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PSEUDO-ISOENZYME FORMS OF LIVER PRENYL TRANSFERASE[1]

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Summary. Prenyl transferase of pig liver exists in two forms, A and B, the latter being more acidic than form A. The ratio of these two forms in initial extracts of the organ varies according to the presence or absence of a thiol reducing agent in the extraction medium. In the presence of 10 mM β-mercaptoethanol the two forms exist as an equilibrium mixture of ca. 70% form A and 30% form B. The latter, when separated and rechromatographed with β-mercaptoethanol reverts to approximate the same 7:3 ratio of forms A and B. In the absence of a thiol reducing agent form B does not change, but form A may be converted almost completely to form B after treatment with oxidized glutathione.

Prenyl transferase of liver, (EC 2.5.1.1), which catalyzes the synthesis of farnesyl pyrophosphate from an allylic pyrophosphate (3,3-dimethylallyl-, or geranyl pyrophosphate) and isopentenyl pyrophosphate,[4] has been recorded to exist in two forms separable by ion-exchange chromatography (1,2,3). Yeh and Rilling (2) noted that these two forms were interconvertible and suggested that the interconversions depended on the counterion used during the gradient-elution of the enzyme from the ion-exchanger. We now show that the interconversions of the two forms of liver prenyl transferase, a dimer consisting of two apparently identical subunits (M_r = 38,500), result from disulfide

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[4] Abbreviations: GPP - geranyl pyrophosphate; IPP = isopentenyl pyrophosphate (3-methylbut-3-enyl pyrophosphate); DTT = dithiothreitol; β-ME = β-mercaptoethanol; GSSG = oxidized glutathione; DTNB = 5,5'-bis-dithio-2-nitrobenzoic acid.

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reductions and thiol oxidations of the same protein. Thus the two forms may be regarded as pseudo-isoenzymes.

METHODS AND MATERIALS

Prenyl transferase was assayed according to Holloway and Popják in 0.5 ml incubations (4) containing 0.1 M Tris-HCl buffer, pH 7.8, 5 mM $MgCl_2$, 50 μM GPP[4] and [1-^{14}C]IPP (specific activity 0.5 Ci/mol), 1 mM DTT and varying amounts of enzyme that gave linear rates for 15 to 30 min. One unit of enzyme catalyzes the synthesis of 1 nmol of farnesyl pyrophosphate per min. Prenyl transferase was partially purified through the first steps of the method of Yeh and Rilling (2) from 50 g lots of pig liver homogenized in a Waring blender for 60 s with 100 ml of distilled water with or without 10 mM β-ME. These small scale preparations assured easily reproducible and rapid experiments. All operations were carried out at 4°C. The pH of the stirred homogenate was adjusted to 5.3 with 3.5 N acetic acid; after 1 h the thick slurry was centrifuged at 16,000 x g for 30 min. The pH of the supernatant was adjusted to 6.0 by the addition of wet DE-52 resin (Whatman), pH 10. Then 35 g of wet DE-52 equilibrated with 10 mM Na acetate buffer, pH 6.0, with or without 10 mM β-ME, were added. The mixture was stirred for 1 h and then filtered through a sintered glass filter funnel. The resin was washed on the filter with the acetate buffer until the washings became colorless and then packed to give a 1.5 x 23 cm column which was eluted with a 300-ml (total volume) linear gradient 0 to 110 mM $(NH_4)_2SO_4$ in 10 mM Na acetate buffer, pH 6.0, with or without 10 mM β-ME. Fractions of 4.5 ml were collected at a flow rate of 17 ml/h and assayed for prenyl transferase, protein content and conductivity. Enzyme fractions were pooled and concentrated to about 5 ml by filtration through Amicon PM-10 membranes. They were then desalted through 1.5 x 50 cm columns of Sephadex G-25 equilibrated with 10 mM Tris HCl buffer, pH 7.6, with or without 10 mM β-ME. The desalted solutions were then applied to fresh 1.5 x 23 cm DE-52 columns equilibrated with the Tris-HCl buffer. A 350 ml (total volume) 0 to 150-mM linear gradient of $(NH_4)_2SO_4$ in 10 mM Tris-HCl buffer, pH 7.6, with or without 10 mM β-ME was then applied. Fractions were collected and assayed as described above.

RESULTS

We had come to suspect that the two forms of liver prenyl transferase represented different oxidation-reduction states of the same protein when we found, during various purification procedures, that the ratios of the two forms depended on the time elapsed between the preparation of the liver extracts and their chromatography on ion-exchangers, and on the presence or absence of a thiol-reducing agent during chromatography.

