Review

Studies on a unique organelle localization of a liver enzyme, serine:pyruvate (or alanine:glyoxylate) aminotransferase

By Arata ICHIYAMA[*1,†]

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Abstract: Serine:pyruvate (or alanine:glyoxylate) aminotransferase (SPT or AGT) in the liver is unique in that its subcellular distribution is entirely peroxisomal in man and herbivores, and largely mitochondrial in carnivores. In rats, this enzyme is located in both mitochondria and peroxisomes and only the mitochondrial activity is markedly induced by glucagon. The mechanism of the species-specific dual organelle localization is either transcription of the gene from two different start sites or loss of upstream translation initiation ATG codon by mutations. In herbivores, peroxisomal localization of SPT appears to be indispensable to prevent excessive oxalate production by removing glyoxylate, an immediate precursor of oxalate, formed from glycolate in this organelle. In carnivores, its mitochondrial localization appears to be needed to metabolize glyoxylate formed from L-hydroxyproline in mitochondria. In addition, SPT contributes substantially to gluconeogenesis from serine in rabbit, human and dog livers, irrespective of its mitochondrial or peroxisomal localization.

Keywords: serine:pyruvate (or alanine:glyoxylate) aminotransferase, peroxisomal and/or mitochondrial localization, alternative transcription initiation from two start sites, gluconeogenesis from L-serine, *in situ* detoxication of glyoxylate, oxalate formation

Introduction

Eukaryotic cells contain cellular organelles such as a nucleus, mitochondria, lysosomes, and peroxisomes, each of which is responsible for an important part of cellular functions. In addition, compartmentation into such organelles of specific metabolic processes or enzymes is believed to facilitate regulation of these processes independent of other processes proceeding elsewhere. In this sense, alanine:glyoxylate (or serine:pyruvate) aminotransferase (AGT or SPT) in the liver is unique in that its subcellular distribution is entirely peroxisomal in man and herbivores and largely mitochondrial in carnivores.[1)–8)] In rats and mice this enzyme is located in both mitochondria and peroxisomes, and only the mitochondrial activity is markedly induced by injection of glucagon.[4),5),9)–12)] Our laboratory in the Department of Biochemistry, Hamamatsu University School of Medicine, Hamamatsu, Japan has been interested in this curious liver enzyme, AGT (or SPT). Our major goal was to elucidate (1) the physiological role of this enzyme, *i.e.*, whether or not it is a bifunctional enzyme actually involved in both the metabolism of L-serine and detoxication of glyoxylate, and (2) why (for what physiological need) the subcellular distribution of this enzyme should be as it is and how (by what molecular mechanism) it can be species-specific and food habit-dependent. Dr. T. Noguchi's group at Kyushu Dental College, Japan and Dr. C. J. Danpure's group at MRC Laboratory for Molecular Cell Biology, University College London, also substantially and creatively contributed to this area of research, but in this review paper the Hamamatsu approach will be mainly introduced.

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Non-standard abbreviations: AGT, alanine:glyoxylate aminotransferase; CBP, CREB-binding protein; CRE, cAMP-responsive element; CREB, CRE-binding protein; LDH, lactate dehydrogenase; PEP-CK, phosphoenolpyruvate carboxykinase; PreSPTm, precursor protein of mitochondrial SPT; PKA, protein kinase A; SDH, serine dehydratase; SPT, serine:pyruvate aminotransferase; SPTm, mitochondrial SPT; SPTp, peroxisomal SPT; SPTm-mRNA, messenger RNA for SPTm; SPTp-mRNA, messenger RNA for SPTp.

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Serine:pyruvate aminotransferase and alanine:glyoxylate aminotransferase

Serine:pyruvate aminotransferase (SPT, EC 2.6.1.51) was first described in dog liver in 1956.[13] At first, this enzyme was assumed to be involved in the biosynthesis of L-serine from 2-phosphoglycerate, a glycolytic intermediate, *via* the non-phosphorylated pathway, the pathway through D-glycerate and hydroxypyruvate.[13] However, a high activity of liver SPT was subsequently observed in animals fed a diet low in carbohydrate such as carnivores,[14,15] neonatal suckling rats[14,16] and rabbits fed an 88% casein diet,[17] as well as after administration into rats of glucagon, cAMP or cortisone[14,16,18] and in alloxan diabetes.[16,18] These results together with the effects of diet and hormones on other enzymes of serine metabolism[17,18] suggested that the non-phosphorylated pathway was associated with gluconeogenesis from L-serine rather than the serine synthesis. On the other hand, serine dehydratase (SDH, EC 4.2.1.13), which catalyzes formation of pyruvate from L-serine, had also been thought to initiate gluconeogenesis from serine in fasted adult rat liver, although its substantial contribution had not been convinced, mainly because of its high K_m (50–70 mM) for L-serine. Then, there was considerable controversy as to the route of gluconeogenesis from L-serine. At that time, the relative flow of serine metabolism through SDH and SPT in rat liver had been studied mainly by the use of inhibitors of phosphoenolpyruvate carboxykinase (PEP-CK) such as quinolinate and 3-mercaptopicolinate, and a contribution of the SPT pathway was suggested from the observations that these inhibitors had less effect on gluconeogenesis from serine than from lactate or alanine.[19–21] Gluconeogenesis from L-serine through pyruvate (SDH-pathway) as well as that from L-alanine and lactate involves conversion of oxaloacetate to phosphoenolpyruvate catalalyzed by PEP-CK, but that by way of hydroxypyruvate (SPT pathway) bypasses this PEP-CK-catalyzed step. However, other studies supported the serine metabolism primarily *via* SDH at least in rat liver under gluconeogenic conditions such as starvation.[22–25] I encountered SPT in 1969 when I was studying abroad at the Enzyme Institute, University of Wisconsin. In my study at that time, data obtained did not support the major role of the SPT pathway in gluconeogenesis from serine as far as fasted rat liver was concerned, leaving the actual physiological role of this enzyme to be studied later. During this study, however, I found that glucagon-induced SPT was predominantly localized in mitochondria making me very curious to know why (for what physiological need) glucagon-induced SPT should be in mitochondria and, assuming the synthetic site of this enzyme to be cytoplasmic ribosomes, how the induced SPT molecule was translocated to this organelle.

