The Enzymology of Short-Chain Fatty Acyl-Coenzyme A Synthetase from Seeds of *Pinus radiata*

KINETIC STUDIES AND A PROPOSED REACTION MECHANISM

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1. Short-chain fatty acyl-CoA synthetase from seeds of *Pinus radiata* was examined by acetate- and propionate-dependent PP_i -ATP exchange. Reaction mixtures came to equilibrium almost instantly as judged by rates of exchange and analysis of an incubation mixture. 2. The activity of the enzyme was correlated with the concentration of $MgP_2O_7^{2-}$ but not with the concentration of Mg^{2+} , as judged by PP_i -ATP exchange and fatty acyl AMP-dependent synthesis of ATP in the presence of PP_i . In PP_i -ATP exchange assays, no clear relationship between activity and any single species of ATP was apparent. 3. High concentrations of fatty acid inhibited PP_i -ATP exchange. PP_i -dATP exchange was less than PP_i -ATP exchange at low concentrations of fatty acid, but at higher concentrations PP_i -dATP exchange exceeded PP_i -ATP exchange. The rate of synthesis of fatty acyl-CoA in the presence of dATP was less than with ATP. 4. ATP and propionate inhibited the synthesis of ATP from propionyl-AMP and PP_i . The inhibition by ATP was competitive with respect to propionyl-AMP and non-competitive with respect to PP_i . The inhibition by propionate was non-competitive with respect to propionyl-AMP and PP_i . 5. AMP was a competitive inhibitor of propionyl-AMP-dependent synthesis of ATP and competitively inhibited propionate-dependent PP_i -ATP exchange when ATP was the variable substrate. 6. It was concluded that the first partial reaction catalysed by the enzyme is ordered; ATP is the first substrate to react with the enzyme and PP_i is probably the only product released.

Seeds of *Pinus radiata* contain a short-chain fatty acyl-CoA synthetase. The enzyme has been partially purified and some of its properties studied (Young & Anderson, 1974). Using yeast acetyl-CoA synthetase, Berg (1956) postulated that the formation of acetyl-CoA occurred in two steps:

Subsequently Webster & Campagnari (1962) and Webster (1963), using the acetyl-CoA synthetase from ox heart mitochondria, demonstrated that acetyl-AMP was an enzyme-bound intermediate. Young & Anderson (1974) concluded that the reaction scheme for the short-chain fatty acyl-CoA synthetase of pine seeds was analogous to that for yeast acetyl-CoA synthetase.

Rate equations for isotope exchange at chemical equilibrium have been described for some enzyme-catalysed reactions, including PP_i -ATP exchange reactions (Boyer, 1959; Alberty *et al.*, 1962; Cole & Schimmel, 1970a). Experiments described below indicate that the fatty acid-dependent PP_i -ATP exchange assay of the pine seed enzyme occurs at

chemical equilibrium. At chemical equilibrium, inhibition of isotopic exchange of one reactant by a second is characteristic of, although not proof of, a mechanism containing a compulsory (ordered) pathway (Boyer, 1959). The inhibition of fatty acid-dependent PP_i -ATP exchange by high concentrations of fatty acid (Young & Anderson, 1974) suggests that the mechanism of reaction (i) is ordered and that ATP is the first substrate to bind to the enzyme for reaction in the forward direction. Further, since purified pine seed enzyme did not catalyse PP_i -ATP exchange in the absence of fatty acid, it is unlikely that the mechanism of reaction (i) is non-sequential with any of the fatty acid substrates.

The present paper reports a study of the kinetics of the reactions catalysed by the pine seed short-chain fatty acyl-CoA synthetase, by using an ordered mechanism involving ATP as the first substrate to bind to the enzyme as a working hypothesis.

Experimental

Short-chain fatty acyl-CoA synthetase was purified from pine seeds and assayed by the procedures described by Young & Anderson (1974), except that in the study of the role of Mg^{2+} in PP_i -ATP exchange (Fig. 1), sodium propionate and Na_4ATP were used

in lieu of potassium propionate and K_2Na_2 ATP, and incubations were conducted at 30°C instead of 35°C.

Chemicals were obtained from the sources described by Young & Anderson (1974).

Results and Discussion

Attainment of chemical equilibrium in PP_i -ATP-exchange assays

Two lines of evidence indicate that PP_i -ATP-exchange incubation mixtures reach chemical equilibrium rapidly. The rate of fatty acid-dependent PP_i -ATP exchange was examined over a range of concentrations of all substrates with sufficient enzyme to effect up to 30% attainment of isotopic equilibrium in 1 h. The rate of exchange for each mixture was constant for at least 1 h; constant rate of exchange (after correction for fractional attainment of isotopic equilibrium) is a necessary condition of chemical equilibrium (Duffield & Calvin, 1946).

