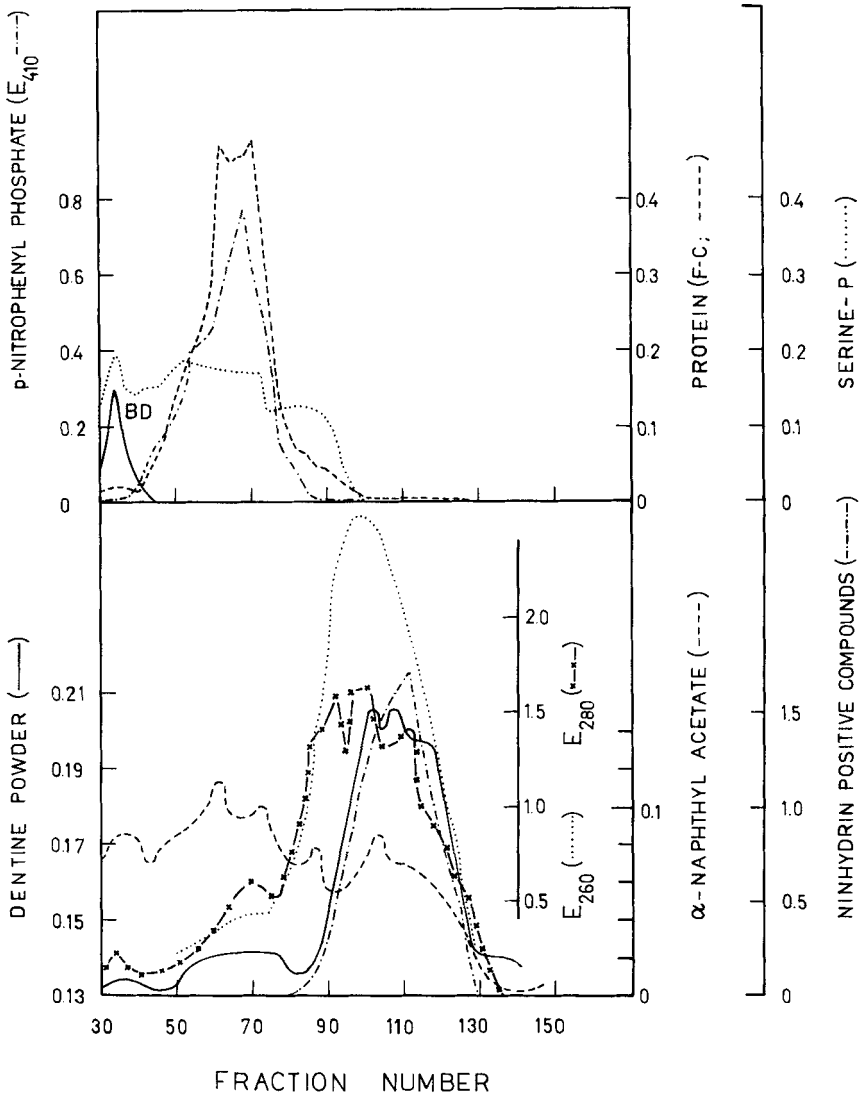


Fig. 1. Molecular exclusion chromatography of the phosphorus-liberating component present in *E. coli* alkaline phosphatase preparation on a Bio-Gel P-300 column (59.0×2.5 cm). Sample: 5.0 ml of the commercial enzyme preparation, containing 100 mg protein. Elution buffer: 0.01 M tris-HCl, pH 7.0. Hydrostatic pressure: 25 cm. Flow rate: 14 ml per h. Temperature: +2°C. The x-axis is intended to represent the dissolution of phosphorus by buffer. Any phosphorus value above x-axis is due to the action of the phosphorus-liberating factor. F-C = Folin-Ciocalteu method ( $E_{500}$ ); serine-P = serine phosphate BD = Blue Dextran.

hydrin-positive compounds in the chromatogram. Total phosphorus assays did not show any measurable phosphorus in fractions 90—130 (see Fig. 1). The phosphorus-liberating peak was accompanied by some esterase activity toward  $\alpha$ -naphthyl acetate. It is thought that this activity is caused by some contaminating compounds and has nothing to do with the liberation of phosphorus. The fractions 90—130 were not seen to contain any measurable amounts of polysaccharides or DNA. The peak in the chromatogram, obtained by measuring absorption at 260 and 280  $m\mu$ , represents a component which evidently contains RNA with its absorption maximum at 258—260  $m\mu$ .

The results indicate that the ninhydrin-positive phosphorus-liberating factor could perhaps be an ammonium group-rich compound whose strong chromogenic potential (when reacting to ninhydrin) results in its high content of either arginine or lysine, or both. This is supported by finding that fractions 90—130 contain considerable amounts of chemical groupings reacting as ammonia and as guanidino groups. This ammonia could be derived from the free side chains of arginine or lysine, or it can be derived from ammonium sulphate present in the enzyme preparation used. The identified factor displays enzyme-like properties. For example, the rate of release of phosphorus is proportional to the amount of enzyme preparation (or purified factor) present in the reaction mixture. Also, the effect of pH on the release of phosphorus greatly resembles that in many enzymic reactions: a fairly typical pH dependence curve was obtained, indicating a system with two successive ionizations.

Identification of the involved factor revives the old problem of the significance of the so called «bone factor» in the mineralization process. If the ammonia-like factor of *E. coli* is able to accept and donate phosphate, it may be asked whether this kind of mechanism is also involved in bone metabolism.

More detailed descriptions of the methods and results will be presented in forthcoming papers.

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#### SUMMARY

*E. coli* alkaline phosphatase preparations were found to contain a factor capable of liberating phosphorus from powdered human dental enamel. It was found too, that the essential alkaline phosphatase is not able to per-

form this liberation of phosphorus. The factor has enzyme-like properties, and chemical analysis shows that it may be a compound containing considerable amounts of ninhydrin-positive groups.

## RÉSUMÉ

LIBÉRATION ENZYMATIQUE DU PHOSPHORE DE L'ÉMAIL DENTAIRE HUMAIN AU MOYEN DE PRÉPARATIONS ENZYMATIQUES D' *E. coli*

La présence d'un facteur capable de libérer le phosphore de l'émail dentaire humain pulvérisé a été mise en évidence dans des préparations de phosphatase alcaline d' *E. coli*. La chromatographie par exclusion moléculaire a révélé que la phosphatase alcaline essentielle n'est pas en état de réaliser la libération du phosphore. Ce facteur a des propriétés analogues à celles des enzymes et il a été établi par analyse chimique qu'il peut s'agir d'une amine contenant des quantités considérables de groupements donnant la réaction de la ninhydrine.

## ZUSAMMENFASSUNG

DURCH *E. coli* ENZYMPRÄPARATE BEDINGTES AUSLÖSEN VON PHOSPHOR IM MENSCHLICHEN ZAHNSCHMELZ

Es wurde festgestellt, dass die alkalischen Phosphatasepräparate von *E. coli* einen Faktor enthalten, der imstande ist, Phosphor vom menschlichen Schmelzpulver auszulösen. Die Gelfiltration zeigte, dass eine absolut alkalische Phosphatase diese Reaktion nicht hervorrufen kann. Der Faktor besitzt enzymähnliche Eigenschaften. Die chemische Analyse zeigte, dass der Faktor seiner Natur nach offensichtlich eine Amine mit vielen Ninhydrin-positiven Gruppen ist.

## REFERENCES

- Paunio I. K., K. K. Mäkinen & A. N. Scheinin, 1968: Liberation of phosphate from human dental enamel by enzymes. *Caries Res.* 2: 317.

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