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HOMOLOGY OF 1-AMINOCYCLOPROPANE-1-CARBOXYLATE SYNTHASE, 8-AMINO-7-OXONONANOATE SYNTHASE, 2-AMINO-6-CAPROLACTAM RACEMASE, 2,2-DIALKYLGLYCINE DECARBOXYLASE, GLUTAMATE-1-SEMIALDEHYDE 2,1-AMINOMUTASE AND ISOPENICILLIN-*N*-EPIMERASE WITH AMINOTRANSFERASES

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Profile analysis showed the title enzymes to be homologous with the aminotransferases. 1-Aminocyclopropane-1-carboxylate synthase is closely related to subgroup I of aminotransferases which includes aspartate, alanine, histidinol-phosphate, tyrosine and phenylalanine aminotransferase. 2,2-Dialkylglycine decarboxylase, glutamate-1-semialdehyde 2,1-aminomutase and 2-amino-6-caprolactam racemase are most similar to subgroup II which comprises aminotransferases with ω-amino acids as substrates. 8-Amino-7-oxononanoate synthase is closely related to both subgroup I and II, and isopenicillin-*N*-epimerase to subgroup IV with serine and phosphoserine aminotransferase. Aminotransferases and the title enzymes belong to a regio-specific family of evolutionarily related pyridoxal-5'-phosphate-dependent enzymes. © 1994 Academic Press, Inc.

A recent comparison of all known amino acid sequences of aminotransferases has shown that they form a group of homologous pyridoxal-5'-phosphate-dependent enzymes (1). The evolutionary relationships, however, are rather distant and most pair-wise comparisons show degrees of sequence identity that are in general considered insignificant. Therefore, the homology was confirmed in a quantitative manner by profile analysis, an algorithm especially suited for the detection of distant relationships, because it takes into account family-specific features (2). On the basis of their mutual similarity, the aminotransferases were subdivided into four subgroups. This paper reports that certain subgroup profiles, i.e. the scoring tables constructed from the set of the aligned sequences of the individual subgroups, were found to indicate homology not only among the members of the respective subgroup and some aminotransferases of other subgroups but also with the title enzymes which catalyze transformations of amino acids other than transamination.

DATA BASE AND METHODOLOGY

The amino acid sequences of 1-aminocyclopropane-1-carboxylate synthase, 8-amino-7-oxononanoatesynthase,2,2-dialkylglycinedecarboxylase,glutamate-1-semial-

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Table I. Known amino acid sequences of the title enzymes

Enzyme	EC number	Source	Abbrev.	Reference
8-Amino-7-oxononanoate synthase	2.3.1.47	*Bacillus sphaericus*	AonSYbs	Gloeckler, R. *et al.*, 1990 (unpublished)
		Escherichia coli	AonSYec	3
2,2-Dialkylglycine decarboxylase	4.1.1.64	*Pseudomonas cepacia*	DagDCpc	4
1-Aminocyclopropane-1-carboxylate synthase	4.4.1.14	*Cucurbita maxima*	AccSYcm	5
		Cucurbita pepo	AccSYcp	6
		Dianthus caryophyllus	AccSYdc	7
		Glycine max	AccSYgm	Liu, D., Li, N., & Matto, A. K., 1992 (unpublished)
		Lycopersicon esculentum	AccSYle	8
		Tomato	AccSYt	9
		Vigna radiata	AccSYvg	10
Glutamate-1-semialdehyde 2,1-aminomutase	5.4.3.8	*Bacillus subtilis*	GsaAMbs	11
		Escherichia coli	GsaAMec	12
		Hordeum vulgare	GsaAMhv	13
		Nicotiana tabacum	GsaAMnt	Axelsen and Grimm, 1992 (unpublished)
		Salmonella typhimurium	GsaAMst	14
		Synechococcus sp.	GsaAMss	12
Isopenicillin-*N*-epimerase	[a]	*Streptomyces clavuligerus*	IpeEPsc	15
2–Amino-6–caprolactam racemase	[a]	*Achromobacter obae*	AclRMao	16

[a] No EC number has been attributed to this enzyme.

dehyde 2,1-aminomutase and isopenicillin-*N*-epimerase have been deposited in the Swiss-Prot (Release 24), EMBL DataLib (Release 33) or GenBank (Release 73) databases; the sequence of 2-amino-6-caprolactam racemase has not yet been included into a database (Table I). Standard comparison and alignment programs as included in the University of Wisconsin GCG software package, version 7.2 (17) were used with default parameters for multiple sequence alignments. The Prettybox program of R. Westerman (unpublished) was used to denote identical residues in presentations of multiple sequence alignments.

