The effect of fatty acids with various structure and length in the hydrocarbon chain on the purified AMP deaminase from bovine brain was investigated. All the saturated fatty acids and unsaturated ones of trans configuration, longer than 16 in the hydrocarbon chain, were activators of the enzyme, while the unsaturated fatty acids of cis configuration including oleic, linoleic, linolenic, arachidonic, and palmitoleic acids acted as inhibitors. The methyl esters of fatty acids showed no effect on the enzyme, suggesting that a free carboxyl group of fatty acid is required for the effect of fatty acid on the enzyme. The activation and inhibition of AMP deaminase by fatty acids are qualitatively and quantitatively accounted for by a "partial noncompetitive" mechanism with respect to the substrate AMP; best fit theoretical curves were obtained for the activation and inhibition of the enzyme by fatty acids. A close correlation was observed between the fatty acid conformation (or the parameter defined by the distance from carboxyl carbon to \( \omega \) carbon atom) and the reaction rate constant for the enzyme substrate fatty acid (ES1) complex. It is suggested that the nonpolar interactions of each fatty acid with the same hydrophobic region induce the conformational change of the enzyme, which results in the increase or decrease in the breakdown rate of the ES1 complex.

The importance of AMP deaminase (AMP aminohydrolase, EC 3.5.4.6) in the purine nucleotide cycle (1, 2), interconversion of adenine, inosine, and guanine nucleotides (3-6), and stabilization of adenylate energy charge (7) in various tissues makes this enzyme an interesting subject for enzymological and physicochemical studies from the regulatory point of view. We have investigated the regulatory mechanism of AMP deaminase reaction (8-15). Recently, we reported the potent inhibition of the enzyme by fatty acyl-CoA and its physiological significance was discussed in connection with the regulation of adenylate energy charge (13). The preliminary results that free fatty acids can also activate or inhibit the activity of AMP deaminase (13) led us to the kinetic study on the interaction of these ligands with the enzyme. Activation and inhibition of various enzymes by free fatty acids or fatty acyl-CoAs have been demonstrated in connection with metabolic control (16-32). However, it seems that knowledge concerning the kinetic analysis of the interactions of enzymes with alkyl derivatives including fatty acids is limited. To clarify the mode of action of fatty acid on AMP deaminase, the kinetic parameters of the enzyme in the presence of fatty acids were investigated and the sequence specificity of fatty acids was analyzed. Regulation of the enzyme activity by fatty acid through a specific nonpolar interaction of the compound with the enzyme molecule was indicated.

**EXPERIMENTAL PROCEDURES**

**Materials**—Fatty acids were obtained from Sigma Chemical Co. Bovine serum albumin was a product of Nutritional Biochemical Corp. All other chemicals were purchased from commercial sources as mentioned in a previous study (13).

**Methods**—Bovine brain AMP deaminase was purified as a homogeneous preparation according to the method described previously (13). The enzyme activity was determined as described previously (13); the reaction mixture of 0.25 ml contained 30 mM potassium phosphate buffer, pH 7.1, 0.1 M NaCl, various concentrations of AMP and effectors, and 0.2 

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**RESULTS**

Table I shows the effects of various fatty acids on the AMP deaminase activity. It is clear that all the saturated fatty acids longer than 16 in the hydrocarbon chain activate the enzyme to the same extent at the concentration of 0.2 mM. At higher concentration of fatty acids (0.78 mM), the degree of activation by palmitate, stearate, and arachidate did not change, while laurate and myristate showed a tendency to inhibit the enzyme.

Figs. 1 and 2 show the effects of increasing concentrations of saturated and unsaturated fatty acids on the enzyme activity. As shown in Table I, all the saturated fatty acids longer than 16 in the hydrocarbon chain increased the enzyme activity. Of the fatty acids tested, stearate, an unsaturated fatty acid of trans configuration, exhibited an activating effect with the increase in its concentration similar to that of saturated fatty acids. However, unsaturated fatty acids of all cis configuration including oleate, linoleate, linolenate, arachidonate, and palmitoleate showed powerful inhibition. The inhibition of the enzyme by fatty acids except linolenate appears to be partial; the activity falls to a definite limit with the increase in inhibitor concentration.

