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TWO ISOPENTENYL PYROPHOSPHATE ISOMERASES FROM PUMPKIN FRUIT

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Abstract—Two isopentenyl pyrophosphate isomerase fractions were separated by hydroxylapatite chromatography of pumpkin fruit extract.

ISOPENTENYL pyrophosphate isomerase [E.C. 5.3.3.2] has been purified from various organisms,[1-4] but isoenzymes are not known. We now report the separation of two differentially moving proteins on hydroxylapatite which are active as isopentenyl pyrophosphate isomerase.

The protein fraction precipitating between 40–60% $(NH_4)_2SO_4$ saturation from 40,000 *g* supernatant of pumpkin extract was applied on a hydroxylapatite column and the elution was performed with a linear gradient of phosphate buffer. Enzyme activity was measured by determining the amount of the conversion of ^{14}C-isopentenyl pyrophosphate into acid-labile dimethylallyl pyrophosphate according to a previously described method.[4] Figure 1 shows a typical chromatogram; in some experiments the first peak (A) was smaller than the second (B), whereas in other experiments the first peak was barely detectable. Prenyltransferase, not shown here, is eluted in fraction numbers 29–32 in Fig. 1. For the

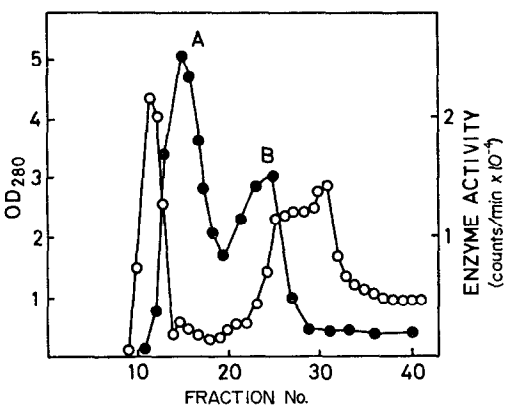


FIG. 1. CHROMATOGRAPHY OF A 40–60% $(NH_4)_2SO_4$ FRACTION ON HYDROXYLAPATITE.
○——○ Absorbance at 280 nm. ●——● Enzyme activity.

[1] B. W. AGRANOFF, H. EGGERER, U. HENNING and F. LYNEN, *J. Biol. Chem.* **235**, 326 (1959).
[2] D. H. SHAH, W. W. CLELAND and J. W. PORTER, *J. Biol. Chem.* **240**, 1946 (1965).
[3] P. W. HOLLOWAY and G. POPJÁK, *Biochem. J.* **106**, 835 (1968).
[4] K. OGURA, T. NISHINO and S. SETO, *J. Biochem.* (*Tokyo*) **64**, 197 (1968).

TABLE 1. EFFECT OF IODOACETAMIDE AND INORGANIC PYROPHOSPHATE ON THE TWO FRACTIONS

Inhibitor	Concentration (M)	Inhibition (%) A	B
ICH_2CONH_2	5×10^{-4}	67	93
$Na_3P_2O_7$	1×10^{-4}	25	24
	1×10^{-3}	57	49

comparison of these two fractions (A and B), the enzyme solutions in fraction numbers 15 and 25 in Fig. 1 were used in all examinations described below.

The formation of ^{14}C-dimethylallyl pyrophosphate by the action of these two fractions was confirmed by gas chromatographic identification of ^{14}C-dimethylallyl alcohol and ^{14}C-dimethylvinylcarbinol formed by acid hydrolysis. Iodoacetamide and inorganic pyrophosphate[5] inhibited both fractions (Table 1). K_m values for isopentenyl pyrophosphate were determined from the Lineweaver–Burk plots as $4{\cdot}5 \times 10^{-5}$ M and $2{\cdot}2 \times 10^{-5}$ M for A and B, respectively.

In order to confirm the reproducibility of the separation, the fractions A and B (fraction Nos. 12–28) were combined, and a portion of the dialyzed protein was chromatographed again on hydroxylapatite using a different gradient elution; separation was again achieved (Fig. 2).

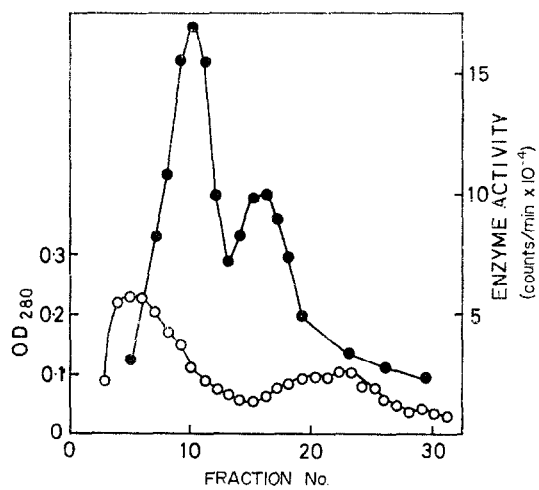


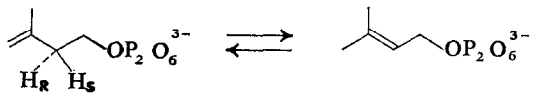
FIG. 2. RECHROMATOGRAPHY OF A COMBINED FRACTION ON HYDROXYLAPATITE.

○—— ○ Absorbance at 280 nm. ●——● Enzyme activity.

Holloway and Popják[3] have suggested that there might be two isopentenyl pyrophosphate isomerases, on the basis of differences in stereospecificity of the reaction catalysed. One of our enzyme fractions catalyses the isomerization of isopentenyl pyrophosphate *via*

[5] K. OGURA, T. KOYAMA, T. SHIBUYA, T. NISHINO and S. SETO, *J. Biochem. Tokyo* **66**, 117 (1969).

the elimination of the H_R hydrogen at C-2 (Scheme 1) but it remains to be seen whether the two isomerases obtained here correspond to the two proposed by Holloway and Popják.



SCHEME 1.

EXPERIMENTAL

Materials

Pumpkin fruits (*Cucurbita pepo*, Cucurbitaceae) were obtained locally. ^{14}C-Isopentenyl pyrophosphate (Sp. act. 1·2 μc/μmole) was chemically synthesized.[4] Hydroxylapatite was a product of Nippon Chemical Co., Tokyo.

Hydroxylapatite Chromatography

A 40–60% $(NH_4)_2SO_4$ fraction obtained from 400 g of pumpkin fruit was placed on a 1·0 × 35·5 cm column of hydroxylapatite. Elution was accomplished with a linear phosphate buffer gradient at pH 6·8; 150 ml of 0·01 M buffer were placed in the mixing flask and 0·25 M buffer in the reservoir and 3 ml fractions were collected. In the second chromatography a 0·9 × 13·5 cm column was used. The mixing flask contained 150 ml of 0·01 M buffer and the reservoir contained 150 ml of 0·20 M buffer. Fractions of 2·5 ml were collected.

Acknowledgement—We thank Nippon Chemical Co., for the generous gift of hydroxylapatite.