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COMPARATIVE SPECIFICITY OF PRENYLTRANSFERASE OF PIG LIVER AND PUMPKIN WITH RESPECT TO ARTIFICIAL SUBSTRATES

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SUMMARY

Comparative studies on the substrate specificity of prenyltransferase (dimethylallyl pyrophosphate:isopentenyl pyrophosphate dimethylallyltransferase, EC 2.5.1.1) from pig liver and from pumpkin showed that the enzyme from liver had broader specificity with respect to artificial substrates than the enzyme from pumpkin. Seven allylic pyrophosphates, which were inactive as substrates for pumpkin enzyme, were found to be active for liver enzyme. These were *cis*-3-methyl-2-octenyl, *trans*-3,4-dimethyl-2-pentenyl, *trans*-3,4-dimethyl-2-hexenyl, *trans*-3,5-dimethyl-2-hexenyl, *trans*-3,6-dimethyl-2-heptenyl, 3-propyl-2-hexenyl and cycloheptylideneethyl pyrophosphates. No difference was observed between the two enzymes, in that geranyl pyrophosphate or its homolog was accumulated in an early stage of the reaction of dimethylallyl pyrophosphate or its homolog with isopentenyl pyrophosphate.

INTRODUCTION

Prenyltransferase (dimethylallyl pyrophosphate:isopentenyl pyrophosphate dimethylallyltransferase, EC 2.5.1.1) has been purified from yeast[1], pig liver[2,3] and pumpkin[4]. It is well established that a purified preparation of this enzyme from any of these sources catalyzes the condensation of isopentenyl pyrophosphate (3-methyl-3-butenyl pyrophosphate) both with 3,3-dimethylallyl pyrophosphate (1)* and with geranyl pyrophosphate (2) to give *trans,trans*-farnesyl pyrophosphate. It has been also shown that some homologs of 1 act as condensing partners with isopentenyl pyrophosphate in the reaction catalyzed by prenyltransferase from pig liver[5–8] and pumpkin fruit[9,10]. However, comparative study of this enzyme from these sources has not been reported. We now report differential specificities of this enzyme from liver and pumpkin with respect to artificial substrates.

* The numbers (1) *etc.* refer to compounds as set out in Tables I and II.

MATERIALS AND METHODS

All allylic pyrophosphates were the same preparations as used in the previous studies[8–10], except that cycloheptylideneethyl pyrophosphate (24) was synthesized from cycloheptanone by a method similar to that for the synthesis of cyclohexylideneethyl pyrophosphate (23)[8]. [^{14}C]Isopentenyl pyrophosphate (spec. act., 1.2 Ci/mole) was prepared by the method of Yuan and Bloch[11]. Prenyltransferase was purified from pig liver according to the procedure of Holloway and Popják[3], and from pumpkin fruit by our method[4]. The specific activities of the enzyme preparations from liver and pumpkin were 18 and 20 units/mg, respectively. One unit of enzyme is defined as the amount of enzyme which converts 1 nmole of isopentenyl pyrophosphate, in the presence of 3,3-dimethylallyl pyrophosphate (1), into farnesyl pyrophosphate per min. Both preparations were free of isopentenyl pyrophosphate isomerase and geranylgeranyl pyrophosphate synthetase.

Enzymatic reaction was assayed as usual[9] by determining the incorporation of [^{14}C]isopentenyl pyrophosphate into the acid-labile allylic pyrophosphates formed by the condensation. The standard incubation mixture contained, in a final volume of 2 ml, 40 μmoles of potassium phosphate buffer (pH 7.0), 10 μmoles of $MgCl_2$, 50 nmoles of [^{14}C]isopentenyl pyrophosphate (60 nCi), 25 nmoles of an allylic pyrophosphate to be examined and 50 μg of enzyme protein. The mixture was incubated at 37 °C for 20 min and was then treated with 0.6 ml 1 M HCl for 15 min to complete the hydrolysis of allylic pyrophosphates, and the mixture was extracted with 4 ml of *n*-hexane. The radioactivity in the extract was routinely determined with a liquid scintillation counter. For the identification of the products of the enzymatic reaction, the reaction mixture was treated with alkaline phosphatase, and the radioactive alcohols thus liberated were analyzed by radiogas chromatography as described previously[10].

