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Enzymatic synthesis of *S*-aminoethyl-L-cysteine from pantetheine

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The recently characterized compound *S*-aminoethylcysteine ketimine can be synthesized from purified *S*-aminoethylcysteine by enzymatic systems (transaminases or L-amino acid oxidase) present in mammalian tissues. *S*-Aminoethylcysteine, which could be considered as the natural precursor of the ketimine, is produced from L-serine and cysteamine by the action of the enzyme cystathionine-β-synthase. We demonstrate in this paper that pantetheine, a normal cellular component, is an efficient cysteamine donor for the synthesis of *S*-aminoethylcysteine and of *S*-aminoethylcysteine ketimine in the place of free cysteamine, and we describe the enzymatic system, composed of partially purified enzymes, for the in vitro synthesis of *S*-aminoethylcysteine ketimine from pantetheine. This seems to indicate a new biological role for pantetheine.

Introduction

A new natural class of sulfur, nitrogen-containing cyclic iminoacids, called ketimines, and their reduced derivatives have been recently reported to occur in mammalian tissues and human urine [1–8]. The presence of detectable amounts of these and of some related compounds [9,10] in vivo suggests a biological role for them. Some of these compounds have been chemically synthesized by reacting cysteamine, L-cysteine or L-homocysteine with bromopyruvate [11] and by spontaneous cyclization of L-cysteine-mercaptopyruvate mixed disulfide [11], allowing some chemical properties to be elucidated. During the search for metabolic pathways involved in ketimine production, we observed that sulfur-containing amino acids such as *S*-aminoethyl-L-cysteine (AEC), L-cystathionine, L-cystine and L-lanthionine are converted in vitro into their corresponding cyclic ketimines (AECK, cystathionine ketimine, cystine ketimine and lanthionine ketimine, respectively) by an oxidative monodeamination reaction catalyzed by LAAO [12–15] or by a transaminase activity isolated from bovine liver, kidney and brain [16–19]. Only L-cystathionine and L-cystine are, however, present in tissues in appreciable amounts. L-Lanthionine has been found occasionally in nonmammalian tissues [20,21]. Although AEC has never been found in tissues, it may be produced enzymically from L-serine and cysteamine [22]. Free cysteamine is very low in tissues [23]. Pantetheine, one of the components of CoA and of acyl carrier proteins, is a natural carrier of cysteamine present in tissues and has been shown in vitro to be a putative metabolic natural precursor of free cysteamine and of some of its derivatives [24]. This work is addressed to the study of the possible biochemical use of pantetheine as a cysteamine donor for the synthesis of AEC in the place of free cysteamine. We test the possibility for pantetheine to be a natural precursor for AEC and we describe the in vitro enzymatic production of AEC and of AECK from pantetheine with partially purified enzymes.

Abbreviations: AEC, *S*-aminoethyl-L-cysteine; AECK, *S*-aminoethyl-L-cysteine ketimine; HPLC, high-performance liquid chromatography; PT-AEC, D-pantothenyl-*S*-aminoethyl-L-cysteine; DAAO, D-amino acid oxidase; LAAO, L-amino acid oxidase; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid).

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Experimental procedures

Materials

D-Pantethine is a Sigma product. D-Pantethine is reduced with a 25-fold excess L-cysteine in 0.3 M bicarbonate buffer, 0.1 mM EDTA, pH 8.5 under nitrogen. After 30 min the solution is loaded into a 1×33 cm column of Dowex 50 X-8 H^+ form. Pantetheine is eluted with water, located and quantitatively determined with Ellman's reagent [25]. Alternatively, pantetheine can be obtained from pantethine by reduction through a 5.5×0.8 cm Reductacryl (Calbiochem) column following the instructions given in the technical information of the manufacturer.

