Avian Liver Prenyltransferase. The Role of Metal in Substrate Binding and the Orientation of Substrates during Catalysis†

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ABSTRACT: Although prenyltransferase (EC 2.5.1.1) requires either Mn^{2+} or Mg^{2+} for catalytic activity, the enzyme will bind either of its substrates in the absence of a divalent cation. Under these conditions the stoichiometry for each substrate is 1:1, substrate for enzyme subunit, with an affinity reduced several-fold compared to that observed in the presence of metal ion. Also, when both substrates are added, they are bound noncompetitively and without catalysis in the absence of metal. It was possible to demonstrate competition for binding to enzyme between isopentenyl pyrophosphate and farnesyl pyrophosphate, the product, in the absence of a divalent cation. Similarly, the analogue 2-fluorofarnesyl pyrophosphate bound competitively with isopentenyl pyrophosphate in the presence of Mg^{2+}. Previous experiments had shown that the product competed with the allylic substrates for binding to the enzyme (Reed, B. C., and Rilling, H. C. (1976), *Biochemistry 15*, 3739). In the absence of substrate, $^{54}Mn^{2+}$ is not bound to the enzyme, but when product or either substrate is present, 2 mol of metal is bound per enzyme subunit. Simultaneous binding of the 2-fluoro analogues of both allylic and homoallylic substrates does not increase the number of metal ions bound. These data are interpreted to show that, when farnesyl pyrophosphate is bound to the enzyme, the pyrophosphate group occupies the isopentenyl pyrophosphate binding site while the hydrocarbon moiety overlaps into the allylic binding site. The metal binding data indicate that the pyrophosphate groups of both substrates are linked via metal ion bridges. This, taken in conjunction with the orientation of substrates required for condensation, provides a model for the alignment of the substrates in the catalytic site.

Prenyltransferase catalyzes the principal carbon-to-carbon bond-forming step in polyisoprenoid biosynthesis, which is the head-to-tail condensation between allylic isoprenyl pyrophosphate and isopentenyl pyrophosphate. The prenyltransferase that participates in cholesterol (sterol) biosynthesis has been purified to homogeneity from several sources (Eberhardt and Rilling, 1975; Reed and Rilling, 1975; Dorsey et al., 1966). The homogeneous proteins have been shown to be dimeric and to catalyze the condensation of isopentenyl pyrophosphate with either dimethylallyl or geranyl pyrophosphate with equal facility. A previous report from this laboratory (Reed and Rilling, 1976) established that the enzyme contained two allylic and four isopentenyl pyrophosphate binding sites per dimer. Since half of the isopentenyl pyrophosphate binding sites were susceptible to competition from an allylic analogue, Reed and Rilling (1976) concluded that two of the isopentenyl pyrophosphate molecules were bound at the allylic sites. These studies were in the presence of Mg^{2+} which is required for enzymatic activity. We have extended these studies to examine the relationship between metal and substrate binding. We find that two substrate binding sites are clearly distinguishable in the absence of metal and that substrate is required for metal binding. Also, allylic and homoallylic substrate bind metal to enzyme in distinctly different patterns. The data are interpreted in terms of the spatial relationship of the substrates in the catalytic site.

Materials and Methods

Preparation of Substrates. The 2-fluoro analogues of isopentenol, geraniol, and farnesol were kindly supplied by Dr. C. Dale Poulter, Department of Chemistry, University of Utah. [1-^{14}C]Isopentenyl pyrophosphate was purchased from Amersham/Searle. The pyrophosphate esters of the various alcohols were prepared and their concentrations and specific radioactivities determined as described previously (Reed and Rilling, 1976). Unlabeled and labeled substrates were stored in water and 50% water/methanol, respectively, at −10 °C in the presence of a slight excess of ammonia. The specific activities were [1-^{3}H]geranyl pyrophosphate, 119 μCi/μmol; [1-^{3}H]farnesyl pyrophosphate, 405 μCi/μmol; and [1-^{14}C]isopentenyl pyrophosphate, 49 μCi/μmol. The $^{54}MnCl_2$ (New England Nuclear) was diluted with $^{55}MnCl_2$ to the desired specific activity.