In order to determine the functional group of fatty acids responsible for the activation or inhibition of AMP deaminase activity, the effect of methyl esters of several fatty acids was investigated. As shown in Table II, all the methyl esters of fatty acids used had no effects at two different concentrations, suggesting that a free carboxyl group is clearly required. We explored the mechanism of activation and inhibition by fatty acids and studied the affinity for the substrate in the absence and presence of palmitate, linoleate, or arachidonate.
Table I
Effect of saturated fatty acids on the activity of AMP deaminase

<table>
<thead>
<tr>
<th>Chain length (number of carbon atoms)</th>
<th>Trivial name</th>
<th>In presence of 0.20 mM fatty acid</th>
<th>In presence of 0.78 mM fatty acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>No addition</td>
<td></td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>6</td>
<td>Caproate</td>
<td>1.05</td>
<td>1.00</td>
</tr>
<tr>
<td>8</td>
<td>Caprylate</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>10</td>
<td>Caprate</td>
<td>1.03</td>
<td>1.00</td>
</tr>
<tr>
<td>12</td>
<td>Laurate</td>
<td>0.97</td>
<td>0.00</td>
</tr>
<tr>
<td>14</td>
<td>Myristate</td>
<td>0.07</td>
<td>0.85</td>
</tr>
<tr>
<td>16</td>
<td>Palmitate</td>
<td>1.40</td>
<td>1.40</td>
</tr>
<tr>
<td>18</td>
<td>Stearate</td>
<td>1.40</td>
<td>1.40</td>
</tr>
<tr>
<td>20</td>
<td>Arachidate</td>
<td>1.40</td>
<td>1.40</td>
</tr>
</tbody>
</table>

*The enzyme activity without fatty acid was expressed as an arbitrary value of 1.00.

Fig. 3 indicates that the activation and inhibition of the enzyme by fatty acids are exerted through a "noncompetitive" mechanism and it involves no change in the affinity of the enzyme for the substrate. The reaction mixture and assay conditions are similar to those described in Fig. 1. Points are experimental data, and lines are theoretically drawn from Equation 4, assuming that \( k'/k \) values are 1.40, 1.40, 0.25, and 0.08 for arachidate (Curve A), palmitate (B), arachidonate (C), and palmitoleate (D), respectively.

Kinetic Interpretation of the Activation and Inhibition of the Enzyme by Fatty Acids—The present results suggest the effects of fatty acids on the AMP deaminase to be a partial inhibition mechanism (35). We may try to analyze the activation and inhibition of the enzyme by fatty acids, and represent the system by partial noncompetitive mechanism as shown in Fig. 4. The predictions of this model, which is based on the Adair-Pauling sequential interaction mechanism (36), are given as follows (Fig. 4): (a) the enzyme has \( n \) binding sites for the substrate, and we may use the notation \( ES_1, ES_2, \ldots, ES_n \), to designate the ES complexes involving 1, 2, \ldots, \( n \) molecules of the substrate, and (b) fatty acid (I) combines with the enzyme at the distinct site, sufficiently far from the substrate site, and thus does not affect the combination of the substrate (S) with the enzyme, but affects only \( V_{max} \). It is formally convenient to regard \( K_i \) as the dissociation constant of the fatty acid-enzyme complexes, and the dissociation constants \( K_1, K_2, \ldots, K_n \) were defined for the successive binding steps of the substrate as described in Fig. 4. (c) EIS complexes such as \( EIS_1, EIS_2, \ldots, EIS_n \), and the different velocity constants \( k \) and \( k' \) apply to the breakdown of \( ES \) and \( EIS \) complexes, respectively. The overall velocity will be given as follows:

\[
v = k \sum_{i=1}^{n} [ES_i] + k' \sum_{i=1}^{n} [EIS_i]
\]

We assume the \( n \) value in Fig. 4 and Equation 1 to be 4,
where \( K' \) is a constant comprising the dissociation constants \( K_1, K_2, K_3, \) and \( K_4 \) defined above for the successive binding steps. Equation 3, which is known as the Hill equation in the absence of inhibitor, qualitatively and quantitatively can account for the sigmoid nature of the saturation curves with respect to the substrate and “noncompetitive” effect of fatty acids on the enzyme (see Fig. 3). When the substrate concentration was fixed at 25 mM in the experiments (Figs. 1 and 2), which is the concentration necessary for 50% activity, Equation 3 may be rearranged into the following form writing \( V_0 \) as the velocity in the absence of fatty acid:

\[
\frac{v}{V_0} = 1 + \frac{k'[\text{S}]}{K'},
\]

or in terms of relative quantities, using \( \alpha \) for \( \frac{[\text{I}]}{K} \) as in Equation 4.