Cystathionine-β-synthase (EC 4.2.1.22) was prepared from chicken liver following the method described for rat liver [26]. The enzyme was purified up to the first DEAE-cellulose chromatography step and dialyzed overnight against 10 mM potassium phosphate buffer (pH 7.8) containing 1 mM mercaptoethanol and 0.1 mM pyridoxal phosphate. Specific activity was about 0.25 U/mg protein. Pantetheinase (EC 3.5.1.-) was prepared according to Wittwer et al. [27] and used at step 6 of the purification procedure, at a specific activity of about 1 unit/mg. *Crotalus adamanteus* LAAO (type IV, 5 U/mg, EC 1.4.3.2), hog kidney DAAO (crystalline suspension in 3.2 M ammonium sulfate, 14 U/mg, EC 1.4.3.3), catalase (2100 units/mg, EC 1.11.1.6), β-chloro-L-alanine, AEC and CoASH are Sigma products. AECK was prepared in glacial acetic acid as previously reported [14]. All other chemicals were of highest reagent grade.

Chemical synthesis and quantitative determination of D-pantothenyl-S-aminoethyl-L-cysteine (PT-AEC)

D-Pantetheine (6.6 mM) and β-chloro-L-alanine (13.2 mM) are reacted at a 1:2 molar ratio in 1.5 ml 0.01 M Tris-glycine buffer (pH 9.0) under nitrogen at 30°C for 48 h, keeping constant the pH during the reaction by 1 M NaOH additions. After concentration in vacuo the PT-AEC-containing solution has been used without further purification. Samples were taken during the reaction time, reduced with excess $NaBH_4$ for 15 min, acidified to destroy the reagent and reacted with DTNB to quantify the amount of pantetheine oxidized to pantethine. Pantetheine, under these conditions, oxidizes to no more than 2% in 4 h. Thiol group disappearance was followed with DTNB over the whole reaction time. In order to monitor the extent of the reaction, samples were hydrolyzed with 6 M HCl in vacuo at 110°C for 24 h and AEC was quantitatively determined by ion-exchange chromatography using the 25×0.46 cm column of Carlo Erba 3A 29 Amino acid Analyzer (Italy), filled with 3AR/6/DC/20 resin, according to conditions previously reported [28]. Samples were also incubated overnight at 37°C with 0.02 units pantetheinase in 0.2 ml final volume of 0.1 M potassium phosphate buffer (pH 8.0). AEC produced was directly assayed by amino acid analysis or oxidatively deaminated to AECK with 0.06 units LAAO and 100 units catalase at 37°C, following the reaction at 296 nm [14] for 4 h. Cysteamine hydrochloride (6.6 mM) and β-Cl-L-alanine (13.2 mM) are reacted in the same conditions.

Detection of AECK

The amount of AEC was routinely estimated by the quantitative determination of AECK produced after the reaction of LAAO and catalase on AEC, under standardized conditions. Determination was either spectrophotometric ($\epsilon_M = 6200\ M^{-1}\ cm^{-1}$ at 296 nm [14]), by HPLC or by the method of DAAO inhibition.

HPLC determination. Quantitative determination of AECK was performed by following the HPLC procedure of Nardini et al. [8], with some modifications. The aqueous sample was acidified with concentrated HCl (0.5 M final concentration), deproteinized with hot ethanol, centrifuged and taken to dryness in a rotary evaporator at 35°C under reduced pressure in the presence of 0.1 mM mercaptoethanol and 0.1 mM EDTA. The dried sample was added to 0.3 ml of a 2:1:2 ethanol/triethylamine/water mixture and taken to dryness again under reduced pressure in a PicoTag Work-station (Waters). The residue was derivatized by addition of 100 μl of a 7:1:1:1 ethanol/triethylamine/water/phenylisothiocyanate solution; after 30 min at room temperature samples were dried under reduced pressure and frozen until use. HPLC analyses were performed on a Violet (Roma, Italy) gradient liquid chromatograph consisting of a model Clar 002 solvent pump and a model GR 30 solvent controller program. Eluates were detected at 254 nm by a Perkin Elmer LC 15 UV detector. The analytical column (250×4 mm) was a C_{18} reversed phase (Hypersil ODS 5 μ). The mobile phase was as follows: solvent A, 0.05 M sodium acetate/acetonitrile (70:30, v/v); solvent B, 0.05 M sodium acetate/acetonitrile (40:60, v/v); solvent C, acetonitrile/water (70:30, v/v). The column was washed for 10 min with solvent C and preconditioned with solvent A for 15 min. Samples, dissolved in 0.5 ml acetonitrile, after loading (50–100 μl) were eluted with a linear gradient from A to 98% B for 30 min. At a flow rate of 1 ml/min at room temperature a sample of pure cristalline AECK-phenylthiohydantoin derivative (obtained from Professor L. Pecci) was eluted with a retention time of 17.5 min.

