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Sequence of cDNA for rat cystathionine γ-lyase and comparison of deduced amino acid sequence with related *Escherichia coli* enzymes

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A cDNA clone for cystathionine γ-lyase was isolated from a rat cDNA library in λ gt11 by screening with a monospecific antiserum. The identity of this clone, containing 600 bp proximal to the 3'-end of the gene, was confirmed by positive hybridization selection. Northern-blot hybridization showed the expected higher abundance of the corresponding mRNA in liver than in brain. Two further cDNA clones from a plasmid pcD library were isolated by colony hybridization with the first clone and were found to contain inserts of 1600 and 1850 bp. One of these was confirmed as encoding cystathionine γ-lyase by hybridization with two independent pools of oligodeoxynucleotides corresponding to partial amino acid sequence information for cystathionine γ-lyase. The other clone (estimated to represent all but 8% of the 5'-end of the mRNA) was sequenced and its deduced amino acid sequence showed similarity to those of the *Escherichia coli* enzymes cystathionine β-lyase and cystathionine γ-synthase throughout its length, especially to that of the latter.

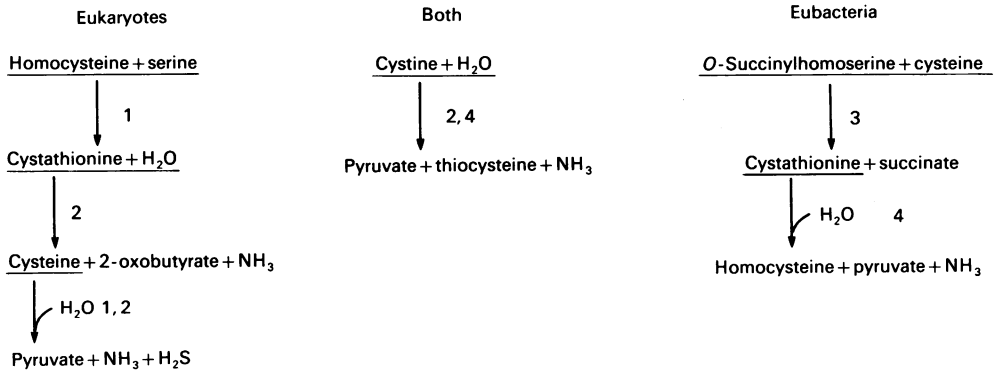
INTRODUCTION

Cystathionine γ-lyase (CSE, EC 4.4.1.1) is the final enzyme in the trans-sulphuration pathway that in mammalian cells synthesizes L-cysteine from L-methionine. This pyridoxal 5'-phosphate-dependent enzyme catalyses the cleavage of L-cystathionine to L-cysteine, as well as other reactions shown in Scheme 1 [1]. Also shown are the reactions catalysed by three other enzymes involved in cysteine and cystathionine metabolism whose amino acid sequences are compared with cystathionine γ-lyase in the present paper. Rat cystathionine γ-lyase is composed of four 43000 Da subunits [1]. Besides its role as an amino acid component of polypeptide chains, L-cysteine is required for the synthesis of GSH [2,3], the major reducing thiol, which protects cells from oxidative stress [2]. The expression of CSE is controlled in a tissue-specific manner [4], and is dramatically increased during late fetal development of the liver [5] and altered during haematopoietic differentiation [6].

In order to begin to investigate the regulation of CSE expression and the possible structural similarities it may have to other proteins, especially those requiring pyridoxal 5'-phosphate for their activity [7], or having cystathionine or cysteine as a substrate [8], it was necessary to clone and sequence cDNA for this key enzyme [9] of the trans-sulphuration pathway. We report that the deduced amino acid sequence of rat cystathionine γ-lyase has a high degree of similarity to those of two eubacterial enzymes involved in cystathionine and cysteine metabolism.

