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Binding of Nucleotides to an Extramitochondrial Acetyl-CoA Hydrolase from Rat Liver

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ABSTRACT: Cold labile extramitochondrial acetyl-CoA hydrolase (dimeric form) purified from rat liver was activated by various nucleoside triphosphates and inhibited by various nucleoside diphosphates. Activation of acetyl-CoA hydrolase by ATP was inhibited by a low concentration of ADP ($K_i \cong 6.8$ μM) or a high concentration of AMP ($K_i \cong 2.3$ mM). ADP and AMP were competitive inhibitors of ATP. A Scatchard plot of the binding of ATP to acetyl-CoA hydrolase (dimer) at room temperature gave a value of 25 μM for the dissociation constant with at least 2 binding sites/mol of dimer. Cold-treated monomeric enzyme also associated with ATP–agarose, suggesting that the monomeric form of the enzyme also has a nucleotide binding site(s), probably at least 1 binding site/mol of monomer. Phenylglyoxal or 2,3-butanedione, both of which modify arginyl residues of protein, inactivated acetyl-CoA hydrolase. ATP (an activator) greatly protected acetyl-CoA hydrolase from inactivation by these reagents, while ADP (an inhibitor), valeryl-CoA (a substratelike, competitive inhibitor), and CoASH (a product) were less effective. However, addition of ADP plus valeryl-CoA (or CoASH) effectively prevented the inactivation by 2,3-butanedione, but that is not the case for phenylglyoxal. These results suggest that one or more arginyl residues are involved in the nucleotide binding site of extramitochondrial acetyl-CoA hydrolase and that their nucleotide binding sites locate near the substrate binding site.

An extramitochondrial acetyl-CoA hydrolase (EC 3.1.2.1) in rat liver hydrolyzes acetyl-CoA to acetate and CoASH. The enzyme may be important in maintenance of the cytosolic acetyl-CoA and CoASH pool (Prass et al., 1980; Matsunaga et al., 1985). It is activated by ATP and inhibited by ADP (nucleotide is not a substrate), and its effect is probably due to allosteric interaction (Prass et al., 1980). ATP and ADP regulate not only the catalytic properties of this enzyme but also the association–dissociation state of its subunits (Isohashi et al., 1983a). The purified enzyme at 25 °C without ATP is present as an active dimer ($M_r \sim 135000$) (Isohashi et al., 1983a). On addition of ATP or ADP, but not AMP, to high concentrations of dimer at 25 °C, the dimer reversibly aggregates to a tetramer ($M_r \sim 240000$) (Isohashi et al., 1983a). The dimer and tetramer are inactivated by exposure to lower temperature, with dissociation of the dimer and tetramer into the monomer (Isohashi et al., 1983a,b). The cold-inactivated enzyme (monomer) reassociates into an active dimer and tetramer when warmed to 37 °C. This reactivation of the enzyme on rewarming is enhanced by 2 mM ATP (Isohashi et al., 1984).

This enzyme is also inactivated in the presence of L-ascorbate and a trace concentration of Cu^{2+} due to oxidation caused by active oxygen, which is generated in the course of autoxidation of L-ascorbate (Nakanishi et al., 1985a). Valeryl-CoA, a substratelike competitive inhibitor, has a strong protective effect against this inactivation, but ATP and ADP have only slight protective effects, suggesting that oxidative modification of this enzyme mainly occurs at or near its substrate binding site (Nakanishi et al., 1985a).

This enzyme has a multisubunit structure and is complicatedly regulated by nucleotides, but little is known about the nucleotide binding site that may constitute the regulatory domain. Thus, the aim of the present experiments is to un-

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derstand the details of the nucleotide binding site(s) and its association with subunit structures. A preliminary report of part of this work has been presented (Nakanishi et al., 1985b).

Materials and Methods

Materials. ATP, GTP, UTP, CTP, TTP, ADP, GDP, UDP, CDP, TDP, AMP, 2,3-butanedione, CoASH, valeryl-CoA, bovine serum albumin (albumin), DL-dithiothreitol (DTT),[1] and phenylmethanesulfonyl fluoride were obtained from Sigma Chemical Co. Phenylglyoxal was obtained from Aldrich Chemical Co. ATP–agarose and ADP–agarose were obtained frm Pharmacia P-L Biochemicals. Acetyl-CoA was prepared from CoASH and acetic anhydride as reported by Simon and Shemin (1953). [2,8,5′-^{3}H]ATP (tetrasodium salt) (51.2 Ci/mmol) was obtained from New England Nuclear. Unless otherwise specified, all other chemicals (reagent grade) were purchased from Wako Pure Chemical Industries.