Radioisotope Determinations. Radiolabeled pyrophosphates were dissolved in 1 mL of water and 10 mL of a 1:2 mixture of Triton X-100 (New England Nuclear) and toluene containing 0.85% Omnifluor. Simultaneous determinations of ^{3}H and ^{14}C were obtained by standard techniques. In all cases counting efficiencies were determined by internal standardization. The radionuclide, ^{54}Mn, was determined by a Beckman Biogamma counter.

Preparation of Enzyme. The enzyme was purified from avian liver by the method of Reed and Rilling (1975). The crystals were stored at 4 °C as a suspension in buffered saturated ammonium sulfate solution containing dithiothreitol.

Enzyme Binding Studies. Substrate and metal binding were measured at 4 °C using a "forced dialysis" technique (Paulus, 1969; Cantley and Hammes, 1973). By this method, eight separate measurements could be made within 30 min of mixing ligand and enzyme, thus eliminating any major error due to hydrolysis of the substrate by the enzyme (Poulter and Rilling, 1976). All binding studies utilized 50 mM potassium Tes[1] buffer (pH 7.0) containing 10 mM 2-mercaptoethanol and 100 mM KCl as solvent. Other additions are described in the individual figure legends. For each measurement, a 200-μL mixture of enzyme and ligand at 4 °C was submitted to forced

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[1] Abbreviation used: Tes, *N*-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid.

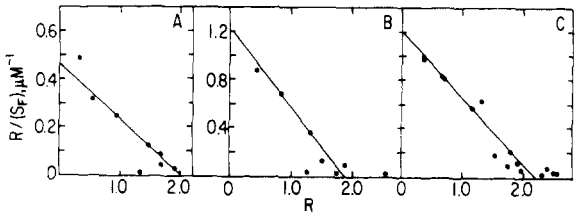

FIGURE 1: Substrate binding by avian liver prenyltransferase in the absence of divalent metal ion. R is the number of moles of substrate bound per mole of enzyme. S_F is the free substrate concentration of isopentenyl pyrophosphate (panels A and B) or geranyl pyrophosphate (panel C). Panel C represents the results of two experiments. The enzyme concentrations were 4.74 μM in A, 3.22 μM in B, and 4.88 and 4.91 μM in C. Mixtures in panel B also contained 20 μM geranyl pyrophosphate.

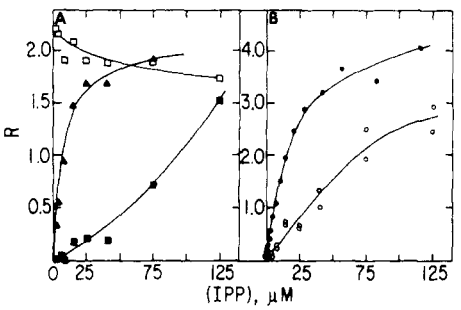

FIGURE 2: (A) Competition between isopentenyl and farnesyl pyrophosphates for binding to prenyltransferase in the absence of divalent metal. R is the number of moles of [1-^{14}C]isopentenyl pyrophosphate (IPP) bound in the presence (■) or absence (▲) of [1-^{3}H]farnesyl pyrophosphate (20 μM), or the number of moles of [1-^{3}H]farnesyl pyrophosphate (□) bound in the presence of varying concentrations of [1-^{14}C]isopentenyl pyrophosphate, per mole of enzyme. (■) and (□) were measured simultaneously. The enzyme concentrations were 4.79 μM (■ and □) or 4.74 μM (▲). (B) Competition between isopentenyl and 2-fluorofarnesyl pyrophosphates for binding to prenyltransferase in the presence of 1 mM $MgCl_2$. R is the number of moles of [1-^{14}C]isopentenyl pyrophosphate bound in the presence of 1 mM $MgCl_2$ (●, data of Reed and Rilling, 1976), or moles of [1-^{14}C]isopentenyl pyrophosphate bound in the presence of 200 μM 2-fluorofarnesyl pyrophosphate and 1 mM $MgCl_2$ (○), per mole of enzyme. Each curve represents the results of two experiments. The enzyme concentrations were 5.79 μM (●) and 4.23 or 4.39 μM (○).

dialysis as described by Reed and Rilling (1976). The membrane contribution to apparent binding was approximately 3% of the free ligand concentration.[2] This value was used for calculating bound and free ligand concentrations.