RESULTS AND DISCUSSION

Tables I and II show the ability of various allylic pyrophosphates to react with isopentenyl pyrophosphate in the reaction catalyzed by the enzymes from liver and pumpkin under the same conditions. The reactivity is expressed as the relative amount of [^{14}C]isopentenyl pyrophosphate converted into acid-labile allylic pyrophosphates by the condensation, 3,3-dimethylallyl pyrophosphate (1) being taken as a standard. No difference was observed between the enzymes from liver and from pumpkin when natural substrates were used; the reactivities of geranyl pyrophosphate (2) in the reaction catalyzed by these two enzymes relative to that of 1 were nearly equal to each other. The general relationships between the chain lengths of the alkyl group of artificial 3-methyl-2-alkenyl pyrophosphates and their reactivities catalyzed by these two enzymes were also essentially similar to each other. Namely, in the cis series (12–15) the reactivity decreased with increase of chain length, and in the trans series (3–11), on the other hand, C_9 (6) or C_{10} compound (7) showed the maximum reactivity. However, a difference was observed in that *cis*-3-methyl-2-hexenyl pyrophosphate (13) was 4 times and *cis*-3-methyl-2-heptenyl pyrophosphate (14) was 9 times more reactive in the reaction catalyzed by the liver enzyme than by the pumpkin enzyme. Even *cis*-3-methyl-2-octenyl pyrophosphate (15) which was

TABLE I

REACTION OF 3-METHYL-2-ALKENYL PYROPHOSPHATES IN THE REACTION WITH ISOPENTENYL PYROPHOSPHATE CATALYZED BY PRENYLTRANSFERASE OF LIVER AND PUMPKIN

Compound $R =$	*Reactivity*	
	Liver	*Pumpkin*
trans-$R(CH_3)C=CHCH_2OP_2O_6^{3-}$		
CH_3 (1)	1	1
$(CH_3)_2C=CHCH_2$ (2)	0.80	0.82
C_2H_5 (3)	0.31	0.18
n-C_3H_7 (4)	0.56	0.16
n-C_4H_9 (5)	0.63	0.50
n-C_5H_{11} (6)	0.65	0.90
n-C_6H_{13} (7)	0.73	0.88
n-C_7H_{15} (8)	0.65	0.74
n-C_8H_{17} (9)	0.35	0.37
n-C_9H_{19} (10)	0.06	0.03
n-$C_{11}H_{23}$ (11)	Inactive*	Inactive*
cis-$R(CH_3)C=CHCH_2OP_2O_6^{3-}$		
C_2H_5 (12)	0.24	0.15
n-C_3H_7 (13)	0.20	0.05
n-C_4H_9 (14)	0.18	0.02
n-C_5H_{11} (15)	0.04	Inactive*

* Inactive indicates that the reactivity is less than 0.01.

TABLE II

REACTIVITY OF ALLYLIC PYROPHOSPHATES IN THE REACTION WITH ISOPENTENYL PYROPHOSPHATE CATALYZED BY PRENYLTRANSFERASE OF LIVER AND PUMPKIN