\[
\frac{v}{V_0} = \frac{1 + \alpha [\text{S}]}{1 + \alpha}
\]

From the data in Figs. 1 and 2, the concentration necessary for 50% inhibition or activation of the enzyme activity (\( I_{50} \) or \( A_{50} \) value) can be estimated to be approximately 0.07 mM for each fatty acid. From the value obtained together with assumed \( k'/k \) values, theoretical curves were computed and compared with the experimental results. The best fit theoretical curves were obtained assuming that \( k'/k \) values are 0, 0.27, 0.77, 1.35, 0.08, 0.25, and 1.40 for linolenate, linoleate, oleate, elaidate, palmitoleate, arachidonate, and saturated fatty acids including palmitate, stearate and arachidate, respectively (Figs. 1 and 2).

We now try to shed more light on the relationship between the effect of fatty acids on the enzyme and the conformation of these ligands. Typical conformation of fatty acids such as linoleate and linolenate is shown in Fig. 5. Since the carboxyl group of fatty acid is necessary for the activation and inhibition as described above, the distance, \( r \), from C-1-carboxyl carbon to \( \omega \) carbon atom was determined from the figure, and the volume of a sphere, which has a diameter \( r \), was calculated. The \( k'/k \) value, which means relative reaction rate constant for each enzyme-substrate-fatty acid complex or the maximal inhibition (or activation) velocity in the presence of excess fatty acid was plotted against the cubic volume of the fatty acid calculated above. All the fatty acids of octadecanoic series, which was a typical example of this plot as shown in Fig. 6, gave a straight line relationship of \( k'/k \) versus “molecular volume” of the fatty acid, suggesting the conformation-dependent effects of fatty acids on the enzyme; the reaction
acids are taken from Fig. 1. The relative reaction rate constants \( k'/k \) in the presence of fatty acids. The "molecular volume" was calculated from the distance, \( r \), from carboxyl carbon to \( \omega \) carbon atom as shown in Fig. 5. The relative reaction rate constants \( k'/k \) in the presence of fatty acids are taken from Fig. 1.

rate of the enzyme-fatty acid-substrate complex is essentially dependent on the conformation of fatty acid.

**Discussion**

A large number of enzymes have been reported to be activated or inhibited by fatty acids and their CoA esters, and the physiological significance of these phenomena was discussed in connection with metabolic control, although the regulatory significance of these ligands as negative effectors has been questioned because of the difficulties in distinguishing specific regulatory effects from possible nonspecific detergents. However, there seems to be many cases which have a physiological meaning in metabolic regulation; these are the inhibition of glycocytic enzymes (16-18), and the activation of gluconeogenic enzymes by fatty acids (22, 23). The present study demonstrates that AMP deaminase is activated or inhibited in vitro by long chain fatty acids. In an attempt to see whether or not the inhibition and activation of AMP deaminase are specific for free fatty acids, we examined the effects of various detergents, such as Triton X-100 (polyoxyethylene t-octylphenyl ether), Tween 20, 40, and 80 (polyoxyethylene sorbitan monolaurate, monopalmitate, and monooctylate, respectively), and found that at comparable concentrations these detergents have little effect on the AMP deaminase activity. Therefore, it is extremely plausible that the activating and inhibitory effects of fatty acids on the enzyme cannot be ascribed simply to the detergent action of fatty acids.

The physiological significance of the effects of fatty acids presented in this paper cannot be assessed directly from these experiments. However, the concentrations for the fatty acids were within the range of physiological concentration of the compounds; the concentrations of free fatty acids in brain, which result mainly from degradation of glycerolipids, range from 0.3 to 0.4 mM, and deactivation, i.e. anoxia results in the rapid increase by over 100% in fatty acid content, which is thought to be caused by phospholipase action (37). Free fatty acids in brain are enriched with unsaturated fatty acids in comparison with those in plasma, and free arachidonate, one of the most potent inhibitors of the enzyme, is further produced selectively during the first few minutes after death; the concentration of this fatty acid increases from 0.04 to 0.18 mM 1 to 6 min postmortem, respectively (37). The inhibition of AMP deaminase by arachidonate newly liberated may participate in the rapid decrease in the adenylate energy charge under the anoxic or ischemic state after deactivation (38). Furthermore, free fatty acids produced from phospholipids in developing brain can also modify the activity of AMP deaminase in brain.