MATERIALS AND METHODS

CSE was purified from rat liver through to the ethanol precipitation step as described in ref. [10]. The dissolved pre-



Scheme 1. Summary from published studies of reactions catalysed *in vitro* by cystathionine γ-lyase and three other enzymes involved in the metabolism of cystathionine, cysteine and cystine

Key to enzymes: 1, cystathionine β-synthase (EC 4.2.1.22); 2, cystathionine γ-lyase (CSE, EC 4.4.1.1); 3, cystathionine γ-synthase [*O*-succinylhomoserine (thiol)-lyase, EC 4.2.99.9]; 4, cystathionine β-lyase (EC 4.4.1.8).

Abbreviations used: CSE, cystathionine γ-lyase; NSE, neuron-specific enolase.

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The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases.

cipitate was further purified by chromatography on a DEAE-cellulose column [11]. The CSE activity was monitored during the purification as described in ref. [10]. CSE for immunization and for amino acid analysis of its tryptic peptides was separated from minor contaminants by SDS/PAGE [12]. Purified CSE and total rat liver proteins were electrophoretically transferred to nitrocellulose as described in ref. [13]. A rabbit was immunized intrasclerally three times over a 2-week period with a total of about 20 μg of CSE that had been immobilized on nitrocellulose and then solubilized [14]. At 3 weeks later the rabbit was boosted intraperitoneally, intramuscularly and subcutaneously with about 100 μg of gel-purified CSE, emulsified with Freund's incomplete adjuvant. Serum was collected after a further 2 weeks. This antiserum stained purified CSE on Western blots and stained a single polypeptide from rat liver total soluble proteins that co-migrated with purified CSE.

A rat liver cDNA library in λ gt11 [15] was screened as described in ref. [16], with the above antiserum, except that the bound anti-CSE antibody was detected with alkaline-phosphatase-conjugated goat anti-(rabbit Ig) antibody (Pro-Mega). For screening the rat liver cDNA library in pcD expression plasmid [17] by colony hybridization [18], the cDNA insert from the λ gt11 clone was labelled as described in ref. [19].

A cDNA clone for neuron-specific enolase (NSE) was obtained from a rat brain cDNA library in λ gt11 (Clontech) by screening with an end-labelled [20] synthetic oligonucleotide corresponding to part of the published sequence for NSE [21].

Rat liver total cytoplasmic RNA, prepared as described in ref. [22], was used to make Northern blots [23] and for hybridization selection [24] of CSE mRNA. Selected mRNA was translated [25] in a rabbit reticulocyte lysate (ProMega) containing [^{35}S]-methionine (New England Nuclear). The radioactive CSE polypeptides were immunoprecipitated [26] with a characterized antiserum [27], resolved by SDS/PAGE [12] and located by fluorography [28].

Tryptic peptides from CSE were resolved by reverse-phase h.p.l.c. Several prominent peptides were separated from minor contaminants by electroelution [29] after SDS/PAGE. These then were microsequenced with the use of an Applied BioSystems 477A sequencer. Two sets of oligodeoxynucleotides of length 17 nucleotide residues with 96-fold (pool 1) or 162-fold (pool 2) degeneracy were synthesized by using an Applied BioSystems 381A synthesizer, these being based on the amino acid sequences obtained. The oligodeoxynucleotides were end-labelled [20] for use in probing [30] Southern blots [20].

For sequencing, deletion subclones [31] of cDNA inserts subcloned in M13 mp19 [32] were prepared in both directions. Sequencing by the chain-termination method was accomplished with a modified bacteriophage T7 DNA polymerase [33]. DNA sequencing by the chemical-degradation method was performed as described in ref. [34]. Compilation and analysis of sequence data was aided by the DNA Inspector II program for the Apple MacIntosh computer.

RESULTS

A monospecific rabbit antibody raised to SDS/PAGE-purified CSE was used to screen a rat liver cDNA library in λ gt11. The most intensely stained plaque, designated λ gt11 CSE-1, did not react with pre-immune serum from the immunized rabbit. The approx. 600 bp cDNA insert from λ gt11 CSE-1 was subcloned into plasmid pUC19 [32], yielding pUC-CSE-1.