Enzymatic determination. The AECK content of samples has been also estimated by an enzymatic coupled procedure [8] similar to the one described for the detection of cysteamine [23] and based on the strong and specific inhibition of DAAO by AECK. Kinetics were followed at 340 nm (20–22°C) with a Kontron

Uvikon 860 spectrophotometer. Percentage of enzyme inhibition against ketimine concentration gives a linear relationship in the range 0.2–10 nmol. Determination of AECK was performed by adding 0.1–0.2 ml of sample to the standardized incubation mixture reported elsewhere [8].

Enzymatic production of AEC

Enzymatic synthesis of PT-AEC was performed as follows. 8 mM D-pantetheine and 8 mM L-serine in 0.75 ml 0.18 M Tris-HCl buffer (pH 8.6) were bubbled with nitrogen. A small sealed dialysis bag containing cystathionine-β-synthase (0.1–1 mg, 26–260 mU) dissolved in 0.2 ml of the same buffer was added and incubated at 37°C under nitrogen. After 4 h the dialysis bag was removed, 0.02 units pantetheinase (in 0.2 ml 0.02 M potassium phosphate buffer, pH 7.6) was added and incubation was continued overnight. After addition of 0.5 M HCl to obtain pH 8.0, LAAO (2 μl containing 0.06 units) and catalase (100 units in 50 μl water) were added and the incubation was continued for 4 h. After deproteinization with hot ethanol, the solution was processed as described above to obtain quantitative evaluation of the AECK content by HPLC. The same procedure was followed with cysteamine or CoASH as substrates instead of pantetheine.

Alternatively a slightly modified procedure was performed as follows. Cystathionine-β-synthase (0.1–1 mg) was added directly to the reagents containing solution. After 4 h incubation the solution was brought to pH 6.0 with a few drops of 0.5 M HCl and 0.05 ml of 0.5 M potassium phosphate buffer (pH 6.0), added with 0.02 units pantetheinase and incubated again overnight at 37°C. After addition of 0.5 M KOH to obtain pH 8.0, LAAO (0.06 units) and catalase (100 units) were added and incubation was continued for 4 h. In a separate experiment this same procedure was performed without changing the initial pH value to pH 6.0.

Control runs were performed by omitting L-serine or one of the thiol substrates, or by performing the first step without adding cystathionine-β-synthase.

Results

PT-AEC is synthesized from D-pantetheine and β-Cl-L-alanine as described in the Experimental procedures section. The amount of pantetheine unreacted at the end of the reaction, evaluated with DTNB after $NaBH_4$ reduction, did not exceed 10%. After 48 h the reaction product was hydrolyzed with 6 M HCl and the amount of AEC present was estimated by amino acid analysis. A reaction yield of about 12% was obtained. We observed a strong inhibition of pantetheinase activity, when the enzyme was added to the PT-AEC containing solution, probably due to the excess of β-Cl-L-alanine present. The conditions for the chemical synthesis of PT-AEC described in this paper, albeit not optimized as far as the reaction yield concerns, allowed, however, the use of pantetheinase and LAAO in detecting PT-AEC by this coupled enzyme system. Free thiol groups disappeared almost completely after 48 h. There was no correspondence between the disappearance of thiol groups and the amount of PT-AEC synthesized. PT-AEC was also submitted to enzymatic hydrolysis by incubation overnight with 0.02 units pantetheinase followed by quantitative determination of AEC produced by amino acid analysis. PT-AEC synthesized starting with 1 μmol pantetheine released about 30 nmol AEC after pantetheinase hydrolysis. A similar sample, after hydrolysis with pantetheinase, was incubated with LAAO and catalase and the reaction was followed at 296 nm, as a function of the incubation time with LAAO. After 4 h reaction with 0.06 units LAAO and 100 units catalase, 13 nmol AECK were found, an amount which agrees with the amount (11 nmol) found with the DAAO inhibition method.

