Stereospecificity of Enzymatic Transamination of γ-Aminobutyrate

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Summary The stereospecific removal of the 4-*pro*-*R*-hydrogen of γ-aminobutyrate (GABA) has been demonstrated with a bacterial ω-amino acid : pyruvate aminotransferase, while stereospecific removal of the 4-*pro*-*S*-hydrogen has been demonstrated with α-oxoglutarate requiring bacterial GABA transaminase.

Two categories of pyridoxal-P dependent aminotransferases can be recognized: those which act at the chiral α-carbon of an α-amino acid substrate[1] or those which act at the prochiral ω-amino carbon of such molecules as lysine,[2] ornithine,[3] or GABA.[4] In the former category homogeneous aminotransferases are known for both L-α-amino acids[1] and for D-α-amino acids.[5] We here report the stereospecificity of a homogeneous bacterial ω-amino acid : pyruvate aminotransferase (from *Pseudomonas* sp F-126,[6] 3·5 U/mg) acting at the prochiral γ-carbon (C-4) of GABA (equation 1), as well as that for a crude

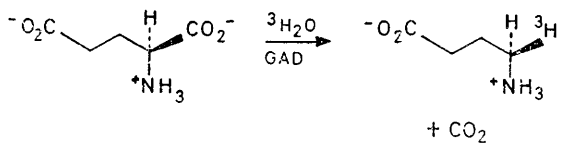
$$\text{GABA} + \text{pyruvate} \overset{\text{E.PLP}}{\rightleftharpoons} \text{succinic semialdehyde} + \text{L-alanine} \quad (1)$$

E.PLP = apoenzyme–PLP complex

preparation of GABA transaminase (GABA-T) from *Pseudomonas fluorescens* ('Gabase,' Sigma), by preparation

and use of 4-*R*-[^{3}H]- and 4-*S*-[^{3}H]-GABA. To our knowledge this is the first such determination for GABA. Chirality assignment depends on the recent demonstration[7] that *E. coli* glutamate decarboxylase replaces CO_2 with H^+ with net retention of configuration.

R-[^{3}H]GABA

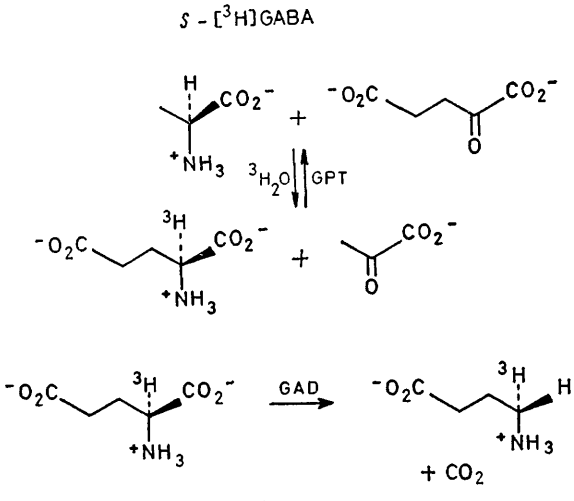


(2)

GAD = glutamate decarboxylase

4-*R*-[^{3}H]GABA was prepared as in equation (2) using 100 μmol L-glutamate, 10 mCi ^3H_2O (New England Nuclear), and 10 U *E.coli* glutamate decarboxylase (25 U/mg, Sigma) in 1 ml at 37 °C, pH 5·0 Radioactive GABA was isolated by Dowex 50 H^+ column chromatography, its purity checked by t.l.c., and its concentration evaluated by Gabase (0·4 U/mg, Sigma). The 4-*S*-[^{3}H]GABA was generated as in equations (3) and (4), with tritium introduced from 10 mCi ^3H_2O by 10 U L-alanine transaminase (84 U/mg, Sigma), using 100 μmol L-alanine in 1 ml at 37 °C, pH 8·0. The 2-*S*-[^{3}H]glutamate was purified by

S - [^{3}H]GABA



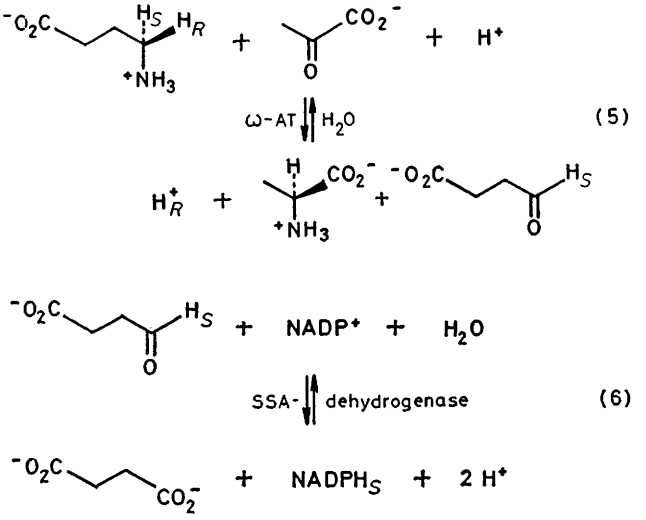
(3)

(4)

GPT = L-alanine transaminase (glutamic : pyruvic transaminase)

Dowex 1 HCO_2^- column chromatography, its purity and concentration evaluated by amino acid analysis, and then decarboxylated in H_2O (acetate buffer); the 4-*S*-[^{3}H]-GABA was purified as above.