The identity of the clone was confirmed in several ways, the first of which was by positive hybridization selection (Fig. 1). Translation of the selected RNA, followed by immunoprecipitation and PAGE, resulted in two bands of 43000 and

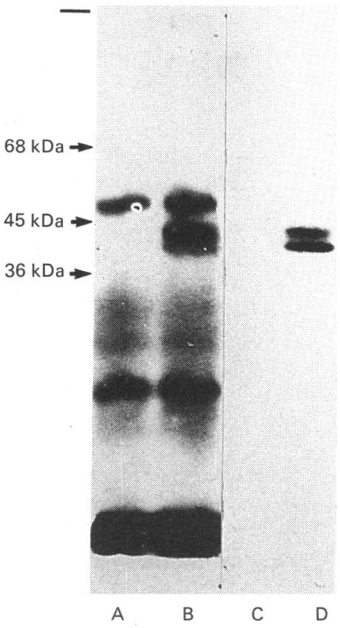

Fig. 1. Immunoprecipitation of polypeptides synthesized by translation *in vitro* of mRNA selected by hybridization with cDNA clone pUC-CSE-1

Fluorogram of SDS/PAGE gel of the translation reaction of RNA selected by pUC19 cloning vector DNA (lane A) or pUC-CSE-1 DNA (lanes B–D). Lanes A and B, total translation products; lanes C and D, products immunoprecipitated by normal rabbit serum (lane C) or antiserum to CSE (lane D).

40000 Da (lanes B and D) that were not found in the translation products of the control pUC19-selected RNA (lane A). These two bands were of the same size as the two polypeptides present in our purified CSE preparations, the latter two having nearly identical partial peptide maps [35] (results not shown). It seems likely the two polypeptides are products of the same gene, the difference in size being due to cleavage by a proteinase specific for pyridoxal 5′-phosphate-containing proteins [36].

The specificity of clone pUC-CSE-1 for CSE was further confirmed in an autoradiograph of a Northern blot of a 1% agarose gel in which equal amounts of total cytoplasmic RNA from rat liver and rat brain were electrophoresed (Fig. 2). Half of the blot was probed with the radioactively labelled putative CSE insert from pUC-CSE-1. Note that lane D (liver) contains a hybridizing RNA whereas lane C (brain) does not. This result is consistent with the fact that CSE is about 100-fold more abundant in liver than brain [4]. The size of the hybridizing band relative to RNA standards is calculated to be about 1950 bp. Since about 1125 bp is needed to code for a 43000 Da protein, this result suggests that CSE mRNA contains about 825 bp of untranslated 3′ and 5′ regions. As a control, half of the blot was probed with an NSE cDNA clone, which identified a hybridizing RNA in brain of the size reported for NSE [21], but not in liver, as was expected from the relative abundance of NSE mRNA in these two tissues [37].

To obtain longer cDNA clones the 600 bp insert from pUC-CSE-1 was used to probe by colony hybridization [18] a rat liver cDNA library in the expression plasmid pcD [17,38]. A clone, designated pcD-CSE-1, was identified that had a 1700 bp *Bam*HI insert (Fig. 3), 101 bp of which is vector DNA and the rest cDNA. Chemical-degradation sequencing of 550 bp *Pst*I–*Bam*HI fragment from the 3′-end of this insert showed that it contained a poly(A) tail of about 150 nucleotide residues (results not shown). Restriction of pcD-CSE-1 with *Pst*I released three small

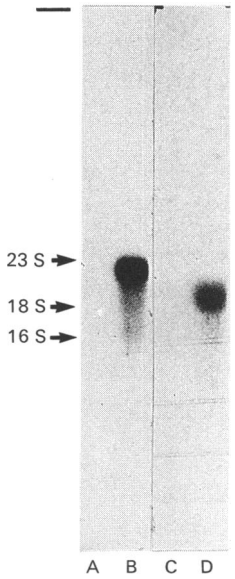

Fig. 2. Northern-blot analysis of tissue-related differences in the abundance of RNA for CSE by hybridization with insert from the cDNA clone pUC-CSE-1

Lanes A and D, 10 μg of rat liver total cytoplasmic RNA; lanes B and C, 10 μg of rat brain total cytoplasmic RNA. Lanes A and B were probed with a cDNA clone for NSE as a control showing a brain-specific mRNA; lanes C and D were probed with pUC-CSE-1.