The concentration of ATP in an incubation mixture containing 1 mM-propionate, 1 mM-ATP, 2 mM- $MgCl_2$ and unlabelled 3 mM- PP_i was monitored during a 1 h incubation. Samples were removed at various times from this primary incubation mixture and transferred into secondary incubation mixtures which contained 1 mM-propionate, 2 mM- $MgCl_2$ and 3 mM- $[^{32}P]PP_i$, but no ATP. Secondary incubation mixtures were incubated for 15 min. The assay conditions and the kinetics of the effect of concentration of ATP on PP_i -ATP exchange are such that radioactivity incorporated into ATP in secondary incubations was a sensitive guide to the concentration of ATP in the primary incubation. Radioactivity incorporated into ATP was independent of the time at which the samples were removed from the primary incubation, which indicated that the concentration of ATP was constant for at least 1 h; in a parallel primary incubation mixture containing $[^{32}P]PP_i$, 23% of the PP_i was exchanged in 1 h. In an analogous experiment the concentration of propionate was monitored during a 1 h incubation. The results indicated that the concentration of propionate was constant for at least 1 h.

Role of Mg^{2+} in reactions of short-chain fatty acyl-CoA synthetase

When propionate-dependent PP_i -ATP exchange was examined in an experiment using 0.25–5 mM-ATP with 1 and 4 mM- PP_i , maximum PP_i exchange occurred between 0.25 and 3 mM- $MgCl_2$ (Fig. 1). Qualitatively similar results were observed when 10 mM-sodium acetate was used in lieu of 1 mM-sodium propionate. The Mg^{2+} requirement of the short-chain fatty acyl-CoA synthetase was low when compared with the Mg^{2+} requirements of the PP_i -ATP exchange reactions catalysed by the amino acyl-

tRNA synthetases (Peterson, 1967) and ATP sulphurylase (Shaw & Anderson, 1972) with similar concentrations of ATP and PP_i .

In view of the unusual Mg^{2+} requirement of the pine seed enzyme in PP_i -ATP exchange reactions, the concentrations of several forms of magnesium were examined within the range of reaction conditions described in Fig. 1. Concentrations were determined by the method of Perrin & Sayce (1967). The chemical species considered and the respective cumulative dissociation constants used are presented in Table 1.

The validity of comparing calculated concentrations of chemical species with experimental results is dependent on the accuracy of the stability constants used for the particular reaction conditions. We chose most constants from Alberty (1969). In general these constants were measured at 25°C at I 0.2; reaction conditions for Fig. 1 were 30°C at values of I between 0.07 and a possible maximum of 0.17. A number of different dissociation-constant values have been

Fig. 1. Effects of $MgCl_2$ concentration on propionate-dependent PP_i -ATP exchange at several concentrations of ATP and PP_i .

The incubation mixtures (assay method 1) contained $MgCl_2$ and ATP as specified, 104 mM-Tris-HCl, pH 8, 20 μ M-EDTA, 1 mM-dithiothreitol, 1 mM-propionate, and either 1 mM- PP_i (a) or 4 mM- PP_i (b). ●, 0.25 mM-ATP; △, 2 mM-ATP; ▲, 5 mM-ATP.

Table 1. Cumulative dissociation constants used to compute the concentrations of species of ATP, PP_i and free Mg²⁺ in incubation mixtures at pH8

Species	Cumulative pK	Reference
HATP ³⁻	6.95	Alberty (1969)
MgATP ²⁻	4.00	Alberty (1969)
MgHATP ⁻	8.43	Alberty (1969)
NaATP ³⁻	1.06	Cole & Schimmel (1970b)
HP ₂ O ₇ ³⁻	8.95	Alberty (1969)
H ₂ P ₂ O ₇ ²⁻	15.07	Alberty (1969)
MgP ₂ O ₇ ²⁻	5.41	Alberty (1969)
MgHP ₂ O ₇ ⁻	12.02	Alberty (1969)
Mg ₂ P ₂ O ₇ ⁻	7.75	Alberty (1969)
NaP ₂ O ₇ ³⁻	1.00	Cole & Schimmel (1970b)
CH ₃ -CH ₂ -CO ₂ H	4.87	Cooper (1969)

reported for the chemical species listed in Table 1, particularly for MgATP²⁻ (O'Sullivan & Perrin, 1964; Phillips *et al.*, 1966). Alteration of several constants, and inclusion of a constant for MgEDTA²⁻ and a range of possible constants for a magnesium-dithiothreitol complex (Cornell & Crivaro, 1972), did not change the overall patterns of concentration of major chemical species.