SPT was later purified to homogeneity from mouse, rat, dog, cat and human livers, and shown to be a homodimer of approximately 40-kDa subunits.[1,2,10,26–30] No notable difference was observed between rat liver mitochondrial and peroxisomal SPTs in their physicochemical and catalytic properties,[29] except that N-terminal amino acid of peroxisomal SPT was blocked whereas that of mitochondrial SPT was methionine.[31] However, substrate specificity of the enzyme from human, dog and cat livers was quite different from that of rodent SPT. Despite the name, serine:pyruvate aminotransferase, rat liver SPT utilized not only L-serine and pyruvate but also many other neutral L-α-amino acids lacking branches at the β-C position and their corresponding α-keto acids as a good amino donor and acceptor, respectively. An exception was that glycine was a very poor substrate, although its corresponding α-keto acid, glyoxylate, served as a most favorable amino acceptor.[26,29–31] We noted that the K_m of rat SPT for glyoxylate was as low as approximately 10 µM, while the constant for other α-keto acids was higher than 0.5 mM.[30] Mouse liver SPT appeared to share these properties with rat enzyme.[27] On the other hand, human, dog and cat liver SPT were shown by Noguchi *et al.*[1,2,27,28] to be specific for L-alanine and L-serine as an amino donor and for pyruvate, hydroxypyruvate and glyoxylate as an amino acceptor. It was noteworthy that human enzyme also showed low K_m for glyoxylate, supposedly the most physiologically important characteristic of this enzyme.

Alanine:glyoxylate aminotransferase (AGT, EC 2.6.1.44) was first purified 800 to 900-fold from human liver and characterized by Thompson and Richardson in 1967, and in this first paper substrate specificity of human AGT was shown to be almost specific to L-alanine and L-serine.[32] In addition, supposing the key role of AGT in glyoxylate and oxalate metabolism, possible involvement of this enzyme in primary hyperoxaluria or oxalosis was already discussed.[32] Subsequently, the catalytic properties and response to hormones and dietary conditions of AGT were shown to be very similar or

almost identical with those of SPT,[2)–4),9),14),33)] and finally SPT and AGT were concluded to be the same enzyme. In 1986, Danpure *et al.* showed that primary hyperoxaluria type 1, a lethal inborn error of glyoxylate metabolism characterized by increased oxalate production, was caused by a deficiency of hepatic peroxisomal AGT (SPT).[34),35)] Since glyoxylate had been known as an immediate precursor of oxalate, a toxic end-product of metabolism, the vital importance of its removal by AGT (SPT) was thus clarified. Hereafter, therefore, this enzyme has been generally called AGT. However, since we used to call this enzyme SPT or serine:pyruvate/alanine:glyoxylate aminotransferase (SPT/AGT), I will use, in this review paper, the name SPT instead of more common name, AGT.

Molecular mechanism underlying the species-specific and food habit-dependent dual organelle distribution of SPT (AGT)

After I moved to Hamamatsu University School of Medicine in 1974 I started, in collaboration with my colleagues, to study how glucagon-induced SPT can be localized in mitochondria in rat liver. First, we demonstrated, as a prerequisite for further studies, that the glucagon-induced increase in the activity of SPT in rat liver occurred mainly in the mitochondrial matrix of parenchymal cells and this increase was due to the accumulation of enzyme protein caused by the rise in the rate of enzyme synthesis.[10)]

While these studies were in progress, Noguchi *et al.* reported that in the liver of normally fed rats the SPT activity was detected not only in mitochondria but also in peroxisomes, and only the mitochondrial activity remarkably increased after administration of glucagon.[9)] The physicochemical, kinetic, and immunological properties of the mitochondrial and peroxisomal enzymes (SPTm and SPTp, respectively) were indistinguishable from each other.[4),5),9),29)] We confirmed these findings and were very much interested in the mechanisms underlying the dual organelle distribution of rat SPT and its selective accumulation in mitochondria when synthesized in response to glucagon stimuli.[12)] We then found in a cell-free protein synthesizing system that SPT was synthesized, being directed by total RNA isolated from glucagon-treated rat livers, as a putative precursor protein which was approximately 2,000 Da larger than the subunit of mature enzyme.[36)] The hepatic level of translatable mRNA coding for the putative precursor was approximately 40 times higher in rats that received a glucagon administration 3.5 h before sacrifice than in control animals.[36)] As to the intracellular site of biosynthesis of SPT, immunoprecipitation of [^{3}H]puromycin-labeled nascent peptides prepared from free and membrane-bound ribosomes of glucagon-treated rat liver showed that nascent peptide of SPT was mainly included in the total nascent peptide on free ribosomes.[37)] These results altogether indicated that SPT destined for mitochondria was synthesized mainly on extra-mitochondrial free ribosomes as the larger precursor (preSPT), as in the case of many other mitochondrial matrix enzymes. Indeed, the precursor (preSPT) synthesized *in vitro* was post-translationally processed to an apparently mature form by isolated rat liver mitochondria, and the processed product was localized in the matrix of mitochondria.[37)]