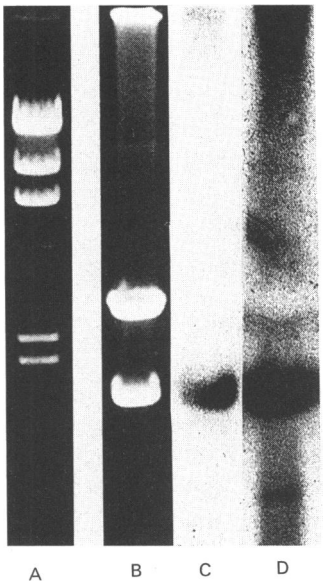

Fig. 3. Hybridization of Southern blots of pcD-CSE-1 with probes consisting of two pools of oligodeoxyribonucleotides synthesized on the basis of partial amino acid sequence data for unrelated peptides of CSE

Lanes B, C and D, each 3 μg of pcD-CSE-1 restricted with *Bam*HI; lane A, 1.5 μg of *Hin*dIII-restricted λ DNA molecular-mass markers. The five prominent bands are 23130, 9416, 6682, 2322 and 2027 bp. Lane B is a photograph of the ethidium bromide-stained plasmid DNA before transfer to nylon filter. Lanes C and D are autoradiographs corresponding to lane B after probing with pools 2 and 1 (see the text) respectively.

fragments (results not shown), which were subcloned in both directions into M13 mp19. In subsequent screening of the pcD library we have obtained a second clone, pcD-CSE-2, with about 1850 bp of cDNA insert, which was used for construction of deletion subclones in both orientations (see below).

The pcD-CSE-1 clone was confirmed as representing CSE in a third way. Purified rat liver CSE was digested with trypsin and several of the resultant peptides were microsequenced. On the basis of two of these sequences we synthesized the following two pools of oligodeoxynucleotides in the anti-message sense: pool 1, 5′-GG-(TGCA)GT-(TC)TC-(TGA)AT-CCA-(AGTC)AC-3′; pool 2, 5′-GG-(TGA)GT-(TGA)AT-(TGA)GC-(TGA)GC-(TC)TC-3′. These end-labelled pools were found to hybridize with cDNA insert of pcD-CSE-1, but not with the vector DNA (Fig. 3, lanes B, C and D).

The *Bam*HI insert from pcD-CSE-2 was subcloned into M13 mp19 in both directions. A set of deletion subclones was prepared from each subclone. Chain-termination sequencing was performed on M13 clones corresponding to the 5′-ends of the pcD-CSE-2 BamH1 insert, the ends of the three *Pst*I fragments of pcD-CSE-1, the ends of pUC-CSE-1 and 11 deletion subclones derived from pcD-CSE-2. A total of 1391 bp of overlapping sequence data (Fig. 4) was obtained starting at the 5′-end of pcD-CSE-2. Starting from the 5′-end of the sequence an open reading frame coding for 364 amino acid residues was found. Four stretches of amino acid residues in the predicted sequence were found that corresponded exactly to amino acid sequence information derived from peptides from the purified CSE, including the two of those having been used to prepare the synthetic oligodeoxynucleotides used in the Southern-blotting experiments (Fig. 3).

The predicted amino acid sequence for rat CSE was compared with those for *E. coli* cystathionine β-lyase and cystathionine γ-synthase, two proteins that have been shown to have similarities of sequence throughout their extent [8]. Overall, rat CSE shows 28% identity with *E. coli* cystathionine β-lyase and 39% identity with cystathionine γ-synthase. In a slightly different measure of their relatedness, when rat CSE is compared with the two *E. coli* enzymes at amino acid residues where the latter are not similar, it is clear that CSE shares more identical residues with cystathionine γ-synthase than with cystathionine β-lyase, 72 and 34 amino acid residues respectively (Fig. 5). The greater similarity of CSE to cystathionine γ-synthase than to cystathionine β-lyase is also evident from the fact that only seven gaps had to be introduced into the amino acid sequences of CSE and cystathionine γ-synthase in order to achieve their alignment, whereas 16 gaps had to be introduced into the sequences of CSE and cystathionine β-lyase.

