COMPARISON OF THE N-TERMINAL SEQUENCES OF ASPARTATE AND ORNITHINE CARBAMOYLTRANSFERASES OF *ESCHERICHIA COLI*

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1. Introduction

Aspartate carbamoyltransferase (ATCase, EC 2.1.3.2), the first enzyme of pyrimidine biosynthesis, catalyzes the formation of carbamoylaspartate (or ureidosuccinate) from aspartate and carbamoylphosphate. In *E. coli*, the enzyme is encoded by the *pyr*B locus. Ornithine carbamoyltransferase (OTCase, EC 2.1.3.3), the sixth enzyme of the arginine pathway, synthesizes citrulline from ornithine and carbamoylphosphate. *E. coli* K12 is endowed with two structural genes for OTCase: *arg*I and *arg*F [1]. Their products interact to produce a family of four trimeric OTCase isoenzymes, two of which are hybrid proteins [2]. As inferred from the latter observation, *arg*F and *arg*I are very similar to each other [3,4].

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Other *E. coli* strains and other bacteria harbour only one OTCase gene which, at least in the Enterobacteriaceae, is homologous to *arg*I [4].

Besides their comparable catalytic functions, ATCase and OTCase exhibit other similarities which suggest a common origin for these two enzymes:

1. Their basic pattern of quaternary structure is the same: the ATCase molecule of *E. coli* (and probably also of *S. typhimurium* [5]) consists of two trimeric catalytic subunits and three regulatory dimers [6]. Moreover a simple trimeric structure has been clearly established for the *B. subtilis* enzyme [7]. All investigated anabolic OTCases are trimers: those of *E. coli* [2], *S. typhimurium* [8], *S. cerevisiae* [9] and ox liver [10]. The significance of this structural similarity between the two carbamoyltransferases is reinforced by the observation that trimeric proteins do not seem to be frequent.

2. The molecular weights of ATCase and OTCase catalytic chains are very similar, 33 500 [6] and 35 000 [2] in *E. coli*.

3. The kinetics of the two carbamoylation reactions are comparable ([11,12], Legrain and Stalon, in preparation).

*pyr*B and *arg*I are strongly linked and probably adjacent to each other in all the Enterobacteriaceae investigated ([1,13] and refs quoted in [4]); they are closely linked in *B. subtilis* [14]. In the light of structural and functional similarities existing between OTCase and ATCase this genetic proximity suggests that *arg*I and *pyr*B are the products of a divergent evolution encountered by tandem duplicates of an ancestral carbamoyltransferase gene. Tandem duplications, which can occur even in the absence of a generalized recombination system [15,16] could be a favoured mechanism for the acquisition of new genetic information.

If the two enzymes share a common origin, they may exhibit some amino acid sequence homology. We therefore determined the amino-terminal sequences of both the catalytic chain of ATCase and the *arg*I chain of *E. coli* K12.

2. Materials and methods

2.1. *ATCase*

The catalytic subunit of ATCase has been purified to homogeneity following the procedure of Gerhart and Holoubek [17] from the *E. coli* K12 strain constructed by these authors, a F′ *pyr*B, *his*, *pyr*F partial revertant.

2.2. *OTCase*

*arg*I OTCase has been purified from strain NCI23, an *arg*G bradytrophic mutant of a *car*B *arg*I$^+$ *arg*F$^-$ (deletion) strain, grown on citrulline as a source of carbamoylphosphate, or from an isogenic *arg*G$^+$ strain carrying an operator constitutive mutation of *arg*I [8]. The purification procedure described by Legrain and Stalon for the OTCase of *E. coli* W [12] has been followed.

2.3. *Sequence determination*

Both enzymes were dissolved in 70% formic acid after dialysis against water and submitted to automatic Edman degradation in a Beckman 890C sequenator. For amino acid identification, a Durrum D-500 analyser, a Hewlett-Packard 7620A gas chromatograph and polyamide thin-layer chromatography were used.

3. Results and discussion

In table 1, the two sequences (37 residues for ATCase, 36 for OTCase) are aligned along each other in such a way that residue x from OTCase faces residue $x + 1$ from ATCase. Ten residues are identical, 4 of which are in a row; 4 additional identities are possible at ATCase residues No. 8, 21, 33 and 35. The overall identity between the two sequences is thus about 30%. Among the other residues, 14 could have resulted from a single nucleotide substitution (see arrows in table 1).

