Kuo, A.-L., & Wade, C. (1979) *Biochemistry 18*, 2301.
Nagle, J., & Wilkinson, J. (1978) *Biophys. J. 23*, 159.
Papahadjopoulos, D., & Miller, N. (1967) *Biochim. Biophys. Acta 135*, 624.
Powers, L., & Pershan, P. S. (1977) *Biophys. J. 20*, 137.
Robles, I. C., & Van Den Berg, D. (1969) *Biochim. Biophys. Acta 187*, 520.
Rubenstein, J., Smith, B., & McConnell, H. M. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 15.
Smith, B. A., & McConnell, H. M. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 2759.
Smith, L. M., Smith, B. A., & McConnell, H. M. (1979) *Biochemistry 18*, 2256.
Vaz, W. L. C., Jacobson, K., Wu, E.-S., & Derzko, Z. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 5645.
Wolf, D. E., Schlessinger, J., Elson, E. L., Webb, W. W., Blumenthal, R., & Henkart, P. (1977) *Biochemistry 16*, 3476.
Wu, E. S., Jacobson, K., & Papahadjopoulos, D. (1977) *Biochemistry 16*, 3936.
Wu, E. S., Jacobson, K., Szoka, R., & Portis, A. (1978) *Biochemistry 17*, 5543.

Effects of Adenosine 5′-Diphosphate on Bovine Glutamate Dehydrogenase: Diethyl Pyrocarbonate Modification†

Alfred George and J. Ellis Bell*

ABSTRACT: Initial rate kinetic studies and reduced coenzyme binding studies with bovine glutamate dehydrogenase have shown that an enzyme–NAD(P)H–glutamate abortive complex is a major participant in the overall enzyme-catalyzed reaction. The allosteric regulator ADP is shown to activate the enzyme by destabilizing this abortive complex. Destabilization of this abortive complex with concomitant activation is also achieved by the ethoxyformylation of a single histidine residue per subunit in the hexameric enzyme. ADP, in addition to its activatory effects, is shown to (a) remove the nonlinearity from the Lineweaver–Burk plots which is attributable to negative cooperativity and (b) inhibit the enzyme as a competitive inhibitor with respect to coenzyme under the appropriate conditions. Ethoxyformylation with diethyl pyrocarbonate, which mimics the activatory effects of ADP, has no effect on the negative cooperativity shown by this enzyme. A model for the action of ADP is proposed in which ADP activates glutamate dehydrogenase by binding to a regulatory binding site and blocks the negative cooperativity by mimicking the natural coenzyme at the active site of the enzyme.

Ox liver glutamate dehydrogenase (EC 1.4.1.3) catalyzes the oxidative deamination of glutamate and a variety of other amino acids (Struck & Sizer, 1960) with either NAD^+ or $NADP^+$ as coenzyme. On the basis of initial rate measurements, Frieden (1959) proposed that each of the six subunits in the glutamate dehydrogenase hexamer had two binding sites for NAD^+ or NADH. Binding of NAD^+ or NADH to the second site produced an activation or an inhibition, respectively, at high concentrations. Since these effects were not observed at high concentrations of the triphosphopyridine nucleotides, which also function as coenzymes, Frieden proposed that the second site was specific for the diphosphopyridine nucleotides. However, more detailed kinetic studies of Engel & Dalziel (1969) demonstrated that the coenzyme activation first observed with NAD^+ as coenzyme (Olson & Anfinsen, 1953) also occurred with $NADP^+$ as coenzyme and that in each case the Lineweaver–Burk plots could be described in terms of a number of linear regions. On the basis of these observations, Dalziel & Engel (1968) proposed that this "coenzyme activation" was due to negative cooperativity. Subsequent studies on the binding of oxidized coenzymes (Egan & Dalziel, 1971) showed no evidence for more than one binding site per polypeptide chain for either NAD^+ or $NADP^+$. In the presence of the substrate analogue glutarate, the data were consistent with either intrinsically nonidentical binding sites or negative homotropic interactions between identical sites in the enzyme–$NAD(P)^+$–glutarate ternary complex. Although the chemical identity of the subunits in the hexamer has been established (Appella & Tomkins, 1966; Smith et al., 1970), there is the possibility that the subunits may be spatially arranged so that they are not in equivalent environments. However, the demonstration that half-saturation of the oligomer with NAD^+ or $NADP^+$ in the presence of glutarate produces a conformational change in the remaining active sites (Bell & Dalziel, 1973) strongly suggests that the nonlinearity found in initial rate studies and in equilibrium binding studies is the result of negative homotropic interactions. This suggestion was substantiated by kinetic studies with thionicotinamide coenzyme analogues (Alex & Bell, 1980).