Since *E. coli* cystathionine β-lyase and cystathionine γ-synthase have subunit molecular masses nearly identical with that of rat cystathionine γ-lyase [39], the proportion of rat cystathionine γ-lyase sequence that remains to be determined can be estimated from the percentage of deduced amino acid residues in the *E. coli* enzymes for which we do not have a corresponding residue. Approx. 6–9% of the 5′-end of the cystathionine γ-lyase sequence has not yet been determined.

Part of the deduced sequence for rat cystathionine γ-lyase, indicated in Fig. 4, is identical with the sequence previously determined [40] for its pyridoxal 5′-phosphate-binding site, including its active-site lysine residue.

DISCUSSION

It has been suggested that several enzymes of cystathionine metabolism probably evolved from a common ancestral gene [8,39]. This hypothesis is based on the following evidence. (1) The

Fig. 4. Nucleotide sequence of the open reading frame of the largest cDNA clone for rat cystathionine γ-lyase

The deduced amino acid sequence is indicated below the nucleotide sequence. The singly underlined sequences are identical with those determined by amino acid sequencing of peptides from rat cystathionine γ-lyase. The doubly underlined sequence is identical with the previously determined amino acid sequence for the pyridoxal 5′-phosphate-binding site of rat cystathionine γ-lyase.

activities of cystathionine γ-lyase, cystathionine γ-synthase and cystathionine β-lyase are each dependent upon the binding of pyridoxal 5′-phosphate through a Schiff base formed with a lysine residue at their respective active sites. These enzymes share the sequences, Thr-Lys(Pxy)-Tyr at their active sites [39,40]. (2) These enzymes are each tetrameric, having identical subunits with molecular masses of about 40000 Da [39]. (3) The sequences of cystathionine γ-synthase and cystathionine β-lyase show a degree of homology throughout their extent [8]. Our data showing the degree of homology of eukaryotic cystathionine γ-lyase with these enzymes further support the concept of a common ancestral gene that gave rise to at least these three known enzymes, although we advance the hypothesis below that the function of this gene may have been in cystine or cysteine metabolism, not cystathionine metabolism.

Our finding of greater sequence similarity between *E. coli* cystathionine γ-synthase and rat CSE than between *E. coli* cystathionine β-lyase and CSE was unexpected in that the latter two have cystathionine as a substrate and have the same function, albeit at different covalent bonds of the cystathionine molecule. This perplexing result suggested the possibility that the greater similarity between cystathionine γ-lyase and cystathionine γ-synthase might be related to other functions or substrates that these enzymes have in common. Since several of the enzymes of cystathionine metabolism are multifunctional and also catalyse reactions with cysteine or cystine as substrates, we summarize previously published K_m values for those enzymes in Table 1. It can be seen that the affinities of these enzymes for cysteine and cystine correlate better with their amino acid sequence similarities than do their affinities for cystathionine. Notice specifically that the more similar pair, CSE and cystathionine γ-synthase, have a lower K_m for cysteine or cystine than does cystathionine β-lyase. Conversely, note that whereas cystathionine is the substrate of cystathionine β-lyase, it is not a substrate for cystathionine γ-

