BBA 61143

Thermodynamic parameters of the α-methyl aspartate–aspartate aminotransferase interaction

Although thermodynamic data are of potential use in understanding the mechanism of enzyme–substrate complex formation, surprisingly few pertinent data are available. In this communication we report the thermodynamic parameters characterizing the formation of a complex between L-α-methyl aspartate and aspartate aminotransferase (EC 2.6.1.1). Although α-methyl aspartate is not an actual substrate, it is known to be a competitive inhibitor of the enzyme and forms a Schiff base with the enzyme-bound pyridoxal phosphate, although transamination cannot occur because of the methyl group in the α position[1-3]. Therefore, the α-methyl aspartate–transaminase reaction appears to be representative of enzyme–substrate complex formation.

The procedure for obtaining the binding constant by spectrophotometric titration has been previously described[3]. All data were obtained at pH 8.0, 0.14 M Tris. The pH was measured with a Beckman Expandomatic or Radiometer pH meter at the temperature at which the spectrophotometric measurements were made. The α subform of aspartate aminotransferase was used[4] and DL-α-methyl aspartate was

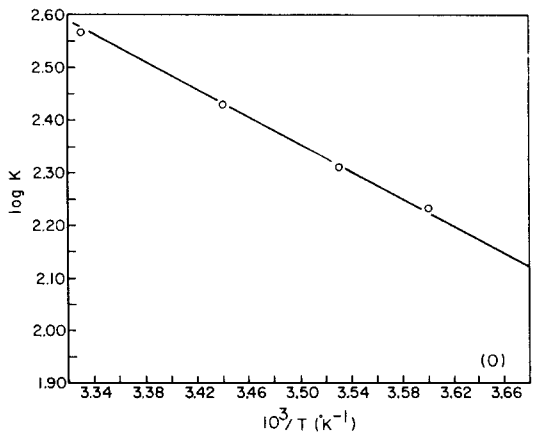

Fig. 1. Plot of the logarithm of the association constant in M^{-1} for the L-α-methyl aspartate–aspartate aminotransferase reaction *versus* the reciprocal absolute temperature. The straight line is that calculated from a least squares analysis. The point at 1.1° (in parentheses) was not used in this analysis.

obtained from Sigma chemicals; as before the assumption was made that only the L isomer binds to the enzyme. Absorption changes were measured at 360 and 430 mμ and the data were analyzed by a weighted least-squares treatment according to the analysis previously presented[3]. Since the form of the enzyme with an absorption maximum at 430 mμ does not bind the amino acid, a small correction ($<5\%$) which varies with temperature was applied to the apparent constants[3]; this required determination of the pK value of the $362 \rightleftharpoons 430$ mμ interconversion of the free

enzyme at various temperatures. The apparent pK value ranged from 6.3 to 6.5 in going from 25° to 5°.

A plot of the logarithm of the binding constant *versus* the reciprocal temperature over the temperature range 5–25° is shown in Fig. 1; a point is also shown for 1.1°, although it is not very reliable because of the difficulties in obtaining spectrophotometric data at such a low temperature. By the usual analysis the standard free energy, enthalpy and entropy changes at 25° were found to be −3.55 (± 0.05) kcal/mole, 5.9 (± 0.8) kcal/mole and 32 (± 3) e.u., respectively. (A least-squares analysis of the data in Fig. 1 was used to obtain the standard enthalpy change.) The positive enthalpy change is rather surprising. The simplest interpretation of this result is that the internal Schiff base of the enzyme is energetically more stable than the Schiff base formed between α-methyl aspartate and the enzyme. The latter forms only because of the favorable entropy change which is probably due to desolvation of the amino acid and increased freedom of the enzyme structure due to the breakdown of the internal aldimine. A loosening of the enzyme structure is also consistent with the reduction of the pyridoxal phosphate Cotton effect in the complex relative to that of the free enzyme[3,5–7]. Thus the enzyme structure appears to be intimately involved in enzyme–substrate complex formation.

The spectrum of the complex is relatively insensitive to temperature: the extinction coefficient at 360 mμ varies from $5.1 \cdot 10^3$ to $4.9 \cdot 10^3$ M^{-1} cm^{-1} in going from 25° to 5°, while the extinction coefficient at 430 mμ changes from $3.8 \cdot 10^3$ to $4.2 \cdot 10^3$ M^{-1} cm^{-1}. These changes suggest that the complex exists in more than one structural form. By analogy with the enzyme itself, the spectral peaks of the complex at 430 and 360 mμ might be expected to represent different ionization states. However, the spectrum of the complex is invarient with pH over the range 5.0–11.0. Thus if different ionization states are involved the associated pK values are either unusually high or low. Finally preliminary kinetic results suggest the actual mechanism of complex formation is quite complex[8]. However, the qualitative interpretation given above to the overall thermodynamic constants still appears to be appropriate and these constants may prove useful in the interpretation of the detailed mechanism.

This work was supported by a grant from the National Institutes of Health (GM 12944).

Department of Chemistry,
Cornell University,
Ithaca, N.Y. (U.S.A.)

GORDON G. HAMMES
JOHN F. TANCREDI

1 W. T. JENKINS, D. A. YPHANTIS AND I. W. SIZER, *J. Biol. Chem.*, 234 (1959) 51.
2 A. BRAUNSTEIN, *Vitamins Hormones*, 22 (1964) 453.
3 P. FASELLA, A. GIARTOSIO AND G. G. HAMMES, *Biochemistry*, 5 (1966) 197.
4 M. MARTINEZ-CARRION, C. TURANO, F. RIVA AND P. FASELLA, *Biochem. Biophys. Res. Commun.*, 20 (1965) 206.
5 Y. TORCHINSKY AND L. G. KORENEVA, *Biochim. Biophys. Acta*, 79 (1964) 426.
6 P. FASELLA AND G. G. HAMMES, *Biochemistry*, 3 (1964) 530.
7 P. FASELLA AND G. G. HAMMES, *Biochemistry*, 4 (1965) 801.
8 G. G. HAMMES AND J. L. HASLAM, in preparation.

Received May 22nd, 1967