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THE EVOLUTION OF PEROXISOMAL AND MITOCHONDRIAL ALANINE: GLYOXYLATE AMINOTRANSFERASE 1 IN MAMMALIAN LIVER

Yoshikazu Takada and Tomoo Noguchi

Department of Biochemistry, Kyushu Dental College, Kitakyushu 803, Japan

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SUMMARY. Immunological distances of alanine:glyoxylate aminotransferase 1 (serine:pyruvate aminotransferase) in mitochondria or peroxisomes from eight different mammalian liver were determined with rabbit antiserum against the mitochondrial enzyme of rat liver by microcomplement fixation. Results suggest that heterotopic alanine:glyoxylate aminotransferase 1 are orthologous proteins and their subcellular localization and substrate specificity changed during rapid molecular evolution.

INTRODUCTION.

Alanine:glyoxylate aminotransferase (EC 2.6.1.44) is distributed in the liver of a wide range of animal species (1-3). Mammalian liver contains two forms of alanine:glyoxylate aminotransferase, designated AGT 1 and AGT 2, respectively. AGT 1 has been reported to be identical with serine:pyruvate aminotransferase (EC 2.6.1.51) (2). All of AGT 1 from mammalian liver consist of two identical subunits with a molecular weight of about 40,000 and are immunologically cross-reactive (2-4). However, intracellular organelles containing hepatic AGT 1 and its substrate specificity vary from species to species: it is located in the peroxisomes in human, monkey, rabbit and guinea-pig (3, 4), in the mitochondria in dog and cat (3-5), and in both organelles in rat, mouse (3, 6-9) and hamster (unpublished data). AGT 1 from rodents (rat, mouse and hamster) catalyse transamination between various L-amino acids and pyruvate or glyoxylate, while AGT 1 from other mammalian species are highly specific for L-alanine and glyoxylate (2, 4, 7,

Abbreviations: AGT, alanine:glyoxylate aminotransferase; ID, immunological distance. Myr: million years.

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8). It is of interest to estimate the degree of structural similarity among AGT 1 in different organelles from different mammalian species.

The present report suggests that on the basis of the immunologica distances (ID) of AGT 1 in different organelles from different mammalian species, heterotopic AGT 1 are orthologous proteins (10) and their subcellular localization and substrate specificity changed during rapid molecular evolution.

EXPERIMENTAL PROCEDURES

Materials Lyophilized guinea-pig serum as the source of complement wa obtained from Kyowa Pharmaceutical Co. (Kyoto, Japan) and sensitized sheep erythrocytes from Ishizu Pharmaceutical Co. (Osaka, Japan). Other reagents used were commercial products of a highest grade available. AGT 1 from dog, cat, guinea-pig, hamster, monkey, human, and dibutyryl cyclic AMP-injected rat and mouse were prepared from liver crude mitochondrial extracts as previously described (3). Peroxisomal fraction without mitochondrial contamination were prepared from rat and mouse liver by the method of Neat and Osmundsen (11). Peroxisomal extracts prepared as in (8) was used as the peroxisomal AGT 1 antigen (rat, mouse). Anti-rat AGT 1 antiserum was prepared and found to be highly specific as described in previous reports (3, 7). The titer of the antiserum, defined as the dilution of antiserum required for 50% complement fixation with homologous antigen, was found to be 1/9600.

Methods Microcomplement fixation experiments were performed according to (12). The complement fixation curves were evaluated as described b Sonderegger et al. (13). ID was calculated according to ID = 100 log (antiserum concentration for 50% complement fixation with heterologous antigen/antiserum concentration for 50% complement fixation with homologous antigen) (12-14).

RESULTS AND DISCUSSION

ID of heterotopic AGT 1 in mitochondria or peroxisomes from different mammalian liver were determined by microcomplement fixation with the antiserum against rat mitochondrial AGT 1 (Table 1). For a set of homologous variants of a given protein, ID has been found to correlate linearly with percentage amino acid sequence difference: ID = 5 x percentage of amino acid sequence difference (15, 16). ID of rat peroxisomal AGT 1 was 0.0 and ID of peroxisomal and mitochondrial AGT 1 from mouse liver were identical (ID=32). This suggests that peroxisomal and mitochondrial AGT 1 from the same mammalian liver have an identical amino acid sequence. ID of peroxisomal and mitochondrial

Table 1. Immunological distances (ID) of AGT 1 in mitochondria or peroxisomes from eight different mammalian liver with rabbit antiserum against the mitochondrial enzyme of rat liver.

