Amino Acids (1993) 5: 33–37

Amino
Acids
© Springer-Verlag 1993
Printed in Austria

Action of the phosphonic analogue of tyrosine and of other tyrosine derivatives on rat liver tyrosine aminotransferase

A. Iron, E. Neuzil, and **A. Cassaigne**

Département de Biochimie Médicale et Biologie Moléculaire, Université de Bordeaux 2, Bordeaux, France

Accepted August 16, 1992

Summary. Tyrosine transamination has been investigated *in vitro* with a preparation of rat liver tyrosine aminotransferase in the presence of several structural derivatives of the substrate, including the phosphonic analogue. The transamination by tyrosine aminotransferase (TAT) needs the presence in the substrate molecule of free amino and carboxylic groups, a three-carbon aliphatic chain, a para-phenolic hydroxylic function and a L-configuration. Some tyrosine analogues can markedly disturb the Tyr-TAT association: the chief structural modifications are (i) the removal of the free amine function in a compound still possessing a para-hydroxylic and a carboxylic group, (ii) the change of the carboxylic function by another acidic group, especially a phosphonic one, (iii) a disubstitution in positions 3 and 5. In every situation, the presence of a para-hydroxylic group is compulsory to observe an inhibitory effect.

Keywords: Amino acids – Rat liver tyrosine aminotransferase – Tyrosine derivatives – Phosphonic analogue of tyrosine

Introduction

The elimination of the amino group of tyrosine is catalyzed by L-tyrosine: 2-oxoglutarate aminotransferase (TAT; EC.2.6.1.5), a pyridoxal phosphate (PLP) dependent and inducible phosphoprotein enzyme, chiefly localized in the liver (Cannelakis and Cohen, 1956a).

The human enzyme is of particular interest since tyrosine aminotransferase deficiency is often implicated in congenital hypertyrosinemia, either isolated (Lemonnier et al., 1979; Kida et al., 1982) or associated with the lack of other enzymes (Lindblad et al., 1977).

In this paper, we study the specificity of the aminoacid substrate of TAT using several tyrosine analogues, among which DL-1-amino-2-(*p*-hydroxyphenyl)-ethylphosphonic acid (Tyr-P); this phosphonic analogue of tyrosine already

appeared to be a useful investigation tool in the field of tyrosine metabolism (Iron et al., 1981)

Material and methods

Liver tyrosine aminotransferase was prepared from male Wistar rats according to Valeriote et al. (1969), after induction by intraperitoneal injection of triamcinolone. An enzyme preparation from four livers was obtained, by going through the different steps of ammonium sulphate fractionation and heat treatement, described by Valeriote et al. (1969).

The activity of the enzyme preparation was determined using an incubation mixture containing: L-Tyr, 0–5.4 mM; α-ketoglutarate, 20 mM; PLP, 0.4 mM; phosphate buffer 0.05 M, pH 7.6. After a preincubation at 37°C (15 min), the reaction was initiated by adding the enzyme preparation containing bovine serum albumin (5 mg/ml). The reaction was stopped after 10 min with 10N KOH. After 30 min, a time interval necessary to decarboxylate *p*-hydroxyphenylpyruvate in an alkaline medium, the resulting *p*-hydroxybenzaldehyde was then determined by a spectrophotometric assay (Diamondstone, 1966). The activity of the enzyme preparation was expressed in units per mg of liver proteins, a unit corresponding to the formation of one micromole of *p*-hydroxyphenylpyruvate per min at 37°C. The Michaelis constant of the enzyme was 1.8 mM, a value close to published data (Hayashi et al., 1967; Voigt and Sekeris, 1978).

Experiments performed to determine the structural specificity of tyrosine analogues as substrates were carried out using similar conditions, with a 10 mM substrate concentration, except for the less soluble compounds which were assayed at a final concentration of 2.5 mM (D-Tyr, DL-*o*-Tyr, DL-*m*-Tyr, L-Phe, APB, AMT). Aliquots were taken after 10 min and 4 h and the occurrence of glutamate formed from α-ketoglutarate checked by paper chromatography (Smith, 1969); positive results were followed by new assays in which the reaction was stopped with HCl and the amount of glutamate quantitatively determined by ion-exchange chromatography (Beckman Analyzer).

Determination of TAT activity, when tyrosine was used as a substrate (1 mM) in the presence of a putative inhibitor (10 mM; I/S = 10), was performed by the spectrophotometric determination of *p*-hydroxybenzaldehyde, except in some cases where the spectral characteristics of the compound tested interfered with a measurement at 331 nm: the enzyme activity was then evaluated by determining the amount of the unreacted tyrosine by ion exchange chromatography.