Several kinetic models have been presented for the qualitative and quantitative explanation of sigmoidal substrate saturation curves of AMP deaminase. Setlow and Lowenstein (5) attempted to explain the sigmoid kinetics of the brain enzyme by the empirical Hill equation. Recently, we proposed a kinetic model of chicken erythrocyte AMP deaminase (11) based on the concerted transition theory of Monod et al. (39). As described in this paper, the Adair-Pauling model (36), which is the more general and extended form of the Hill equation, can also yield best fit theoretical curves for all experimental saturation curves with respect to AMP in the absence or presence of the inhibitor.

Activation and inhibition of AMP deaminase by fatty acids can be accounted for qualitatively and quantitatively by a "partial noncompetitive" mechanism with respect to the substrate AMP. The partial inhibition is not simply due to the solubility of fatty acid: the fatty acid concentration, at which the activity falls to a definite limit, is below each critical micellar concentration. We further investigated the role of the hydrocarbon chain and polar carboxyl group of fatty acid on the activation or inhibition of AMP deaminase; the polar portion of fatty acid (the carboxyl group) is necessary for the effects, and the kinetic parameters of the activation or the inhibition are determined through the nonpolar interaction of the hydrocarbon chain of the compound with the enzyme.

Our previous paper (13) has also shown that both the polar and nonpolar portions of fatty acyl-CoAs are necessary for the inhibition of AMP deaminase by these ligands. Although the polar groups are responsible for the regulation by fatty acids and the inhibition by fatty acyl-CoAs of the enzyme, it should be noted that these two mechanisms differ from one another: the former is a partial type but the latter a complete inhibition. "Partial noncompetitive" effects presented in this paper suggest that the reaction rate of the enzyme-AMP-fatty acid complex is determined by the conformation of the fatty acid. The conformation is represented by the volume of a sphere, the diameter of which is the distance from the carboxyl carbon to the \( \omega \) carbon atom of fatty acid. A linear relation was observed between the breakdown rate of EIS complex and the "molecular volume" of fatty acid (Fig. 6). A possible explanation of the mechanism for these phenomena would be that the conformational change of the enzyme molecule is dependent on the structure of the hydrophobic portion of fatty acids which get into a specific "pocket," distinct from the catalytic site of the enzyme; the conformational change of the enzyme molecule may result in the increase or decrease in the breakdown rate of the EIS complex. Of particular interest is the finding on the complete inhibition of the enzyme by laurate, whereas other fatty acids showed no complete inhibition (Table I). The distance, \( r \), from the carboxyl carbon to \( \omega \) carbon atom of laurate is 13.9 Å, the value of which is comparable to that of linolenate (13.3 Å), the most potent inhibitor of the enzyme. The values of the "molecular volume," on which the breakdown rate of the enzyme-AMP-fatty acid complex depends, of laurate and linolenate are thus in agreement with each other. These findings can account for the inhibition of the enzyme by laurate.

Several lines of evidence have indicated that the hydrophobic interactions of a homologous series of alkyl derivatives with various other enzymes are responsible for the control of enzyme activity. Some of these examples are the inhibition of cholinesterases (EC 3.1.1.8) by n-alkyltrimethylammonium salts (40), the interaction of substrate with L-cysteine sulfoxide lyase (EC 4.4.4.4) (41), and the binding of alkylammonium salts to trypsin (EC 3.4.4.4) (42). Mitochondrial nicotinamide nucleotide transhydrogenase (EC 1.6.1.1) was shown to be
competed hydri

case of CoA with saturated fatty acids, i.e. pal-

en the degree of hydro-

ism. A close relation between the degree of hydro-

of the inhibitor and inhibitory efficiency suggests

that the hydrophobic portion of the inhibitor is responsible

for the binding to the NADP(H)-sites of the enzymes. Andersen's group has reported the kinetic analysis on the enzyme

regulation through the nonpolar interaction of several dehy-

drogenases with alkyl substituents including aliphatic carbox-

ylic acids, N'-alkylnicotinamide chlorides and alkylammon-

ium chlorides (44-47). These inhibitors were shown to be

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