While many studies [for a review of these, see Dalziel (1975)] have been directed toward establishing the number and types of binding sites for coenzymes and substrates, little attention has been paid to studies of the effects of the nucleotide regulators of glutamate dehydrogenase, ADP and GTP. Previous reports have shown that the binding site for ADP can be covalently modified by the binding site directed affinity label 3′-[*p*-(fluorosulfonyl)benzoyl]adenosine (Pal et al., 1975) which decreases both activation by ADP and the substrate inhibition which is observed at high NADH concentrations, suggesting some overlap of the second NADH binding site and the ADP binding site. We report here the results of initial rate studies, fluorescence binding studies, and chemical modification studies with diethyl pyrocarbonate which allow further definition of the effects of ADP on glutamate dehydrogenase and examine the potential role of the ADP:second NADH binding site in the negative homotropic interactions shown by glutamate dehydrogenase.

† From the Department of Biochemistry, University of Rochester Medical Center, Rochester, New York 14642. *Received June 5, 1980.*

0006-2960/80/0419-6057$01.00/0

Experimental Procedures

Materials and Methods. Bovine liver glutamate dehydrogenase was obtained from Sigma Chemical Co. as an ammonium sulfate suspension. The enzyme was dialyzed extensively against 0.1 M phosphate buffer containing 10 μM EDTA, pH 6.0, prior to use. Enzyme concentrations were determined by using an extinction coefficient at 280 nm of 0.93 for a 1 mg/mL solution (Egan & Dalziel, 1971). A subunit molecular weight of 56 000 was used in all calculations. All coenzymes, nucleotides, diethyl pyrocarbonate, and *N*-acetylhistidine were also obtained from Sigma. The rate of oxidative deamination of glutamate was measured as described previously (Alex & Bell, 1980). Binding of reduced coenzymes to glutamate dehydrogenase was determined by using the enhancement of coenzyme fluorescence on binding, as described previously (Melzi D'Eril & Dalziel, 1973), using a Perkin-Elmer MPF-3 fluorescence spectrophotometer.

Modification Studies with Diethyl Pyrocarbonate. Modification experiments were performed in 0.1 M phosphate buffer, pH 6.0, containing 10 μM EDTA at 0 °C unless otherwise stated. Dilutions of diethyl pyrocarbonate were prepared and assayed immediately prior to use. A small volume of reagent was diluted at least 5-fold with ice-cold ethanol and subsequently diluted again with ice-cold buffer containing 10% ethanol for solubility. the concentration of diethyl pyrocarbonate was determined by addition of a small aliquot of the diethyl pyrocarbonate solution to 20 mM *N*-acetylhistidine, and the increase in absorbance at 242 nm was measured. After complete reaction, the diethyl pyrocarbonate concentration was calculated by using a molar extinction coefficient of 3600 cm^{-1}. Modification reactions were initiated by addition of diethyl pyrocarbonate to enzyme in the absence or presence of the indicated substrates or nucleotides. Periodically, 15-μL aliquots were removed, diluted, and assayed as described previously (Alex & Bell, 1980).