Propionate-dependent PP_i-ATP exchange (Fig. 1) was not related to the concentration of free Mg²⁺, but the possibility that this species is necessary for enzyme activity cannot be excluded. When the concentration of PP_{i(T)} or ATP_(T) [the subscript (T) refers to total concentrations] was increased (Fig. 2), the concentration of Mg²⁺ necessary to give the optimum concentration of MgP₂O₇²⁻ also increased and was correlated with optimum propionate-dependent PP_i-ATP exchange (Fig. 1). Conditions affecting the concentration of MgP₂O₇²⁻ similarly affected PP_i-ATP exchange activity. The concentration of MgHP₂O₇⁻ varied with Mg_(T)²⁺ in a similar manner to that of MgP₂O₇²⁻, but the concentrations of MgHP₂O₇⁻ were small by comparison. Apart from MgP₂O₇²⁻, no clear relationship between species of PP_i and exchange activity was evident from this analysis.

The optimum concentration of MgCl₂ for the propionyl-AMP-dependent synthesis of ATP was 1 mM when 6 mM-PP_i was used. Higher concentrations of MgCl₂ (2–10 mM) were inhibitory. The effect of increasing PP_i concentration on propionyl-AMP-dependent ATP formation in the presence of 1 mM-MgCl₂ is shown in Figs. 3 and 4. The concentrations of individual species of PP_i and free Mg²⁺ were calculated for the conditions 0–10 mM-Mg_(T)²⁺ with 0–12 mM-PP_{i(T)}, by using the method of Perrin & Sayce (1967) and the constants listed in Table 1. This study demonstrated that MgP₂O₇²⁻, a postulated

Fig. 2. Effects of the total concentration of magnesium on the calculated concentrations of MgP₂O₇²⁻ at several total concentrations of ATP and PP_i

Concentrations of MgP₂O₇²⁻ were calculated as described in the text, by using the constants listed in Table 1. The total concentrations of ATP used to derive each curve are specified. The total concentration of PP_i was 1 mM in (a) and 4 mM in (b).

biochemically reactive form of magnesium and PP_i for the PP_i-ATP exchange assay, was a major chemical species present in incubation mixtures for assay method 2; HP₂O₇³⁻, however, was the dominant species at PP_{i(T)} concentrations greater than 2 mM in the presence of 1 mM-Mg²⁺.

The study of concentration of chemical species of PP_i, ATP and Mg²⁺ in relation to PP_i-ATP exchange activity provided limited information on the reactive form of the ATP substrate. Between 4 and 10 mM-Mg_(T)²⁺ with 0.25 mM-ATP_(T) and 1 mM-PP_{i(T)}, ATP occurred almost exclusively as MgATP²⁻; propionate-dependent PP_i-ATP exchange occurred under these conditions (Fig. 1a), suggesting that MgATP²⁻ could serve as a substrate. In other enzyme-catalysed reactions involving ATP and

Fig. 3. Double-reciprocal plots of the effect of PP_i concentration on propionyl-AMP-dependent ATP synthesis with and without ATP

Activity was measured by assay method 2 except that the amount of PP_i was replaced with the amounts specified, and ATP was included as specified. The concentration of propionyl-AMP was $22 \mu\text{M}$. ●, No ATP; △, 0.1 mM-ATP; ○, 0.5 mM-ATP.

Fig. 4. Double-reciprocal plots of the effect of PP_i concentration on propionyl-AMP-dependent ATP synthesis with and without propionate

Activity was measured by assay method 2 except that the amount of PP_i was replaced with the amounts specified, and propionate was included as specified. The concentration of propionyl-AMP was $21 \mu\text{M}$. ●, No propionate; △, 1 mM-propionate; ○, 2 mM-propionate.

Mg^{2+} , MgATP^{2-} was concluded to be the active form of ATP (Cole & Schimmel, 1970b; Wohlhueter, 1971). However, between 0 and 1 mM- Mg^{2+} , with 0.25 mM- $\text{ATP}_{(\text{T})}$ and 4 mM- $\text{PP}_{\text{i(T)}}$, the concentration of MgATP^{2-} was very low, suggesting that if MgATP^{2-} is the reactive form of ATP then the affinity of the enzyme for MgATP^{2-} must be very high (Fig. 1b). If MgATP^{2-} , however, is not the reactive species, then another ATP species must similarly be effective at low concentrations, since no single species of ATP occurs in appreciable concentrations over the whole range of MgCl_2 concentrations at which the enzyme catalyses propionate-dependent PP_i -ATP exchange. The possibility that several species of ATP are reactive cannot be excluded.

