THE INVOLVEMENT OF A TRYPTOPHAN RESIDUE OF GLUTAMATE DEHYDROGENASE IN THE BINDING OF L-GLUTAMATE

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We have previously shown that L-glutamate combines with the active GDH*--DPNH complex in such a way as to hinder the dissociation of the coenzyme from the enzyme surface (Fisher and McGregor, 1960). The existence of this ternary complex was first demonstrated spectrophotometrically by Winer and Schwert (1958) and later proven to be the catalytically active complex by Fisher (1960). We show here that some part of this bound glutamate molecule lies in intimate contact with a tryptophan residue on the surface of the enzyme.

The phenomenon leading to this conclusion is shown in Figure 1. Curve A is the difference spectrum between a solution containing GDH and DPNH alone, and a solution containing identical amounts of GDH and DPNH, but with L-glutamate added. Curve B represents a typical solvent perturbation difference spectrum of the N-acetyl, ethyl ester of tryptophan (with D_2O as the perturbing solvent).** The peak-for-peak correspondence of the two spectra is apparent. (The complete difference spectrum, of which Curve A represents only a portion, contains small contributions resulting from red

*(E.C.1.4.1.3)

**Because D_2O has a refractive index less than that of H_2O, it produces perturbation difference spectra whose ordinates are reversed in sign with respect to such spectra produced by most solutions. Curve B has therefore been plotted on a negative ordinate to facilitate comparison with Curve A.

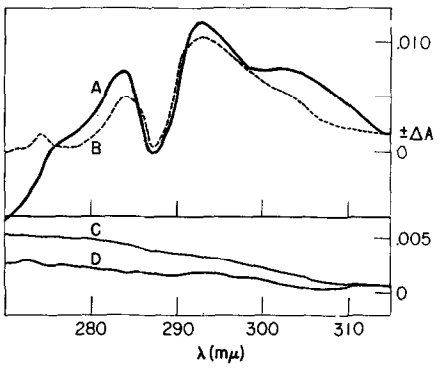

Figure 1. Curve A: Difference spectrum between 1.00 mg/ml GDH and 125 μM DPNH in the front cuvette, and 1 mM L-glutamate in the rear cuvette, both in the sample compartment versus 1.00 mg/ml GDH, 125 μM DPNH and 1 mM L-glutamate in the front cuvette and solvent in the rear cuvette, both in the reference compartment of a Cary 14 spectrophotometer. This tandem arrangement of cells, as described by Herskovits and Laskowski (1962), with their respective components can be represented by the notation: [(GDH, DPNH) (L-glutamate)] - [(GDH, DPNH, L-glutamate) (solvent)] where brackets indicate the respective sample and reference compartments of the spectrophotometer and parentheses indicate individual cells within the compartments. Curve B: [(GDH) (D_2O)] - [(GDH, D_2O) (solvent)]. Curve C: [(GDH) (L-glutamate)] - [(GDH, L-glutamate) (solvent)]. Curve D: [(DPNH) (L-glutamate)] - [(DPNH, L-glutamate) (solvent)]. Difference spectra A, C, and D have been plotted on a negative ΔA ordinate to facilitate comparison with Curve B which was plotted on a positive ordinate. For difference spectra A, C, and D the solvent used was 0.2 M potassium phosphate buffer pH 7.60, and in each case the concentrations of the constituents were: 1.00 mg/ml GDH, 125 μM DPNH and 1 mM L-glutamate. The constituents in Curve B were 46.5 μM N-acetyl-L-tryptophan ethyl ester and 89.5% deuterium oxide, both in 0.2 M potassium phosphate buffer pH 7.60. Matched quartz cuvettes with 1.000 cm pathlengths were used in every case. All difference spectra were recorded on a Cary 14 double-beam, recording spectrophotometer with sample and reference compartments thermostated to 25° C. Slit widths ranged from 0.08 mm to 1.2 mm. Dilution errors were avoided by using Lang Levy micro pipettes prerinsed with the stock solution to be pipetted and by making volumetric dilutions in a constant temperature bath.

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shifts (Leach and Scheraga, 1960) of the 260 and 340 mμ peaks of DPNH. Investigations of this phenomenon will be reported elsewhere.)

Curves C and D of Figure 1 are difference spectra due to interaction of L-glutamate with GDH, and of L-glutamate with DPNH, respectively.

They are seen to be essentially null signals and thus cannot account for the spectrum given by the mixture of all three components. The tryptophan signal, then, occurs only under conditions where the GDH-coenzyme-substrate ternary complex is formed. (This lack of interaction of L-glutamate with enzyme alone as shown by Curve C is the first direct evidence for an obligatory, ordered reaction sequence in the GDH reverse reaction.)

It has been argued elsewhere (Leach and Scheraga, 1960; Williams and Foster, 1959; Yanari and Bovey, 1960; Fisher and Cross, 1965) that any difference in the environment of a given chromophoric residue will produce a difference spectrum characteristic only of that particular chromophore itself; the shape of the difference spectrum by itself yields no detailed information as to the nature of the change in environment. Applying this reasoning to the experimental results shown in Figure 1, we conclude that in the presence of L-glutamate, a tryptophan residue of the GDH-DPNH complex has undergone a change in environment.

While the shape of a difference spectrum is independent of the specific cause of the change in environment producing that spectrum; its sense does depend on the cause of that change. Because the signal observed, Curve A of Figure 1, corresponds to a red shift of the original spectrum of the tryptophanyl chromophore, we can immediately state that its origin cannot be due to an unfolding of the protein in such a way as to expose a previously buried tryptophanyl residue. The observed signal is consistant with any set of circumstances which increases the polarity of the medium immediately surrounding a tryptophanyl residue.

The tryptophan perturbation seen in Curve A cannot be attributed to the increase in refractive index of the gross solvent caused by the addition of glutamate; the glutamate added causes a refractive index change

less than one-fiftieth of the refractive index change responsible for the similar spectrum of Curve B. The phenomenon could, however, be attributed to a fifty-fold increase in the local concentration of glutamate in the vicinity of a tryptophanyl residue of GDH. Such a local increase in concentration is, of course, equivalent to binding.

The demonstration of the nature of the ternary complex previously referred to provides a straightforward explanation of the phenomenon we have reported here: in the glutamate--DPNH--GDH complex, the α-carbon end of the glutamate molecule lies over the nicotinamide ring of the coenzyme, sterically hindering its dissociation; the carboxyl end of the glutamate molecule, then, lies immediately over a tryptophanyl residue of the protein. This tryptophanyl residue must be located about 4 Å from the site of the bound nicotinamide ring--close enough to interact with bound glutamate, but not close enough to be perturbed by bound DPNH.

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