The 25 molecules screened as substrate and/or inhibitor on the TAT preparation were: L-Tyr, *p*-hydroxyphenylacetic acid (pHPA), DL-*p*-hydroxyphenylglycine from Fluka, Switzerland; DL-1-amino-2-phenyl-ethylphosphonic acid (Phe-P), DL-1-amino-2-(*p*-hydroxyphenyl)-ethylphosphonic acid (Tyr-P), L-Phe, DL-1-amino-4-phenylbutyric acid (APB), D-Tyr, L-3-iodo-Tyr (MIT), L-3,5 diiodo-Tyr, 2 H_2O (DIT), DL-3,4 dihydroxyphenylalanine (DOPA) from Calbiochem, USA; L-tyrosinamide from Mann, USA; tyramine hydrochloride from Prolabo, France; L-N-acetyl-Tyr from California Corp., USA; DL-α-methyl-Tyr (AMT) from Aldrich, USA; L-3-fluoro-Tyr (MFT) from Bayer, Germany; DL-phenyl-Gly, DL-*p*-fluoro-Phe, DL-β-phenyl-β-Ala, DL-*p*-amino-phenylglycine from California Foundation, USA; DL-*o*-Tyr, DL-*m*-Tyr, DL-*p*-bromo-Tyr from Cyclo, USA; L-O-methyl-Tyr from Hoescht, Germany; DL-*p*-tolylalanine was from the laboratory collection.

Results and discussion

Only two tyrosine derivatives, out of the 24 tested, could be readily transaminated (Table 1): MIT and MFT both possess, as tyrosine itself, a free amino and a free carboxylic groups on the three-carbon atoms side-chain, as well as an unsubstituted *p*-phenolic group on the benzene ring. The relatively broad specificity of TAT versus *m*-halogenated tyrosine confirms previous results (Mason

Table 1. Transamination by TAT of L-tyrosine and some substitution products. Final concentration of the substrate, 2.5 mM; the transamination level is given as percentage of the tyrosine value

Compound	Transamination yield
L-Tyrosine	100
L-3-Iodo-tyrosine (MIT)	67
L-3-Fluoro-tyrosine (MFT)	45
L-3,5-Diiodo-tyrosine (DIT)	4
DL-3-Hydroxy-tyrosine (DOPA)	2
L-O-Methyl-tyrosine	traces

and Deshmukh, 1985); however, 3,5-diiodotyrosine appeared to be a weak substrate. DIT and furthermore DOPA, which shows a transamination rate of only 2%, still possess the three above mentioned structural features. The faint glutamate ninhydrin positive spot observed with O-methyl-L-Tyr may be related to a partial hydrolysis of the methoxy group.

The transamination yields obtained with mixtures of L-Tyr and the different derivatives are given in Table 2.

The racemic phosphonic analogue exhibited a particular interest since it appeared to be one of the most potent competitive inhibitors of tyrosine transamination with a K_I of 0.5 mM for a K_M (L-Tyr) of 1.8 mM.

The effect of tyrosine aminotransferase upon several structural analogues of tyrosine has only been studied by Cannelakis and Cohen (1956b) and by Jacoby and La Du (1964). Our discussion takes those previous results and our own findings into account to emphasize the interference of the functional groups of tyrosine analogs in their interaction with tyrosine aminotransferase.

p-Hydroxyphenylacetate, a deaminated compound produced in the catabolic pathway of Tyr, showed a ca. 50% inhibition, a value comparable to the data reported by Jacoby and La Du (1964), who observed a more marked inhibition with *p*-hydroxyphenyllactate. N-acetyl-Tyr, which still possesses a nitrogenous group on the side chain was not transaminated by TAT, but inhibited slightly the interaction between Tyr and the enzyme, probably by means of a steric disturbance.

Blocking or removing the carboxylic group, as in tyrosinamide and tyramine respectively, provokes an almost complete disappearance of interaction. On the contrary, replacing the carboxylic by a phosphonic group (Tyr-P) highly facilitates the fixation as, among the different tyrosines modified on the acidic function, the phosphonic analogue was the best inhibitor of tyrosine transamination ($K_I = 0.5$ mM).

Whereas, as a substrate, L-Tyr is stereospecific, the stereospecificity does not appear so strict in inhibition studies: in the presence of D-Tyr, the TAT activity for transamination of L-Tyr decreased by one third.

Regarding the side-chain, the suppression of a carbon atom leads to *p*-hydroxyphenylglycine, a compound which has lost the ability to be a substrate for TAT, but which is still a potent inhibitor.

Table 2. Influence of tyrosine analogues on L-tyrosine by TAT

Putative inhibitor [I]/[S] = 10	TAT transamination, % (L-tyrosine alone = 100)	
	Method A*	Method B**
Phenolic compounds		
D-tyrosine	69	
L-N-acetyl-tyrosine	73	
L-tyrosine amide	88	
L-O-methyl-tyrosine	86	
L-3-iodo-tyrosine (MIT)		166
L-3-fluoro-tyrosine (MFT)	134	
DL-3-hydroxy-tyrosine (DOPA)		0
L-3,5-diiodo-tyrosine (DIT)		0
DL-*o*-tyrosine	95	
DL-*m*-tyrosine	61	
DL-1-amino-2-(p-hydroxyphenyl)-ethylphosphonic acid (Tyr-P)	0	0
DL-α-methyl-tyrosine	110	
tyramine, HCl	99	
DL-*p*-hydroxyphenylglycine		21
p-hydroxyphenylacetic acid	55	
Non phenolic compounds		
L-phenylalanine	106	
DL-*p*-fluoro-phenylalanine	102	
DL-*p*-bromo-phenylalanine	106	
DL-*p*-tolyl-alanine	83	
DL-1-amino-2-phenyl-ethylphosphonic acid (Phe-P)	102	
DL-β-phenyl-β-alanine		55
DL-2-amino-4-phenylbutyric acid	103	
DL-phenylglycine	113	
DL-*p*-amino-phenylglycine		52