Effect of concentration of substrates and alternative substrates on some reactions catalysed by short-chain fatty acyl-CoA synthetase

Propionate-dependent PP_i -ATP exchange activity increased with concentrations of PP_i up to 4 mM irrespective of the concentration of ATP (0.25–5 mM) provided that the concentration of MgCl_2 was constant (Fig. 1). Concentrations of PP_i greater than 4 mM did not inhibit exchange, implying that the reactive species of PP_i did not inhibit.

At certain concentrations of MgCl_2 and PP_i (Figs. 1 and 5), ATP inhibits exchange. If the inhibition is caused, even in part, by the reactive species of ATP, then the hypothesis that ATP is the first substrate to react with the enzyme in a simple ordered mechanism is untenable. Similarly, other simple mechanisms proposed for analogous PP_i -ATP exchange reactions (Cole & Schimmel, 1970a) would also be untenable for the pine seed enzyme.

Other evidence is consistent with the proposed mechanism. Enzyme stability was markedly increased by storage in ATP, whereas propionate was ineffective (Young & Anderson, 1974); this suggests that ATP can combine with the enzyme in the absence of propionate.

dATP is an alternative substrate of ATP in the PP_i -exchange assay (Young & Anderson, 1974). The effect of fatty acid concentration on PP_i exchange with ATP and dATP as nucleotides was compared (Fig. 6). Fatty acid-dependent PP_i -dATP exchange was less than PP_i -ATP exchange at low fatty acid concentrations (Fig. 6); this was confirmed in an independent study of PP_i exchange at concentrations of propionate less than 0.5 mM. At higher fatty acid concentrations PP_i -dATP exchange exceeded PP_i -ATP exchange. The inhibition of PP_i exchange at high concentrations of fatty acid was less with dATP than with ATP.

According to the proposed reaction mechanism, high concentrations of fatty acid should inhibit PP_i exchange by combining with an enzyme-ATP

Fig. 5. Effect of ATP concentration on propionate-dependent PP_i -ATP exchange with and without AMP

The incubation mixtures (assay method 1) contained ATP and AMP as specified, 84mM-Tris-HCl, pH 8, 20 μ M-EDTA, 1mM-dithiothreitol, 1mM-propionate, 2mM-MgCl₂ and 3mM- PP_i . ●, No AMP; ○, 0.5mM-AMP; ▲, 1mM-AMP; △, 5mM-AMP.

Fig. 6. Effects of concentration of acetate and propionate on fatty acid-dependent PP_i -ATP and PP_i -dATP exchange

The incubation mixtures (assay method 1) contained fatty acid as specified, 84mM-Tris-HCl, pH 8, 20 μ M-EDTA, 1mM-dithiothreitol, 2mM-MgCl₂, 1mM-K₂Na₂ATP or 1mM-K₂Na₂dATP, and 3mM- PP_i . (a) ●, Acetate-dependent PP_i -ATP exchange; ○, acetate-dependent PP_i -dATP exchange; (b) ▲, propionate-dependent PP_i -ATP exchange; △, propionate-dependent PP_i -dATP exchange.

complex. If ATP is the preferred nucleoside triphosphate for the enzyme, then the affinity of the enzyme-ATP complex for fatty acid could be greater than the affinity for the enzyme-dATP complex. A further consequence of the model would be that acetate and propionate would be less effective inhibitors of PP_i exchange with dATP than with ATP. The data in Fig. 6 are consistent with this hypothesis. The model was tested further. If ATP is the preferred nucleoside triphosphate then the synthesis of acetyl-CoA and propionyl-CoA should be stimulated more by ATP than by dATP. At concentrations up to 32mM-acetate and 24mM-propionate, fatty acyl-CoA formation (method 3) was greater with 2mM-ATP than with 2mM-dATP (Young & Anderson, 1974). Similar results were obtained with 48mM-acetate and 40mM-propionate. This test, however, is not unequivocal. One objection is that the relative composition of reactive chemical species might alter when dATP is substituted for ATP, and so alter the kinetic patterns, but this is unlikely, since the metal complexes of ATP and dATP have similar stability constants (O'Sullivan, 1969).

