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# EXCHANGE REACTIONS CATALYZED BY γ-AMINOBUTYRIC–GLUTAMIC TRANSAMINASE

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## SUMMARY

γ-Aminobutyrate-glutamate transaminase catalyzes exchange reactions between γ-aminobutyrate and succinic semialdehyde and between L-glutamate and α-ketoglutarate. Several analogs were tested for inhibition of each of the exchange reactions; for a given analog, approximately equal inhibitions were obtained in both exchange reactions. Glutarate and butyrate were the most effective analogs tested.

## INTRODUCTION

The purification of a soluble γ-aminobutyric-glutamic transaminase from a strain of *Pseudomonas fluorescens* has permitted a kinetic analysis of the course of the reaction described by equation (1). The results of this work were consistent with the hypothesis

that only binary complexes of the transaminase and its substrates are involved and permitted the formulation of a mechanism[1] summarized by equations (2) to (5).

$$\gamma\text{-aminobutyrate} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{succinic semialdehyde} + \text{L-glutamate} \quad (1)$$

$$RCH_2NH_2 + ECHO \rightleftharpoons E' \quad (2)$$

$$E' \rightleftharpoons RCHO + ECH_2NH_2 \quad (3)$$

$$RCOCOOH + ECH_2NH_2 \rightleftharpoons E'' \quad (4)$$

$$E'' \rightleftharpoons RCH_2NH_2COOH + ECHO \quad (5)$$

The enzyme may be pictured as an aldehyde (ECHO), as an amine ($ECH_2NH_2$) and as two enzyme–substrate complexes (E′, E″) of unspecified structure. Since pyridoxal phosphate has not been definitely identified as the prosthetic group of the transaminase it is only by analogy to other transaminases that the aldehyde and amino forms of the enzyme may be equated with the equivalent forms of the coenzyme. The symbol, R, represents $-CH_2COOH$, the radical common to all four substrates.

Implicit in this formulation is the capacity of the enzyme to catalyze an exchange of isotope between either of the substrate amino acids and their respective carbonyl analogues. Thus, incubation of the enzyme with γ-[$^{14}$C]aminobutyrate and succinic semialdehyde should lead to the partition of the isotope between the pair in a manner determined[2] by their respective concentrations (equations (2) and (3)). In an entirely analogous fashion an exchange reaction between L-glutamate and α-ketoglutarate (equations (4) and (5)) would be expected.

In the present report, it is demonstrated that γ-aminobutyric-glutamic transaminase catalyses isotopic exchange between γ-aminobutyrate and succinic semialdehyde as well as between glutamate and α-ketoglutarate. The inhibitory properties of several structural analogs of the substrates are examined with respect to the exchange reactions and the implications of these results for the mechanism of the overall transaminase reaction are discussed.

## EXPERIMENTAL

### *Methods and materials*

γ-[1-$^{14}$C]aminobutyric acid (specific activity 1 μC/μmole) and α-[1,2-$^{14}$C]ketoglutaric acid (0.9 μC/μmole) were obtained from Merck and Co., Ltd. of Canada. The enzyme preparation was Fraction II obtained from a strain of *Pseudomonas fluorescens* by methods described previously[1].

Succinic semialdehyde, α-ketoglutarate and γ-aminobutyrate concentrations were determined spectrophotometrically[3].

Measurements of the transamination and exchange reactions were based upon a method for the separation of the amino acid substrate by adsorption on Dowex 50 and subsequent elution with ammonia. The enzyme incubations were carried out in a total volume of approx. 0.1 ml at 30° and terminated by the addition of 0.1 ml of 0.1 *M* sodium bisulfite. The samples were transferred quantitatively to a water-washed column of 200–400 mesh Dowex 50 X-8 ($H^+$ form) consisting of 1 ml of wet resin in a 1-cm diameter, coarse, sintered-glass funnel. After addition of the incubation mixture, the columns were eluted with 7 ml of distilled water; the eluates were

collected in graduated test tubes. When the radioactivity of the amino acid was to be determined, a second elution with 7.0 ml of 10 % $NH_4OH$ (concentrated ammonium hydroxide diluted ten-fold) was performed. Eluate volumes were recorded and 100-μl aliquots were added to 10 ml of liquid scintillation phosphor solution. The phosphor solution contained 0.3 % 2,5-diphenyloxazole in toluene–methanol (3:1, v/v). A Packard Model 314 liquid scintillation counter was used for $^{14}C$ determinations.

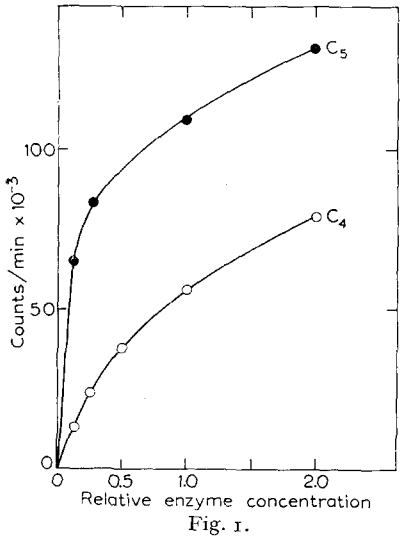



Fig. 1.