Results

Effects of ADP on the Oxidative Deamination of Glutamate. At pH 7.0, with high glutamate concentrations (50 mM), Lineweaver–Burk plots with $NAD(P)^+$ as the varied substrate consist of a series of linear regions (Engel & Dalziel, 1969) with discontinuities at 15, 90, and 400 μM NAD^+. In the presence of 1 mM ADP, however, a completely linear Lineweaver–Burk plot is observed (Figure 1A), with activation at high NAD^+ concentrations and inhibition at low NAD^+ concentrations. With much lower glutamate concentrations (0.25 mM), where in the absence of ADP linear Lineweaver–Burk plots are observed, ADP acts as a strict competitive inhibitor with respect to NAD^+ (Figure 1B). Qualitatively, similar results are seen at pH 8.0. When glutamate is the varied substrate, the effects of ADP are more complex. At pH 7.0 (data not shown), with high NAD^+ concentrations, a small but quite definitive activation is seen, which appears to coincide with a slight substrate inhibition seen at high glutamate concentrations in the absence of ADP. At lower glutamate concentrations, inhibition by ADP is observed. At pH 8.0, a quite marked substrate inhibition in the absence of ADP is observed. This substrate inhibition is seen with greater than 7–10 mM glutamate concentrations, depending on the NAD^+ concentration. In the presence of 1 mM ADP, a marked activation is seen at glutamate concentrations above about 5 mM, with inhibition being seen at lower glutamate concentrations. In the presence of ADP, the substrate inhibition at high glutamate concentrations is markedly decreased; a slight substrate inhibition is seen at glutamate concentrations above 50 mM. In all the above experiments, similar results are observed with $NADP^+$ as coenzyme.

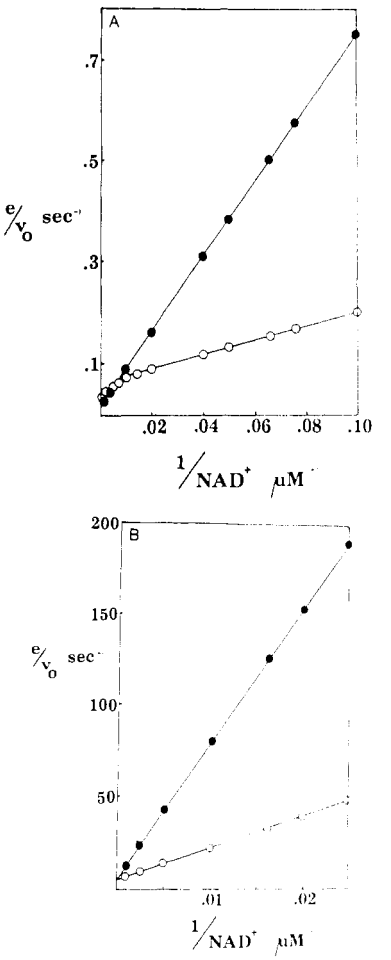

FIGURE 1: Effects of ADP on the oxidative deamination of L-glutamate. Lineweaver–Burk plots with NAD^+ as the varied substrate in the absence (○) and presence (●) of 1 mM ADP. (A) With 50 mM L-glutamate. (B) with 0.25 mM L-glutamate. Other conditions: pH 7.0, 0.1 M phosphate buffer containing 10 μM EDTA.

Effects of ADP on the Binding of NAD(P)H to Glutamate Dehydrogenase. The enhancement of NAD(P)H fluorescence with excitation at 340 nm and emission at 465 nm upon binding to glutamate dehydrogenase was determined by titration of a 1 μM coenzyme solution with increasing concentrations of enzyme (Bell & Dalziel, 1973). The fluorescence enhancement was determined under the various conditions used in this study; these results are given in Table I. Binding titrations, with a fixed concentration of enzyme active sites (8.9 μM), in the presence of 0.2 M L-glutamate indicated (Figure 2) that both NADH and NADPH bound to the enzyme in a manner indicative of negative cooperativity (or nonidentical binding sites). With both coenzymes, extrapolation indicated one binding site per subunit. The results with NADH gave two dissociation constants, 0.5 and 1.8 μM. With NADPH, values of 0.25 and 0.9 μM were calculated. In the presence of 1 mM ADP (Figure 2), two linear regions of the Scatchard plots were again observed, with somewhat larger dissociation constants (0.8, 2.4 μM and 0.4, 1.4 μM) for NADH and NADPH, respectively. Qualitatively, similar results were obtained in the absence of glutamate at pH 8.0 and in the presence of glutamate at pH 7.0. These results are tabulated in Table I. Binding studies were not performed at pH 7.0 in the absence of glutamate as the fluorescence enhancement is too low to permit accurate measurements.