Figures 1 to 5 illustrate a series of consecutive experiments done with the same liver extract. Fig. 1 shows that when the liver extract is made without β-ME, chromatography of the crude extract at pH 6.0 gives two

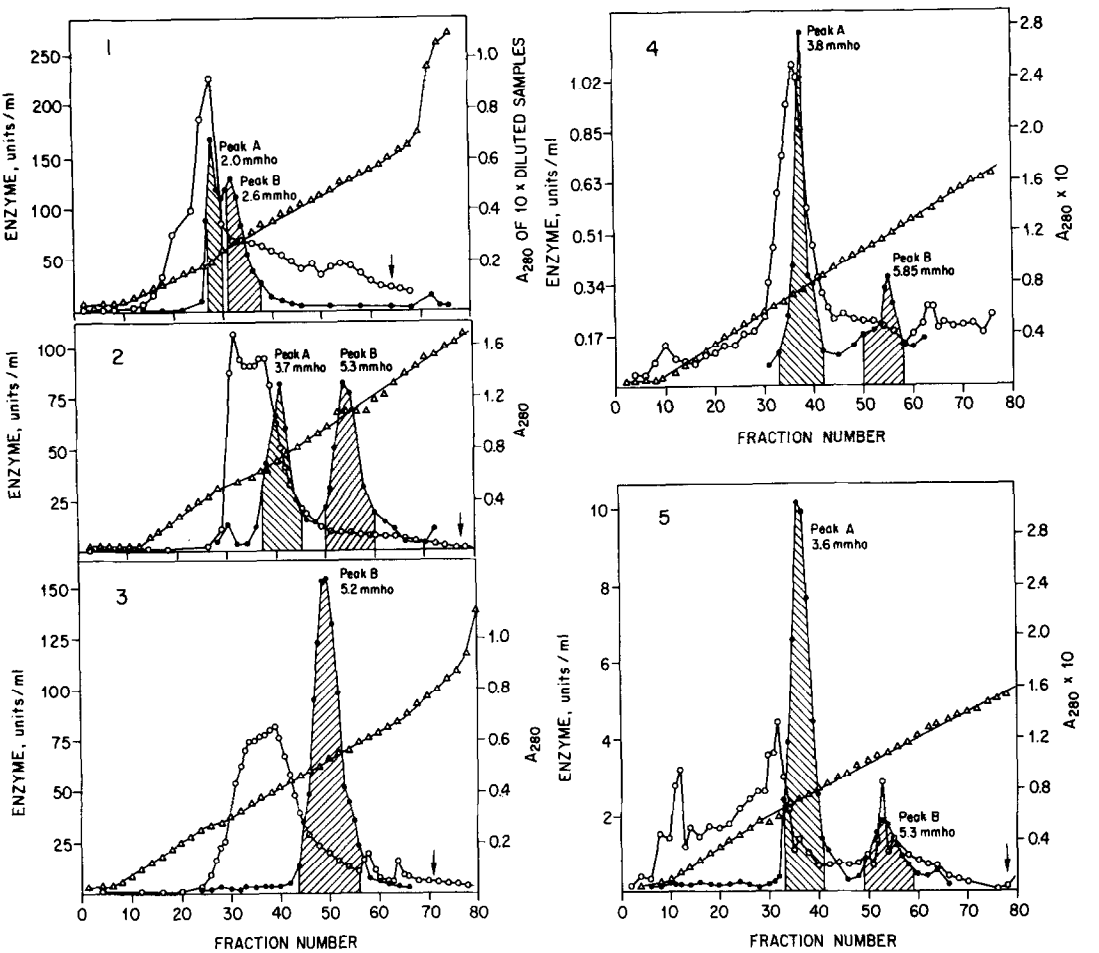


Fig. 1 Chromatography at pH 6.0 of liver extract, made without β-ME. The symbols in all the figures are the same: o, protein as measured at 280 nm; • and shaded areas, prenyl transferase; Δ, conductivity of effluent. The arrow seen in some of the figures indicates the application of 200 mM $(NH_4)_2SO_4$, which did not elute further amounts of prenyl transferase.

Fig. 2 Pooled and concentrated fractions of Peak A (1-A) from the experiment of Fig. 1 rechromatographed at pH 7.6 without β-ME as described in Methods and Materials, and in the text.

Fig. 3 Pooled and concentrated fractions of Peak B (1-B) from the experiment of Fig. 1 rechromatographed at pH 7.6 without β-ME.

Fig. 4 Pooled and concentrated fractions of Peak A (2-A) from the experiment of Fig. 2 rechromatographed at pH 7.6 with buffer and $(NH_4)_2SO_4$ gradient containing 10 mM β-ME.