Species	Intracellular Localization	Index of Dissimilarity	Immunological Distance
Rat	Mitochondria	1	0
	Peroxisomes	1.00	0
Mouse	Mitochondria	2.09	32
	Peroxisomes	2.09	32
Hamster	Mitochondria	6.17	79
Guinea-pig	Peroxisomes	60.3	178
Monkey	Peroxisomes	37.2	157
Human	Peroxisomes	79.4	190
Dog	Mitochondria	43.7	164
Cat	Mitochondria	49.0	169

Index of dissimilarity = antiserum concentration for 50% complement fixation with heterologous antigen/antiserum concentration for 50% complement fixation with homologous antigen. ID = 100 x log(Index of dissimilarity) (12-14).

AGT 1 from the indicated mammalian species were plotted against the paleontologically estimated times of divergence of ancestors of rat from the other mammalian species. ID was not corrected for multiple mutations at the same site (Fig. 1). The order of ID of AGT 1 reflects the phylogenic branching order of the species in which they are found. The strong correlation was found between ID and paleontological estimates of divergence time (r=0.94). The evolution of peroxisomal AGT 1 were at constant and nearly identical rate. These findings suggest that peroxisomal and mitochondrial AGT 1 were orthologous proteins, and were not formed by divergent evolution after gene duplication (10).

The evolutionary constraint on the structure of enzyme had been suggested for heterotopic aspartate aminotransferase isoenzymes, a homologous isoenzymes which have arisen by divergent evolution from a common ancestral protein: it was suggested that since the emergence of mammals the cytosolic isoenzymes have been evolving at twice the rate

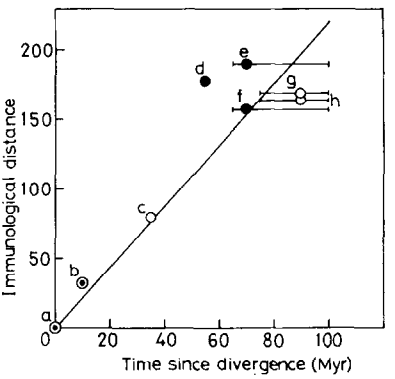

Fig. 1. Evolution of peroxisomal (●) and mitochondrial (○) AGT 1. The immunological distances (ID) of AGT 1 in mitochondria or peroxisomes from eight different mammalian liver with rabbit antiserum against mitochondrial enzyme of rat liver were plotted against the paleontologically estimated times of divergence of the ancestores of rat from the various mammalian species. ID were taken from Table 1 and were not corrected for multiple mutations at the same site. The assumed times of divergence are in Myr: dog or cat, 90(75-100) (10, 17); human or monkey, 70(65-100) (10, 17); guinea-pig, 55(18); hamster, 35(18); mouse, 10(18). The slope of the line was ~ 2.2 (r=0.94). (a) rat; (b) mouse; (c) hamster; (d) guinea-pig; (e) human; (f) monkey; (g) cat; (h) dog.

of that of the mitochondrial form, and the slower rate of evolution of mitochondrial isoenzymes has been suggested to imply the existence of additional evolutionary constraint on the structure of organelle-confined enzyme (13, 14). The finding that the rate of evolution for peroxisomal and mitochondrial AGT 1 in mammalian species was identical suggests that no evolutionary constraint was operative on the structure of AGT 1 confined to different organelles.

The time required for a 1% change in the amino acid sequence of AGT 1 to arise between two lineage can be estimated to be ~ 2.2 Myr, suggesting that the evolution of AGT 1 was relatively rapid (10).

It has been reported that hepatic AGT 1 from rat, mouse and hamster (unpublished data) liver are remarkably different from those from the other mammalian species in substrate specificity (2, 4, 7, 8). The present study suggests that the subcellular distribution and substrate specificity of AGT 1, an orthologous protein, changed during rapid molecular evolution.

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