Table I: Binding of Reduced Coenzymes to Glutamate Dehydrogenase: Effects of ADP

conditions	fluorescence enhancement	apparent dissociation constants (μM)
pH 8.0, no glutamate		
NADH, no ADP	3.60	8.5, 20
NADH + 1 mM ADP	2.64	20, 62
NADPH, no ADP	3.91	7.6, 16
NADPH + 1 mM ADP	2.74	14.3, 30
pH 8.0, 0.2 M glutamate		
NADH, no ADP	3.47	0.5, 1.8
NADH + 1 mM ADP	3.13	0.8, 2.4
NADPH, no ADP	3.89	0.25, 0.9
NADPH + 1 mM ADP	3.53	0.4, 1.4
pH 7.0, 0.2 M glutamate		
NADH, no ADP	3.47	1, 2.3
NADH + 1 mM ADP	3.60	4.4, 9.8
NADPH, no ADP	3.96	0.5, 2.0
NADPH + 1 mM ADP	4.20	2.1, 8.6

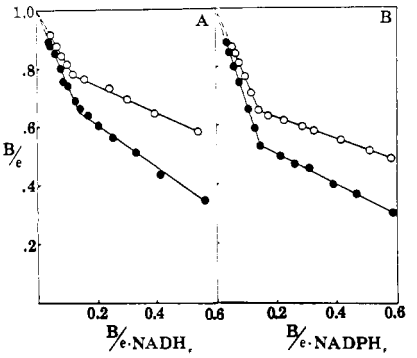

FIGURE 2: Equilibrium binding of reduced coenzyme to glutamate dehydrogenase: effects of ADP. Binding determined by fluorescence titrations in the absence (○) or presence (●) of 1 mM ADP. (A) With NADH; (B) with NADPH. Other conditions: pH 8.0, 0.1 M phosphate buffer containing 10 μM EDTA with 0.2 M L-glutamate and 8.9 μM active sites.

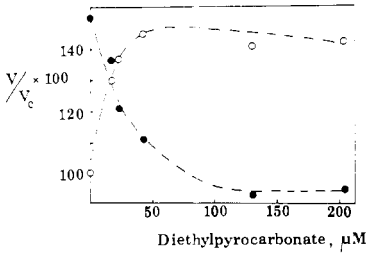

FIGURE 3: Modification of glutamate dehydrogenase by diethyl pyrocarbonate. Glutamate dehydrogenase (13.8 μM active sites) was modified at 4 °C for 20 min at pH 6.0. Enzyme assays were at pH 7.6; activity, v/v_c, is expressed as a percentage of the unmodified enzyme assayed under the same conditions. Assays were performed in the absence (○) or the presence (●) of 0.4 mM ADP.

Effects of Modification with Diethyl Pyrocarbonate. Glutamate dehydrogenase (13.8 μM active sites) was modified at pH 6.0, 4 °C, for 20 min with increasing concentrations of diethyl pyrocarbonate. The modified enzyme was then assayed, at pH 7.6, in either the absence or the presence of ADP. As shown in Figure 3, when assayed in the absence of ADP (open circles), a quite marked activation is seen as the diethyl pyrocarbonate concentration is increased. However, when assayed in the presence of ADP (closed circles), which under these conditions is activatory, a quite definite inhibition is observed. When the effects of diethyl pyrocarbonate are studied as a function of time of incubation with the reagent (Figure 4), it is seen that the maximal effect is seen within 30 min of incubation. When 50 mM glutamate is included

