A SELECTIVE INACTIVATION OF DIMETHYLALLYL-TRANSFERING ACTIVITY OF PRENYLTRANSFERASE

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SUMMARY

In a heat treatment of prenyltransferase, geranyl-PP which is one of the substrates served as a selective stabilizer of the activity for the condensation reaction of geranyl-PP with isopentenyl-PP, while the other substrates, dimethylallyl-PP and isopentenyl-PP did not show any protective effect. The presence of magnesium ion was essential for the stabilization by geranyl-PP.

Prenyltransferase [EC 2.5.1.1] which catalyzes the formation of farnesyl-PP from isopentenyl-PP and prenyl pyrophosphates has been purified from yeast, pig liver and pumpkin fruit, and it is shown that every enzyme preparation has both dimethylallyl- and geranyl-transfering activities, namely activities for the reactions of isopentenyl-PP with dimethylallyl-PP and with geranyl-PP, and that the ratio of these two activities remains unchanged throughout the course of purification (1-5). A purified enzyme from pig liver which is thought to be nearly homogeneous from the sedimentation pattern and disc electrophoresis is also shown to be active for both reactions, and it is taken as a single enzyme protein that catalyzes these two consecutive reactions, whether one or two catalytic sites may be involved (3).

We have been interested in the difference in the property of the catalytic site(s) responsible for dimethyalallyl- and geranyl-transfering activities, and observed previously that dimethylallyl monophsopahte was a potent inhibitor for dimethylallyl-transfering activity, but not for geranyl-transfering activity (6). The result suggests that two separate sites might be involved in the prenyl-transferase reaction, and we examined the possibility of a selective inactivation of the enzyme by heat treatment in the presence of one of the substrates.

Abbreviations used : isopentenyl-PP, isopentenyl pyrophosphate; dimethylallyl-PP, dimethylallyl pyrophosphate; geranyl-PP, geranyl pyrophosphate; farnesyl-PP, farnesyl pyrophosphate.

MATERIALS AND METHODS

Enzyme was obtained from pumpkin fruits by ammonium sulfate fractionation followed by Sephadex G-200 and hydroxylapatite chromatographies according to our previous paper (5). Dimethylallyl-PP, geranyl-PP and ^{14}C-isopentenyl-PP were prepared by the method as reported in the same paper. Heat treatment of the enzyme were carried out as described under the figures, and the measurement of enzyme activity was made by the method reported previously (5).

RESULTS AND DISCUSSION

After prenyltransferase was incubated with one of the substrates in the presence of magnesium chloride at 50° for an appropriate period, the substrate which is a partner in the condensation reaction was add-

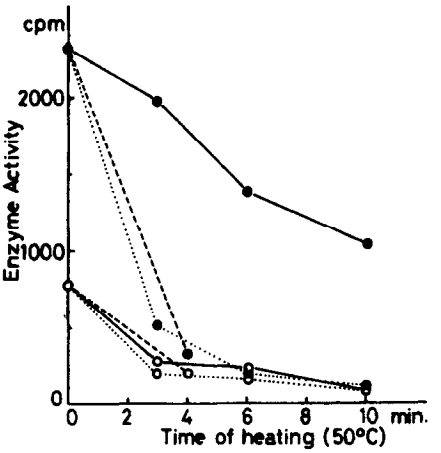

Figure 1. Effect of heating in the presence of substrate on enzyme activity

A mixture of 165 μg of prenyltransferase was preincubated with one (25 mμmoles) of the three substrates, isopentenyl-PP, dimethylallyl-PP and geranyl-PP and magnesium chloride (5 μmoles) in a final volume of 1 ml of 0.1 M phosphate buffer, pH 7.0 at 50°. After heating for indicating period, the mixture was cooled in an ice bath and ^{14}C-isopentenyl-PP (25 mμmoles) was added to a mixture already containing dimethylallyl-PP (o————o) or geranyl-PP (●————●). To a mixture already containg ^{14}C-isopentenyl-PP, 25 mμmoles of dimethylallyl-PP (o------o) or geranyl-PP (●------●) was added. For the control experiments, 165 μg of the enzyme was preincubated with 5 μmoles of magnesium chloride in the same buffer. After heating , 25 mμmoles of each dimethylallyl-PP and ^{14}C-isopentenyl-PP (o.....o) or 25 mμmoles of each geranyl-PP and ^{14}C-isopentenyl-PP (●.....●) was added.