On chiral oxidation of GABA by the ω-amino acid : pyruvate aminotransferase, one of the two prochiral hydrogens at C-4 will be exchanged into water (some fraction may be transferred to alanine[8]) and the other will remain in the product succinic semialdehyde. To trap this latter hydrogen in a stable and readily isolable form, the transamination was coupled to the NADP-dependent succinic semialdehyde dehydrogenase (equations 5 and 6) to achieve direct transfer of the aldehyde hydrogen to C-4 of NADPH.



(5)

(6)

ω-AT = ω-aminoacid : pyruvate aminotransferase; SSA = succinic semialdehyde

The dehydrogenase activity is present in Gabase from Sigma, a crude *Pseudomonad* preparation containing GABA-T activity as well. The undesired GABA-T molecules were irreversibly inactivated with gabaculine[9] (gift of Dr. R. R. Rando), and excess of reagent was separated from succinic-semialdehyde dehydrogenase activity by Sephadex G-25 gel filtration. In the case of the GABA-T determination, Gabase was used directly. Incubations of 4-*R*-[^{3}H]- and 4-*S*-[^{3}H]-GABA were conducted according to the Table and

TABLE. Stereochemical selectivity in GABA transamination[a]

Enzyme	Substrate	Specific activity /nCi μmol^{-1}	Product	μmol formed	Specific activity/nCi μmol^{-1} Observed	Predicted
ω-Amino acid: pyruvate aminotransferase	4-*R*-[^{3}H]GABA	39·4	NADPH	2·50	5·89[b]	0·00
			H_2O	2·50	19·0	<39·4[c]
	4-*S*-[^{3}H]GABA	65·9	NADPH	2·75	66·1	65·9
			H_2O	2·75	9·19[d]	0·00
GABA-T (α-oxoglutarate requiring)	4-*R*-[^{3}H]GABA	39·4	NADPH	2·50	36·7	39·4
			H_2O	2·50	11·6[d]	0·00
	4-*S*-[^{3}H]GABA	65·9	NADPH	2·50	5·52	0·00
			H_2O	2·50	48·2	<65·9[c]

[a] Incubations of ω-amino acid : pyruvate aminotransferase (0·3 mg) proceeded at 25 °C in 100 mM KPP_i buffer, pH 8·6, containing 1·0 mM β-mercaptoethanol. Pyruvate was added in aliquots to maintain the concentration at 1·5—2·0 mM. Incubations of GABA-T 0·044 U) proceeded at 37 °C in the same buffer with 10 mM α-oxoglutarate. In each case 5·0 μmol of NADP and stereo-labelled GABA were initially present. Product formation (NADPH) was monitored at 340 nm. [b] Charcoal was added to this solution to absorb NADPH and then filtered. The filtrate contained 26% of the radioactivity, indicating that this tritium was on exchangeable positions of the NADPH and not the nicotinamide ring. [c] This value is subject to isotope selection, solvent–solute exchange, and internal transfer to alanine or glutamate formed. [d] This may contain some [^{3}H]succinic semialdehyde (see text).

equations (5) and (6). Product NADP^{3}H was separated from other tritiated molecules on 0·6 × 6·0 cm DEAE Sephadex A-25 columns (H_2O wash, elution of NADP^{3}H with 2 N NH_4Cl, pH 8·2), while unchanged [^{3}H]GABA was separated from ^3H_2O on Dowex 50 H^+ columns.

The Table shows that the ω-amino acid : pyruvate aminotransferase has a clear preference for removal of the *pro-R* hydrogen at C-4 of GABA (shown in equation 5) during oxidative deamination, while the *pro-S* hydrogen remains bound to C-4 in succinic semialdehyde, available for subsequent hydride transfer to NADP. This stereoselectivity corresponds to that experienced by glycine when it is processed by L-specific α-amino acid aminotransferases.[10] On the other hand, it may be seen in the Table that GABA-T exhibits the opposite stereochemical preference. This is in agreement with the recent observation that the (+)-*S* isomer of γ-acetylenic-GABA inactivates GABA-T.[11] An *R,S* mixture of γ-acetylenic-GABA does not inactivate the ω-amino acid aminotransferase. It may be that these opposing stereochemical preferences reflect differing modes of binding of GABA in the active sites of the two enzymes.

The 'predicted' column in the Table indicates the specific activities one would expect to find in the products, given the stereochemical outcome observed, if the reactions were terminated and products were separated instantaneously. However, in reality, the reactions were allowed to proceed to *ca.* 50% completion (indicated by μmol formed from 5·0 μmol of starting GABA) and then applied directly to the DEAE columns, without quenching, to avoid destruction of NADPH. Thus, the reactions could continue to some extent on the DEAE columns with production of untrapped succinic semialdehyde which would be eluted with H_2O. Also it should be noted that the specific activity of product H_2O would be diminished by any isotopic selection during the course of the reaction and by proton exchange both during the reaction and on the columns.

(*Received, 5th March* 1979; *Com.* 216)

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