BBA 66315

REACTIVITY OF ARTIFICIAL SUBSTRATES FOR PRENYLTRANSFERASE

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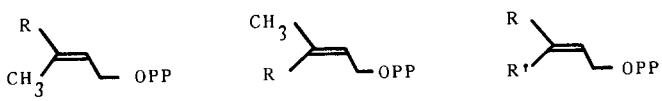
(Received December 31st, 1970)

SUMMARY

Four compounds out of 6 new allylic pyrophosphates synthesized were found to act as artificial substrates for pig liver prenyltransferase (dimethylallylpyrophosphate:isopentenylpyrophosphate dimethylallyltransferase, EC 2.5.1.1). These were cyclopentylideneethyl, cyclohexylideneethyl, *trans*-3-ethyl-2-hexenyl, and *cis*-3-ethyl-2-hexenyl pyrophosphates. 2-Heptenyl and 2-octenyl pyrophosphates which have no substituent at the 3 position were inactive. The reactivities of these artificial substrates including 4 known compounds, *trans*- and *cis*-3-methyl-2-hexenyl pyrophosphates, and *trans*- and *cis*-3-methyl-2-heptenyl pyrophosphates were compared in terms of K_m and v_{max}, and it was found that the *trans* structure was favored for the binding to the enzyme.

It has been reported that certain homologues of dimethylallyl pyrophosphate could be substrates for prenyltransferase (dimethylallylpyrophosphate:isopentenylpyrophosphate dimethylallyltransferase, EC 2.5.1.1) of either pig liver or pumpkin[1–3]. We showed that the structural requirement for the substrate of the pumpkin enzyme was that the substituent R in Compound A (Scheme 1) could be as large as n-C_7H_{15}, and that R in Compound B could not be larger than n-C_4H_9 (ref. 3). These results led us to investigate in more detail the effects of the geometry of the carbon chain on substrate specificity. This paper describes the reactivities of various allylic pyrophosphates of Type C including A and B. A comparison of the reactivity of compounds of the same carbon number but of different geometry was made using the pig liver enzyme.



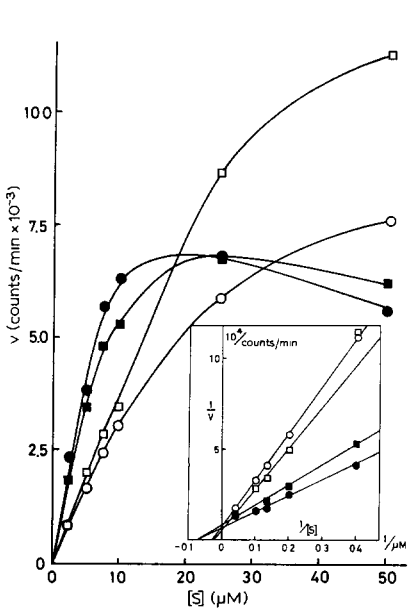
(A) (B) (C)

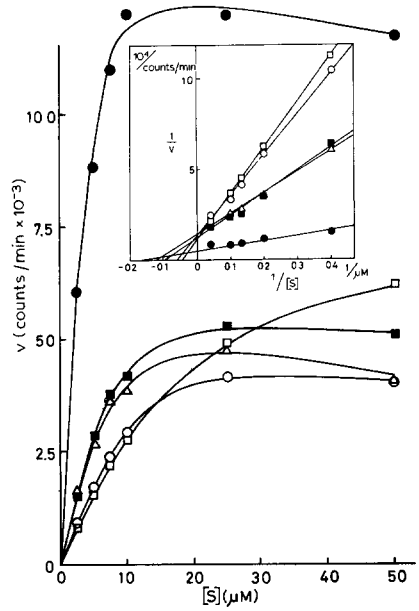
$-OPP = OP_2O_6^{3-}$

Scheme 1

The enzymic reaction was followed by determining the radioactivity present in acid-labile allylic pyrophosphate into which [^{14}C]isopentenyl pyrophosphate was incorporateed by condensation[3]. [^{14}C]Isopentenyl pyrophosphate and Compounds II, IV, VII and X were the same preparations as used in the previous study[3]. Other new compounds were obtained by phosphorylation of the alcohols synthesized as described below. The syntheses of the alcohols and their phosphorylation were carried out on a 1-mmole and a 0.1–0.2-mmole scale, respectively. The products were characterized by the same method as reported previously[3]. For the synthesis of V and XI, cyclopentanone and cyclohexanone were treated with diethyl methoxycarbonylmethyl phosphonate in the presence of sodium methoxide to give methyl esters of cyclopentylideneacetic acid and cyclohexylideneacetic acid. In order to remove a minor impurity in the esters, they were hydrolyzed to the free acids, which were purified by recrystallization. Cyclopentylideneethanol and cyclohexylideneethanol were obtained by $LiAlH_4$ reduction of the acids. For the synthesis of III (ref. 2), diethyl ketone was subjected to the same treatment as described above, and 3-ethyl-2-pentenol was obtained. In the synthesis of VIII and IX, the separation of *cis* and *trans* isomers of methyl 3-ethyl-2-hexenoate, obtained by the Wittig reaction with ethyl *n*-propyl ketone, was achieved essentially by the same procedure as in the previous report[3]. The mixture of the *cis* and *trans* esters was treated with alkali to give a mixture of the free acids, from which the *trans* isomer was purified by recrystallization. The filtrate, which had been enriched with the *cis* acid, was treated with diazomethane, and the *cis* ester was purified by chromatography on a column of silica gel with *n*-hexane. The *cis* ester and the *trans* acid were reduced with $LiAlH_4$ to the corresponding alcohols. The characterization of the *cis* and *trans* isomers was based on their NMR spectra. Methyl *cis*-3-ethyl-2-hexenoate showed signals at τ 7.81 (quartet, 2H) and 7.36 (triplet, 2H) for the methylenes adjacent to the double bond, whereas the *trans* isomer showed lines at τ 7.88 (triplet, 2H) and 7.38 (quartet, 2H). For the synthesis of 2-heptenyl (I) and 2-octenyl pyrophosphate (VI), *trans*-heptenol and *trans*-octenol were obtained by the $LiAlH_4$ reduction of the corresponding acids synthesized by the reaction of malonic acid with *n*-pentylaldehyde and *n*-hexylaldehyde. The two alcohols obtained contained about 45% of *n*-heptanol and *n*-octanol, but the phosphorylation was carried out without further purification.