Possible homologies in secondary structure have been investigated by using the conformational parameters computed by Chou and Fasman [19] and applying the predictive rules derived by the same authors [20]. Noteworthy are the stretches from residues 18–33 in ATCase and 17–34 in OTCase both of which exhibit a strong probability to adopt an α-helix configuration. In addition α helices appear likely to include residues 3–8 in ATCase, 5–12 in OTCase.

The noted sequence similarities strengthen the suggestion [2] that ATCase and OTCase share a common origin, having evolved from tandem duplicates of an ancestral gene. As the biosynthetic pathways must have been acquired at a still primitive stage of metabolic evolution, the alleged divergence of the duplicated transferase gene may be considered as a primeval case of 'enzyme recruitment' [21,22]: a primitive carbamoyltransferase, active mainly towards aspartate or ornithine and possibly endowed with a certain degree of substrate ambiguity could have differentiated into the sister enzyme.

The determination of the total amino acid sequences of ATCase and OTCase will be a further test of this hypothesis. In the case of ATCase, an almost complete sequence has been obtained for the catalytic chain (Konigsberg, W., manuscript in preparation); it bears no obvious relationship to that of the regulatory chain [23].

Table 1
N-terminal amino acid sequences of *E. coli* K12 aspartate and ornithine carbamoyltransferases catalytic chains

Aspartate carbamoyltransferase (ATCase)					Ornithine carbamoyltransferase (OTCase)			
Residue no.	α	β	Amino acid		Amino acid	α	β	Residue no.
1	H	I	Ala					
2	b	b	Asn	⟷	Ser	i	b	1
3	B	b	Pro		Gly	B	i	2
4	H	h	Leu	⟷	Phe	h	h	3
5	b	h	Tyr		Tyr	b	h	4
6	h	h	Gln		(Arg)	(i)	(i)	5
7	I	b	Lys		Lys	I	b	6
8	h	b	His		(His)	(h)	(b)	7
9	I	H	Ile	⟷	Phe	h	h	8
10	I	H	Ile	⟷	Leu	H	h	9
11	i	b	Ser		Lys	I	b	10
12	I	H	Ile	⟷	Leu	H	h	11
13	b	b	Asn		Leu	H	h	12
14	i	i	Asp		Asp	i	i	13
15	H	h	Leu	⟷	Phe	h	h	14
16	i	b	Ser	⟷	Thr	i	h	15
17	i	i	Arg	⟷	Pro	B	b	16
18	i	i	Asp	⟷	Ala	H	I	17
19	i	i	Asp	⟷	Glu	H	B	18
20	H	h	Leu		Leu	H	h	19
21	b	b	Asn		Asx	i/b	i/b	20
22	H	h	Leu	⟷	Ser	i	b	21
23	h	H	Val	⟷	Leu	H	h	22
24	H	h	Leu		Leu	H	h	23
25	H	I	Ala		Gln	h	h	24

Table 1 (continued)

Aspartate carbamoyltransferase (ATCase)				Ornithine carbamoyltransferase (OTCase)			
Residue no.	α	β	Amino acid	Amino acid	α	β	Residue no.
26	i	h	Thr	Leu	H	h	25
27	H	I	Ala	Ala	H	I	26
28	H	I	Ala ⟷	Ser	i	b	27
29	I	b	Lys	Lys	I	b	28
30	H	h	Leu	Leu	H	h	29
31	I	b	Lys	Lys	I	b	30
32	H	I	Ala	Ala	H	I	31
33	b	b	Asn	Asx	i/b	i/b	32
34	B	b	Pro	Lys	I	b	33
35	h	h	Gln	Glx	H/h	B/h	34
36	B	b	Pro				35
37	H	B	Glu ⟷	Gly	B	i	36

α and β assignments refer to helical potential (α) and β-sheet potential (β) according to Chou and Fasman [20]. Arrows indicate pairs of residues of which the codons could differ by one single base. A certain degree of ambiguity remains for a few amino acids which have been marked by parentheses.

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