AECK is unstable in alkaline solution. It decomposes oxidatively and the decomposition product(s) absorb negligibly at 296 nm (Ref. 29, and unpublished results). In order to obtain a correct estimate of the amount of AECK produced, a correction for this decomposition in alkaline medium could be introduced. The absorbance changes at 296 nm of dilute solutions of pure synthetic AECK were followed as a function of concentration, and are linear for at least 10 min in the conditions of the in vitro enzymatic incubation. A pseudo-first-order decomposition rate constant of $1.2 \cdot 10^{-3}$ min^{-1} was calculated from the slope of the linear plot of initial rates vs. AECK concentration. We expect, therefore, that the spontaneous decomposition of AECK during the incubation time with LAAO did not exceed 30%.

Table I collects quantitative data, obtained by different methods, of AEC or AECK recovery after chemical hydrolysis or enzymatic incubation of various starting compounds. In order to assay AEC produced enzymically from PT-AEC or AEC chemically synthesized from cysteamine and β-Cl-L-alanine, various samples were incubated with LAAO and catalase, with or without being first incubated overnight with pantetheinase. Identification as AECK of the product obtained after incubation of PT-AEC with the above enzyme system was supported by the peculiar absorption peak at 296 nm, by the specific DAAO inhibition [30] and by HPLC analysis as outlined above. Results obtained with four different determination methods are comparable. These methods represent valuable tools for evaluating AEC content in solution and have different sensitivity ranges. The yield of the oxidative deamination of pure synthetic AEC by LAAO and catalase was in our conditions about 50%, and this value should be corrected for the spontaneous decom-

position of AECK in alkaline solution. The yield in AEC which was obtained in the chemical synthesis starting with D-pantetheine is about 20% of the yield in AEC obtained when starting with cysteamine as substrate. AECK content was below the detection level in control incubation experiments performed without adding pantetheinase or LAAO, and without D-pantetheine or β-Cl-L-alanine.

The in vitro enzymatic synthesis of PT-AEC from D-pantetheine and L-serine in the presence of purified cystathionine-β-synthase was assayed with LAAO and catalase after hydrolysis with pantetheinase and compared with the enzymatic synthesis of AEC starting with cysteamine and L-serine. It was not possible to follow this reaction spectrophotometrically at 296 nm, due to the low amount of AECK produced compared with other interfering compounds absorbing at 296 nm, nor by the DAAO inhibition method. Quantitative determination was obtained by HPLC detection after phenylisothiocyanate derivatization [8].

HPLC analysis of the products of the reaction (Fig. 1A) starting with D-pantetheine and L-serine, after the incubation with cystathionine-β-synthase and pantetheinase and after LAAO and catalase treatment and derivatization with phenylisothiocyanate, showed the presence of a component coeluting with authentic AECK. The identification was corroborated by comparison with internal standard samples of recrystallized AECK-phenylthiohydantoin derivative (fig. 1B).

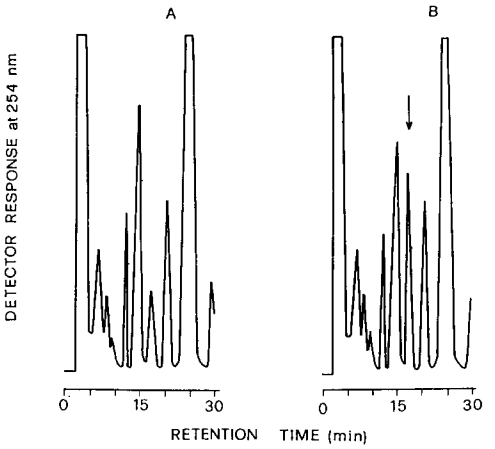

Fig. 1. HPLC elution pattern of the reaction products of the in vitro enzymatic synthesis of PT-AEC. After incubation with pantetheinase and LAAO plus catalase, samples of the solution were reacted with phenylisothiocyanate. Detection at 254 nm. Chromatographic conditions as reported in Experimental procedures. (A) Chromatogram corresponding to 20 μl of incubation mixture; (B) as (A) plus 4 nmol of authentic AECK derivative.