Fig. 5 Pooled and concentrated fractions of Peak B (2-B) from the experiment of Fig. 2 rechromatographed in the same way as described in Fig. 4.

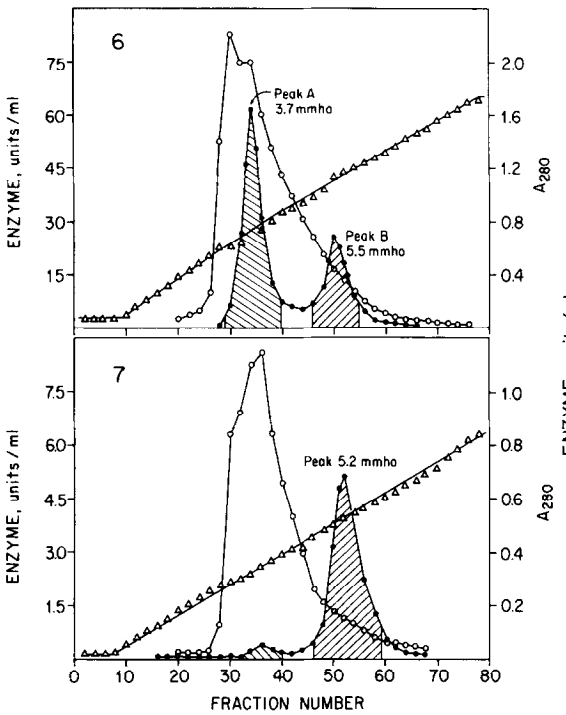
poorly separated peaks of the transferase. Application of peak 1-A[5] onto a fresh DE-52 column, equilibrated at pH 7.6, and elution again without β-ME, resulted in two well separated enzyme forms, 2-A and 2-B, with peaks eluted at 3.7 and 5.3 mmho respectively (Fig. 2). On the other hand, similar rechromatography of enzyme 1-B (Fig. 3) gave only a single form of the enzyme eluted at a conductivity of 5.2 mmho in a position very close to that of form B seen in Fig. 2.

The separately pooled fractions of peak 2-A and 2-B, after addition of 1 mM DTT, were chromatographed at pH 7.6 with buffer and gradient containing 10 mM β-ME. As shown in Fig. 4 and 5 this experiment gave mostly form A with only a relatively small amount of form B. Thus enzyme form B is readily convertible in the presence of a thiol reducing agent into form A (Fig. 5), which under similar conditions remains mostly in its original form (Fig. 4).

When the extract of the liver is made with water containing 10 mM β-ME and the crude extract is chromatographed at pH 6.0 with buffer and gradient containing β-ME only a single band of enzyme is obtained (not shown) as was observed by Yeh and Rilling (2). When such an enzyme preparation was re-chromatographed at pH 7.6 in the presence of β-ME, forms A and B were obtained in a ratio of 2.2:1 (Fig. 6). The fractions comprising peak 6-A were pooled, concentrated and passed through a Sephadex G-25 column (cf. Methods and Materials) equilibrated with 10 mM Tris-HCl buffer, pH 7.6, to remove β-ME and $(NH_4)_2SO_4$. Now 1 mM neutralized GSSG was added to the preparation, which was left at 4°C for 3 days. As is seen from Fig. 7, nearly all the recovered enzyme was in form B.

We examined further whether the conversion of enzyme form A into B was truly attributable to the effect of GSSG and not to a time dependent "air-oxidation" of the protein. For this purpose a preparation of enzyme form A (2400 units) was obtained in an experiment similar to that shown in Fig. 1

[5] Each pooled enzyme fraction will be named by the experiment figure number followed by either form A or B, e.g., 2-B.



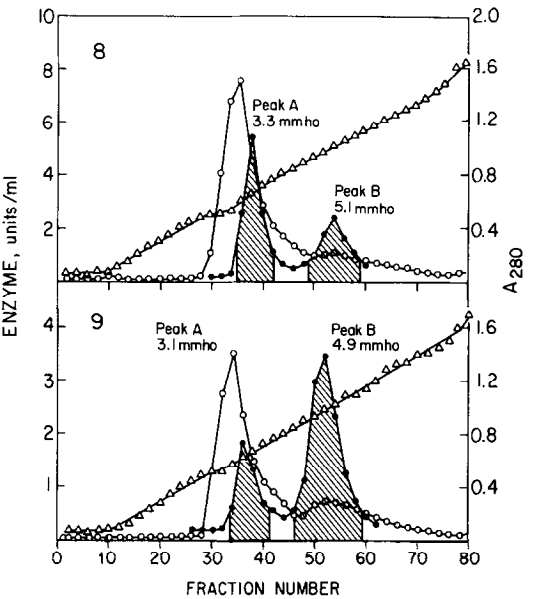


Fig. 6 Rechromatography at pH 7.6 and in the presence of 10 mM β-ME of the single peak of prenyl transferase obtained by extraction of liver with 10 mM β-ME at pH 6.0.

