Homologous Amino Acid Sequences in Enzymes Mediating Sequential Metabolic Reactions

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Muconolactone A-isomerase (EC 5.3.3.4) and \( \beta \)-keto-adipate enol-lactone hydrolase (EC 3.1.1.24) mediate sequential metabolic steps in the \( \beta \)-keto-adipate pathway. This catabolic sequence is used by members of the biologically distant bacterial genera Acinetobacter and Pseudomonas. Different regulatory mechanisms are used to govern the synthesis of the enzymes in the two genera. For example, isofunctional enol-lactone hydrolases I and II are formed in response to different inducers in Acinetobacter calcoaceticus whereas a single enol-lactone hydrolase is induced in Pseudomonas putida.

The muconolactone isomerases of the two bacterial genera share 28 of the first 50 residues in their NH\(_2\) terminal amino acid sequences, indicating that the proteins are evolutionarily homologous. Introduction of a single gap permits 10 of the first 29 residues of the NH\(_2\) terminal amino acid sequence of Acinetobacter enol-lactone hydrolase II to be aligned with identical residues in the Pseudomonas muconolactone isomerase sequence. The similar sequences, corresponding to about 30% of the primary structure of the proteins, indicate that these enzymes, endowed with different catalytic functions, share a common ancestral gene. Alignment of the Acinetobacter muconolactone isomerase and the Acinetobacter enol-lactone hydrolase II sequences reveals only 5 identical residues in the 29-residue comparison (after introduction of a single gap). This evidence indicates that the genes for the enzymes diverged as they were co-selected within the genus.

The evolution of metabolic pathways demands the acquisition of enzymes with novel functions. In part, the accretion of metabolic capabilities may be achieved by cells "borrowing" enzymes from one pathway to serve a function demanded in an evolving metabolic sequence; the catalytic site of the enzyme is selected, and its substrate specificity or biosynthetic regulation is altered by mutation (1, 2). This form of evolution has been observed in laboratory experiments with microorganisms (3, 4), and abundant evidence indicating the inter-pathway borrowing of enzymes has come from the demonstration of homologous amino acid sequences in "families" of enzymes that subject different substrates to identical chemical type reactions (5).

The concept of borrowed enzymes leaves open the question of how new catalytic capabilities evolved. This question was addressed by Horowitz (6, 7) in his retroevolution hypothesis. In general form, the hypothesis states that evolution initially selects the site that binds the substrate to an enzyme and that novel catalytic properties are acquired by mutation. Since enzymes bind their products as well as their substrates, a duplicated gene for an enzyme catalyzing one step in a metabolic pathway may serve as the ancestral gene for the evolution of the enzyme catalyzing the next step in the pathway (8). According to the retroevolution hypothesis, mutations alter the newly evolved enzyme so that it subjects the product of the ancestral enzyme to a different chemical transformation. The hypothesis has been applied to the complex catabolic pathways of bacteria by Dagley (9) who observed that similar transition state intermediates are likely to be formed within some sequences of catabolic reactions. A common ancestral enzyme might be altered to give rise to enzymes that mediate different chemical transformations via analogous transition states. Jeffcoat and Dagley (10) demonstrated that \( \gamma \)-glucuronate hydrolyase from Klebsiella aerogenes and Pseudomonas acidovorans slowly catalyzes the aldolase cleavage of its product. Thus, it appears that the hydrolyase could resemble the evolutionary precursor of the aldolase. In another study, Truffa-Bachi et al. (11) used immunoabsorbent columns to demonstrate common antigenic determinants in enzymes catalyzing sequential biosynthetic reactions in Escherichia coli.

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FIG. 1. Sequential metabolic reactions mediated by muconolactone isomerase and \( \beta \)-keto-adipate enol-lactone hydrolase. The isomerase mediates the intralactonic migration of a double bond, and enol-lactone hydrolase catalyzes the conversion of a lactone to a dicarboxylic acid.
Evolutionary Origin of Metabolic Diversity

1.2.3.4.5.6.7.8.9.10.11.12.13.14.15.16.17.18.19.20.21.22.23.24.25.26.27.28.29.30.31.32.33.34.35.36.37.38.39.40.41.42.43.44.45.46.47.48.49.50.

The procedures and results are described in the supplement.1

Discussion

Muconolactone Isomerases from Acinetobacter and Pseudomonas are Evolutionarily Homologous—As shown in Fig. 2, 28 of the first 50 residues in the muconolactone isomerase and enol-lactone hydrolase sequences at residue 11. Identical residues are enclosed in boxes, and residues that are underlined differ by single base changes in their codons.