For these studies, crystalline enzyme was pelleted, then dissolved in 100 μL of buffer, and desalted on a 0.5 × 25 cm Sephadex G-25 column within 30 min of the start of an experiment.

Isopentenyl and Geranyl Pyrophosphate–Metal Dissociation Constants. The dissociation constant for the complex of Mg^{2+} or Mn^{2+} with isopentenyl or geranyl pyrophosphates was determined by the method of Burton (1959), except that 50 mM potassium Tes buffer (pH 7.0) was used instead of triethanolamine buffer. Measurements of the absorption of the hydroxyquinoline–metal complex were at 4 °C using a Cary recording spectrophotometer.

Results

Substrate Binding to Prenyltransferase in the Absence of Divalent Metal Ion. The previous substrate binding studies carried out with this enzyme were in the presence of 1 mM Mg^{2+}, an ion required for catalytic activity. To obtain metal-free enzyme for binding studies, the enzyme was chromatographed on Sephadex G-25 in buffer devoid of divalent metal. After this chromatographic step, the specific activity of the enzyme without metal was 1.3×10^{-7} times that when assayed under standard conditions in the presence of Mg^{2+} and is of questionable significance. Thus, the enzyme is essentially devoid of catalytic activity in the absence of divalent metal. Other experiments showed that the enzyme thus prepared contained only traces of metal ions (B. C. Reed, unpublished observations). Prenyltransferase, in the absence of metal ion, still interacts strongly with its substrates. A Scatchard plot (Scatchard, 1949) of the binding of geranyl pyrophosphate shows that, at saturation, 2.2 mol of this substrate are bound per mol of enzyme (Figure 1A). At high substrate concentration there is an additional low affinity association of geranyl pyrophosphate with the enzyme. The dissociation constant for geranyl pyrophosphate binding is 1.8 μM. Prenyltransferase also binds isopentenyl pyrophosphate in the absence of divalent metal ion. A Scatchard plot of the data (Figure 1B) is linear and the intercepts indicate that 2.0 mol of isopentenyl pyrophosphate is bound per mol of enzyme dimer with a dissociation constant of 4.3 μM. The saturation value of 2 mol of isopentenyl pyrophosphate bound per mol of enzyme dimer is half the previously reported value which was determined in the presence of 1 mM $MgCl_2$ (Reed and Rilling, 1976) and indicates that divalent metal ion is instrumental in the binding of 2 additional mol of isopentenyl pyrophosphate to the enzyme. Since divalent metal ion is required for catalytic activity, it was possible to study the binding of both substrates in the absence of metal. For this experiment, the enzyme was brought to 90% of saturation with 20 μM [1-^{3}H]geranyl pyrophosphate, and the binding of both substrates was measured as a function of [1-^{14}C]isopentenyl pyrophosphate concentration. The amount of geranyl pyrophosphate bound was relatively constant at 1.7–2.1 mol/mol of enzyme dimer at the concentrations of [1-^{14}C]isopentenyl pyrophosphate tested (data not shown). The Scatchard plot of the [1-^{14}C]isopentenyl pyrophosphate binding (Figure 1C) shows that geranyl pyrophosphate has no effect on the extrapolated saturation value obtained for [1-^{14}C]isopentenyl pyrophosphate in the absence of divalent metal ion. However, under these conditions there is an increase in the affinity of the enzyme for isopentenyl pyrophosphate from a dissociation constant of 4.3 to 1.5 μM. This indicates that, in the absence of divalent metal ion, geranyl pyrophosphate facilitates the binding of isopentenyl pyrophosphate to the enzyme.