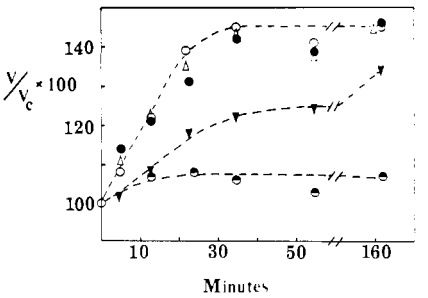

FIGURE 4: Substrate protection against ethoxyformylation of glutamate dehydrogenase. Glutamate dehydrogenase (14 μM active sites) was modified by using 20 μM diethyl pyrocarbonate at 4 °C, pH 6.0, either alone (●) or in the presence of 50 mM L-glutamate (○), 1 mM ADP (△), 1 mM NADH (▼), or 1 mM NADH and 50 mM L-glutamate (⊖). Enzyme assays were as described under Materials and Methods.

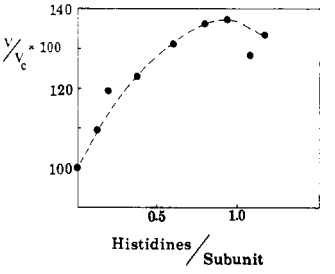

FIGURE 5: Extent of histidine ethoxyformylation by diethyl pyrocarbonate. Histidine ethoxyformylation in glutamate dehydrogenase was determined from absorbance changes at 242 nm, and the extent of activation of the oxidative deamination of L-glutamate was determined as a function of the histidine modification.

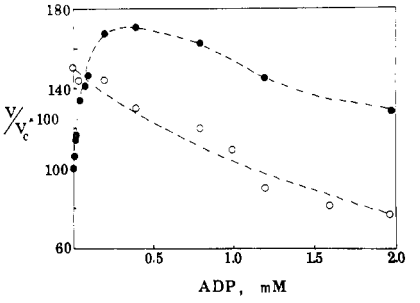

FIGURE 6: Effects of ADP concentration on native and ethoxyformylated enzyme. Assays were performed at pH 7.6 with 50 mM glutamate and 0.5 mM NAD^+, and the results are expressed as a percentage of the activity determined in the absence of ADP. (●) Unmodified enzyme; (○) enzyme modified to the extent of one (ethoxyformyl)histidine per subunit.

in the incubation mixture, no additional effect is seen. However, when 50 mM glutamate and 1 mM NADH are included, almost complete protection against modification is seen. The presence of 1 mM ADP, or 0.1 mM GTP, did not affect the modification effects.

The modification of hisitidine residues by diethyl pyrocarbonate was measured by using absorbance changes at 242 nm. When these measurements were correlated with the activatory effect of diethyl pyrocarbonate modification (Figure 5), it is apparent that maximal activation is observed when one histidine residue per subunit of glutamate dehydrogenase has been modified. A second-order rate constant for the activation by modification at 4 °C and pH 6.0 was measured as 5.5×10^2 M^{-1} min^{-1} (data not shown).

The kinetic properties of the maximally modified enzyme were studied. At pH 7.6, the effects of varied ADP concentrations with modified and unmodified enzyme are shown in Figure 6. With the control, a typical activation by ADP is

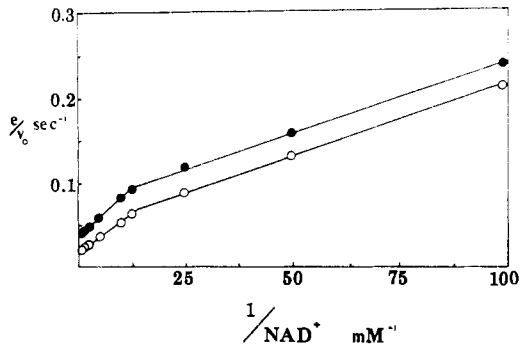

FIGURE 7: Negative cooperativity in ethoxyformylated glutamate dehydrogenase. Lineweaver–Burk plots with NAD^+ as the varied substrate, with either native enzyme (●) or enzyme containing one (ethoxyformyl) histidine per subunit (○). Other conditions: pH 7.6, 0.1 M phosphate buffer containing 10 μM EDTA and 50 mM L-glutamate.

seen, with a slight decrease in the effect observed above 0.5 mM ADP. With ethoxyformylated enzyme, no activation by ADP is observed, but an inhibition, which mimics the effects of high ADP concentrations with the control enzyme, is observed.