Fig. 7 Rechromatography at pH 7.6 without β-ME of the pooled, concentrated and desalted fractions of Peak A (6-A) from the experiment shown in Fig. 6 that was treated for 3 days at 4°C with 1 mM GSSG.

Fig. 8 and 9 A specimen of prenyl transferase, form A, was obtained first in an experiment similar to that presented in Fig. 1. One-half of the pooled, concentrated and dialyzed Peak A was left for 4 days and at 4°C in a sealed tube and then chromatographed at pH 7.6 in the absence of β-ME (Fig. 8). The other half of the specimen was treated with 1 mM GSSG also for 4 days and at 4°C in a sealed tube and chromatographed at pH 7.6 in the absence of β-ME (Fig. 9).

and was concentrated to 6 ml and then dialyzed overnight against 10 mM Tris-HCl buffer, pH 7.6 without β-ME. To one half of this preparation neutral GSSG was added to a final concentration of 1 mM; nothing was added to the other half. Both specimens were left for 4 days in sealed tubes at 4°C and were then rechromatographed at pH 7.6 in the absence of β-ME. Whereas the "air-oxidized" sample gave forms A and B in a ratio of 1.5:1 (Fig. 8), the

one treated with GSSG gave a ratio of 1:3 (Fig. 9) proving that GSSG accelerated the conversion of form A to B.

DISCUSSION

Our experiments demonstrate that the interconversions of the two forms of liver prenyl transferase depend most probably on disulfide reductions and thiol oxidations in the same protein rather than on the presence of PO_4^{3-} and absence of SO_4^{2-} ions during chromatography as concluded by Yeh and Rilling (2). Koyama et al. (3) came to a conclusion similar to ours as to the relation of the two forms of the enzyme. Specifically they claimed the conversion of form A into an inactive B by cupric ions and partial reactivation of the latter by DTT. We were unable to repeat these experiments, as we found only total inactivation of the enzyme by cupric ions without any reactivation by DTT.

The substrate and product specificities of the two forms of enzyme are identical as are their specific activities (700 to 800 units/mg when purified.[6] Our experiments have shown that in the presence of a thiol reducing agent forms A and B exist as an equilibrium mixture of about 70% form A and 30% B, but form B once purified does not revert to A without a thiol reducing reagent. We have found that DTT enhances the activities of the freshly prepared forms of the enzyme only slightly if at all, but aged preparations of both forms of the enzyme, kept without a thiol reducing agent and which lose much of their activity are nearly fully reactivated by 1 mM DTT if preincubated at 37°C for 30 min. Apparently free SH-groups are essential for maintaining active enzyme structure as was also implied by previous alkylation experiments with iodoacetamide (3,4).

The effect of the transformation of form A into B is an apparent increase of the surface negative charge on the protein, presumably by some conformational change, as evidenced by a stronger binding to the ion-exchanger and an increased mobility of form B as compared to form A on polyacrylamide gel

[6] G. F. Barnard and G. Popják, unpublished observations.

electrophoresis at pH 8.6.[6] Yeh and Rilling (2) reported isoelectric points at pH 4.85 and 4.99 for the two forms of the enzyme.

Amino acid analysis of the pure liver prenyl transferase showed that it contained 5 to 6 half-cystinyl residues per subunit and titration of a pure preparation of the enzyme, predominantly in form A, with DTNB indicated 3 free SH-groups per subunit.[6] Therefore free cysteinyl residues are available for intramolecular disulfide-bond formation. It has been determined by Yeh and Rilling (2), Koyama et al. (3), and by us[6] that the two forms have identical molecular weights and hence intermolecular disulfide bond formation is unlikely.

REFERENCES

(1) Parker, T.S. (1975) Ph.D. Thesis, University of California Los Angeles, Los Angeles.
(2) Yeh, L., and Rilling, H.C. (1977) Arch. Biochem. Biophys., 183, 718-725.
(3) Koyama, T., Saito, Y., Ogura, K., and Seto, S. (1977) J. Biochem., 82, 1585-1590.
(4) Holloway, P.W., and Popják, G. (1967) Biochem. J., 104, 57-70.