Compound $R =$	*Reactivity*	
	Liver	*Pumpkin*
trans-$R(CH_3)C=CHCH_2OP_2O_6^{3-}$		
$(CH_3)_2CH$ (16)	0.13	Inactive*
$(C_2H_5)(CH_3)CH$ (17)	0.19	Inactive*
$(CH_3)_2CHCH_2$ (18)	0.14	Inactive*
$(CH_3)_2CHCH_2CH_2$ (19)	0.06	Inactive*
$(CH_3)_2CHCH_2CH_2CH_2$ (20)	0.51	0.52
$(CH_3)_2CHCH_2CH_2CH_2CH_2$ (21)	0.29	0.24
Cyclopentylideneethyl-$OP_2O_6^{3-}$ (22)	0.41	0.24
Cyclohexylideneethyl-$OP_2O_6^{3-}$ (23)	0.29	0.04
Cycloheptylideneethyl-$OP_2O_6^{3-}$ (24)	0.16	Inactive*
$(n\text{-}C_3H_7)_2C=CHCH_2OP_2O_6^{3-}$ (25)	0.24	Inactive*

* Inactive indicates that the reactivity is less than 0.01.

no longer active as a substrate for the pumpkin enzyme[9], was found to be still accepted by the liver enzyme. This C_9 compound (15) was almost as reactive for the liver enzyme as *cis*-3-methyl-2-hexenyl pyrophosphate (13) was for the pumpkin enzyme. A more remarkable difference in the specificity of these two enzymes was observed with allylic pyrophosphates having a branched chain or cyclic structure (Table II). 3,4-Dimethyl-2-pentenyl (16), 3,4-dimethyl-2-hexenyl (17), 3,5-dimethyl-2-hexenyl (18) and 3,6-dimethyl-2-heptenyl pyrophosphate (19), which were all

inactive for the pumpkin enzyme, acted as substrates for the liver enzyme. As to cyclic compounds, cyclohexylideneethyl pyrophosphate (23) reacted 7 times more rapidly in the reaction catalyzed by the liver enzyme than by the pumpkin enzyme, and cycloheptylideneethyl pyrophosphate (24) was active as a substrate only for the liver enzyme. 3-Propyl-2-hexenyl pyrophosphate (25), which was not accepted by the pumpkin enzyme, was also a fairly good substrate for the liver enzyme. Thus, allylic pyrophosphates which were reactive as substrates for the pumpkin enzyme were also all reactive for the liver enzyme, but some substrates were found to be reactive only for the latter enzyme.

Holloway and Popják[3] and Popják *et al.*[6] reported that geranyl pyrophosphate (2) was never accumulated on incubation of liver prenyltransferase with 3,3-dimethylallyl pyrophosphate (1) and isopentenyl pyrophosphate, and that the reaction of isopentenyl pyrophosphate with *trans*-3-methyl-2-pentenyl (3) and with *trans*-3-methyl-2-hexenyl pyrophosphate (4) similarly gave the homologs of farnesyl pyro-