The profile-analysis package is part of the University of Wisconsin GCG sequence analysis package. It was used with the default parameters. The profile, a position-specific scoring table, is constructed from a set of aligned homologous sequences, the probe, and an amino acid substitution matrix. It attributes scores to each of the 20 amino acids and gaps at all positions in the alignment. The total score of the target sequence corresponds to the algebraic sum of the scores attributed to all its residues, insertions and gaps. The Z score corresponds to the difference between the score attributed to the target sequence and the mean score of unrelated sequences expressed in terms of the standard deviation and is normalized for the length of the sequences compared. A target sequence attaining a Z score > 6.0 is considered to be related to the probe sequences (18); Z score values between 3.0 and 6.0 are considered still meaningful as they might indicate distant relationships.

RESULTS

The profile constructed from the sequences of the five enzymes of aminotransferase subgroup I, i.e. aspartate, alanine, histidinol-phosphate, tyrosine and phenylalanine aminotransferase (1), attributes high Z scores (> 6) to the sequences of 1-aminocyclopropane-1-carboxylate synthase. Accordingly, in the reciprocal profile analysis the profile constructed from the five sequences of 1-aminocyclopropane-1-carboxylate synthase attributes Z scores to the sequences of subgroup I that are in part well above the significance limit (Table II). Profiles constructed from sequences of the other aminotransferase subgroups yielded lower Z score values for 1-aminocyclopropane-1-carboxylate synthase than the subgroup I profile.

TABLE II. Reciprocal profile analyses of title enzymes and aminotransferase subgroups

Probe	Target enzyme(s)	Z score	Sequence with maximum score
Subgroup I (5 enzymes, 16 sequences)	Acc synthase	6.4 - 6.7	Acc synthase *Lycopersicon esculentum*
Acc synthase (5 sequences)	Subgroup I	2.8 - 13.2	Aspartate aminotransferase *Bacillus species*
Subgroup II (5 enzymes, 10 sequences)	Aon synthase	5.6	Aon synthase *Escherichia coli*
Aon synthase (2 sequences)	Subgroup I	0.04 - 5.4	Histidinol-phosphate aminotransferase *Haloferax volcanii*
	Subgroup II	0.08 - 3.7	ω-Amino acid-pyruvate aminotransferase *Pseudomonas putida*
Subgroup II (5 enzymes, 10 sequences)	Dag decarboxylase	21.2	Dag decarboxylase *Pseudomanas cepacia*
Dag decarboxylase (1 sequence)	Subgroup II	6.8 - 22.1	4-Aminobutyrate aminotransferase *Escherichia coli*
Subgroup II (5 enzymes, 10 sequences)	Gsa aminomutase	13.8 - 16.8	Gsa aminomutase *Escherichia coli*
Gsa aminomutase (6 sequences)	Subgroup II	4.5 - 18.9	4-Aminobutyrate aminotransferase *Escherichia coli*
Subgroup II (5 enzymes, 10 sequences)	Acl racemase	21.8	Acl racemase *Achromobacter obae*
Acl racemase (1 sequence)	Subgroup II	7.6 - 25.3	4-Aminobutyrate aminotransferase *Escherichia coli*
Subgroup IV (2 enzymes, 6 sequences)	Ipe epimerase	4.7	Ipe epimerase *Streptomyces clavuligerus*
Ipe epimerase (1 sequence)	Subgroup IV	0.1 - 7.2	Serine aminotransferase Rat

The subgroup profiles were based on the previously published alignments (1). The abbreviations are explained in Table I. The profiles of the title enzymes are based on the alignments in Fig. 1. In all profile searches, none of the unrelated sequences in the data base reached a Z score > 4. Scores > 4 were obtained among 2,2-dialkylglycine decarboxylase, glutamate-1-semialdehyde 2,1-aminomutase, 2-amino-6-caprolactam racemase themselves as well as with the profiles of 1-aminocyclopropane-1-carboxylate synthase and isopenicillin-*N*-epimerase for the mal*Y* and the nif*S* gene product, respectively, both of which have been shown previously to be homologous to aminotransferases (19).

Similar profile analyses indicated definitive structural relationships of the aminotransferases of subgroup II with 8-amino-7-oxononanoate synthase, 2,2-dialkylglycine decarboxylase, glutamate-1-semialdehyde 2,1-aminomutase and 2-amino-6-caprolactam racemase, as well as between the aminotransferases of subgroup IV and isopenicillin-*N*-epimerase. 8-Amino-7-oxononanoate synthase proved a special case, it appears to be related to both subgroup I and II. In all analyses with profiles constructed from the sequences of the title enzymes, the maximum Z scores obtained by sequences other than those of the aminotransferases of the most closely related subgroup were < 4.