Purification of Acetyl-CoA Hydrolase. Male albino Donryu rats, weighing 300–400 g, were starved for 2 days and then sacrificed (Isohashi et al., 1983a). The purified, dimeric enzyme was prepared without ATP as described previously (Isohashi et al., 1983a). The final specific activity was comparable with that reported previously (Isohashi et al., 1983a). The concentrated, purified sample (0.1 mL) was suspended in 0.2 mL of 100 mM potassium phosphate buffer (pH 7.2 at 25 °C) containing 1 mM phenylmethanesulfonyl fluoride, 0.02% NaN_3, 1 mM EDTA, 2.3 M sucrose, and 2 mM DTT and stored at −80 °C. In this condition the purified enzyme was stable for at least 12 months. Before use for experiments, the samples were reactivated by incubation at 37 °C for 30 min.

Assay of Acetyl-CoA Hydrolase. Enzyme activity was assayed by determining the rate of formation of CoASH from acetyl-CoA with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) (Prass et al., 1980; Isohashi et al., 1983a). The reaction mixture routinely consisted of 0.1 M Tris-HCl buffer (pH 7.8), 0.5 mM EDTA, 0.5 mM acetyl-CoA, 2 mM ATP, and 0.1 mM DTNB in a total volume of 0.5 mL. Reactions werre started by adding the enzyme. The rate of CoASH formation was recorded at 412 nm and 25 °C. A molar extinction coefficient of 13 600 M^{-1} cm^{-1} was used to calculate the amount of CoASH released in the reaction.

Binding Studies. The capacity of the acetyl-CoA hydrolase to bind ATP was measured by a modification (Frey & Utter, 1977) of the flow dialysis technique of Colowick and Womack (1969). The protocol for this technique follows.

The flow dialysis cell (1-cm diameter) consisted of upper and lower chambers, separated by a membrane of Visking dialysis tubing of 2.5-cm flat width. Membranes were washed with 1 mM EDTA, pH 7.0, at 75 °C and then equilibrated with buffer I (0.1 M potassium phosphate buffer, pH 7.8, containing 0.5 mM EDTA and 2 mM DTT). Small magnetic stirring bars were placed in both chambers, and the entire cell was placed over a magnetic stirrer under N_2 gas because the enzyme rapidly lost activity under oxidative conditions (Nakanishi et al., 1985a). Buffer I was pumped through the lower chamber (1 mL in volume) at a constant rate of 1 mL/min. Highly purified acetyl-CoA hydrolase (90 μL, 0.7 mg) and [^{3}H]ATP (10 μL) were introduced into the upper chamber. The effluent from the lower chamber was collected automatically in 1-mL fractions. After ~4 mL of buffer had passed through, a steady-state level (initial steady state) was reached, which was proportional to the concentration of unbound [^{3}H]ATP in the upper chamber (Colowick & Womack, 1969). After a steady state was reached, an excess of unlabeled ATP was added to the upper chamber in order to release the bound [^{3}H]ATP. A new higher steady state (final steady state) was reached when another 4 mL of buffer had passed through the lower chamber. Aliquots (0.1 mL) from the lower chamber effluent fractions were diluted in toluene-based scintillation mixture containing NCS tissue solubilizer and counted in a liquid scintillation counter. The number of moles of ATP bound per mole of enzyme, r, was determined from the effluent steady-state values (dpm initial, dpm final), the molar concentration of enzyme based on a molecular weight of 135 000 (dimer) (Isohashi et al., 1983), and the known molar concentration of [^{3}H]ATP.

$$r = \left(\frac{\text{dpm final} - \text{dpm initial}}{\text{dpm final}}\right)\frac{[\text{ATP}]}{[\text{enzyme}]}$$

The final steady-state value was corrected, both for volume changes due to the addition of unlabeled ATP and for unreleased [^{3}H]ATP after the addition of excess unlabeled ATP. The results of a binding experiment in which seven concentrations of ATP were used were shown as a Scatchard plot (Scatchard, 1949).