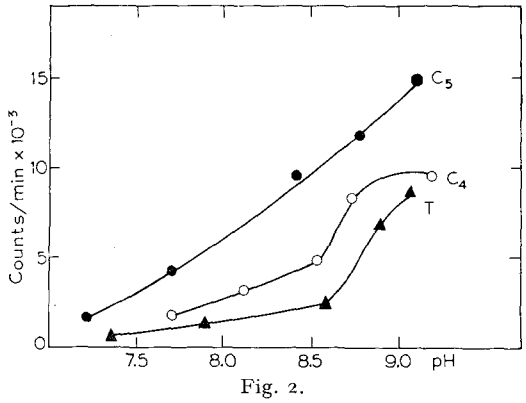



Fig. 2.

Fig. 1. Isotope exchange as a function of enzyme concentration. ($C_4$) Each tube contained 0.126 μmole of γ-aminobutyrate, 1.35 μmoles of succinic semialdehyde, 5 μmoles of sodium pyrophosphate buffer, pH 8.4, and 10 μl of enzyme in a final volume of 105 μl. ($C_5$) Each tube contained 0.24 μmole of α-ketoglutarate, 1.25 μmoles of glutamate, 5 μmoles of sodium pyrophosphate buffer at pH 8.4 and 10 μl of enzyme in a final volume of 100 μl. In $C_4$, the radioactivity was initially in the γ-aminobutyrate and the curve represents the accumulation of radioactivity in the water eluates, *i.e.*, in succinic semialdehyde. In $C_5$, the radioactivity was initially in the α-ketoglutarate and the curve represents the accumulation of radioactivity in the ammonia eluates, *i.e.*, in glutamate. The value of 1.0 on the abscissa corresponds to the amount of enzyme used in the experiments reported in Table I. Incubation was for 12 min at 25°.

Fig. 2. Effect of pH on exchange reactions. ($C_4$) Each tube contained 10 μmoles of pyrophosphate buffer, 0.25 μmole of γ-[$^{14}C$]aminobutyrate, 5 μmoles of succinic semialdehyde, and 1.6 μl of enzyme in a total volume of 115 μl. ($C_5$) Each tube contained 10 μmoles of pyrophosphate buffer, 0.5 μmole of α-[$^{14}C$]ketoglutarate, 1.25 μmoles of glutamate, and 1.6 μl of enzyme in a total volume of 110 μl. Incubation was for 10 min at 25°. The pH of each sample was measured on an aliquot of the incubation mixture.

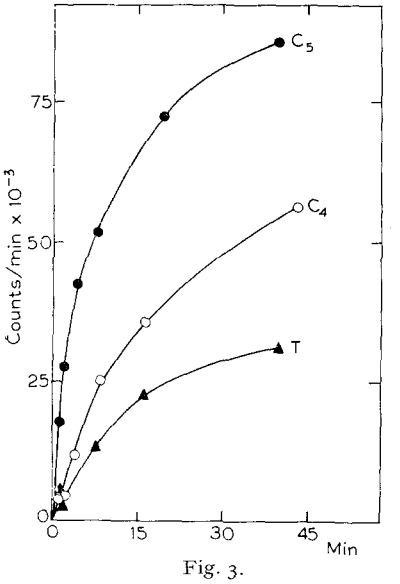



Fig. 3.

Fig. 3. Time course of the exchange reactions and of transamination. Each incubation mixture contained 50 μmoles of potassium pyrophosphate at pH 8.4 and 40 μl of enzyme in a total volume of 0.79 ml. Flask $C_4$ (○) contained, in addition, 1.26 μmoles of γ-[1-$^{14}C$]aminobutyrate and 13.5 μmoles of succinic semialdehyde. Flask $C_5$ (●) contained 3.0 μmoles of α-[1,2-$^{14}C$]ketoglutarate and 12.5 μmoles of L-glutamate. Flask T (▲) contained 3.0 μmoles of α-[1,2-$^{14}C$]ketoglutarate and 12.5 μmoles of γ-aminobutyrate. Each point represents the radioactivity found in that substrate which was non-radioactive initially, a 50-μl aliquot being withdrawn at the indicated time. Incubation was at 25°.

The relative rates of the two exchange reactions as functions of enzyme concentration, pH and incubation time are shown in Figs. 1, 2 and 3, respectively. The $\gamma$-aminobutyrate-succinic semialdehyde exchange ($C_4$ exchange reaction) proceeds more slowly than the glutamate-$\alpha$-ketoglutarate exchange ($C_5$ exchange reaction). In Fig. 3, the initial rate of the over-all transamination reaction may be noted to approximate the initial rate of the $C_4$ exchange reaction*.

The enzyme preparation was ineffective in catalyzing the transamination of $\alpha$-ketoglutarate with aspartate, alanine or pyridoxamine phosphate; neither glutamate nor $\gamma$-aminobutyrate transaminated with added pyridoxal phosphate.