* Method A: evaluation of *p*-hydroxybenzaldehyde at 331 nm
** Method B: determination of unreacted L-Tyr by ion exchange chromatography

The importance of the substituents in the benzene ring has been studied with numerous modified tyrosines. The only ring substituent in Tyr is the para-phenolic group; its elimination, giving Phe, suppresses any interaction possibility. The same remark applies to Phe-P where $-COOH$ of Phe is replaced by $-PO_3H_2$, the inhibitory potentiality of which has already been mentioned. A similar constatation concerns phenylglycine which is absolutely inert, unlike *p*-hydroxyphenylglycine; in this latter compound, the replacement of the $-OH$ group by $-NH_2$, leading to *p*-aminophenylglycine does not totally suppress the inhibitory effect. Moving the phenolic hydroxylic group to another place in the aromatic ring leads either to the conservation of an interaction (*m*-Tyr) or to its disapperance (*o*-Tyr). The replacement of the para-OH group by others substituents give molecules which show no interference with the enzyme (*p*-fluoro-Phe, *p*-bromo-Phe) or only a weak inhibition (O-methyl-Tyr, *p*-tolyl Ala). The para-hydroxylic group thus appears to be essential to obtain the interaction, as

even molecules with modified side chain (*p*-hydroxyphenylglycine, dopamine, norepinephrine, epinephrine) are excellent inhibitors. An additional substitution in meta-position may in some cases (MIT, MFT) facilitate the transamination or in other cases (DOPA, 3 amino Tyr) considerably disturb the enzyme-tyrosine interaction; a double substitution in 3 and 5 positions on the tyrosine ring suppresses the ability of transamination (DIT) but highly hinders the fixation of tyrosine on TAT.

Among the four different aromatic non phenolic aminoacids tested, three of them, Phe, phenylglycine and 2-amino-4-phenylbutyric acid were inert compounds. The inhibitory properties of β-phenyl-β-alanine which possesses the same side-chain length as Phe and the amino group in juxtanuclear position (as in phenylglycine), need a further explanation.

References

Cannelakis ZN, Cohen PP (1956a) Purification studies of tyrosine α-ketoglutaric acid transaminase. J Biol Chem 222: 53–62

Cannelakis ZN, Cohen PP (1956b) Kinetic and substrate specificity study of tyrosine α-ketoglutaric acid transaminase. J Biol Chem 222: 63–71

Diamondstone TI (1966) Assay of tyrosine transaminase activity by conversion of *p*-hydroxyphenyl-pyruvate to *p*-hydroxybenzaldehyde. Anal Biochem 16: 395–401

Hayashi S, Granner DK, Tomkins GM (1967) Tyrosine aminotransferase. Purification and characterisation. J Biol Chem 242: 3998–4006

Iron A, Ruart M, Duboy JP, Béranger M, Cassaigne A, Neuzil E (1981) The phosphonic analogue of tyrosine: a tool in metabolic studies. Biochem Soc Trans 9: 246

Jacoby GA, La Du BN (1964) Studies on the specificity of tyrosine-α-ketoglutarate transaminase. J Biol Chem 239: 419–424

Kida K, Takahashi M, Fujisawa Y, Matsuda H, Machino H, Miki Y (1982) Hepatic tyrosine aminotransferase in tyrosinaemia type II. J Inher Metab Dis 5: 229–230

Lemonnier F, Charpentier C, Odièvre M, Larrègue M, Lemonnier A (1979) Tyrosine aminotransferase isoenzyme deficiency. J Pediatr 94: 931–932

Lindblad B, Lindstedt S, Steen G (1977) On the enzyme defects in hereditary tyrosinemia. Proc Natl Acad Sci (USA) 74: 4641–4645

Mason M, Deshmukh DR (1985) Aminotransferases acting on aromatic substrates. In: Christen P, Metzler DE (eds) Transaminases. Wiley, New York, p 439

Smith I (1969) Chromatographic and electrophoretic techniques, vol I, 3rd edn. Heinemann, London, p 104

Valeriote FA, Auricchio F, Tomkins GM, Riley D (1969) Purification and properties of rat liver tyrosine aminotransferase. J Biol Chem 244: 3618–3624

Voigt J, Sekeris CE (1978) A simplified procedure for the purification of rat liver tyrosine aminotransferase. Hoppe-Seyler's Z Physiol Chem 359: 1363

Authors' address: A. Iron, Département de Biochimie Médicale et Biologie Moléculaire, Université de Bordeaux 2, 146, rue Léo-Saignat, 33076 Bordeaux Cedex, France.

Received January 2, 1992