After the addition of substrates the mixture was incubated at 37° for 20 minutes, and the enzyme acivity was measured by the method as described in the previous paper (5). Enzyme activity was expressed by counts per minute of ^{14}C-isopentenyl-PP converted into acid labile prenyl pyrophosphate.

ed and the enzyme activity was measured. The presence of isopentenyl-PP and magnesium chloride in the heat treatment had no effect on the stability of the enzyme, since the enzyme activity for both reactions was lost as rapidly as in the absence of substrate. On the other hand, the enzyme treated with geranyl-PP in the presence of magnesium ion showed against inactivation, showing 87% recovery of geranyl-transfering activity after 3 minites. Omission of magnesium chloride resulted in only 22% recovery of geranyl-transfering activity. Dimethylallyl-PP did not show such a stabilizing effect (Figure 1). In order to compare both activities in the enzyme treated in the presence of geranyl-PP and magnesium chloride, an incubation of larger scale was made in the same condition, and after the

Table 1. Sephadex G-25 filtration of the enzyme heated in the presence of geranyl-PP and magnesium chloride

After a mixture of prenyltransferase (2.14 mg), geranyl-PP (75 mμmoles), magnesium chloride(15 μmoles) and phosphate buffer, pH 7.0 (150 μmoles) in a final volume of 1.8 ml was heated at 50° for 5 minutes, the mixture was filtered on a column of Sephadex G-25 (0.4 x 25 cm) which had been equilibrated with 0.1 M phosphate buffer, pH 7.0, and eluted with the same buffer. The filtration was carried out at 4°. The enzyme fractions were combined and assayed for enzyme concentration and for geranyl-transfering activity. Enzyme concentration was estimated by the measurement of optical density at 280 mμ.

Enzyme Fraction	Substrate Added	Enzyme Activity *
Untreated enzyme	^{14}C-Isopentenyl-PP + Geranyl-PP	100.0
Treated enzyme before filtration	^{14}C-Isopentenyl-PP	80.0
Treated enzyme after filtration	^{14}C-Isopentenyl-PP	2.9
Treated enzyme after filtration	^{14}C-Isopentenyl-PP + Geranyl-PP	36.5
Treated enzyme after filtration**	^{14}C-Isopentenyl-PP	89.2

* Enzyme activity is given by relative value of the amount of the product formed per optical density unit (280 mμ) of enzyme.

**The enzyme fraction obtained by the filtration with a solution of geranyl-PP (75 mμmoles) and magnesium chloride (15 μmoles) in 1.8 ml of 0.1 M phosphate buffer, pH 7.0 in steatd of 0.1 M phosphate buffer alone was used.