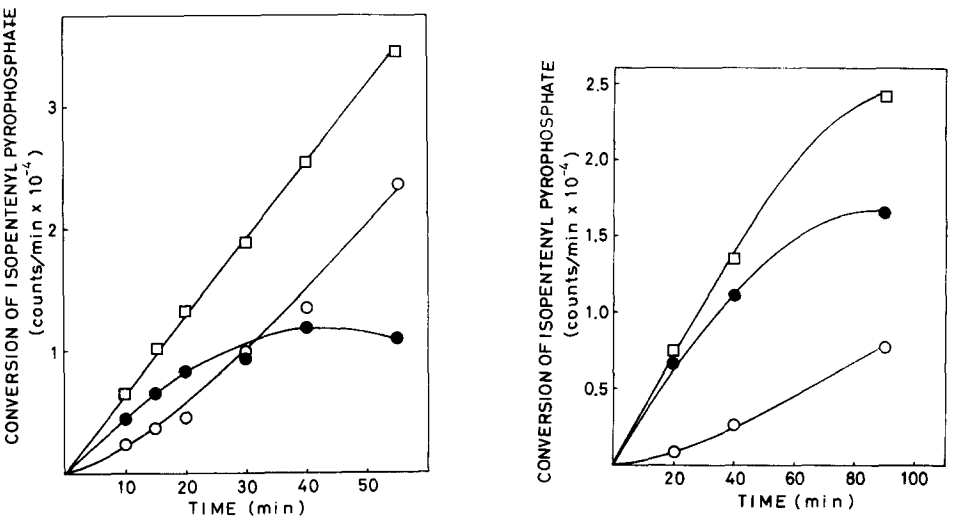

Fig. 1. Conversion of [^{14}C]isopentenyl pyrophosphate into geranyl and farnesyl pyrophosphate as a function of time. The incubation mixture contained, in a final volume of 14 ml, 280 μmoles of potassium phosphate buffer (pH 7.0), 70 μmoles of $MgCl_2$, 350 nmoles of [^{14}C]isopentenyl pyrophosphate, 175 nmoles of 3,3-dimethylallyl pyrophosphate (1) and 200 μg of liver prenyltransferase. At the end of each time interval as indicated, two 1-ml aliquots were withdrawn. One aliquot was treated with acid as usual for the measurement of the total conversion of radioactivity into acid-labile allylic pyrophosphates, and the other aliquot was heated at 100 °C for 2 min and was treated with alkaline phosphatase for the product analysis. Individual conversion of radioactivity into geranyl and farnesyl pyrophosphates was estimated on the basis of the peak area for geraniol and farnesol on the radiogas chromatogram. □—□, total radioactivity in allylic pyrophosphates; ●—●, radioactivity in geranyl pyrophosphate; ○—○, radioactivity in farnesyl pyrophosphate. Two isopentenyl residues are incorporated into a farnesyl pyrophosphate molecule, and hence for comparison of the amount of the products the value for farnesyl pyrophosphate should be divided by 2.

Fig. 2. Conversion of [^{14}C]isopentenyl pyrophosphate into trishomogeranyl and trishomofarnesyl pyrophosphates as a function of time. Incubation was carried out in the same manner as described under Fig. 1 except that 3-methyl-2-heptenyl pyrophosphate (5) was used in place of 1 and that the scale of incubation was half. After the reaction 1-ml aliquots were treated as described under Fig. 1. □—□, total radioactivity in allylic pyrophosphates; ●—●, radioactivity in trishomogeranyl pyrophosphate; ○—○, radioactivity in trishomofarnesyl pyrophosphate.

phosphate without accumulation of any geranyl pyrophosphate homologs. On the other hand, we showed that geranyl pyrophosphate (2) was accumulated in the reaction of 1 with isopentenyl pyrophosphate catalyzed by the pumpkin enzyme, and that the reaction of 4 with isopentenyl pyrophosphate gave both homologs of geranyl pyrophosphate and of farnesyl pyrophosphate[4,9]. It seems attractive to explain this discrepancy by assuming that prenyltransferase of plant origin has two separate sites, a dimethylallyl-transferring ($C_5 \rightarrow C_{10}$) and a geranyl-transferring site ($C_{10} \rightarrow C_{15}$) for the synthesis of geranyl pyrophosphate which is a precursor for the monoterpene biosynthesis as well as farnesyl pyrophosphate, and that the liver enzyme has one common site which catalyzes the completely consecutive condensation, $C_5 \rightarrow C_{10} \rightarrow C_{15}$ only for the synthesis of farnesyl pyrophosphate.

However, comparison of the reaction products revealed that this discrepancy was not due to the difference in the source of the enzyme, but to the difference in the condition of incubation. In an early stage of the reaction, the accumulation of geranyl pyrophosphate or its homolog was dominant in the reaction catalyzed by the liver enzyme as well as by the pumpkin enzyme, and the formation of farnesyl pyrophosphate or its homolog became conspicuous as the reaction proceeded. Time courses of the reaction are exemplified in Figs 1 and 2.

It was also confirmed that the C_{18} compound derived from 5, 15, or 10, the former two being capable of reacting with two molecules of isopentenyl pyrophosphate, was the longest homolog formed by the liver enzyme as well as by the pumpkin enzyme.

General properties of prenyltransferase from liver and pumpkin were similar to each other except that the former had broader substrate specificity with respect to several artificial substrates.

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