Inactivation of Acetyl-CoA Hydrolase by Chemical Modifiers. Modification reactions were carried out under the conditions given in the figure and table legends. Briefly, the purified dimeric enzyme (4 mg/mL) was adjusted to an enzyme protein concentration of ~14 μg/mL with the indicated buffer containing 1 mg/mL albumin and the chemical modifying agent (phenylglyoxal or 2,3-butanedione). In some cases, various ligands of acetyl-CoA hydrolase were added to the dilution medium. The mixtures were incubated at 37 °C for the indicated times, and small aliquots were withdrawn for measurement of remaining activity.

Binding of Acetyl-CoA Hydrolase to ATP– or ADP–Agarose. ATP– or ADP–agarose columns (0.5 × 1 cm) were equilibrated with 50 mM potassium phosphate buffer (pH 7.8) containing 2 mM DTT. Purified dimeric enzyme or cold-treated monomeric enzyme (residual activity 5%, which was incubated at 0 °C for 30 min) was applied to each column at 25 or 4 °C, and the column was washed with equilibrium buffer and eluted with buffer containing 2 mM ATP or ADP. Fractions of 0.2 mL were collected. Other conditions are indicated in the figure legends.

Protein Determination. Proteins was determined by a modification (Hartree, 1972) of the method of Lowry et al. (1951) with albumin as a standard. When specified, the Coomassie blue method was used (Bradford, 1976).

Results and Discussion

Regulation of Enzymatic Activity by Various Nucleotides. Prass et al. (1980) reported that extramitochondrial acetyl-CoA hydrolase from rat liver is activated by ATP and inhibited by ADP. Thus, we were interested in the regulation of acetyl-CoA hydrolase activity by various nucleotides. Addition of 2 mM ATP to the assay mixture increased the hydrolytic rate to about 17 times that in its absence. Under the same conditions, GTP (2 mM) also increased the activity 10-fold. These values were comparable to earlier reports (Prass et al., 1980; Söling & Rescher, 1985). However, UTP, CTP, and TTP have little effect. ATP and GTP both contain a purine base, so these results show that nucleoside triphosphates having a purine base are potential activators of the extramitochondrial

[1] Abbreviations: DTT, DL-dithiothreitol; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); CoA, coenzyme A; CoASH, reduced coenzyme A; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)-aminomethane.

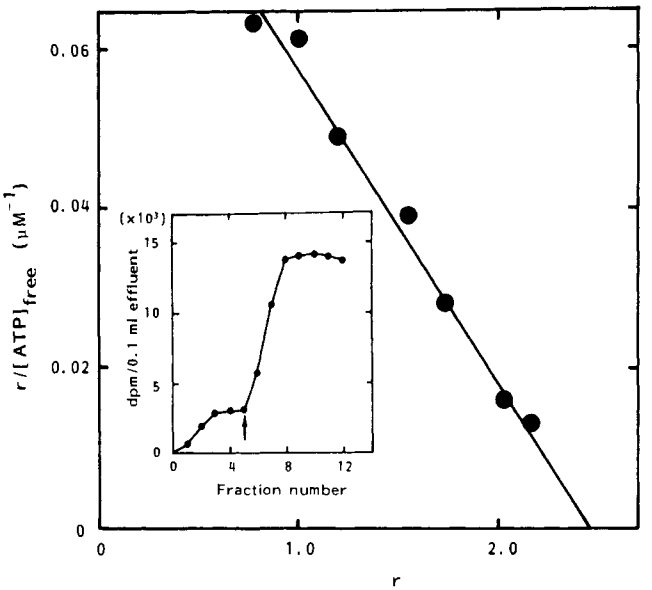

FIGURE 1: Scatchard plot of the binding of ATP to acetyl-CoA hydrolase (dimer) at 25 °C. Each point represents the determinations by the flow dialysis technique. The experiment was performed in buffer I (see Materials and Methods) over a 5-fold range of ATP concentration (50–280 μM). The inset shows results of a typical binding measurement showing the dpm/0.1 mL of lower chamber effluent as a function of fraction number for the flow dialysis technique. Details of the procedure are described under Materials and Methods. Acetyl-CoA hydrolase was at a concentration of 51.8 μM (as dimer, M_r 135 000), and [^{3}H]ATP was added at a concentration of 50 μM (1515 mCi/mmol) to initiate the experiment. After a steady state was reached, 2 mM unlabeled ATP was added to release the bound labeled ATP (shown by arrow).