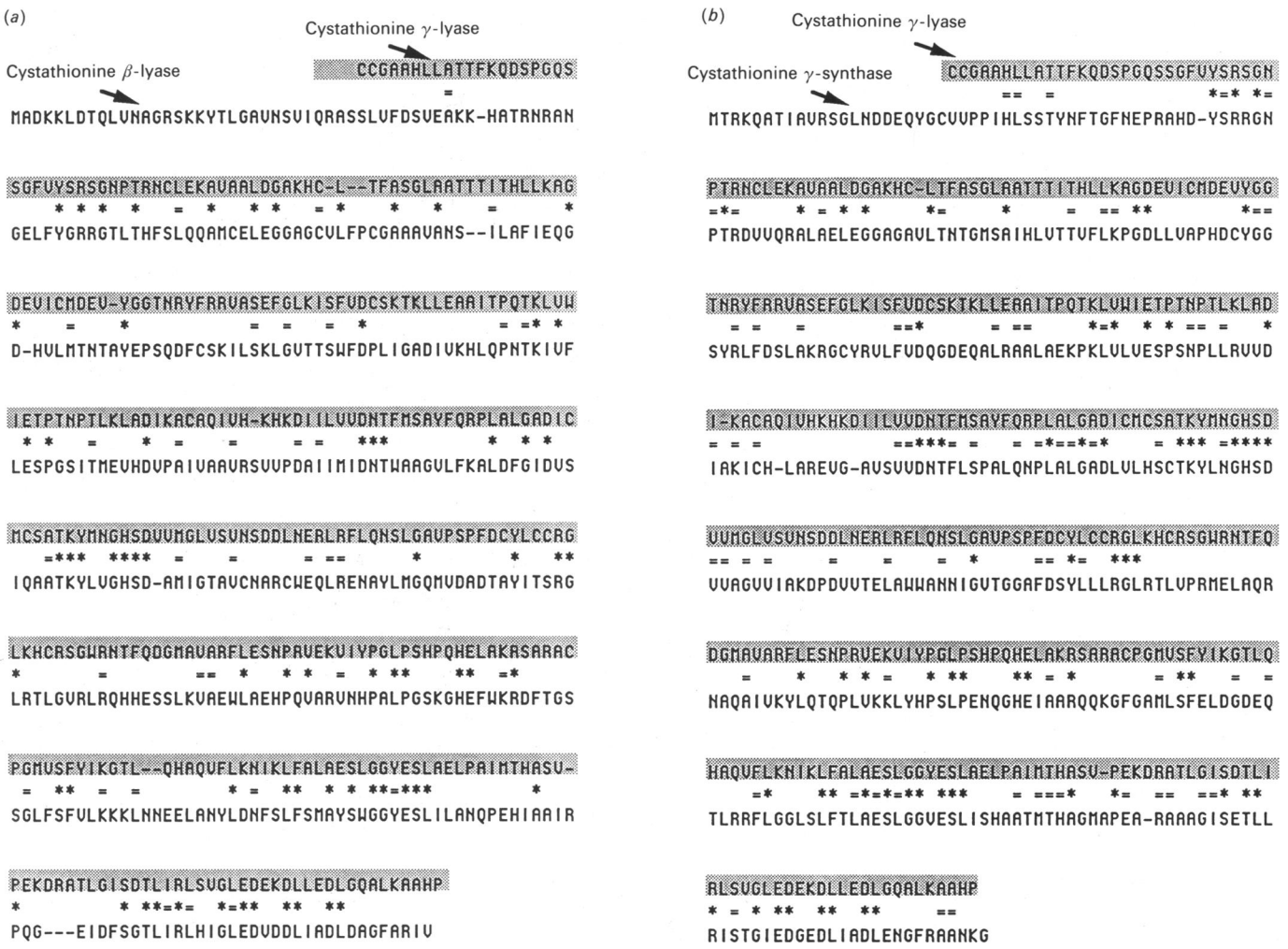

Fig. 5. Comparison of rat cystathionine γ-lyase with *E. coli* cystathionine β-lyase (*a*) and cystathionine γ-synthase (*b*)

The deduced amino acid sequences are presented in the one-letter code and have been aligned by introducing gaps (–) in order to maximize identities. The amino acid sequences of the *E. coli* proteins are whole, whereas the cystathionine γ-lyase is lacking approx. 8% of 5′-end. =, Identity of rat cystathionine γ-lyase amino acid residues with those of the *E. coli* protein under comparison; *, identity of rat cystathionine γ-lyase amino acid residues with those of both *E. coli* proteins.