We then cloned, being instructed by Prof. Shigetada Nakanishi of Kyoto University, cDNA for SPT from rat livers.[38)] Nineteen cDNA clones were isolated by screening of a cDNA expression bank of rat liver with an antibody against the enzyme, and one of the clones named pRspt10 contained the entire coding region for the mature enzyme and expressed in bacteria a specific 43-kDa protein having the immunoprecipitable activity of SPT. In RNA blot analysis performed with a [^{32}P]-labeled cDNA fragment as a probe, two types of SPT-mRNA with different sizes were detected. The larger mRNA was composed of approximately 1900 nucleotides and induced by glucagon, while the smaller one of approximately 1700 nucleotides was not affected by the hormone.[38)] In good agreement with the two types of SPT-mRNA, two immunoprecipitable products of different size (45 kDa and 43 kDa) were detected in the *in vitro* translation, and when RNA from glucagon-treated rats were used to direct protein synthesis the formation of the 45-kDa product markedly increased.[38),39)] These results indicated that mitochondrial SPT (SPTm) was synthesized through the 1900-nucleotide mRNA (SPTm-mRNA) and the 45-kDa precursor (preSPTm) and that glucagon caused the induction of the enzyme by increasing the level of the 1900-nucleotide mRNA. The remaining 1700-nucleotide mRNA and 43-kDa translation product were presumed to be connected with the synthesis of peroxisomal SPT (SPTp) (*cf.* Fig. 1-B).

Although pRspt10 covered the entire coding region of the mature enzyme, it lacked the nucleotide sequence encoding the N-terminal extension peptide essential for the proper translocation of the amino-