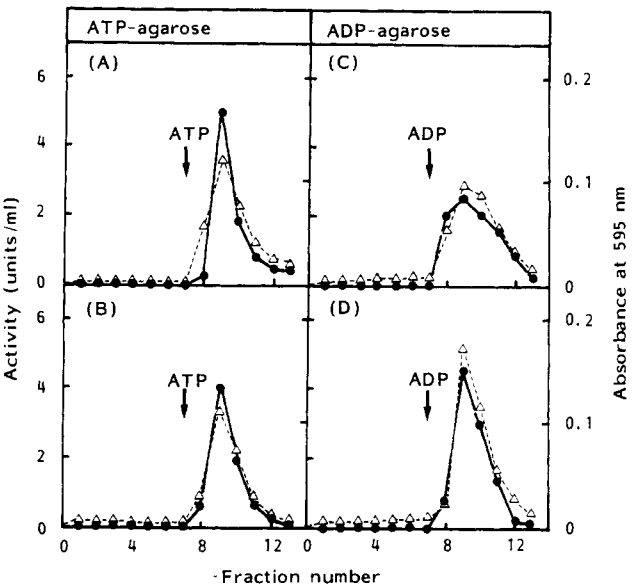

FIGURE 2: ATP- or ADP-agarose binding of various forms of acetyl-CoA hydrolase. Purified dimeric enzyme (29 μg/0.5 mL) (A, C) or cold-treated monomeric enzyme (29 μg/0.5 mL) (B, D) was applied to an ATP- or ADP-agarose column at 25 °C (A, C) or 4 °C (B, D). The columns were washed with equilibrium buffer and material was eluted with buffer containing 2 mM ATP (A, B) or 2 mM ADP (C, D) (arrows). For (B) and (D), fractions were incubated at 37 °C for 30 min with 0.45 M potassium pyrophosphate buffer (pH 7.8) containing 1 mg/mL albumin to reactivate the cold-inactivated enzyme. Protein (△) was stained with Coomassie blue, and absorbance was determined at 595 nm. The enzyme activity (●) was measured at 25 °C in the presence of 2 mM ATP.

acetyl-CoA hydrolase. On the other hand, 2 mM nucleoside diphosphates with a purine base (e.g., ADP and GDP) almost completely inhibited the acetyl-CoA hydrolase. These results suggest that the extramitochondrial acetyl-CoA hydrolase is potentially regulated by nucleotides containing a purine base and especially adenine nucleotides (ATP, ADP).

Effect of Adenine Nucleotides (ATP, ADP, and AMP) on Enzyme Activity. The activity of extramitochondrial acetyl-CoA hydrolase is greatly affected by adenine nucleotides such as ATP and ADP. Therefore, we next analyzed the interactions between ATP and ADP by Lineweaver–Burk plots. Double-reciprocal plots of activity vs the concentration of ATP at various concentrations of ADP gave straight lines that intersected at a point on the ordinate, suggesting that ATP and ADP are competitors. This is in good agreement with the results of Dixon plots by Söling and Rescher (1985). Since the K_i of ADP (6.8 μM) is very low, ADP is a strong inhibitor.

AMP at 2 mM had little effect on the rate of acetyl-CoA hydrolysis (Prass et al., 1980). However, Lineweaver–Burk plots showed that a high concentration of AMP (50 mM) inhibited the stimulation of enzyme activity by ATP and that AMP is also a competitor ($K_i \cong 2.3$ mM) for ATP binding to the enzyme.

Scatchard Plot Analysis of the Binding of ATP to Acetyl-CoA Hydrolase. We measured the binding of ATP to acetyl-CoA hydrolase by a modification (Frey & Utter, 1977) of the flow dialysis technique of Colowick and Womack (1969).

The results of a typical binding measurement at a single concentration of ATP are shown in the inset of Figure 1. The radioactivity in the effluent reached a steady state in less than 4 mL. After the level had been established, an excess of unlabeled ATP was added to obtain the total radioactivity present. The difference between the two plateaus is used to determine the concentration of bound ATP as discussed under Materials and Methods. The results of a binding experiment in which seven concentrations of ATP were used are shown in Figure 1 as a Scatchard plot. The data show that the dimeric enzyme (M_r ~135 000 at 25 °C) has at least two binding sites for ATP with a dissociation constant of 25 μM.