Table 1. Affinities of cystathionine γ-lyase and three other enzymes involved in the metabolism of cystathionine, cysteine and cystine for these amino acids

Enzyme	Substrate	K_m (M)	Reference
Cystathionine γ-synthase			
O-Succinylhomoserine + cysteine → cystathionine + succinate	Cysteine	7.0×10^{-5}	[41]
Cystathionine γ-lyase (CSE)			
(*a*) Cystine + H_2O → pyruvate + NH_3 + thiocysteine	Cystine	9.3×10^{-5}	[42]
(*b*) Cysteine + H_2O → pyruvate + NH_3 + H_2S	Cysteine	5.2×10^{-4}	
(*c*) Cystathionine + H_2O → cysteine + NH_3 + 2-oxobutyrate	Cystathionine	3.7×10^{-3}	
Cystathionine β-lyase			
(*a*) Cystine + H_2O → pyruvate + NH_3 + thiocysteine	Cystine	2.5×10^{-4}	[43]
(*b*) Cystathionine + H_2O → NH_3 + homocysteine + pyruvate	Cystathionine	4.0×10^{-5}	
Cystathionine β-synthase			
Cysteine + H_2O → pyruvate + NH_3 + H_2S	Cysteine	3.6×10^{-2}	[44]

synthase. The primary structure of rat cystathionine β-synthase, also a pyridoxal 5′-phosphate-dependent enzyme, appears to be consistent with this correlation. This enzyme, which has the lowest affinity for cysteine of the four, exists in two forms, both different in size from the other three enzymes. The predominant form is a tetramer of 63000 Da subunits and the minor, but more catalytically active, form is a proteolytically processed dimer of 48000 Da subunits [45]. In addition, it appears that the deduced amino acid sequence of cystathionine β-synthase has little, if any, similarity to those of *E. coli* cystathionine β-lyase or cystathionine γ-synthase (Jan Kraus, personal communication).

If one accepts the similarities between rat cystathionine γ-

lyase, *E. coli* cystathionine γ-synthase and *E. coli* cystathionine β-lyase as evidence for a common ancestral gene, a remaining issue is to infer the properties of this earlier gene product from a more extensive group of homologous contemporary genes than have so far been studied. This will require the identification, cloning and sequencing of such genes, as well as an investigation of the catalytic properties of the enzymes that are expressed by them.

Towards elucidating whether the function of the ancestral gene in question was in cystathionine metabolism or, alternatively, in cysteine or cystine metabolism, informative studies might be based on genes from archaebacteria, the third kingdom of living organisms that along with eukaryotes and eubacteria such as *E. coli* descended from an earlier living organism, or progenote [46]. The blocks of amino acid sequence homologies identified herein between rat cystathionine γ-lyase and the two *E. coli* enzymes should allow the construction of synthetic oligodeoxynucleotide hybridization probes to clone candidate genes from archaebacteria. This seems an achievable goal, since genomic libraries have already been prepared for three representative archaebacterial genera. These, in fact, allowed a comparison to be made between the sequence of the same genes (components of DNA-dependent RNA polymerase) isolated from each of them and their counterparts isolated from eukaryotes and eubacteria [47].