Simultaneous interaction of [1-^{14}C]isopentenyl pyrophosphate and [1-^{3}H]farnesyl pyrophosphate with prenyltransferase was also studied in the absence of divalent metal ion. The binding of [1-^{14}C]isopentenyl pyrophosphate by the enzyme was inhibited by the addition of 20 μM [1-^{3}H]farnesyl pyrophosphate, as is shown in Figure 2A. The converse, however, is not true since even 125 μM isopentenyl pyrophosphate failed to reduce the amount of farnesyl pyrophosphate bound (1.7–2.1 mol/mol of enzyme dimer).

Binding of Isopentenyl Pyrophosphate and 2-Fluorofarnesyl Pyrophosphate in the Presence of $MgCl_2$. The previous experiment indicates competitive binding between farnesyl pyrophosphate and isopentenyl pyrophosphate in the absence of metal, while earlier experiments (Reed and Rilling, 1976) had demonstrated that farnesyl pyrophosphate was competitive with the allylic substrates when Mg^{2+} was present. Thus, it seems possible that farnesyl pyrophosphate is interacting at

[2] When binding was measured in the presence of $MnCl_2$ and geranyl or farnesyl pyrophosphates, substrate concentrations often had to be lowered to the 2-μM range to avoid erroneously high membrane retention due to apparent aggregation.

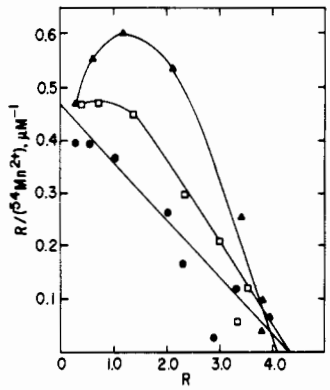

FIGURE 3: Binding of $^{54}Mn^{2+}$ by prenyltransferase in the presence of pyrophosphate substrates. R is the moles of $^{54}Mn^{2+}$ bound per mole of enzyme. Mixtures contained 50 μM geranyl pyrophosphate and 4.50 μM enzyme (●), 50 μM isopentenyl pyrophosphate and 4.97 μM enzyme (▲), or 20 μM 2-fluorogeranyl pyrophosphate, 20 μM 2-fluoroisopentenyl pyrophosphate, and 3.55 μM enzyme (□).

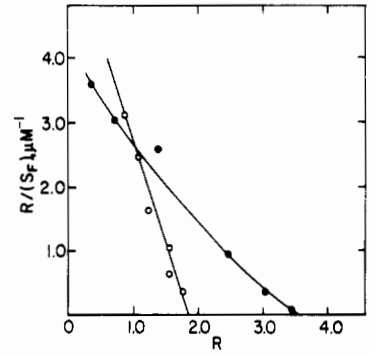

FIGURE 4: Substrate binding by prenyltransferase in the presence of 1 mM $MnCl_2$. R is the moles of substrate bound per mole of enzyme, and S_F is the free substrate concentration of geranyl (○) or isopentenyl pyrophosphate (●). The enzyme concentrations were 4.05 μM (○) and 5.40 μM (●).

one binding site in the absence of metal and at the other in the presence of metal. To resolve this we measured the binding of [^{14}C]isopentenyl pyrophosphate to the enzyme in the presence of 1 mM Mg^{2+} and a relatively nonreactive analogue of farnesyl pyrophosphate, 2-fluorofarnesyl pyrophosphate. The results obtained (Figure 2B) clearly demonstrate competition between these two ligands in the presence of Mg^{2+}.[3]