Inhibition of ATP formation from propionyl-AMP and PP_i by propionate, ATP, dATP and AMP

Young & Anderson (1974) reported that the pine seed enzyme catalysed the formation of [³²P]ATP from propionyl-AMP and [³²P] PP_i in the presence of MgCl₂. ATP and propionate inhibited this reaction. The kinetics of inhibition by ATP and propionate were studied at non-saturating concentrations of the unvaried substrate. The inhibition of activity by ATP was competitive with respect to propionyl-AMP

Fig. 7. Double-reciprocal plots of the effect of propionyl-AMP concentration on propionyl-AMP-dependent ATP synthesis with and without ATP

Activity was measured by assay method 2 except that the concentration of PP_i was 2 mM and ATP was included as specified. ●, No ATP; △, 0.1 mM-ATP; ○, 0.5 mM-ATP.

(Fig. 7) and non-competitive with respect to PP_i (Fig. 3). The inhibition of activity by propionate was non-competitive with respect to propionyl-AMP (Fig. 8) and PP_i (Fig. 4). The kinetics of inhibition are consistent with the kinetics of an ordered sequential mechanism (Cleland, 1963). The kinetics indicate that the order of addition of substrates and release of products is:

This mechanism is consistent with the working hypothesis proposed from concentration studies of acetate- and propionate-dependent PP_i -ATP exchange. dATP was a less effective inhibitor of $[\text{P}^{32}]$ -ATP formation from propionyl-AMP and $[\text{P}^{32}]\text{PP}_i$ than was equimolar ATP (Fig. 9); this observation is consistent with the hypothesis that ATP is the preferred nucleotide for the enzyme.

AMP was also an inhibitor of propionyl-AMP-dependent ATP synthesis. Inhibition by AMP complicates interpretation of Figs. 3, 4, 7, 8 and 9, particularly in those cases where propionyl-AMP was the variable substrate (Figs. 7, 8 and 9) since

Fig. 8. Double-reciprocal plots of the effect of propionyl-AMP concentration on propionyl-AMP-dependent ATP synthesis with and without propionate

Activity was measured by assay method 2 except that the concentration of PP_i was 2 mM and propionate was included as specified. ●, No propionate; △, 0.5 mM-propionate; ○, 1.0 mM-propionate.

Fig. 9. Double-reciprocal plots of the effects of ATP and dATP on propionyl-AMP-dependent ATP synthesis

Activity was measured by assay method 2 except that the concentration of PP_i was 2 mM and $\text{K}_2\text{Na}_2\text{ATP}$ or $\text{K}_2\text{Na}_2\text{dATP}$ was included as specified. ●, No addition; △, 0.2 mM-dATP; ○, 0.2 mM-ATP.

propionyl-AMP was contaminated with AMP (Young & Anderson, 1974). However, at each concentration of propionyl-AMP in Figs. 7, 8 and 9, endogenous AMP will, within limits, affect all curves to the same extent. Further, Young & Anderson (1974) estimated that the highest possible inhibition caused by contaminating AMP was 7%. At a non-saturating concentration of PP_i (2 mM), the inhibition by AMP was competitive with respect to propionyl-AMP; AMP must therefore compete with propionyl-AMP for free enzyme as does ATP. The possible competitive inhibition of propionate-dependent PP_i -ATP exchange by AMP when ATP was the variable substrate (Fig. 5) supports this hypothesis.

Concluding discussion

Considered collectively, the kinetics of PP_i exchange and end-product inhibition of the back reaction indicate that the reaction mechanism of reaction (i) is ordered. Expressing the order in the forward direction, some form(s) of ATP (possibly $MgATP^{2-}$) is the first substrate to react with the enzyme and some form of PP_i (most likely $MgPP_i^{2-}$) is the first product released. In the absence of CoA it is probable that fatty acyl-AMP is not released (Young & Anderson, 1974). After the release of PP_i , presumably the next step is addition of CoA in reaction (ii). The reaction mechanism of the pine seed enzyme is thus analogous to the reaction mechanism of potato acetyl-CoA synthetase (Huang & Stumpf, 1970) up to the point of CoA addition. Fatty acyl-CoA is the first product released in reaction (ii) by the potato enzyme (Huang & Stumpf, 1970), but we are unable to comment on the order of release of fatty acyl-CoA and AMP in reaction (ii) for the pine seed enzyme.