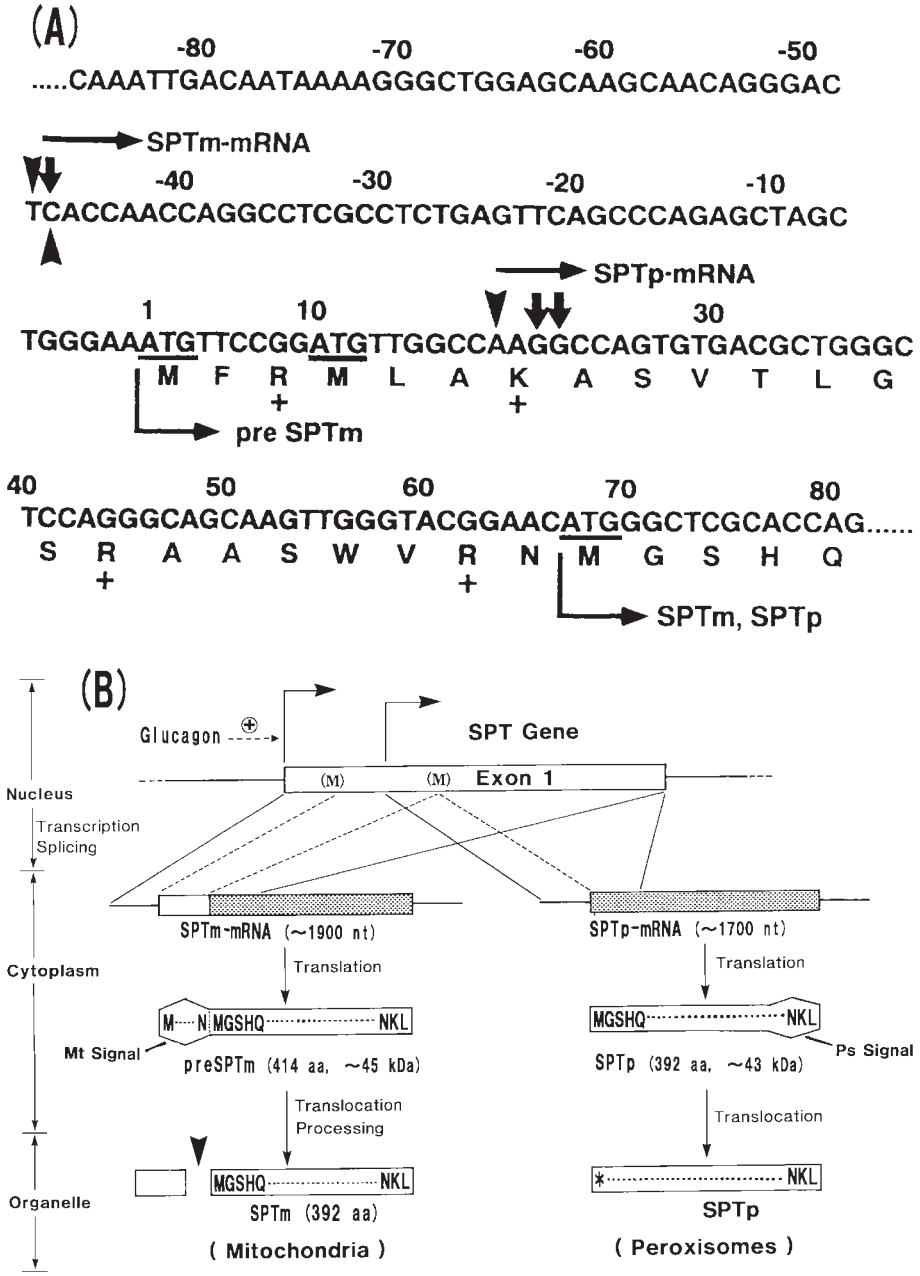

Fig. 1. Transcription of the rat SPT gene from two different initiation sites (A) and schematic representation of the biosynthesis of SPTm and SPTp from a single rat SPT gene (B). In (A), the two transcription initiation sites detected on primer extension and S1 nuclease mapping[41] are indicated by arrows and arrowheads, respectively. A (adenine) of ATG corresponding to the first AUG translation initiator codon of SPTm-mRNA is numbered 1. Methionine codons (ATG) are underlined. The amino acid sequence is represented by one-letter abbreviations, and basic amino acid residues in the mitochondria-targeting N-terminal extension sequence of the 45 kDa-precursor of SPTm (preSPTm) are denoted by +. This region has been predicted to fold as an amphiphilic α-helix. Figure 1-A is reprinted from Funai *et al.*[44] In (B), the two transcription start sites and translation initiation ATG triplets in exon 1 of the rat SPT gene are indicated by hooked arrows and (M), respectively. PreSPTm also contains C-terminal peroxisomal targeting sequence (PTS), but N-terminal mitochondrial targeting sequence (MTS) was shown to be functionally dominant over the C-terminal PTS.[46]

transferase precursor into mitochondria. Therefore, cloned cDNAs encoding an entire sequence of the 45-kDa precursor were then isolated by screening an Okayama–Berg library by using a cloned cDNA fragment as a probe.[40] Sequence analysis of one of the cloned cDNAs isolated, pRspt321, enabled us to deduce the primary structure of the 45-kDa precursor and the mature form of the enzyme. The precursor was revealed to be a protein of 414 amino acids containing an extra-peptide (pre-sequence) of 22 amino acids at the NH_2-terminus of the mature enzyme of 392 amino acids.[40] Upon RNA blot analysis and S1 nuclease protection assay no differences were detected between the two mRNAs other than that about 100 nucleotides of the 5′-terminal sequence of the 1900-nucleotide mRNA (SPTm-mRNA) were lacking in the 1700-nucleotide mRNA (SPTp-mRNA), and the length of the poly(A) tail was different.[41] Southern blot analysis of genomic DNA extracted from rat liver strongly suggested that the SPT gene was single, that was, both the SPTm- and SPTp-mRNAs were transcribed from a single gene.[41] Indeed, we have isolated genomic clones harboring the entire rat SPT gene,[41] characterized them,[42] and later determined the location of the single rat SPT gene to be in the q34–q36 region of chromosome 9 by fluorescence *in situ* hybridization.[43] Primer extension and S1 nuclease mapping analysis, using a DNA fragment of a genomic clone, revealed that the SPTm- and SPTp-mRNAs were transcribed from different initiation sites, about 65 nucleotides apart, in the same exon, exon 1 (Fig. 1-A).[41] Ribonuclease protection assay performed with an RNA hybridization probe corresponding to the 5′-terminal portion of SPTm-mRNA also gave the same results. These results altogether indicated that different organelle distribution of SPTm and SPTp, the products of the same SPT gene, arose from transcription from different initiation sites. Transcription from the upstream start site generates the 1900-nucleotide mRNA for a 45-kDa precursor for SPTm containing a cleavable N-terminal mitochondrial targeting signal of 22 amino acids. The precursor is translocated into mitochondria and converted to the mature size (43 kDa) by processing. On the other hand, transcription from the downstream start site (+66 relative to the upstream start site) generates the 1700-nucleotide mRNA that encodes a product of mature size. The product is then imported into peroxisomes by an intramolecular peroxisomal targeting signal type 1 (PTS1) at the C-terminal region (Fig. 1-B).[45–47] Indeed, when a truncated cDNA was constructed to encode the 43-kDa translation product and expressed in cultured monkey kidney COS-1 cells SPT immunoreactivity was exclusively peroxisomal. When the cDNA encoding the 45 kDa-product was expressed, on the other hand, SPT immunoreactivity was largely localized in mitochondria.[48]

Studies on the effect of glucagon on SPT gene expression in primary cultured rat hepatocytes showed that activation by glucagon of transcription from the upstream initiation site was responsible for the hormone-induced increase in the cellular level of SPTm-mRNA.[49] This effect of glucagon was shown to be mediated by the cAMP/protein kinase A (PKA) system,[49] but in this gene, a CRE (cAMP-responsive element)-like sequence was found only at −673/−666 (relative to upstream transcription start site),[42] and there was no evidence that this CRE-like sequence was involved in the transcription activation. Instead, Sp1 site at −113/−106 and AP-2 site at −1/+9 were shown to be essential for the upstream promoter activity of the rat SPT gene.[50] Expression of AP-2 caused a marked increase in the basal promoter activity, and both the basal and PKA-induced activities were elevated by over-expression of Sp1, its effect on the PKA-induced activity being more pronounced with co-expression of CBP (CREB-binding protein) and repressed by E1A oncoprotein.[50] These results suggested that AP-2 and Sp1 regulated basal activity of the upstream promoter, and Sp1 was also involved in the PKA-mediated expression of the rat SPT gene in concert with the transcriptional co-activator CBP. The downstream promoter activity appeared to be constitutively regulated by C/EBPα and C/EBPβ which bound around the downstream transcription start site (+66 relative to the upstream start site) and still unidentified protein factors bound to a short inverted repeat located 20–30 bp upstream of the downstream start site.[51]