Nucleotide Binding Sites of Monomeric and Dimeric Acetyl-CoA Hydrolase. Previously we found that cold treatment of extramitochondrial acetyl-CoA hydrolase caused dissociation of the dimer into the monomer (Isohashi et al., 1983). However, it is unknown whether the monomeric form has a nucleotide binding site, although the dimeric form of this enzyme has at least two binding sites for ATP (Figure 1). To test this, we used ATP- or ADP–agarose at 25 or 4 °C. Both dimeric (Figure 2, parts A and C) and cold-treated monomeric (Figure 2, parts B and D) enzymes associated with ATP- and ADP–agarose, suggesting that the monomer has a nucleotide binding site(s). However, it is possible that the condition of the ATP–agarose column (the ATP–agarose contains 0.5–2 μmol of ATP/mL of gel) causes dimerization of the monomers at 4 °C. To eliminate the possibility, two sets of experiments were performed. In one, the cold-treated enzyme was applied at 4 °C to a gel filtration column equilibrated with buffer containing 2 mM ATP and eluted with the same buffer. Under these conditions, the enzyme was eluted mainly in the region of the monomer (data not shown). In the other, when the cold-treated enzyme was applied to diluted ATP–agarose with Sepharose 4B (ATP–agarose:Sepharose 4B = 1:9), the cold-treated enzyme also bound to ATP–agarose. These results also suggest that the monomeric form of the enzyme has a nucleotide binding site(s), probably one binding site judging from Figure 1.

Inactivation of Acetyl-CoA Hydrolase by Chemical Modifiers of Arginyl Residues. Next, we studied the amino acid residue related to the nucleotide binding site of acetyl-CoA

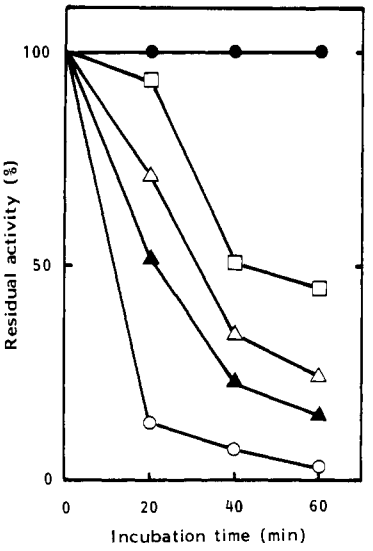

FIGURE 3: Inactivation of acetyl-CoA hydrolase by phenylglyoxal. The purified dimeric enzyme was adjusted to an enzyme protein concentration of ~14 μg/mL with 100 mM potassium phosphate buffer (pH (7.8) containing 1 mg/mL albumin and the following concentrations of phenylglyoxal: (●) 0 mM; (□) 0.2 mM; (△) 0.4 mM; (▲) 0.6 mM; (○) 1 mM. The mixtures were incubated at 37 °C for the indicated times, and then the activities of 5-μL samples in 0.5 mL of reaction mixture were assayed at 25 °C. Activity determined at 25 °C immediately after dilution (0 times at 37 °C) of the enzyme with 100 mM potassium phosphate buffer (pH 7.8) containing 1 mg/mL albumin alone was taken as 100%.

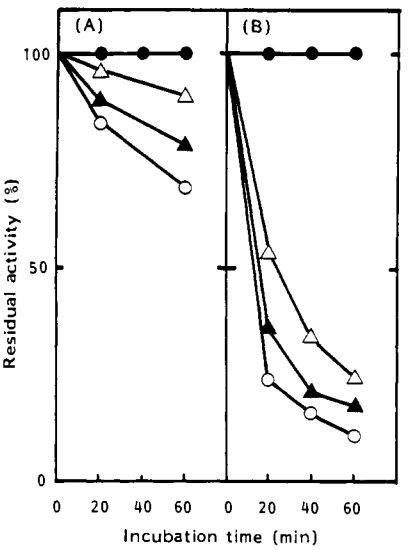

FIGURE 4: Inactivation of acetyl-CoA hydrolase by 2,3-butanedione. The purified dimeric enzyme was diluted to an enzyme protein concentration of ~14 μg/mL with 100 mM potassium phosphate buffer (pH 7.8) (A) or 50 mM borate buffer (pH 7.8) (B) containing 1 mg/mL albumin and the following concentrations of 2,3-butanedione: (●) 0 mM; (△) 0.4 mM; (▲) 0.6 mM; (○) 1 mM. Incubation conditions and 100% activity were as described for Figure 3.