One consequence of the mechanism proposed for the pine seed enzyme is that it should be possible to demonstrate an enzyme-ATP complex in the manner of Rouget & Chapeville (1968b). The purified pine seed enzyme, however, was not available in sufficient quantity for these studies.

The reaction mechanisms of several activation reactions involving ATP as one substrate and PP_i as one product have been reported. The reaction mechanisms of the leucyl- and prolyl-tRNA synthetases from *Escherichia coli* (Rouget & Chapeville, 1968a,b; Papas & Mehler, 1971), the threonyl-tRNA synthetase from rat liver (Allende *et al.*, 1970), the tryptophyl-tRNA synthetase from ox pancreas (Zinoviev *et al.*, 1972) and the acetyl-CoA synthetase from potato tuber (Huang & Stumpf, 1970) are ordered; in each case ATP is the first substrate to react with the enzyme. Reaction (i) catalysed by pine seed short-chain fatty acyl-CoA synthetase is analogous to these reactions. A general rate equation

applicable to ordered PP_i -ATP exchange reactions in which ATP is the first substrate to react with an enzyme has been derived by Cole & Schimmel (1970a). The equation contains an apparent (overall) rate constant which has a concentration-dependent term such that high concentrations of the second substrate could lead to inhibition of PP_i -ATP exchange depending on the values of other terms also in the apparent rate constant. Considering the PP_i -ATP exchange reaction catalysed by *E. coli* leucyl-tRNA synthetase (Rouget & Chapeville, 1968a), high concentrations of leucine do not inhibit exchange as predicted by the general rate equation (Cole & Schimmel, 1970a). High concentrations of fatty acids, however, inhibit PP_i -ATP exchange catalysed by short-chain fatty acyl-CoA synthetase from pine seeds. It is probable that the apparent rate constant of the PP_i -ATP exchange reaction of leucyl-tRNA synthetase is very close to constant over a wide range of leucine concentrations, thereby precluding inhibition. Conversely, because of uncertainty as to the true nature of the apparent rate constant of the PP_i -ATP exchange reaction of isoleucyl-tRNA synthetase, Cole & Schimmel (1970a) could make no clear choice between a number of possible reaction mechanisms for this enzyme. Similarly, the reaction mechanisms of other activation reactions as studied by PP_i -ATP exchange may have been obscured by the nature of the overall rate constant.

To our knowledge, only three previous instances of inhibition of PP_i -ATP exchange by the second substrate have been reported. High concentrations of 3-hydroxytyrosine inhibited 3-hydroxytyrosine-dependent PP_i -ATP exchange catalysed by two bacterial tyrosyl-tRNA synthetases, but tyrosine and other alternative substrates did not inhibit exchange (Calendar & Berg, 1966); it is possible that the inhibition by a single alternative substrate may not be of mechanistic significance. Jencks & Lipmann (1957) demonstrated *n*-octanoate-dependent PP_i -ATP exchange with medium-chain fatty acyl-CoA synthetase from pig liver; concentrations of *n*-octanoate greater than $10\mu M$ inhibited exchange. The inhibition could, however, be due to a non-specific detergent effect of *n*-octanoate on the enzyme (Pande & Mead, 1968; Pande, 1972). Berg (1956) reported that acetate-dependent PP_i -ATP exchange catalysed by the acetyl-CoA synthetase of yeast was inhibited by acetate concentrations of 3 mM or higher; high concentrations of acetate did not inhibit the formation of acetyl-CoA. The results of studies with the pine seed enzyme are analogous to the results of Berg (1956), suggesting that the reaction mechanism of the yeast acetyl-CoA synthetase is the same as the reaction mechanism of the pine seed enzyme.

Aminoacyl-tRNA synthetases catalyse amino acid-dependent PP_i -dATP exchange in addition to PP_i -ATP exchange. These enzymes also catalyse the

synthesis of amino acyl-tRNA when dATP is used as the nucleotide in lieu of ATP (Mitra & Mehler, 1969). Similarly, dATP can replace ATP in the PP_i -exchange reaction catalysed by ATP sulphurylase (Shaw & Anderson, 1972) and in the reactions catalysed by the pine seed enzyme. The higher rate of PP_i exchange by the pine seed enzyme with dATP relative to ATP, observed at concentrations of fatty acid which inhibit PP_i -ATP exchange, is unusual; we suspect that this phenomenon is correlated with inhibition of exchange by the second substrate and therefore with the nature of the apparent rate constant governing exchange rates. The important point is that as fatty acid concentrations tend to zero, where inhibition of exchange is presumably negligible, the exchange with ATP exceeds that with dATP.

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