Binding of $^{54}Mn^{2+}$ by Prenyltransferase. The binding of $^{54}Mn^{2+}$ to prenyltransferase (3.0–4.3 μM) without added substrate was measured both by forced dialysis and equilibrium dialysis.[4] Binding was quite low and variable at $MnCl_2$ concentrations from 2 to 125 μM, with an average of 0.38 ± 0.35 mol of Mn^{2+} bound per mol of enzyme dimer. A Scatchard plot of these data (not shown) indicated that the binding was weak and probably not specific. However, when geranyl pyrophosphate was included as a ligand, Mn^{2+} was tightly bound to the enzyme. The binding of Mn^{2+} to prenyltransferase in the presence of 50 μM geranyl pyrophosphate is shown in Figure 3. The extrapolated saturation point is 4.3 mol of Mn^{2+} per mol of enzyme with an apparent dissociation constant of 9.3 μM. Similar results are obtained if isopentenyl pyrophosphate (50 μM) rather than geranyl pyrophosphate is included with the enzyme. A Scatchard plot of the data (Figure 3) extrapolates to a saturation value of 4.0 mol of Mn^{2+} bound per mol of enzyme dimer but is markedly convex at low Mn^{2+} concentration. The linear portion of this curve can be extrapolated to an apparent dissociation constant of approximately 3 μM. The nonlinear nature of this curve indicates that at low $MnCl_2$ concentrations the dissociation constant is greater than 3 μM and decreases as $MnCl_2$ is raised.

Mn^{2+} binding to prenyltransferase also was obtained in the presence of farnesyl pyrophosphate. The curve obtained (not shown) was linear and extrapolated to four Mn^{2+} bound per mol of enzyme with a dissociation constant of 9.2 μM.

Prenyltransferase catalyzes the reaction of a normal allylic cosubstrate with the substrate analogue, 2-fluoroisopentenyl pyrophosphate, at a rate 4% of that obtained with the normal homoallylic cosubstrate. The analogous allylic compound, 2-fluorogeranyl pyrophosphate, is also a substrate for the enzyme but condenses with isopentenyl pyrophosphate at a rate 1200 times less than with geranyl pyrophosphate. By using these substrate analogues simultaneously, catalysis is sufficiently suppressed to allow measurement of the binding of $^{54}Mn^{2+}$ to the enzyme in the presence of both substrates. A Scatchard plot of these binding data is presented in Figure 3. The extrapolated saturation value is again 4.3 mol per dimer, which demonstrates that there are a total of four metal binding sites per enzyme dimer and all sites are available for binding whenever either or both of the allylic and homoallylic binding sites are occupied.

Substrate Binding of Prenyltransferase in the Presence of $MnCl_2$. Since metal binding studies were done with radioisotopic Mn^{2+} and the earlier binding studies with Mg^{2+}, it was necessary to repeat the substrate binding experiments in the presence of Mn^{2+} instead of Mg^{2+}.

The data indicate that [1-^{3}H]geranyl pyrophosphate (Figure 4) and [1-^{14}C]isopentenyl pyrophosphate (Figure 4) are bound by prenyltransferase in the presence of 1 mM $MnCl_2$ with saturation values of 1.9 and 3.6 mol bound per mol of enzyme dimer and with dissociation constants of 0.23 and 0.8 μM, respectively. These saturation values are in good agreement with those determined in the presence of 1 mM $MgCl_2$ (Reed and Rilling, 1976).

The enzyme substrate binding data are summarized in Table I.

Dissociation Constants of Geranyl and Isopentenyl Pyrophosphate with Divalent Metal Ion. The association between divalent metal ion and the substrates was examined in order to determine the relative concentration of free substrate and metal–substrate complex present during binding experiments with enzyme. The dissociation constants for the 1:1 complexes between geranyl pyrophosphate and Mg^{2+} or Mn^{2+} are 140 and 30 μM, respectively, while those between isopentenyl pyrophosphate and Mg^{2+} or Mn^{2+} are 520 and 120 μM, respectively. The dissociation constants indicate that geranyl pyrophosphate binds approximately 4 times more strongly to divalent metal than does isopentenyl pyrophosphate and that Mn^{2+} binds 4 to 5 times more tightly to both substrates than does Mg^{2+}. These data are consistent with metal binding to ADP in which the binding of ADP with Mn^{2+} is tighter than with Mg^{2+} (Smith and Alberty, 1956).

Discussion

Binding of Substrate in the Absence of Divalent Cations. Previous studies of substrate binding to prenyltransferase were

[3] Neither the dissociation constant nor the inhibition constant for this analogue has been determined. However, the 2-fluoro analogues of both geranyl and isopentenyl pyrophosphates interact strongly with this enzyme (Poulter et al., 1977; H. C. Rilling and C. D. Poulter, unpublished data).