hydrolase. Riordan and co-workers (Riordan et al., 1977; Borders & Riordan, 1975) have shown that arginyl residues participate in binding the carboxyl group, phosphate ester, NADH, ATP, and RNA template to several enzymes. Thus, they suggested that, in general, enzymes acting on anionic substrates or cofactors may contain arginyl residues as components of their ligand binding sites (Riordan et al., 1977). Other groups have also identified many enzymes in which arginyl residues interact with negatively charged moieties of substrates or cofactors (Patthy & Smith, 1975; Fushiki et al., 1983; Subramanian et al., 1983; Pullan et al., 1983; Kotlyar & Vinogradov, 1984). Figure 3 shows the time course of the inactivation of acetyl-CoA hydrolase by various concentrations of phenylglyoxal, which modifies arginyl residues. The rate of inactivation of acetyl-CoA hydrolase depended on the incubation time and the concentration of modifier employed. The half-life of this enzyme ($t_{1/2}$) in 0.6 mM phenylglyoxal was esimated at 20 min, and this concentration was used in subsequent experiments. Figure 4 shows the time course of the inactivation of acetyl-CoA hydrolase by 2,3-butanedione, another modifier of arginyl residues. The modification of arginyl residues by 2,3-butanedione is known to be enhanced in borate buffer, which probably stabilizes the adduct between the guanidino group and butanedione (Riordan, 1973). This buffer had a similar effect with acetyl-CoA hydrolase; that is, inactivation of the enzyme by 2,3-butanedione was more enhanced in borate buffer (Figure 4B) than in phosphate buffer Figure 4A). The data in Figures 3 and 4 suggest that arginyl residues are essential in the enzymatic mechanism of acetyl-CoA hydrolase.

We also tested the effects of phenylglyoxal and 2,3-butanedione on the enzyme using ATP– and ADP–agarose. Results showed that phenylglyoxal- or 2,3-butanedione-treated acetyl-CoA hydrolase could not bind to ATP– or ADP–agarose (data not shown). From these results we conclude that an arginyl residue is essential for binding of nucleotides to acetyl-CoA hydrolase.

Table I: Effects of Various Ligands on the Rate of Inactivation of Acetyl-CoA Hydrolase by Phenylglyoxal and 2,3-Butanedione[a]

additions	residual activity (%)	
	phenyl-glyoxal (0.6 mM)	2,3-butane-dione (0.6 mM)
none	16	21
ATP (2 mM)	79	92
ATP (0.5 mM)	68	
ATP (0.1 mM)		75
ADP (2 mM)	32	64
ADP (0.1 mM)		53
AMP (2 mM)	21	34
valeryl-CoA (0.5 mM)	21	53
valeryl-CoA (0.1 mM)		37
CoASH (0.5 mM)	31	50
CoASH (0.1 mM)		43
acetate (2 mM)		21
acetate (0.5 mM)	12	
DTT (2 mM)	71	23
ATP (2 mM) + DTT (2 mM)	98	95
ADP (2 mM) + CoASH (0.5 mM)	35	94
ADP (2 mM) + valeryl-CoA (0.5 mM)	36	95

[a] Purified dimeric enzyme was adjusted to an enzyme protein concentration of ~14 μg/mL with 100 mM potassium phosphate buffer (pH 7.8) (inactivation by phenylglyoxal) or 50 mM borate buffer (pH 7.8) (inactivation by 2,3-butanedione) containing 1 mg/mL albumin and the indicated materials. The mixtures were incubated at 37 °C for 60 min, and aliquots of 5 μL were used in the 0.5-mL assay mixture at 25 °C. Activity determined at 25 °C immediately after dilution (0 time at 37 °C) of the enzyme with each buffer containing 1 mg/mL albumin and the various additions without modifiers was taken as 100%.