heat treatment the enzyme fraction was freed from geranyl-PP and magnesium chloride by the filtration on a column of Sephadex G-25. The removal of geranyl-PP and magnesium ion was confirmed by the observations that addition of ^{14}C-isopentenyl-PP alone to the enzyme fraction gave only a neglegible amount of the product and that addition of the two components as well as ^{14}C-isopentenyl-PP was necessary to start the reaction (Table 1). Then both dimethylallyl- and geranyl-transfering activities were measured by using this enzyme fraction obtained by the Sephadex G-25 filtration. The ratio of enzyme activities with geranyl-PP and with dimethylallyl-PP was found to be 2.7 times as much as that of untreated enzyme at least up to enzyme concentration of 150 μg/ml, indicating that the heat treatment of prenyltransferase in the presence of geranyl-PP had caused a selective inactivation of dimethylallyl-transfering activity (Figure 2). When the enzyme treated with geranyl-PP and magnesium chloride was made free of geranyl-PP and magnesium chloride by the Sephadex filtration, the specific enzyme activity decreased as compared with that in the mixture before the filtration. However, when the mixture was filtered on the same column which had been equilibrated with a solution of geranyl-PP and magnesium chloride of the same concentration as that in the incubation mixture and eluted with the same solution, the specific activity was kept unchanged (Table 1). This fact suggests that once the enzyme was treated for the selective inactivation it becomes so unstable that geranyl-PP and magnesium ion are required to surround the enzyme for the maintenance of complete activity.

Kandutsch et al. have observed that farnesyl-PP has a stabilizing effect at least on farnesyl-transfering activity of geranylgeranyl pyrophosphate synthetase which is responsible for farnesyltransfering activity as well as dimethylallyl- and geranyl-transfering activities and that dimethylallyl-PP has no effect, though it is unknown whether the effect of farnesyl-PP is specific only for farnesyl-transfering activity (7).

Our results described above showed the effect of geranyl-PP was specific for geranyl-transfering activity of farnesyl pyrophosphate synthetase. One could expect that the effect of farnesyl-PP in the heat treatment of geranylgeranyl pyrophosphate synthetase might be also specific.

The possibility that two separate sites for dimethylallyl- and geranyl-transfering activities the latter of which is protected by

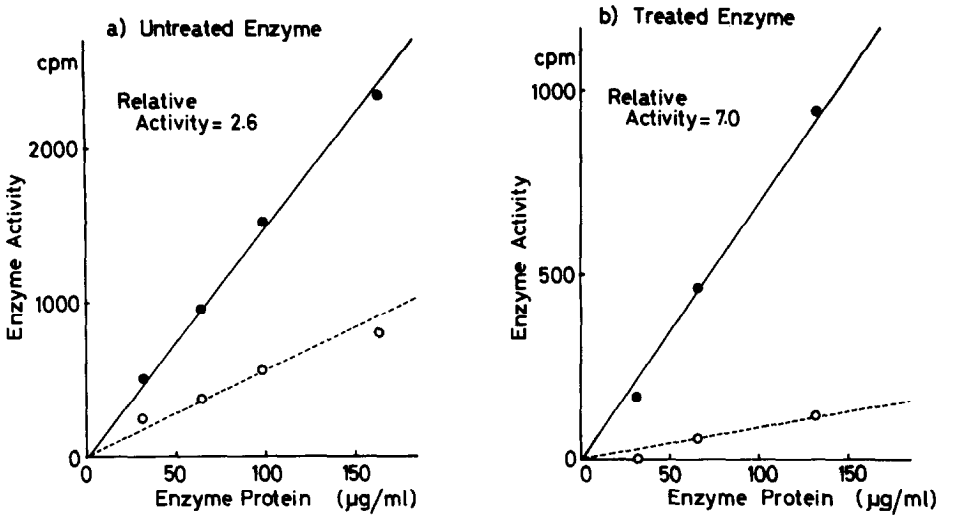

Figure 2. Enzyme activity of treated and untreated prenyltransferase

●——● Enzyme activity when ^{14}C-isopentenyl-PP and geranyl-PP were substrates.

o——o Enzyme activity when ^{14}C-isopentenyl-PP and dimethylallyl-PP were substrates.

Enzyme activity is given in the same way as described under Figure 1. Therefore, for the estimation of dimethylallyl-transfering activity, the enzyme activity indicated (o——o) should be divided by 2, since the product is always almost farnesyl-PP alone.

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geranyl-PP may be involved or that one common site for both activities may be deformed in such a way that it fits geranyl-transfering reaction better than dimethylallyl-transfering reaction would explain this differential effect.

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