[4] Either Mn^{2+} or Mg^{2+} will support the catalytic activity of prenyltransferase. Under standard assay conditions, the enzyme activity measured in the presence of Mn^{2+} is about half that obtained when Mg^{2+} is the cation.

TABLE I: Ligand Binding, Dissociation Constants, and Saturation Values.[a]

Fixed ligand	Fixed ligand concn (mM)	Measured ligand	S.V.[b]	K_{diss}[c] (μM)	Enzyme concn (μM)
Mg[d]	1.0	DMAPP	2	2.4	6.5
Mg[d]	1.0	GPP	2	0.28	6.0
Mg[d]	1.0	FPP	2	0.73	5.8
Mg[d]	1.0	IPP	3.8	2.5	5.8
Mn	1.0	GPP	1.9	0.23	4.05
Mn	1.0	IPP	3.6	0.8	5.4
None	0	GPP	2.2	1.8	4.9
None	0	IPP	2.0	4.3	4.7
GPP	0.02	IPP	1.9	1.5	3.2
GPP	0.05	Mn	4.3	9.3	4.5
FPP	0.002[e]	Mn	4.2	9.2	8.8
IPP	0.05	Mn	4.0	3[f]	5.0
None	0	Mn	N.S.		4.3
FIPP + FGPP	0.02 + 0.02	Mn	4.3	6.3	3.5

[a] See Results section for conditions. [b] Abbreviations used are: S.V., saturation value, moles of ligand bound per mole of enzyme dimer; K_{diss}, dissociation constant; DMAPP, dimethylallyl pyrophosphate; GPP, geranyl pyrophosphate; FPP, farnesyl pyrophosphate; IPP, isopentenyl pyrophosphate; FGPP, 2-fluorogeranyl pyrophosphate; FIPP, 2-fluoroisopentenyl pyrophosphate; N.S., not significant. [c] The apparent dissociation constants reported are larger than the true dissociation constants, since all concentrations of fixed ligands must be saturating to obtain true constants. This was not possible in many cases due to limited solubilities and availability of the substrates. [d] Data of Reed and Rilling, 1976. [e] The unusually low concentration of FPP and high concentration of enzyme were employed in order to avoid aggregation of Mn^{2+} and FPP while obtaining easily measurable binding data. [f] Extrapolated value from the linear portion of the Scatchard plot in Figure 3.

in the presence of Mg^{2+} (Reed and Rilling, 1976), since divalent cation, Mg^{2+} or Mn^{2+}, is required for catalysis by this enzyme. However, it is clear from the experiments reported in this paper that these divalent cations are not required for substrate binding. In these experiments we have shown that prenyltransferase binds one molecule of geranyl pyrophosphate per enzyme monomer (Table I) with a dissociation constant of 1.8 μM. Similarly, in the absence of metal, the enzyme binds two molecules of the homoallylic substrate per enzyme dimer with a dissociation constant of 4.3 μM.

When the divalent cations are omitted, the rate of the enzyme-catalyzed reaction is depressed sufficiently so that the binding of these substrates can be measured when both are present. Under these circumstances, isopentenyl and geranyl pyrophosphate are simultaneously bound without competition (Table I, Figure 1C). These results also demonstrate that, since both substrates can be bound to the enzyme in the absence of Mn^{2+} or Mg^{2+}, these ions are essential for catalysis rather than for binding of substrates.

Reed and Rilling (1976) demonstrated that the three allylic substrates, dimethylallyl, geranyl, and farnesyl pyrophosphate, compete for binding with prenyltransferase. In the present experiments we have shown that farnesyl pyrophosphate, the principal product of prenyltransferase catalysis, also binds competitively with isopentenyl pyrophosphate in the absence of divalent cations. The form of the competition between these two ligands is unusual. The presence of farnesyl pyrophosphate drastically reduces the affinity of the enzyme for isopentenyl pyrophosphate. However, at high concentrations of the homoallylic substrate, 2 mol is bound per mol of enzyme *without* displacement of farnesyl pyrophosphate (Figure 2A).