TABLE I

Quantitative determination of AEC from various sources

D-Pantetheine (1 μmol) or cysteamine (1 μmol) are reacted with β-Cl-L-alanine (2 μmol) at pH 9.0 under nitrogen for 48 h at 30°C. Incubation with pantetheinase (0.02 units) was overnight at pH 8.0 and 37°C; incubation with LAAO (0.06 units) and catalase (100 units) was for 4 h at 37°C. Incubations with β-Cl-L-alanine, D-pantetheine and AEC were performed with 1 μmol each. Further details of the analytical procedures for the determination of AEC by amino acid analysis and of AECK by DAAO inhibition and HPLC analysis after phenylisothiocyanate derivatization are reported in Experimental procedures. Values are means of three separate samples. C.V. is no more than 10%.

Reagents	Conditions	nmol AECK recovered			nmol AEC recovered amino acid analyzer
		ΔA (296 nm)	DAAO inhibition	HPLC	
D-Pantetheine + β-Cl-L-alanine	hydrolysis 6 M HCl 24 h	–	–	–	120
D-Pantetheine + β-Cl-L-alanine	pantetheinase	< 1	< 0.1	< 0.5	30
D-Pantetheine + β-Cl-L-alanine	pantetheinase LAAO + catalase	13	11	9	–
D-Pantetheine + β-Cl-L-alanine	LAAO + catalase	< 1	< 0.1	< 0.5	–
Cysteamine + β-Cl-L-alanine	LAAO + catalase	75	71	60	–
Cysteamine + β-Cl-L-alanine	pantetheinase LAAO catalase	71	64	58	–
β-Cl-L-alanine	pantetheinase LAAO + catalase	< 1	< 0.1	< 0.5	–
D-Pantetheine	pantetheinase LAAO + catalase	< 1	< 0.1	< 0.5	–
AEC	pantetheinase LAAO + catalase	500	533	426	–

TABLE II

Quantitative determination of AECK obtained after incubation of various substrates with cystathionine-β-synthase, pantetheinase and L-amino acid oxidase

Results are given in nmol AECK obtained after incubation performed as described in Experimental procedures and determined by HPLC after derivatization. Each incubation was performed starting with 6 μmol substrate. Data are means of triplicate determinations. C.V. are less than 10%.

Substrate (+6 μmol L-serine)	Cystathionine-β-synthase	pH 8.6 dialysis bag	pH 6.0	pH 8.6
Pantetheine	0.1 mg	64	60	58
Pantetheine	1 mg	520	485	470
Cysteamine	0.1 mg	8	8	7
Cysteamine	1 mg	55	55	60
CoA	0.1 mg	< 2	< 2	< 2
CoA	1 mg	< 2	< 2	< 2

Table II shows quantitative data as averages of three determinations. The preliminary data obtained with the three step experiment at pH 8.6 (last column of table II) are consistent with two different possibilities. In the first pathway the enzyme cystathionine-β-synthase catalyzes the synthesis of PT-AEC from D-pantetheine and L-serine, and PT-AEC is then hydrolyzed by pantetheinase. Alternatively, in the second pathway pantetheinase hydrolyzes pantetheine giving cysteamine and only after this reaction cystathionine-β-synthase catalyzes the synthesis between cysteamine and L-serine to give AEC. The three step experiment, in which cystathionine-β-synthase is added first at pH 8.6 and then after 4 h pantetheinase is added at pH 6.0, at which pH cystathionine-β-synthase is inefficient [31] and pantetheinase still retains activity [32] and the experiment in which cystathionine-β-synthase is removed from the solution after its reaction time show that the first route is quantitatively the most important and that AEC originates mainly from PT-AEC. Furthermore, the reaction of cystathionine-β-synthase represents the rate-limiting step of this enzyme chain, at least in vitro. Cysteamine bound to pantothenic acid, i.e., in pantetheine, is a more efficient precursor of AEC compared to free cysteamine, as substrate of cystathionine-β-synthase. AECK is below the detection sensitivity in the presence of CoASH as substrate, showing that cysteamine in CoASH is not a precursor of AEC. Control experiments performed either by adding cystathionine-β-synthase inactivated by boiling or by omitting L-serine or D-pantetheine or cysteamine showed that AECK is not formed in these conditions.