It was found that mono-substituted allyl pyrophosphates, *trans*-2-heptenyl (I) and *trans*-2-octenyl pyrophosphate (VI), were not accepted by the enzyme at all. The contamination of I and VI with their dihydro derivatives is presumably not an obstacle, at least for the examination as to whether or not they are enzymically active as substrates, because the effect of citronellyl pyrophosphate in such a low concentration on the reaction of geranyl pyrophosphate with isopentenyl pyrophosphate was found to be negligible. Other allylic pyrophosphates of C_7 and C_8 including the cyclic derivatives reacted enzymically with [^{14}C]isopentenyl pyrophosphate to give acid-labile [^{14}C]allylic pyrophosphates. The effect of substrate concentration on the initial velocity is shown in Fig. 1 and 2. The K_m and v_{max} values obtained by the Lineweaver–Burk method are given in Table I. It appears that the geometry of the substrate has a marked effect on the reactivity. In either the C_7 or C_8 series the K_m value decreases in the order of increasing *trans* character of substrate; the K_m for IV is 3 times greater than that for its *trans* isomer II, and in the C_8 series the K_m





Figs. 1 and 2. The reaction mixture contained, in a final volume of 1 ml, 20 μmoles of phosphate buffer (pH ,7.0), 5 μmoles of $MgCl_2$, 0.025 μmole of [^{14}C]isopentenyl pyrophosphate (specific activity, 1.2 μC/μmole), an allylic pyrophosphate to be examined, and 50 μg of farnesyl pyrophosphate synthetase prepared from pig liver according to the procedure of HOLLOWAY AND POPJÁK[4]. The reaction velocity was measured by the same method as previously reported[3], and was expressed in counts/min as the amount of the conversion of [^{14}C]isopentenyl pyrophosphate into acid labile substance during 20 min at 37°. Fig. 1 (left): Effect of substrate concentration on reaction velocity of C_7 compounds. ●—●, *trans*-3-methyl-2-hexenyl (II); ■—■, 3-ethyl-2-pentenyl (III); ○—○, *cis*-3-methyl-2-hexenyl (IV); □—□, cyclopentylideneethyl pyrophosphate (V). Fig. 2 (right). Effect of substrate concentration on reaction velocity of C_8 compounds. ●—●, *trans*-3-methyl-2-heptenyl (VII); ■—■, *trans*-3-ethyl-2-hexenyl (VIII); ○—○, *cis*-3-ethyl-2-hexenyl (IX); □—□, *cis*-3-methyl-2-heptenyl (X); △—△,cyclohexylideneethyl pyrophosphate (XI).

TABLE I

K_m AND v_{max} VALUES OF ARTIFICIAL SUBSTRATES

OPP = $OP_2O_6^{3-}$.

Compound	K_m *(μM)*	v_{max} *(10⁻⁴ × counts/min)*	*Compound*	K_m *(μM)*	v_{max} *(10⁻⁴ × counts/min)*
OPP (I)	—	—	OPP (VI)	—	—
OPP (II)	13	1.49	OPP (VII)	7	2.20
OPP (III)	13	1.22	OPP (VIII)	10	0.83
OPP (IV)	40	1.60	OPP (IX)	16	0.76
OPP (V)	50	2.08	OPP (X)	22	0.87
			OPP (XI)	8	0.73

increases in the order of VII, VIII, IX and X. However, the relationship between v_{max} and *cis–trans* character is not straightforward. The cyclic derivative V showed a reactivity of *cis*-type profile in the C_7 series, showing the largest K_m, but it had the largest v_{max} of this series. In the C_8 series, cyclization did not cause unfavorable change in the reactivity with respect to K_m; the cyclohexylidene derivative, XI, showed a K_m near to that for *trans*-3-methyl-2-heptenyl pyrophosphate (VII), but the v_{max} was only one-third of that for VII.

The capacity of the binding site of prenyltransferase for the hydrophobic moiety of the substrate has been discussed mainly from the standpoint of the size of the alkyl group of the substrate[1–3]. In the present study the reactivities of allylic pyrophosphates containing the same number of carbon atoms were compared, and it was observed that the *trans* structure was favored for the binding to the enzyme. Four out of six new allylic pyrophosphates synthesized were found to be enzymically active. Mono-substituted allyl pyrophosphates, I and VI, were inactive.

ACKNOWLEDGMENT

We thank Mr. T. Koyama for his assistance in part of this work. This research was supported by Grant-in-Aid 40702 from the Ministry of Education.

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