While we were devoted to studying the transcriptional regulation responsible for the hormone-induced and constitutive synthesis of rat mitochondrial and peroxisomal SPT, Lumb, Purdue and Danpure[52] showed that transcription of the AGT (SPT) gene in cats occurs almost entirely from a single site corresponding to the upstream start site in the rat SPT gene, consistent with the largely mitochondrial localization of SPT. In rabbits and humans, similar start sites were also found, but the upstream AUG codon for translation of the N-terminal mitochondrial targeting sequence had been

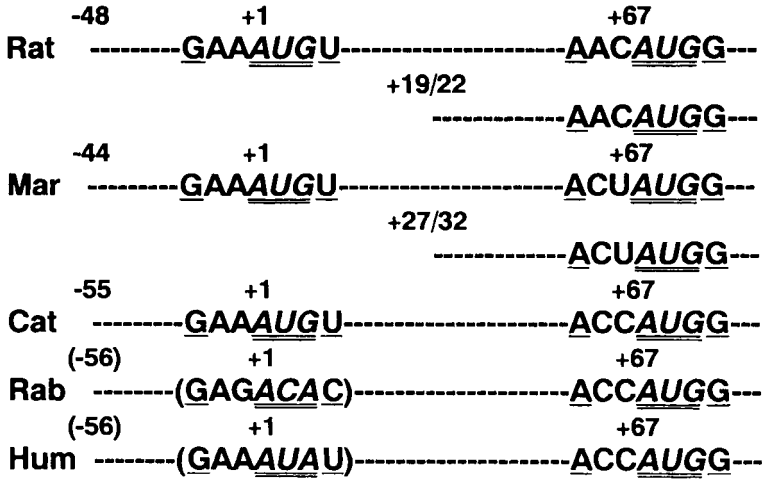

Fig. 2. Initiation of transcription and translation. The SPT-mRNA of different animal species is schematically shown. A of the first AUG initiator codon of SPTm-mRNA is numbered +1. Methionine codons (AUG) are double underlined, and nucleotides at positions −3 and +4 (numbers are relative to A of AUG) are underlined. Kozak[55] has shown that nucleotide at positions −3 and that at +4 should be purines (A or G) and G, respectively, for efficient translation initiation from the AUG codon. In rat SPTm-mRNA, there is another AUG triplet at position +10, but this AUG is omitted from the figure, because nucleotide at position −3 and that at +4 of this AUG are C and T, not A or G and G, respectively (*cf.* Fig. 1), and there is no evidence that it is used for initiation of translation. The first AUG codon of rat SPTm-mRNA is in a suboptimal context in that nucleotide at position −3 is G but that at +4 is U. In the translation of the longer mRNA, therefore, some ribosomal subunits bypass the first AUG codon and reach the third AUG codon at +67, which is in a favorable context, and initiate translation to produce the 43-kDa product.[44] Mar, marmoset; Rab, rabbit; Hum, human. Reprinted from Ichiyama *et al.*[56]

mutated to ACA and AUA, respectively.[53),54] In this case, the first methionine codon encountered in translation should be the downstream AUG at +67 (relative to A of upstream AUG), consistent with the entirely peroxisomal localization of the enzyme in these animal species (Fig. 2). Marmoset, a New World monkey, has AGT (SPT) in both mitochondria and peroxisomes in the liver, and in this animal species two SPT-mRNAs are formed by transcription from upstream and downstream start sites, as in the case of the rat.[54]

It was thus revealed that the major determinant of the SPT organelle destination was the AUG codon first encountered in translation, as we had first proposed to explain the organelle distribution of rat liver SPT in the presence and absence of glucagon stimuli.[41] When the longer mRNA (SPTm-mRNA) was translated from the upstream AUG codon at +1 position preSPTm containing a mitochondria-targeting N-terminal signal sequence was formed. Mutation of the upstream AUG codon and generation of the shorter mRNA (SPTp-mRNA) were used as a means to allow usage of the downstream AUG codon at +67 position as an initiation codon for synthesis of peroxisomal SPT (Fig. 2). It appears that the transcription from the downstream start site to produce SPTp-mRNA has been used to distribute SPT to peroxisomes in addition to mitochondria.