Discussion

The apparent yield of chemically synthesized PT-AEC is very poor and is only slightly increased at higher β-Cl-L-alanine/pantetheine molar ratios. The presence of β-Cl-L-alanine or of other unidentified compounds in the PT-AEC-containing solution probably explains the observed partial inhibition of pantetheinase in these conditions. Inhibition by other alkylating agents and a strong protection by substrate have been reported [33]. The difference between the yield in AEC after acid hydrolysis and after hydrolysis by pantetheinase, which shows that the enzymatic hydrolysis is not exhaustive, can be due to this partial inhibition of pantetheinase. The different apparent yield of the chemical syntheses starting with D-pantetheine or with cysteamine can be due either to a lower reactivity of D-pantetheine with β-Cl-L-alanine compared to the reactivity of cysteamine or because PT-AEC is a poor substrate for pantetheinase. Determination of kinetic parameters of PT-AEC as substrate of pantetheinase could clarify this specific point.

The specificity of cystathionine-β-synthase has been studied extensively [34] and it is known that cysteamine can replace homocysteine with good efficiency. Experiments reported in this paper demonstrate that D-pantetheine may replace homocysteine even better than cysteamine. With D-pantetheine as substrate more AECK as product is obtained, which means that the reaction between D-pantetheine and L-serine catalyzed by cystathionine-β-synthase is 10-times more efficient than the reaction between cysteamine and L-serine. Cysteamine is reported [35] to be less actively transformed (about 42%) as homocysteine, in experimental conditions which are, however, not comparable with those present in our experiments. Some properties of the product of the enzymatic synthesis between D-pantetheine and L-serine have been compared with those of the chemically synthesized PT-AEC. The product arising from the reaction with the enzyme 'chain' pantetheinase, LAAO and catalase was identified as AECK by spectral evidences, by HPLC identification after phenylisothiocyanate derivatization and by inhibition of DAAO activity. Pantetheine can be, therefore, considered as an in vitro precursor of both compounds AEC and AECK, by the action of enzyme activities present in mammalian tissues. Thioether derivatives obtained by reacting pantetheine with bromopyruvate or bromopyruvate ethyl ester have been shown to be hydrolyzed by the enzyme pantetheinase (Refs. 36 and 37, and unpublished results). We demonstrated [37] that one of these pantetheine derivatives, *S*-pantetheine-3-pyruvate, may be a precursor of AECK in the presence of bovine liver extracts. AEC (called by some authors L-thialysine) has been demonstrated in vitro to be substrate of a mammalian transaminase activity [17]. AEC, however, has never been detected in animal tissues. LAAO, which has been found in rat liver [38], as well as in other tissues, may be considered operative in mammalian biosynthetic pathways. The

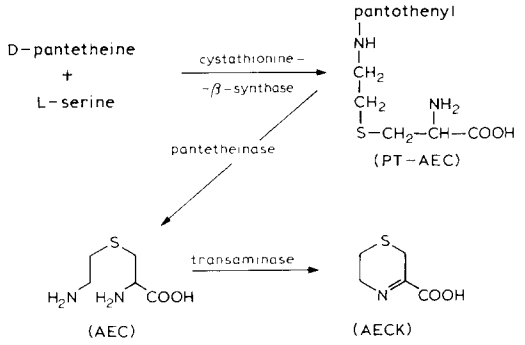

Fig. 2.

scarce data available show that free pantetheine is present in tissues at concentrations ranging from 2 to 14 nmol/g acetone powder [39], whereas bound forms either as phosphopantetheine, CoA and CoA derivatives or as protein components are present at much higher concentrations. The steady-state concentration of free cysteamine is also apparently very low, of the order of 2–20 nmol/g fresh tissue [23].

In vivo the pathway described in (Fig. 2) may be operative.