The Effect of Divalent Cations on Substrate Binding. The presence of 1 mM $MnCl_2$ or $MgCl_2$ leads to a six- to eightfold enhancement of the binding of geranyl pyrophosphate to the enzyme but does not alter the number of sites (two) available to this substrate. In contrast, the binding pattern of the other substrate, isopentenyl pyrophosphate, is markedly influenced by divalent cations. In the absence of this ligand, each enzyme monomer binds one isopentenyl pyrophosphate molecule (Figure 1B). Divalent cations not only enhance the affinity of the substrate for the enzyme (twofold for Mg^{2+} and fivefold for Mn^{2+}) but also double the number of sites available for binding isopentenyl pyrophosphate. This observation confirms the previous conclusion that isopentenyl pyrophosphate can bind at the allylic site as well as the homoallylic site (Reed and Rilling, 1976) and shows that metal is required for binding of this ligand at the allylic site.

Also, the data show that the relative affinity of the enzyme for its substrate, in the presence of 1 mM metal, is proportional to the dissociation constant for the substrate–cation complexes. For example, the dissociation constants for Mg–isopentenyl pyrophosphate and for isopentenyl pyrophosphate with enzyme in the presence of Mg^{2+} are substantially greater than constants observed for Mn–geranyl pyrophosphate and for geranyl pyrophosphate with enzyme in the presence of Mn^{2+}.

Competitive binding between farnesyl pyrophosphate and isopentenyl pyrophosphate in the presence of Mg^{2+} was not attempted since these two compounds are condensed by the enzyme at an appreciable rate. However, by substituting the relatively unreactive 2-fluoro analogue for farnesyl pyrophosphate, we were able to demonstrate competition between these two ligands in the presence of Mg^{2+} (Figure 2B).

Metal Binding. In the absence of substrate, a small and variable amount of Mn^{2+} is bound to enzyme, and we attribute this to nonspecific interaction rather than binding at a specific site. However, Mn^{2+} is bound tightly in the presence of the substrates. Two moles of Mn^{2+} is bound per monomer in a mixture of enzyme and geranyl pyrophosphate. The Scatchard plot of the data (Figure 3) is linear, with no indication of sites with different affinities for Mn^{2+}. The data obtained for Mn^{2+} binding to enzyme in the presence of isopentenyl pyrophosphate (Figure 3) give a similar saturation value of two Mn^{2+} per catalytic site. In this case, the Scatchard plot is not linear and shows positive cooperativity in the binding.

Mn^{2+} binding to the enzyme was also measured in the presence of nonreactive fluoro analogues of both substrates, and again 2 mol of Mn^{2+} is per catalytic site. A Scatchard plot of the data qualitatively looks like an average of the data obtained for Mn^{2+} binding in the presence of the individual natural substrates (Figure 3). Thus, two molecules of Mn^{2+} can bind to each catalytic site of the enzyme as long as the product or either substrate (or both) are present. Apparent cooperative binding is observed whenever the homoallylic substrate is present.

Spatial Relationship between the Substrates. These binding data can be used to define more closely the spatial relationship between the substrates and between substrates and products in the catalytic site of prenyltransferase.

The present experiments demonstrate that farensyl pyrophosphate competes with isopentenyl pyrophosphate for binding to the enzyme in the absence of metal. Also, the 2-fluoro analogue of the product is competitive with the homoallylic substrate when divalent metal ions are present. This, taken in conjunction with the previous observation that farnesyl pyrophosphate was competitive with both allylic substrates, indicates that the product can simultaneously occupy both substrate binding sites. We envision that the pyro-

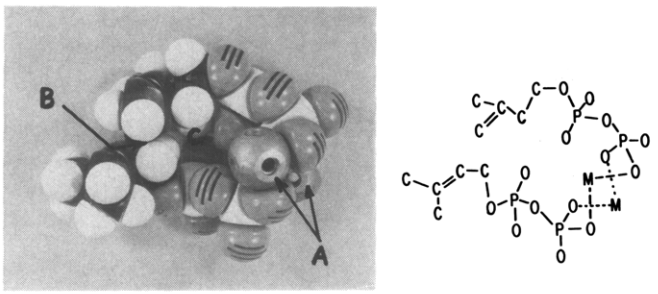

FIGURE 5: Orientation of substrates and divalent cations during binding to prenyltransferase. The positions of the divalent cations (A, left panel) are indicated by the letter M on the right. During catalysis, a new carbon–carbon bond is formed at B and a hydrogen atom (C) is lost. Hydrogen atoms have been eliminated from the right panel for simplicity.