Finally, the effects of ethoxyformylation on the negative cooperativity exhibited by glutamate dehydrogenase were examined. Lineweaver–Burk plots (Figure 7) with NAD^+ as the varied substrate were biphasic with either native enzyme or enzyme containing one (ethoxyformyl)histidine per subunit.

Discussion

Quite clearly, the effects of ADP on glutamate dehydrogenase are complex. In the initial rate studies reported here, it is apparent that ADP can act as both an activator and an inhibitor of the oxidative deamination of L-glutamate with either NAD^+ or $NADP^+$ as coenzyme. Moreover, high concentrations of ADP remove the indications of negative cooperativity from the Lineweaver–Burk plots obtained at high glutamate concentrations. We have sought to understand the effects of ADP by examining its effects not only on initial rate measurements but also on the equilibrium binding of the reduced coenzyme. We have also examined the effects of ethoxyformylation of glutamate dehydrogenase, which in some ways mimics the effects of ADP but in other ways behaves quite differently.

The effects of ADP on the oxidative deamination of L-glutamate can be detailed as follows: (i) With low glutamate concentrations (0.25 mM), where the characteristic indications of negative cooperativity are not seen due to the relative insignificance of the ϕ_0 and ϕ_1 terms in eq 1, the effects of ADP

$$\frac{e}{v_0} = \phi_0 + \frac{\phi_1}{[\text{NAD(P)}^+]} + \frac{\phi_2}{[\text{glutamate}]} + \frac{\phi_{12}}{[\text{NAD(P)}^+\text{–glutamate}]} \quad (1)$$

are simply as a competitive inhibitor with respect to the coenzyme. Similar effects (data not shown) are observed with norvaline as the amino acid substrate, which has been shown previously not to exhibit negative cooperativity (Engel & Dalziel, 1969). (ii) At high glutamate concentrations, where the ϕ_0 and ϕ_1 terms of eq 1 contribute significantly, ADP activates at high $NAD(P)^+$ concentrations, the activation being greater at pH 8 than at pH 7, and has the effect of removing the indications of negative cooperativity from the Lineweaver–Burk plots at saturating ADP concentrations. At lower ADP concentrations (data not shown), the Lineweaver–Burk plots with coenzyme as the varied substrate still show the characteristic breaks observed in the absence of ADP. When the effects of ADP are examined with L-glutamate as the varied substrate, it is clear that ADP drastically reduces the substrate inhibition seen at high glutamate concentrations. The activatory effects of ADP appear to result completely from the relief of this substrate inhibition. At low glutamate concentrations, where the effects of ADP as a competitive inhibitor with respect to the coenzyme would be of greater significance, inhibition of ADP is in fact seen.

There are several mechanisms to explain the substrate inhibition seen at high glutamate concentrations. At high glutamate concentrations, nonproductive binding of glutamate could occur at the active site, giving an enzyme–2(glutamate) nonproductive complex. Alternatively, glutamate could bind to the enzyme in the product phase of the reaction, giving an abortive enzyme–product–glutamate complex. The most likely candidate for such an abortive complex would be an enzyme–NAD(P)H–glutamate complex. The equilibrium binding studies reported here of reduced coenzyme binding to either free enzyme or enzyme–glutamate and the effects of ADP on the stability of these complexes strongly support the suggestion that the substrate inhibition seen at high glutamate concentrations and the activatory effects of ADP are a result of there being a significant contribution of an enzyme–NAD(P)H–glutamate abortive complex in the glutamate dehydrogenase reaction. The binding studies (Figure 2 and Table I) show that glutamate stabilizes the enzyme–NAD(P)H complex, making it harder for reduced coenzyme to be released during the product phase of the reaction. In the presence of glutamate, ADP quite clearly destabilizes the abortive complex, making release of reduced coenzyme easier. These results are consistent with the proposed role of the enzyme–NAD(P)H–glutamate abortive complex in the glutamate dehydrogenase reaction.