Protective Effects of Nucleotides and Other Ligands against Enzyme Inactivation by Phenylglyoxal and 2,3-Butanedione. Loss of enzyme activity by modifiers of arginyl residues should be counteracted by nucleotides. As shown in Table I, 2 mM ATP had a significant protective effect against inactivation of acetyl-CoA hydrolase by phenylglyoxal. After incubation for 60 min at 37 °C in the presence of 2 mM ATP, 79% of the original activity remained. However, inactivation of acetyl-CoA hydrolase by 2,3-butanedione was effectively prevented by ATP (residual activity, 92%) (Table I). The possible reasons why inactivation by phenylglyoxal was not

completely prevented by addition of ATP are as follows: Phenylglyoxal reacts highly specifically with guanidino groups of arginyl residues, but it also reacts slightly and reversibly with sulfhydryl groups (Takahashi, 1977). On the other hand, 2,3-butanedione is not known to react with sulfhydryl groups. Thus, the effects of phenylglyoxal on the acetyl-CoA hydrolase observed in this work could have been partly due to modification of sulfhydryl groups. Furthermore, we found that the inactivation by phenylglyoxal was completely protected by 2 mM ATP plus 2 mM DTT, supporting the above view (Table I) and suggesting that ATP protected the arginyl residue and DTT protected the sulfhydryl group. We reported previously that sulfhydryl groups are essential for the enzymatic activity of acetyl-CoA hydrolase from rat liver cytosol (Nakanishi et al., 1984). Presumably, this sulfhydryl group which was affected by phenylglyoxal is also essential for activity.

Table I also shows that ADP, AMP, valeryl-CoA (a substratelike, competitive inhibitor), CoASH (a product), and acetate (a product) have only slight protective effects against the inactivation by phenylglyoxal. A combination of ADP and valeryl-CoA (or CoASH) did not improve the effects. However, addition of ADP plus valeryl-CoA (or CoASH) to the 2,3-butanedione modification system effectively prevented the inactivation, although either ADP or valeryl-CoA (or CoASH) alone had little protective effect. The reason why 2 mM ADP had little effect on inactivation of the enzyme by phenylglyoxal and 2,3-butanedione is unknown. However, it seems likely that the γ-phosphate group of ATP plays an essential role in this protection against inactivation by both modifiers. The effective protection by ADP plus valeryl-CoA (or CoASH) against inactivation by 2,3-butanedione raised the possibility that the nucleotide binding site of acetyl-CoA hydrolase locates near the substrate binding site. The differences in effects on protection of these compounds against inactivation by phenylglyoxal and 2,3-butanedione may be due to differences in accessibility of the modifiers to the nucleotide binding site of acetyl-CoA hydrolase and to differences in their specificity in the modification. From these results, we conclude that one or more arginyl residues are involved in the binding of nucleotide to extramitochondrial acetyl-CoA hydrolase. However, we could not exclude the possibility that accessibility of the modifier to an arginyl residue at a site remote from the nucleotide binding site would be changed by ATP binding, allowing a necessary conformation change in the activation process.

General Discussion. In the present study we have found that each subunit constituting the acetyl-CoA hydrolase molecule contains the nucleotide binding site (at least 1 binding site/subunit) and one or more arginyl residues play an essential role in binding of nucleotides to acetyl-CoA hydrolase.

Nucleotide appears to be involved with both the catalytic properties of the enzyme and the association–dissociation state of its subunits (Isohashi et al., 1983a). Binding of ATP to the monomer at 25 °C shifts the equilibrium between an inactive monomeric state and an active polymeric state (dimer and tetramer) toward the latter, apparently by inducing a conformational change in the monomer that favors polymerization, although the monomer does not aggregate at low temperature in the presence of ATP (Isohashi et al., 1984). Interestingly, this enzyme can exist in both a nearly inactive dimeric state in the absence of ATP and a catalytically active dimeric state in the presence of ATP, presumably owing to conformational changes induced by ATP (Isohashi et al., 1983a). The change from the dimeric to the tetrameric state on addition of ATP may increase the affinity for the substrate. The apparent K_m values for acetyl-CoA of the dimeric and tetrameric forms are different, being 170 μM and 60 μM, respectively (Isohashi et al., 1983a). On the other hand, binding of ADP to the enzyme also accelerates the polymerization of the enzyme, but in assay medium with 2 mM ADP, neither the tetrameric nor dimeric enzyme showed enzymatic activity (Isohashi et al., 1983a). These results indicate that the nucleotide interactions are not simple competitive displacements in their binding sites but presumably induce conformational changes, involving different polymeric states of the enzyme with different catalytic levels and affinities for substrate.

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