Physiology of the species-specific and food habit-dependent dual organelle distribution of SPT

It has been generally accepted from the known over-production of oxalate in primary hyperoxaluria type 1, an inborn error of glyoxylate metabolism caused by a functional deficiency of peroxisomal SPT,[34),35] that one of the major physiological roles of SPT in this organelle was removal of glyoxylate by catalysis of its conversion to glycine, at least in humans. SPT had also been presumed to participate in gluconeogenesis from serine but supporting evidence was still insufficient. In this connection, it was noteworthy that the liver SDH activity was known to be inversely related to body size in mammals,[57] suggesting a possibility that the contribution of SDH to the serine metabolism, if any, sharply decreased with increasing body size of animals. Therefore, we studied L-serine metabolism in rat (24-h starved and glucagon-treated), rabbit, human and dog livers, focusing on the relative contribution of SPT.[58),59] The flux of serine metabolism in the liver by way of the three pathways, one initiated by SDH, another by SPT, and the other the pathway through glycine

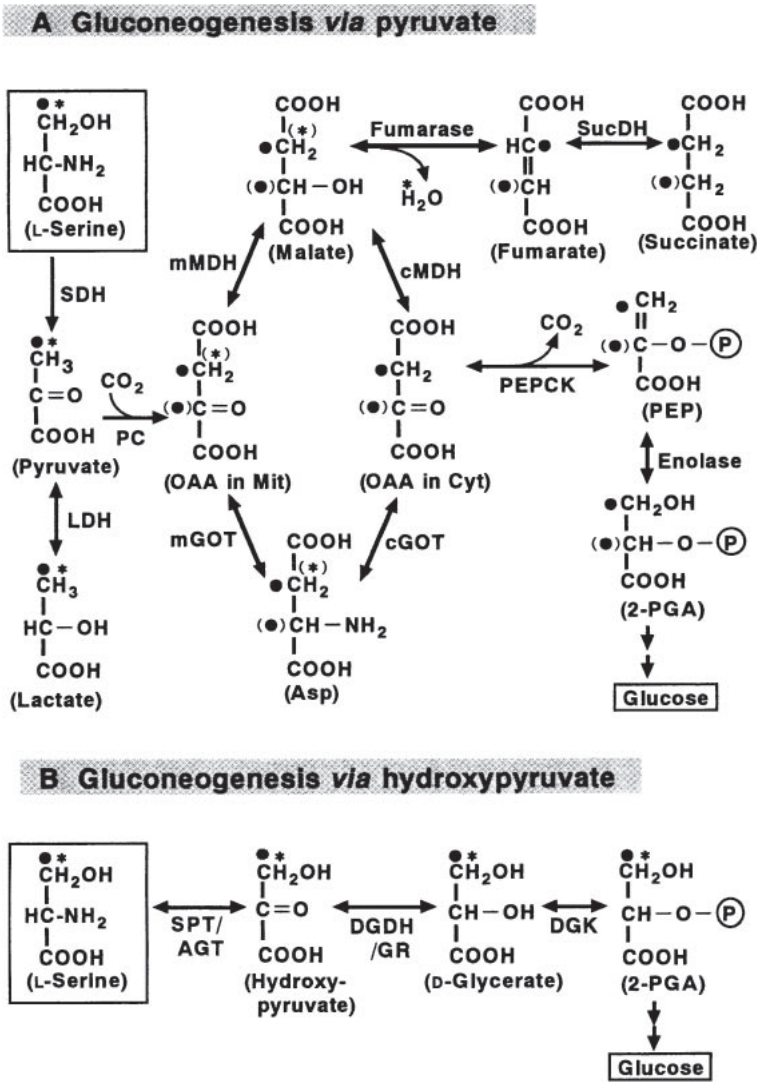

Fig. 3. Presumed metabolic fates of carbon and hydrogen at the 3-position of L-serine in gluconeogenesis *via* pyruvate (A) and hydroxypyruvate (B). • and ∗: carbon and hydrogen originating from those at the 3-position of L-serine, respectively. (∗): ^{3}H that is expected to be lost on the fumarase-catalyzed shuttling between malate and fumarate. In the gluconeogenic pathway *via* pyruvate (A), no intermediates after oxaloacetate in the cytosol are labeled with ∗, emphasizing the removal of this hydrogen in mitochondria. (•): ^{14}C labeling attributable to randomization at the succinate dehydrogenase step. LDH: lactate dehydrogenase, OAA: oxaloacetate, PC: pyruvate carboxylase, mGOT and cGOT: mitochondrial and cytosolic aspartate aminotransferase, respectively, mMDH and cMDH: mitochondrial and cytosolic malate dehydrogenase, respectively, PEP: phosphoenolpyruvate, PEPCK: phosphoenolpyruvate carboxykinase, SucDH: succinate dehydrogenase, 2-PGA: 2-phosphoglycerate, DGDH/GR: D-glycerate dehydrogenase/glyoxylate reductase, DGK: D-glycerate kinase, Mit: mitochondria, Cyt: cytosol. Reprinted from Xue *et al.*[58]

was measured *in vitro*, and relative contribution of SDH and SPT to gluconeogenesis from L-serine was estimated *in vivo* and in perfused liver using L-[3-^{3}H, ^{14}C]serine as substrate. The principle of the *in vivo* and perfusion experiments is shown in Fig. 3. The carbon derived from the 3-position of L-serine is thought to be retained throughout the gluconeogenic reactions in either pathway. On the other hand, the hydrogen at the 3 position is expected to be largely removed in the gluconeogenesis *via* pyruvate (SDH-pathway), whereas it is retained most in gluconeogenesis *via* hydroxypyruvate (SPT-pathway). The results of the *in vitro* and *in vivo* experiments are summarized in Table 1. In rat liver, flux through SDH was predominant, and the contribution of SPT was about 1/10–1/7 of that through SDH even after