DISCUSSION

The profile searches show that the six title enzymes, which all have been identified previously as pyridoxal-5'-phosphate-dependent enzymes, are structurally related to specific subgroups of aminotransferases. The sequences of 1-amino-cyclopropane-1-carboxylate synthase, 2,2-dialkylglycine decarboxylase and isopenicillin-*N*-epimerase have been aligned previously with sequences of subgroup I aminotransferases (22), ornithine aminotransferase (4), and serine and phosphoserine aminotransferase (21), respectively. However, the degrees of sequence identity deduced from the alignments were relatively low. The homology of these enzymes with aminotransferases is now confirmed in a quantitative manner by profile analysis. Homology of 2,2-dialkylglycine decarboxylase with aspartate aminotransferase has also been indicated by similar folding patterns of their polypeptide chains (23). No complete sequence alignment of glutamate-1-semialdehyde 2,1-aminomutase with aminotransfera-ses has been published nor has their homology been quantitatively established (14). 8-Amino-7-oxononanoate synthase has been aligned previously with 7,8-diaminopelargonate aminotransferase (3) which, however, is not the aminotransferase most closely related with it. The relationship of 2-amino-6-caprolactam racemase with aminotransferases has not been recognized before.

Can the homology of the title enzymes with aminotransferases be rationalized in terms of the reactions they catalyze? The common denominator of these enzymes is that the transformations of their substrates depend on cleavage of a C-H or C-C bond at the carbon atom that carries the amino group forming the imine linkage with pyridoxal-5'-phosphate (for reviews of the mechanisms of action of pyridoxal-5'-phosphate-dependent enzymes, see Refs 24, 25). Each title enzyme, with the exception of 8-amino-7-oxononanoate synthase, is most closely related to a particular subgroup of aminotransferases (Table II). Apparently, the title enzymes, though they do not catalyze transamination reactions, diverged from the aminotransferases only after these had specialized into subgroups.

The relationship of 8-amino-7-oxononanoate synthase and glutamate-1-semialdehyde 2,1-aminomutase to aminotransferase subgroup II seems plausible. In the substrates of both enzymes, the amino group is distal of the carboxylate group, and subgroup II comprises indeed all aminotransferases acting on ω-amino acids (1). In the alignment of glutamate-1-semialdehyde 2,1-aminomutase, 2,2-dialkylglycine decarboxylase, 2-amino-6-caprolactam racemase and 4-aminobutyrate aminotransferase only two residues, i.e. the coenzyme-binding lysine residue and the glutamate residue interacting with the pyridine-N of the coenzyme, were found to remain invariant out of the four invariant residues in the comprehensive alignment of aminotransferases (1). The absence of a conserved arginine residue downstream of the pyridoxal-5'-phosphate-binding lysine residue in the previously reported alignment of glutamate-1-semialdehyde 2,1-aminomutase sequences with an overall identity of 39 % (11) supports the conclusion that the equivalent of Arg562AT (Arg386 in cytosolic aspartate aminotransferase) which is invariant in aminotransferases (1) is missing in glutamate-1-semialdehyde 2,1-aminomutase. In the aminotransferases, Arg562AT binds the α-carboxylate group of the substrates. The residue is also found invariant in the aminotransferases of subgroup II which accept ω-amino acids as substrate (2), very likely because these enzymes act with 2-oxoglutarate and glutamate as the substrate/product pair of the second half reaction. The absence of an equivalent to Arg562AT in glutamate-1-semialdehyde 2,1-aminomutase seems thus explainable by the fact that this enzyme does not act on dicarboxylic substrates. 2-Amino-6-caprolactam racemase is closer to subgroup II than to any of the other subgroups very likely because its amino group is again not at a carbon atom substituted with a carboxylate group. 2,2-Dialkylglycine decarboxylase functions in fact as an aminotransferase with L-amino acids that are not alkylated at Cα (26). It remains to be explained why it is most closely related with subgroup II. Perhaps both subgroup II aminotransferases and 2,2-dialkylglycine decarboxylase possess two different binding sites, namely for α-amino acids on the one hand and for ω-amino acids and dialkylglycine, respectively, on the other. For the close relationship of 1-aminocyclopropane-1-carboxylate synthase with aminotransferase subgroup I, the decisive common feature might be the ring system in the substrate; subgroup I comprises among others histidinol-phosphate aminotransferase and the aromatic amino acid aminotransferases. The basis for the affiliation of isopenicillin-*N*-epimerase with subgroup IV is unclear.

The present data show that the aminotransferases and the title enzymes which catalyze reactions of amino acids other than transamination constitute a regio-specific

enzyme family, the members of which use a homologous protein scaffold to catalyze different reactions.

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