Cystathionine-β-synthase activity has been reported in many tissues, ranging from 0.05 to 1 μmol cystathionine synthesized/h per mg protein [40]. The distribution of pantetheinase activity has been studied intracellularly [41] and activities of about 0.03 μmol pantothenate produced/h per mg protein were reported for rat kidney [42].

In view of our data we believe that the contribution of the alternative pathway:

$$\text{D-pantetheine} \xrightarrow{\text{pantetheinase}} \text{cysteamine}$$

$$\text{cysteamine} + \text{L-serine} \xrightarrow{\text{cystathionine-}\beta\text{-synthase}} \text{AEC}$$

$$\text{AEC} \xrightarrow{\text{transaminase (or LAAO)}} \text{AECK}$$

to the total in vitro AEC and AECK synthesis is negligible. These results, which could reflect also the in vivo metabolic steady state, are in agreement with the measured activities of cystathionine-β-synthase and of pantetheinase and with the fact that AEC has not been found until now in vivo, being present probably only at very low steady-state concentrations. Apparently other more efficient pathways scavenge cysteamine, for instance for the synthesis of hypotaurine and taurine.

Results reported in this paper seem to indicate a new role for pantetheine and a possible preferential biological role of the reaction for the production of AEC through cystathionine-β-synthase and pantetheinase if these enzymes are present in the same organs and in the same cellular fraction. Work is in progress to elucidate the distribution of these enzymes.

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References

1 Cavallini, D., Pecci, L., Matarese, R.M., Ricci, G. and Achilli, M. (1985) J. Biol. Chem. 260, 15577–15579.
2 Cavallini, D., Matarese, R.M., Pecci, L. and Ricci, G. (1985) FEBS Lett. 192, 247–250.
3 Matarese, R.M., Pecci, L., Ricci, G., Nardini, M., Antonucci, A. and Cavallini, D. (1987) Proc. Natl. Acad. Sci. USA 84, 5111–5114.
4 Pecci, L., Antonucci, A., Nardini, M. and Cavallini, D. (1988) Biochem. Int. 17, 877–883.
5 Matarese, R.M., Solinas, S.P., Montefoschi, G., Ricci, G. and Cavallini, D. (1989) FEBS Lett. 250, 75–77.
6 Ricci, G., Vesci, L., Nardini, M., Arduini, A., Storto, S., Rosato, N. and Cavallini, D. (1989) Biochim. Biophys. Acta 990, 211–215.
7 Ricci, G., Vesci, L., Matarese, R.M., Antonucci, A., Maggio, A., Pecci, L. and Cavallini, D. (1990) J. Neurochem. 55, 1599–1602.
8 Nardini, M., Matarese, R.M., Pecci, L., Antonucci, A., Ricci, G. and Cavallini, D. (1990) Biochem. Biophys. Res. Commun. 166, 1251–1256.
9 Tominaga, F. and Oka, K. (1963) J. Biochem. 54, 222–224.
10 Granroth, B. (1970) Ann. Acad. Sci. Fennicæ, A. II. 154, 9–71.
11 Cavallini, D., Ricci, G. and Federici, G. (1983) in Sulfur Amino Acids: Biochemical and Clinical Aspects (Kuriyama, K., Huxtable, R.J. and Iwata, H., eds.), pp. 355–363, Alan R. Liss, New York.
12 Ricci, G., Federici, G., Achilli, M., Matarese, R.M. and Cavallini, D. (1981) Physiol. Chem. Phys. 13, 341–346.
13 Ricci, G., Federici, G., Lucente, G., Achilli, M. and Cavallini, D. (1982) Physiol. Chem. Phys. 14, 193–199.
14 Cavallini, D., Ricci, G., Federici, G., Costa, M., Pensa, B., Matarese, R.M. and Achilli, M. (1982) in Structure and Function Relationships in Biochemical Systems (Bossa, F., Chiancone, E., Finazzi-Agrò, A. and Strom, R., eds.), pp. 359–374, Plenum Press, New York.
15 Ricci, G., Nardini, M., Santoro, L., Achilli, M., Caccuri, A.M., Federici, G. and Cavallini, D. (1983) Ital. J. Biochem. 32, 49–50.
16 Costa, M., Pensa, B., Fontana, M., Foppoli, C. and Cavallini, D. (1986) Biochim. Biophys. Acta 881, 314–320.
17 Ricci, G., Nardini, M., Federici, G., and Cavallini, D. (1986) Eur. J. Biochem. 157, 57–63.
18 Costa, M., Pensa, B., Di Costanzo, B., Coccia, R. and Cavallini, D. (1987) Neurochem. Int. 10, 377–382.
19 Pensa, B., Achilli, M., Fontana, M., Caccuri, A.M. and Cavallini, D. (1989) Neurochem. Int. 15, 285–291.
20 Sloane, N.H. and Untch, K.G. (1966) Biochemistry 5, 2658–2665.
21 Rao, D.R., Ensor A.H. and Thorpe, B. (1967) Biochemistry 6, 1208–1216.
22 Costa, M., Pecci, L., Pensa, B., Fontana, M. and Cavallini, D. (1989) J. Chromatogr. 490, 404–410.
23 Ricci, G., Nardini, M., Chiaraluce, R., Duprè, S. and Cavallini, D. (1983) J. Appl. Biochem. 5, 320–329.
24 Cavallini, D., Scandurra, R., Duprè, S., Federici, G., Santoro, L., Ricci, G. and Barra, D. (1976) in Taurine (Huxtable, R.J. and Barbeau, A., eds.), pp. 59–66, Raven Press, New York.
25 Ellman, G.L. (1959) Arch. Biochem. Biophys. 82, 70–77.

