## Binding Sites in the Active Center of Glutamic-Aspartic Transaminase

Combination of substrates or inhibitors with glutamic-aspartic transaminase can be studied by measuring their effectiveness as substrates or inhibitors in transamination and also by their effects on the absorption spectrum of the enzyme[1]. Previous studies with keto substrates and dicarboxylic acid analog inhibitors[2,3] have shown that for binding to the active center of transaminase a compound should have two carboxyl groups in *cis* position separated by more than one and less than five carbon atoms. Amino substrates also react with the enzyme and change its spectrum[4]. In the present investigation we have measured the ability of amino compound analogs of amino substrates to act as substrates or inhibitors of the transamination reaction. At the same time their effects on the enzyme spectrum were measured. An attempt was then made to correlate chemical structure with reactivity in the transaminase system. Transaminase activity was measured by the determination of the $C^{14}$-glutamate formed in 1 h at 37° after addition of $C^{14}$-α-ketoglutarate to a solution of aspartate and enzyme. The $C^{14}$-glutamate formed was measured by its radioactivity and the intensity of its color with ninhydrin after separation of the reaction mixture by paper electrophoresis (1000 V, 80 mA). Radioactivity was measured by scanning the paper strip with a low background autoscanner, Model 880 (Vanguard Instrument Company).

Certain conclusions relating chemical structure of amino compounds to their effectiveness as substrates or inhibitors of the transamination reaction and to their influence on the enzyme spectrum can be drawn from the Table. The size of the molecule is critical for all effects and is optimal for four to five carbon atoms. Aminomalonate is neither a substrate nor inhibitor nor does it influence the enzyme spectrum. At the opposite extreme, α-aminopimelate is a very poor substrate and inhibitor and has a small effect on the spectrum. α-Aminoadipate is a fair substrate, a good inhibitor, and is fairly active in producing a spectral change. The indispensable role played by the two carboxyl groups of aspartate or glutamate or other analogs can be seen from studies made with derivatives such as asparagine, isoglutamine, α-aminobutyrate and aspartic acid diethylester, which are not substrates and do not influence the enzyme spectrum (Table). Any group with an acidity equivalent to a carboxyl group functions as well as a carboxyl group (L-cysteine sulfinate and L-cysteate, Table).

It appears that an amino substrate binds to the active center by its two carboxyl groups. These should be separated by more than one and less than five carbon atoms (three seems to be optimal). In addition, amino substrates should have available for binding: (1) hydrogen of the amino group (negative results for binding and inhibition by glycyl-L-aspartate and glycyl-L-glutamate, Table); (2) hydrogen in the α-position (failure of α-methyl aspartate and α-methyl glutamate to act as substrates); and (3) hydrogen in the β-position (difference in behavior of the derivatives of β-hydroxyaspartate and β-methylaspartate and β,β-dimethylaspartate). The inhibition of the transamination reaction by these amino substrate analogs suggests that the same sites are involved in the binding of these compounds and of regular substrates.

In column I is shown the transamination of the analogs with $C^{14}$-α-ketoglutarate as measured by $C^{14}$-glutamate formed; in column II, their effect on the transaminase spectrum; and in column III, their behavior as inhibitors[a]

| Amino acid | $C^{14}$-glutamate formed (μmoles) | Increase in absorption (333 mμ) | Inhibition of transaminase % |
|---|---|---|---|
| L-aspartate | 0.31 | 0.320 | |
| L-asparagine | 0.02 | 0.020 | 20 |
| Erythro-DL-β-hydroxyaspartate | 0.42 | [b] | 90 |
| Threo-DL-β-hydroxyaspartate | 0.02 | 0.090 | 10 |
| α-methyl-DL-aspartate | – | [b] | 50 |
| β-methylaspartate | 0.41 | 0.120 | 40 |
| β-β-dimethylaspartate | – | – | – |
| Glycyl-L-aspartate | – | – | – |
| L-glutamate | 0.34 | 0.390 | 70 |
| α-methyl-DL-glutamate | | – | |
| L-isoglutamine | – | – | – |
| Glycyl-L-glutamate | – | – | – |
| L-cysteine sulfinate | 0.72 | 1.100 | 95 |
| L-cysteate | 0.18 | 0.280 | 40 |
| α-Aminomalonate | – | – | – |
| L-α-aminobutyrate | – | – | – |
| L-α-aminoadipate | 0.22 | 0.270 | 18 |
| DL-α-aminopimelate | 0.01 | 0.180 | 5 |

[a] Transamination reaction was studied in a solution of 0.3 ml 0.05 *M* Tris buffer, pH 8.5, containing 1 μmole $C^{14}$-α-ketoglutarate, 2 μmoles of the corresponding amino compound and 10 μg of transaminase. After incubation for 1 h at 37°, the system was analyzed as described in the text. – Inhibition of transamination was studied by adding 100 μmoles of the corresponding amino compound to the usual transamination system[5]. – The effect on the transaminase spectrum was studied in an enzyme solution (30 mg protein/ml) in 0.02 *M* phosphate buffer, pH 5.5. The final concentration of the amino compound was $1 \cdot 10^{-2}$ *M*. [b] For erythro-DL-β-hydroxyaspartate there is a sharp maximum at 492 mμ, while for α-methyl-DL-aspartate this maximum is at 430 mμ.

*Résumé.* La capacité de plus que vingt des amines composées (analogues aux acides glutamique et aspartique) d'agir comme substrates ou inhibiteurs de la transamination a été étudiée par l'emploi d'acide $C^{14}$-α-kétoglutarique. La comparaison de la structure chimique des amines composées avec leur réactivité comme substrates ou inhibiteurs, a fourni des données sur la specificité de la transaminase.

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