The chemical modification studies that used diethyl pyrocarbonate to modify glutamate dehydrogenase provide further insight on the role of ADP in the glutamate dehydrogenase reaction. Diethyl pyrocarbonate has been shown to react with a variety of amino acids, including the side chains of cysteine, tyrosine, lysine, and histidine (Pradel & Kassab, 1968; Melchior & Fahrney, 1970; Thome-Beau et al., 1971; Tudball et al., 1972; Blumberg et al., 1973; Burstein et al., 1974; Lee et al., 1976). However, diethyl pyrocarbonate is particularly useful as a histidine modification reagent as the (ethoxyformyl)histidine derivative can be monitored at 242 nm (Ovadi et al., 1967). In the studies reported here, it is quite clear that modification with diethyl pyrocarbonate results in an activation of glutamate dehydrogenase when assayed in the absence of ADP (Figure 3). The assay conditions used in the modification studies are those under which a contribution from the enzyme–NAD(P)H–glutamate abortive complex would be expected (see earlier discussion). When the modified enzyme is assayed in the presence of ADP, however, an inhibition is observed. The enzyme is clearly protected from modification by diethyl pyrocarbonate by the presence of NADH and glutamate together (Figure 4); however, either on their own or with ADP, they do not protect the enzyme from modification, although 1 mM NADH appears to slow the rate of modification somewhat. The rate of modification by diethyl pyrocarbonate is quite fast, a second-order rate constant of 5.5×10^2 M^{-1} min^{-1} being measured at pH 6, 4 °C. This rate characterizes a very reactive residue. Cousineau & Meighen (1977) report that rates above 10 M^{-1} min^{-1} at pH 6, 25 °C, exclude reactivity of residues other than histidine. Also con-

sistent with ethoxyformylation of histidine residues is the observation that absorbance measurements at 242 nm indicate that the maximal effects of modification by diethyl pyrocarbonate coincide with the ethoxyformylation of a single histidine residue per subunit of glutamate dehydrogenase (Figure 5).

We have examined the kinetic properties of glutamate dehydrogenase containing one (ethoxyformyl)histidine per subunit. When the effects of increasing ADP concentrations are examined (Figure 6), it is apparent that of the two ADP effects discussed earlier the ethoxyformylated enzyme shows only a gradual inhibition at high ADP concentrations. The unmodified enzyme shows an initial activation, followed by inhibition at high ADP concentrations which parallels that observed with the ethoxyformylated enzyme. The ethoxyformylated enzyme, when compared on a mole to mole basis, has a specific activity about 50% higher than that of the native enzyme. Ethoxyformylation produces the full activation that ADP is capable of eliciting; once the enzyme is maximally activated by ethoxyformylation, ADP shows only inhibitory effects. We have also examined whether ethoxyformylation has the same effects on Lineweaver–Burk plots with coenzyme as the varied substrate as does ADP. Figure 7 shows quite clearly that ethoxyformylated enzyme shows the same discontinuities in the Lineweaver–Burk plot as does native enzyme. Ethoxyformylation does not mimic the effect of ADP in removing indications of negative cooperativity from the Lineweaver–Burk plots.