phosphate moiety of farnesyl pyrophosphate is bound to the pyrophosphate region of the isopentenyl pyrophosphate site while the hydrocarbon portion fills the hydrocarbon binding segment of both the isopentenyl and geranyl pyrophosphate binding sites. At relatively high concentrations isopentenyl pyrophosphate, farnesyl pyrophosphate is displaced from the isopentenyl pyrophosphate site and now occupies only the allylic binding site. This interpretation is consistent with kinetic experiments which show that farnesyl pyrophosphate is an inhibitor against both isopentenyl and geranyl pyrophosphate (Holloway and Popjak, 1967).

Our observation on metal binding along with previous stereochemical studies on prenyltransferase (summarized in Popjak, 1971) can be used to depict the orientation of the two substrates with respect to each other during condensation. The stereochemical studies had shown that carbon-to-carbon bond formation is between the *si–si* face of the double bond in isopentenyl pyrophosphate and the back side of the carbinol carbon of the allylic substrate. This requires the orientation of the plane of the double bond of isopentenyl pyrophosphate to the perpendicular to a line passing through the C–O bond of the allylic substrate. On the basis of these considerations, Popjak (Popjak, 1971; Holloway and Popjak, 1967) proposed a model for the orientation of the substrates which is very similar to the one proposed below. However, since only one point of interaction between the substrates was fixed, a 360° rotation of these molecules with respect to each other was possible in their model.

The present data clearly show that two metal ions are bound at each catalytic site whenever product or either substrate (or both) is present. These divalent cations also enhance the affinity of the enzyme for its substrates. To account for these observations, any model must show both metal ions interacting with both substrates. The most reasonable way to accomplish this interaction would be for the metal ions to act as bridges between the pyrophosphate moieties of the two substrates. The result of this interaction is to fix the orientation of the substrates with respect to each other. Several models can be constructed that have the metal ions bound to the pyrophosphate groups and have the organic portion of the substrates properly aligned. One of these models has both metal ions bridging oxygens attached to P_2 phosphorus (Figure 5). This model shows the 2-*pro-R* hydrogen of isopentenyl pyrophosphate surrounded by oxygens of the allylic pyrophosphate. This is in accord with the previous postulate that this pyrophosphate, while leaving, would provide the base necessary to remove this proton (Poulter and Rilling, 1976; Popjak, 1971).

References

Burton, K. (1959), *Biochem. J. 71,* 388–1083.

Cantley, L. C., and Hammes, G. G. (1973), *Biochemistry 12,* 4900–4904.

Dorsey, J. K., Dorsey, J. A., and Porter, J. W. (1966), *J. Biol. Chem. 241,* 5353–5360.

Eberhardt, N. L., and Rilling, H. C. (1975), *J. Biol. Chem. 250,* 863–866.

Holloway, P. W., and Popjak, G. (1967), *Biochem. J. 104,* 57–70.

Paulus, H. (1969), *Anal. Biochem. 32,* 91–100.

Popjak, G. (1971), *Harvey Lect. 65,* 127–156.

Poulter, C. D., Argyle, J. C., and Mash, E. A. (1977), *J. Am. Chem. Soc. 99,* 957–959.

Poulter, C. D., and Rilling, H. C. (1976), *Biochemistry 15,* 1079–1083.

Reed, B. C., and Rilling, H. C. (1975), *Biochemistry 14,* 50–54.

Reed, B. C., and Rilling, H. C. (1976), *Biochemistry 15,* 3739–3745.

Scatchard, G. (1949), *Ann. N.Y. Acad. Sci. 51,* 660–672.

Smith, R. M., and Alberty, R. A. (1956), *J. Am. Chem. Soc. 78,* 2376–2380.