The effect of a number of structural analogs was examined with respect to the ability of such compounds to influence the rate of the exchange reactions. The analogues were tested at a concentration of 0.016 *M* in a system similar to that described for Fig. 1. Insignificant effects were found with $\alpha$-methylglutamate, $\beta$-aminobutyrate, $\alpha,\gamma$-diaminobutyrate, putrescine and ethanolamine phosphate. Both DL- and allo-$\beta$-hydroxyglutamate at a concentration of 0.032 *M* were similarly ineffective. However, both exchange reactions were markedly inhibited by $\beta$-hydroxy-$\gamma$-aminobutyrate, butyrate and glutarate (Table I).

TABLE I

EFFECT OF STRUCTURAL ANALOGS ON VELOCITY OF EXCHANGE REACTIONS

$\beta$-hydroxyglutamate (DL, allo) was present at a concentration of 0.032 *M*. All other analogs were present at a concentration of 0.016 *M*. ($C_4$) Each tube contained 0.126 $\mu$moles $\gamma$-[$^{14}$C]aminobutyrate, 1.35 $\mu$moles succinic semialdehyde, 5 $\mu$moles sodium pyrophosphate buffer at pH 8.4, and 5 $\mu$l of enzyme in a final volume of 100 $\mu$l. ($C_5$) Each tube contained 0.30 $\mu$mole $\alpha$-ketoglutarate, 1.25 $\mu$moles glutamate, 5 $\mu$moles sodium pyrophosphate buffer at pH 8.4, and 5 $\mu$l enzyme in a final volume of 100 $\mu$l. Samples were incubated 12 min at 25°.

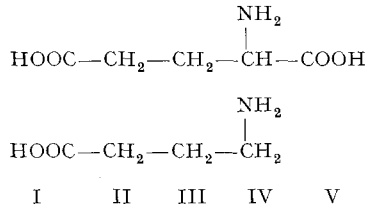
| *Analog* | *% of control* | |
|---|---|---|
| | $C_4$ | $C_5$ |
| DL-$\beta$-hydroxy-$\gamma$-aminobutyrate | 50 | 38 |
| $\beta$-aminobutyrate | 99 | 118 |
| Butyrate | 37 | 62 |
| Glutarate | 37 | 47 |
| Succinate | 85 | 98 |
| $\alpha$-methyl glutamate | 90 | 78 |
| $\beta$-hydroxy glutamate | 93 | 74 |

## DISCUSSION

Previous analysis of the $\gamma$-aminobutyric-glutamic transaminase reaction indicated the binary nature of the enzyme-substrate complexes involved[1]. The isotope exchange technique employed here permitted a study of the characteristics of the partial

* In Fig. 3 the rate of reaction T is seen to decrease more rapidly than the rate of $C_4$. It may be noted that the equilibrium constant for $C_4$ and $C_5$ is 1 while that for reaction T is 0.1 and thus less favorable to the accumulation of succinic semialdehyde. Moreover, the initial rates of $C_4$, $C_5$ and T cannot be expected to become identical even if more adequate methods were available to measure them. The steady state equations describing the initial velocities may be shown to contain different constants in each case. Not only are rate constants combined in T from $C_4$ and $C_5$, but a substrate inhibition of $\alpha$-ketoglutarate on T has been demonstrated[1]. Although the data seem to support the rate limiting role of the initial reaction between $\gamma$-aminobutyrate and enzyme, the rate constants of one or more intermediate complexes may be of significance.

reactions, *i.e.*, those involving interaction of the enzyme with either the four-carbon or the five-carbon substrate pair.



I II III IV V

If the two substrate pairs can be assumed to interact with the coenzyme at the carbonyl–amine position, their interaction with the apoenzyme must be predominantly through the carboxyl functions. The stereospecificity of the enzyme for L-glutamic acid requires that the α-carboxyl (V) must participate in the orientation of the five-carbon pair. Since the enzyme also distinguishes between glutamic acid and other α-amino acids, it is evident that the distal carboxyl (I) is a further requirement. The bacterial enzyme distinguishes between γ-aminobutyrate and both β-alanine and Δ-aminovalerate, implying that the distance between I and IV is critical. The slow transamination of β-hydroxy-γ-aminobutyrate* indicates that substitution at III is compatible with activity.

An attempt was made to inquire into the question of the number of sites involved for each of the exchange reactions since it is evident that the common carboxyl function (I) might occupy the same or a different locus on the enzyme surface for the two substrate pairs. If the interaction of the substrates differs only at V, any competitive interaction at the site for I should interfere with both exchange reactions. Competitive interactions which involved only V would be expected to interfere largely with the glutamate–ketoglutarate exchange. In no case (Table I) was a selective action elicited which could inhibit one exchange reaction and not the other. Carboxylic acids which can interact at I or at I and V might be expected to inhibit the $C_4$ and $C_5$ exchange reactions, respectively. In fact, butyrate and glutarate inhibit both exchange reactions and are about equally effective. In contrast, succinate is relatively ineffective. With the exception of β-hydroxy-γ-aminobutyrate, a substrate of the enzyme, other closely related compounds had only minor effects on the exchange reactions.

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