THE ROLE OF THE AMMONIUM MOIETY IN THE GLUTAMIC DEHYDROGENASE REACTION*

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Application of the law of microscopic reversibility to a number of possible mechanisms for the glutamic dehydrogenase reaction would suggest that the ammonia moiety participating in the forward direction of the reaction be bound to the enzyme at the same site as the α-amino group of the amino acid participating in the reverse reaction. However, not only is proof of the identity of the site of binding of these two groups lacking in the literature, but no direct evidence has been presented to show that either of them is bound to the enzyme at all.

Figure 1 shows conclusively that the ammonia moiety is a competitive inhibitor of L-glutamate. The K_I values calculated for curves B and C are 2.7×10^{-3} M and 2.2×10^{-3} M respectively. The average of these two values agrees within experimental error with the K_S value of ammonium as a substrate (Frieden, 1959) in the reductive amination reaction.

The glutamic dehydrogenase reaction is customarily written as involving the NH_3 molecule (Dixon and Webb, 1958; Meister, 1959). Figure 2, however, shows that the per cent inhibition of the reverse reaction by the ammonia moiety remains practically independent of pH from about 7 to 9, and then falls sharply with increasing pH. The inhibition has completely disappeared at pH 9.5. The midpoint of this drop in inhibition coincides with the pK_a of ammonia (Harned and Owen, 1930). The conclusion drawn from Figure 1 can now be restated that ammonium ion is a competitive inhibitor for L-glutamate.

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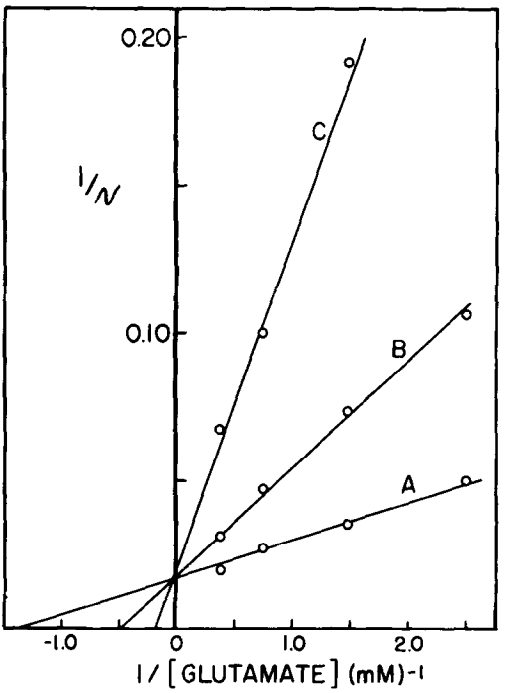

Figure 1. Inhibition of oxidative deamination of L-glutamate by NH_4Cl. Initial reaction velocities were determined at 27.5° C in 0.1 M Tris-HCl buffer, pH 8.25. The initial concentration of DPN was 1 X 10^{-3} M; NH_4Cl concentrations were: (A) 0; (B) 5 X 10^{-3} M; (C) 15 X 10^{-3} M.

This competition between NH_4^+ and the $-NH_3^+$ group of L-glutamate is evidence that each of these groups can be bound to the enzyme-DPN complex, and that such binding must occur at the same site on that complex.

Since the stoichiometry of the reaction requires that an ammonia moiety become attached to α-ketoglutarate in the same position and configuration as the α-amino group of L-glutamate; and since we have shown here that an ammonium ion must be bound to the same site as that α-amino group; it follows that ammonium ion must be the species participating in the reductive amination of α-ketoglutarate catalyzed by glutamic dehydrogenase.

The agreement of the value of K_I for ammonium ion as an inhibitor of the reverse reaction with the value of K_S for ammonium ion as a substrate in the forward reaction suggests that this K_S is a true dissocia-

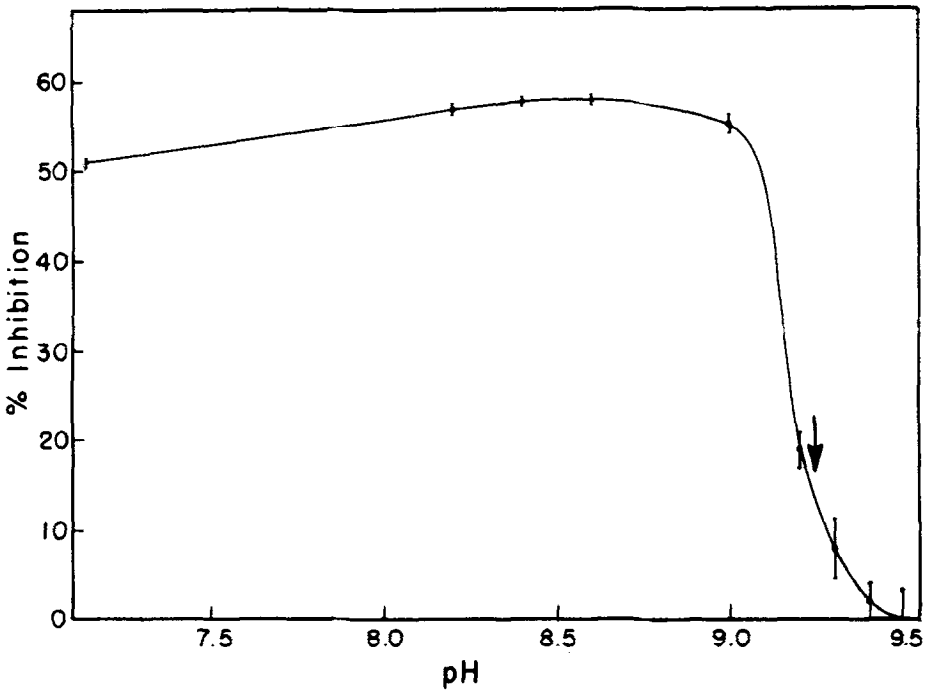

Figure 2. Effect of pH on inhibition by NH_4Cl. Per cent inhibition calculated from initial velocities measured both in the presence and in the absence of 1×10^{-2} M NH_4Cl. Reactions were run in 0.1 M Tris buffer, adjusted to pH as indicated. The initial concentration of L-glutamate was 2.67×10^{-3} M. Other conditions were as described in Figure 1.

tion constant; and further, that the ammonium ion is bound to a site whose properties are the same in the enzyme-DPN complex as in the enzyme-TPNH complex. In view of the pronounced differences in charge distribution in oxidized and reduced pyridine nucleotides (Pullman and Pullman, 1958), it would seem likely that this $-NH_3^+$ binding site is located on the protein itself.

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