We have used automated techniques to extend the comparison of the isomerase NH\_2-terminal amino acid sequences. In addition, we have determined the NH\_2-terminal amino acid sequence of Acinetobacter enol-lactone hydrolase II. The results, presented in this report, reveal similar sequences, suggesting that all three proteins share a common evolutionary origin.

Experimental Procedures and Results

The procedures and results are described in the supplement.1

Fig. 2. NH\_2-terminal amino acid sequences of muconolactone isomerases from Acinetobacter and Pseudomonas. Identical residues are enclosed in boxes, and residues that are underlined differ by single base changes in their codons.

Fig. 3. NH\_2-terminal amino acid sequences of Pseudomonas muconolactone isomerase and Acinetobacter enol-lactone hydrolase. A single gap has been introduced into the enol-lactone hydrolase sequence at residue 7. Identical residues are enclosed in boxes, and residues that are underlined differ by single base changes in their codons.

Fig. 4. NH\_2-terminal amino acid sequences of Acinetobacter muconolactone isomerase and enol-lactone hydrolase. A single gap has been introduced into the enol-lactone hydrolase sequence at residue 7. Identical residues are enclosed in boxes, and residues that are underlined differ by single base changes in their codons.

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1 Portions of this paper (including "Experimental Procedures and Results," Tables I to III, and additional references) are presented in miniprint at the end of this paper. Miniprint can easily be read with the aid of a standard magnifying glass. Pull size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document No. 78M132, cite author(s) and include a check or money order for $1.05 per set of photocopies.
similar catalytic sites from different evolutionary precursor proteins could not have produced such widespread sequence similarities, so we conclude that the two proteins share an ancestral gene.

The close similarity of the structures of the two isomerases raises the possibility that genes for the enzymes may have been exchanged between Acinetobacter and Pseudomonas at a recent point in evolutionary history. Weighted against this interpretation must be the rigorous conservation of antigenic determinants for enzymes of the β-ketoacid pathway within fluorescent Pseudomonas biotypes (18). The serological evidence (18) suggests that genes for the pathway have not been exchanged among Pseudomonas biotypes subsequent to their evolutionary divergence, and intragenic transfer of the genes between Acinetobacter and Pseudomonas is even less likely to have occurred.

Pseudomonas Muconolactone Isomerase and Acinetobacter Enol-lactone Hydrolase II, Enzymes Catalyzing Sequential Metabolic Reactions, Possess Homologous NH₂-Terminal Amino Acid Sequences—The NH₂-terminal amino acid sequences of Pseudomonas muconolactone isomerase and Acinetobacter enol-lactone hydrolase are depicted in Fig. 3. A single gap has been introduced into the hydrolase sequence, aligning its 29 residues with the first 30 residues of the isomerase sequence. Six of the 19 residues following the gap at position 11 are identical (Fig. 3). That this is strong evidence for structural homology is shown by the data of Haber and Koshland (19) who used random number tables to demonstrate that the probability of 6 residues appearing at identical positions in two random 20-residue sequences is less than 0.001. Additional evidence for structural homology comes from the identity of 4 of the first 10 residues in the NH₂-terminal amino acid sequences (Fig. 3). If the observed homology extends over the entire sequence of the proteins, the conclusion that a single ancestral enzyme gave rise to the isomerase and the hydrolase may be drawn. At present, another possibility must be entertained; the homologous NH₂-terminal segment might have been introduced into one of the enzymes by genetic recombination after the catalytic activity of each enzyme had been established.

Acinetobacter Muconolactone Isomerase and Enol-lactone Hydrolase II, Co-selected within a Single Ancestral Line, Have Diverged Widely—The NH₂-terminal amino acid sequences of Acinetobacter muconolactone isomerase and enol-lactone hydrolase II are compared in Fig. 4. As in the comparison of the Pseudomonas isomerase and the Acinetobacter hydrolase, a single gap has been introduced into the hydrolase sequence in order to align its first 29 amino acid residues with the first 30 residues of the isomerase. Only 5 of the 29 residues are identical in both sequences (Fig. 4). Indeed, the homology between the isomerase and the hydrolase would not have been apparent if the comparison had been restricted to enzymes from Acinetobacter. Why does the isomerase from the biologically remote Pseudomonas resemble the Acinetobacter hydrolase more closely than does the Acinetobacter isomerase? The available evidence does not offer a conclusive answer to this question, but attention should be drawn to selective forces that might favor the evolutionary divergence of genes as they co-exist within an organism. These forces include (i) the stable replication of a genome containing duplicated genes and (ii) the accurate self-assembly of subunits for homologous enzymes within a cell (12). A general conclusion that may be drawn is that the relatedness of different enzymes may not be apparent if they are drawn from narrowly circumscribed biological sources.

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Additional references will be found on p. 4923.
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