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REFERENCES

1. Braunstein, A. E. & Goryachenkova, E. V. (1984) Adv. Enzymol. Relat. Areas Mol. Biol. **56**, 1–89
2. Meister, A. (1983) Science **220**, 472–477
3. Beatty, P. W. & Reed, D. J. (1980) Arch. Biochem. Biophys. **204**, 80–87
4. Finkelstein, J. D. (1979) Dev. Neurosci. **5**, 49–58
5. Heinonen, K. (1973) Biochem. J. **136**, 1011–1015
6. Link, D., Drebing, C. & Glode, L. M. (1983) Blut **47**, 31–39
7. Obaru, K., Nomiyama, H., Shimada, K., Nagashima, F. & Morino, Y. (1986) J. Biol. Chem. **261**, 16976–16983
8. Belfaiza, J., Parsot, C., Martel, A., Bouthier de la Tour, C., Margarita, D., Cohen, G. N. & Saint-Girons, I. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 867–871
9. Weber, G. (1977) N. Engl. J. Med. **296**, 486–493
10. Uren, J. R., Ragin, R. & Chaykovsky, M. (1978) Biochem. Pharmacol. **27**, 2807–2814
11. Bikel, I., Pavlatos, T. N. & Livingston, D. M. (1978) Arch. Biochem. Biophys. **186**, 168–174
12. Laemmli, U. K. (1970) Nature (London) **227**, 680–685
13. Erickson, P. F., Minier, L. N. & Lasher, R. S. (1982) J. Immunol. Methods **51**, 241–249
14. Knudsen, K. A. (1985) Anal. Biochem. **147**, 285–288
15. Schwarzbauer, J. E., Tamkun, J. W., Lemischka, I. R. & Hynes, R. O. (1983) Cell **35**, 421–431
16. Young, R. A. & Davis, R. W. (1983) Proc. Natl. Acad. Sci. U.S.A. **80**, 1194–1198
17. McPhaul, M. & Berg, P. (1987) Mol. Cell. Biol. **7**, 1841–1847
18. Grunstein, M. & Hogness, D. S. (1975) Proc. Natl. Acad. Sci. U.S.A. **72**, 3961–3965
19. Feinberg, A. P. & Vogelstein, B. (1983) Anal. Biochem. **132**, 6–13
20. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor
21. Sakimura, K., Kushiya, E., Obinata, M., Odani, S. & Takahashi, Y. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 7453–7457
22. Maxwell, I. H., Maxwell, F. & Hahn, W. E. (1979) Anal. Biochem. **99**, 146–160
23. Thomas, P. S. (1980) Proc. Natl. Acad. Sci. U.S.A. **77**, 5201–5205
24. Ricciardi, R. P., Miller, J. S. & Roberts, B. E. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 4927–4931
25. Pelham, H. R. B. & Jackson, R. J. (1976) Eur. J. Biochem. **67**, 247–256
26. Kessler, S. W. (1981) Methods Enzymol. **73**, 442–459
27. Bikel, I., Faibes, D., Uren, J. R. & Livingston, D. M. (1978) Biochemistry **17**, 5181–5187
28. Laskey, R. A. (1980) Methods Enzymol. **65**, 363–371
29. Allington, W. B., Cordry, A. C., McCullough, G. A. & Nelson, J. W. (1978) Anal. Biochem. **85**, 188–196
30. Wood, W. I., Gitschier, J., Lasky, L. A. & Lawn, R. M. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 1585–1588
31. Dale, R. M. K., McClure, B. A. & Houchins, J. P. (1985) Plasmid **13**, 31–40
32. Yanisch-Perron, C., Vieira, J. & Messing, J. (1985) Gene **33**, 103–119
33. Tabor, S. & Richardson, C. C. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 4767–4771
34. Maxam, A. M. & Gilbert, W. (1980) Methods Enzymol. **65**, 499–506
35. Cleveland, D. W., Fischer, S. G., Kirschner, M. W. & Laemmli, U. K. (1977) J. Biol. Chem. **252**, 1102–1106
36. Katunuma, N. & Kominami, E. (1977) in Proteinases in Mammalian Cells and Tissues (Barrett, A. J., ed.), pp. 164–180, North-Holland Publishing Co., Amsterdam
37. Sakimura, K., Yoshida, Y., Nabeshima, Y. & Takahashi, Y. (1980) J. Neurochem. **34**, 687–693
38. Okayama, H. & Berg, P. (1983) Mol. Cell. Biol. **3**, 280–289
39. Martel, A., Bouthier de la Tour, C. & LeGoffie, F. (1987) Biochem. Biophys. Res. Commun. **147**, 565–571
40. Fearon, C. W., Rodkey, J. A. & Abeles, R. H. (1982) Biochemistry **21**, 3790–3794
41. Flavin, M. & Slaughter, C. (1967) Biochim. Biophys. Acta **132**, 400–405
42. Kato, A., Ogara, M. & Masami, S. (1966) J. Biochem. (Tokyo) **59**, 40–48
43. Dwivedi, C. M., Ragin, R. C. & Urn, J. R. (1982) Biochemistry **21**, 3064–3069
44. Stipanuk, M. H. & Beck, P. W. (1982) Biochem. J. **206**, 267–277
45. Kraus, J. P. (1987) Methods Enzymol. **143**, 388–394
46. Woese, C. R. (1981) Sci. Am. **244** (6), 98–125
47. Puhler, G., Leffers, A., Grapp, F., Palm, P., Klenk, H.-P., Lottspeich, F., Garrett, R. A. & Zillig, W. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 4569–4573

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