The results with diethyl pyrocarbonate modification, together with the effects of ADP in both initial rate studies and equilibrium binding discussed earlier, allow one to propose a mechanism for the various effects of ADP in the oxidative deamination of L-glutamate. Under conditions where an enzyme–glutamate–NAD(P)H abortive complex plays a role in the overall mechanism, ADP clearly activates the enzyme by destabilizing such a complex. A role for such an abortive complex in the overall reaction catalyzed by glutamate dehydrogenase has been indicated in rapid kinetic studies (di Franco & Iwatsubo, 1971). Ethoxyformylation appears to activate the enzyme in a similar manner, suggesting that a histidine residue either in or near the active plays a role in maintaining such an abortive complex. Since ethoxyformylation of glutamate dehydrogenase does not affect the negative homotropic interactions shown by the enzyme, it is apparent that such abortive complexes do not play an essential role in the mechanism of this cooperativity. Proposals for the mechanism of the effects of ADP on the negative cooperativity are more speculative. However, it seems reasonable to suggest that, since ADP is a competitive inhibitor with respect to $NAD(P)^+$ as shown in the present studies, it may, at high concentrations, simply bind to active sites unoccupied by coenzyme and elicit a similar response in the glutamate dehydrogenase oligomer as does saturation with coenzyme, i.e., lock the enzyme into its most saturated form. Such a mechanism would of course imply that the nicotinamide moiety of the coenzyme was not essential in the negatively cooperative response to coenzyme binding. It has recently been shown (Alex & Bell, 1980) that coenzyme analogues with altered nicotinamide rings do show the same negative homotropic effects in initial rate kinetics as do the natural coenzymes, showing that some alteration of the nicotinamide ring is in fact permissible.

References

Alex, S., & Bell, J. E. (1980) *Biochem. J.* (in press).

Appella, E., & Tomkins, G. M. (1966) *J. Mol. Biol. 18*, 77–89.

Bell, J. E., & Dalziel, K. (1973) *Biochim. Biophys. Acta 309*, 237–242.

Blumberg, S., Holmquist, B., & Vallee, B. L. (1973) *Biochem. Biophys. Res. Commun. 51*, 987–992.

Burstein, Y., Walsh, K. A., & Neurath, H. (1974) *Biochemistry 13*, 205–210.

Cousineau, J., & Meighen, E. (1977) *Can. J. Biochem. 55*, 433–441.

Dalziel, K. (1975) *Enzymes, 3rd Ed. 11*, 1–60.

Dalziel, K., & Engel, P. C. (1968) *FEBS Lett. 1*, 349–352.

di Franco, A., & Iwatsubo, M. (1971) *Biochimie 53*, 153–159.

Egan, R. R., & Dalziel, K. (1971) *Biochim. Biophys. Acta 147*, 32–38.

Engel, P. C., & Dalziel, K. (1969) *Biochem. J. 115*, 621–631.

Frieden, C. (1959) *J. Biol. Chem. 234*, 809–814.

Lee, C. Y., Houston, L. L., & Himes, R. H. (1976) *Biochem. Biophys. Res. Commun. 70*, 50–57.

Melchior, W. B., & Fahrney, D. (1970) *Biochemistry 9*, 251–258.

Melzi D'Eril, G., & Dalziel, K. (1973) *Monogr. Biochem. 26*, 33–46.

Olson, J. A., & Anfinsen, C. B. (1953) *J. Biol. Chem. 202*, 841–856.

Ovadi, J., Libor, S., & Elod, P. (1967) *Acta Biochim. Biophys. Acad. Sci. Hung. 2*, 445–458.

Pal, P. K., Wechter, W. J., & Colman, R. F. (1975) *Biochemistry 14*, 707–715.

Pradel, L., & Kassab, R. (1968) *Biochim. Biophys. Acta 167*, 317–325.

Smith, E. L., Landon, M., Piszkiewicz, D., Brattin, W. J., Langley, T. J., & Melamed, M. D. (1970) *Proc. Natl. Acad. Sci. U.S.A. 67*, 724–730.

Struck, J., & Sizer, I. W. (1960) *Arch. Biochem. Biophys. 86*, 260–266.

Thome-Beau, F., Le-Thi-Lan, Olomuchi, A., & Thoai, N. V. (1971) *Eur. J. Biochem. 19*, 270–275.

Tudball, N., Bailey-Wood, R., & Thomas, P. (1972) *Biochem. J. 129*, 419–425.