Table 1. Flux of serine metabolism in the liver

	Rat		Rabbit	Human	Dog
	Starved	Glucagon-treated			
	nmol/60 min/40 mg liver equiv. (%)				
In vitro experiment					
SDH pathway	144	220	~0	~0	~0
(%)	(98)	(89)			
SPT pathway	~0	19	50	38	26
(%)		(8)	(96)	(88)	(57)
Via glycine	2.6	6	2	5	20
(%)	(2)	(3)	(4)	(12)	(43)
Contribution to gluconeogenesis *in vivo*					
SDH (%)	~97	~88	~10	—	—
SPT (%)	~3	~12	~90	—	—

Data from Xue *et al.*[58),59)]

glucagon injection. In rabbit, man and dog, on the other hand, SPT was the major enzyme of hepatic serine metabolism, and in rabbit the contribution of SPT to gluconeogenesis from serine was estimated to be as much as about 90%. These results were in good agreement with the observations of Beliveau and Freedland[60)] that serine is mainly metabolized *via* transamination in hepatocytes isolated from cats, and also compatible with the results of Rowsell *et al.*[61)] that in rabbit and dog livers SPT activity is more than six times higher, and SDH activity is much lower than the respective activities in rat liver. It was noteworthy that SPT was involved in L-serine metabolism, no matter whether the enzyme is largely located in mitochondria (dog liver) or entirely in peroxisomes (rabbit and human livers).

Therefore, the species-specific and food habit-dependent organelle distribution of SPT might be required for proper metabolism of glyoxylate at the subcellular site of its formation. In herbivores, a major source of glyoxylate has been believed to be oxidation of glycolate by glycolate oxidase in liver peroxisomes. Glycolate is an intermediate of photorespiration and is thus much higher in content in plants than in animal tissues,[62)] although its content in vegetables and fruits is not very high, around 2 to 3 mg/100 g of wet weight. However, because the energy available from a given wet weight of plants is much lower than that from meat, herbivores should be heavy eaters to supply enough energy to maintain body functions, growth, and so on. A portion of the ingested glycolate is excreted unchanged in the urine, but a significant portion is oxidized to glyoxylate by glycolate oxidase in peroxisomes. Therefore, the peroxisomal localization of SPT may be indispensable for herbivores to convert glyoxylate thus formed into glycine *in situ*, preventing undesirable overflow of it into oxalate, a dangerous endproduct of metabolism. Glyoxylate is also formed in liver and kidney mitochondria from 4-hydroxy-2-ketoglutarate, an intermediate of L-hydroxyproline metabolism (Fig. 4).[63),64)] Mitochondrial production of glyoxylate from hydroxyproline is assumed to be significant in carnivores, because the hydroxyproline

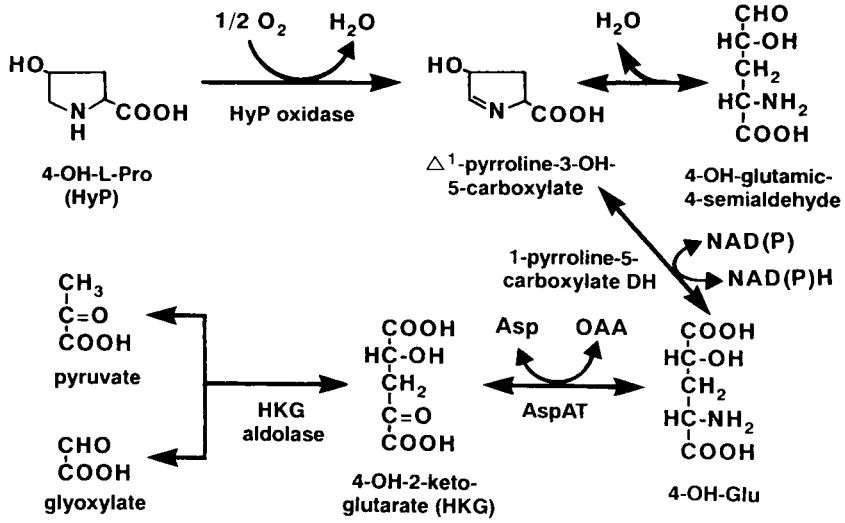

Fig. 4. Catabolic pathway of L-hydroxyproline in mitochondria. AspAT: aspartate aminotransferase, DH: dehydrogenase, Hyp: hydroxyproline, OAA: oxaloacetate, 4-OH-Glu: 4-hydroxy-L-glutamate.

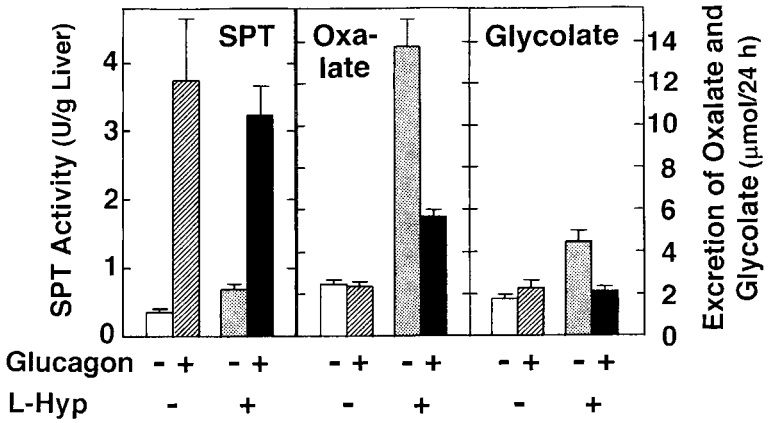

Fig. 5. Urinary excretion of oxalate and glycolate after administration of L-hydroxyproline. Where indicated, 630 mg of L-hydroxyproline in 2 ml water was administered to glucagon-treated rats and control fasted rats *via* a stomach tube, followed by collection of 24-h urine. Then the SPT activity in liver homogenate and oxalate in the 24 h-urine were determined. L-Hyp: L-hydroxyproline. Data from Takayama *et al.*[66]