26 Borcsok, E. and Abeles, R.H. (1982) Arch. Biochem. Biophys. 215, 695–707.
27 Wittwer, C.T., Burckard, D., Ririe, K., Rasmussen, R., Brown, J., Wyse, B.W. and Hansen, R.G. (1983) J. Biol. Chem. 258, 9733–9738.
28 Di Girolamo, M., Di Girolamo, A., Cini, C., Coccia, R. and De Marco, C. (1986) Physiol. Chem. Phys. Med. NMR 18, 159–164.
29 Cini, C., Foppoli, C. and De Marco, C. (1978) Ital. J. Biochem. 27, 233–246.
30 Ricci, G., Nardini, M., Caccuri, A.M. and Federici, G. (1983) Biochim. Biophys. Acta 748, 40–47.
31 Kashiwamata, S. and Greenberg, D.M. (1970) Biochim. Biophys. Acta 212, 488–500.
32 Wittwer, C.T., Wyse, B.W. and Hansen, R.G. (1982) Anal. Biochem. 122, 213–222.
33 Ricci, G., Nardini, M., Chiaraluce, R., Duprè, S. and Cavallini, D. (1986) Biochim. Biophys. Acta 870, 82–91.
34 Braunstein, A.E. and Goryachenkova E.V. (1984) Adv. Enzymol. (Meister, A., ed.), Vol. 56, pp. 1–89, John Wiley & Sons, New York.
35 Braunstein, A.E. and Goryachenkova, E.V. (1976) Biochimie 58, 5–17.
36 Duprè, S., Chiaraluce, R., Nardini, M., Cannella, C., Ricci, G. and Cavallini, D. (1984) Anal. Biochem. 142, 175–181.
37 Chiaraluce, R., Nardini, M., Duprè, S., Ricci, G. and Cavallini, D. (1983) IRCS Med. Sci. 11, 1119–1120.
38 Nakano, M., Tsutsumi, Y. and Danowski, T.S. (1967) Biochim. Biophys. Acta 139, 40–48.
39 Brown, G.M. (1959) J. Biol. Chem. 234, 379–382.
40 Sturman, J.A., Rassin, D.K. and Gaull, G.E. (1970) Int. J. Biochem. 1, 251–253.
41 Abiko, Y. (1975) in Metabolic Pathways (Greenberg, D.M., ed.), 3rd Edn., Vol. 7, pp. 12–25, Academic Press, New York.
42 Wittwer, C.T., Wyse, B.W. and Hansen, R.G. (1986) Methods Enzymol. 122, 36–43.