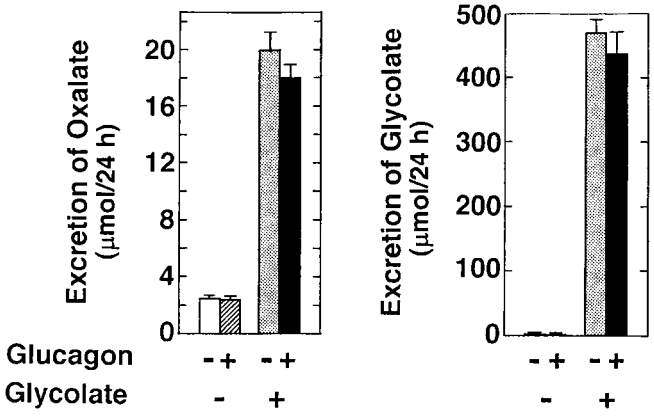

Fig. 6. Urinary excretion of oxalate and glycolate after administration of glycolate. Where indicated, 100 mg of Na-glycolate in 1 ml water was administered to glucagon-treated rats and control fasted rats *via* a stomach tube. Data from Takayama *et al.*[66]

content of collagen is about 10 to 13%[65] and collagen accounts for about 30% of total animal protein. We thus examined whether mitochondrial SPT plays a role in removing hydroxyproline-derived glyoxylate from oxidation to oxalate using rats with or without glucagon induction of liver mitochondrial SPT.[66] When a large dose of L-hydroxyproline was administered orally to 48 h-fasted control rats urinary excretion of oxalate and glycolate was significantly increased (oxalate, 5.6-fold; glycolate, 2.5-fold), and this increase was effectively prevented by prior induction of liver mitochondrial SPT by glucagon (Fig. 5). Administration of glycolate also caused a marked increase (about 8-fold) in oxalate excretion into urine, but the glycolate-derived oxalate was not significantly affected by the glucagon induction of mitochondrial SPT, as expected (Fig. 6). These results suggested that mitochondrial presence of SPT in carnivores is important for the *in situ* metabolism of glyoxylate formed from L-hydroxyproline in mitochondria. The necessity of peroxisomal localization of SPT in humans for efficient removal of glyoxylate in this organelle was demonstrated by Danpure *et al.*[67] by showing that SPT misrouted to mitochondria in a group of patients with primary hyperoxaluria type 1 cannot fulfill its metabolic role of detoxicating glyoxylate properly.

As to the formation of oxalate from glyoxylate, it is important that glyoxylate in aqueous solutions exists largely in a hydrated form and is structurally similar to an α-hydroxy acid. The oxidation of glyoxylate to oxalate is catalyzed *in vitro* by

glycolate oxidase and lactate dehydrogenase (LDH), a short-chain α-hydroxy acid oxidase and dehydrogenase, respectively, between which LDH may be mainly responsible for the oxalate production *in vivo*.[68] As already stated by Richardson and Tolbert for glycolate oxidase,[69] the oxalate formation from glyoxylate by LDH or glycolate oxidase may be an unnecessary occurrence, because for both these enzymes their presence is needed for other purposes; for LDH dehydrogenation of L-lactate to pyruvate with generation of NADH and for glycolate oxidase oxidation of glycolate to glyoxylate. We agree with Richardson and Tolbert[69] that the oxalate production from glyoxylate may occur as a consequence of an evolutionary limit in the development of an enzymatic site of LDH which would react with L-lactate but would not attack the hydrated glyoxylate molecule of nearly similar structure.

Oxalate is thought to be a useless end-product of metabolism in mammals and is excreted into urine largely as a water-insoluble calcium salt. Thus crystallization of calcium oxalate can cause pathology by obstructing tubular lumens, disrupting intercellular and possibly intracellular interactions, or simply killing cells within which or next to which crystallization occurs. What happens when the unnecessary production of oxalate is not properly curtailed may be represented by the symptoms and prognosis of primary hyperoxaluria type 1. This hereditary disease caused by a functional deficiency of peroxisomal SPT is characterized by progressive calcium oxalate urolithiasis, nephrocalcinosis and systemic oxalosis due to increased oxalate production, and the patients usually die before the third decade of age, unless enzyme replacement therapy such as hepatorenal transplantation was properly performed.[35),70] It is attractive to speculate that the removal of glyoxylate, an immediate precursor of oxalate, at the subcellular site of its production would be a practical way to reduce oxalate formation, and those animal species that succeeded in equipping the proper subcellular site with SPT, an enzyme with low K_m for glyoxylate, survived evolutionary.

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Profile

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