BIOCHEMICAL JOURNAL LETTERS

The primary structure of monomeric yeast glyoxalase I indicates a gene duplication resulting in two similar segments homologous with the subunit of dimeric human glyoxalase I

The glyoxalase system plays an important biological role in scavenging methylglyoxal and other toxic 2-oxoaldehydes and converting them into innocuous 2-hydroxyacids [1,2]. The primary cellular substrate appears to be methylglyoxal, which is an obligatory by-product in the triosephosphate isomerase reaction of glycolysis [3]. The transformation of the 2-oxoaldehydes into the corresponding 2-hydroxyacids takes place in two steps catalysed by the distinct enzymes glyoxalase I and glyoxalase II [4]. The first step involves the formation of an *S*-2-hydroxyacylglutathione, and the second step is the hydrolysis in the thiol ester intermediate.

Glyoxalase I from *Pseudomonas putida* [5] and human tissues [6,7] as well as glyoxalase II from human tissues [8] and *Arabidopsis thaliana* (thale cress) (M. Ridderström and B. Mannervik, unpublished work) have been cloned and expressed in *Escherichia coli*. Glyoxalase I has been subjected to detailed investigations concerning kinetics and mechanism of action using enzyme from a variety of biological sources [9]. The enzyme is a zinc protein [10] with an unusual, apparently octahedral, metal co-ordination [11–14]. A prominent difference between glyoxalase I from *Saccharomyces cerevisiae* (baker's yeast) and glyoxalase I from the well-characterized mammalian sources is that the former enzyme from yeast is a monomer with an estimated M_r of 32000 [15], whereas the mammalian enzyme is a dimer of two similar subunits of M_r 23000 [16]. This difference in quaternary structure is not reflected in functional properties such as the complex non-Michaelis–Menten kinetics [17,18]. The present letter gives a possible explanation for the functional similarities in spite of the differences in quaternary structure.

In a database search for primary structures with possible homology with glyoxalase I an unidentified sequence from *S. cerevisiae* [19] was found with obvious structural similarity to human glyoxalase I over a region of approx. 150 amino acid residues (Figure 1). Even though the corresponding protein has not been expressed, the yeast sequence most likely represents glyoxalase I. The deduced M_r of 37209 and the calculated pI of 6.8 match the experimentally determined values for glyoxalase I from *S. cerevisiae* [16] within experimental error. Subsequently, another yeast sequence from *Schizosaccharomyces pombe* [20] (51% sequence identity with the *S. cerevisiae* structure) was found to have structural similarity to human glyoxalase I (Figure 1).

In the sequence alignments it was found that the N-terminal half of each of the yeast sequences has extensive similarities to its corresponding C-terminal portion (Figure 1). Each of the two halves of the yeast protein displayed similar degrees of sequence similarity with human as well as *Ps. putida* glyoxalase I. The sequence identity between residues 1–182 and 183–326 of the *S. cerevisiae* sequence was 47%, and inclusion of conservative replacements further underscored the similarities. The repetitive structure strongly indicates that the yeast proteins have arisen by gene duplication. This conclusion raises questions of functional significance. For example, do the two halves of the yeast sequence fold into domains with similarities to the two subunits of the mammalian enzyme? Does the yeast enzyme have two active sites, like the mammalian enzyme, or is there just one active site? These and other problems have to be addressed by studies of the three-dimensional structure of the protein.

The previously available data suggested that glyoxalase I from mammalian sources was structurally unrelated to its counterpart in yeast [16]. The comparison of the primary structures presented here suggest that the similarities are extensive. A pronounced sequence similarity between human glyoxalase I and

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      1                                                  50
SpoA  .......... .......... MASTTDMSTY KLNHTMIRVK DLDKSLKFYT
SpoB  .......... .......... .......... .FNHTMVRVK DPEPSIAFY.
SceA  .........M STDSTRYPIQ IEKASNDPTL LLNHTCLRVK DPARTVKFYT
SceB  .......... .......... .......... .FNHTMIRIK NPTRSLEFYQ
Hsa   MAEPQPPSGG LTDEAALSCC SDADPSTKDF LLQQTMLRVK DPKKSLDFYT
Ppu   .......MSL NDLNTLPGVT AQADPATAQF VFNHTMLRVK DIEKSLDFYT

      51                                                 100
SpoA  EVFGMKLID. ..QWVFEENE FSLSFLAFDG ...PGALNHG VE..RSKREG
SpoB  EKLGMKVID. ..KADHPNGK FTNYFLAYPS .........D LP..RHDREG
SceA  EHFGMKLLS. ..RKDFEEAK FSLYFLSFPK DDIPKNKNGE PD..VFSAHG
SceB  NVLGMKLLR. ..TSEHESAK FTLYFLGYG. ........VP KTDSVFSCES
Hsa   RVLGMTLIQ. ..KCDFPIMK FSLYFLAYED KNDIPKEKDE KIAWALSRKA
Ppu   RVLGFKLVD. ..KRDFVEAK FSLYFLALVD PATIPADDDA RHQWMKSIPG

      101                                                150
SpoA  ILELTYNFGT EKKEGPVYIN GNTEPKRGFG HICFTVDNIE SACAYLE...
SpoB  LLELTHNWGT EKESGPVYHN GNDGDEKGYG HVCISVDNIN AACSKFE...
SceA  VLELTHNWGT EKNPDYKINN GNEEPHRGFG HICFSVSDIN KTCEELE...
SceB  VLELTHNWGT ENDPNFHYHN GNSEP.QGYG HICISCDDAG ALCKEIEVKY
Hsa   TLELTHNWGT EDDATQSYHN GNSDP.RGFG HIGIAVPDVY SACKRFE...
Ppu   VLELTHNHGT ERDADFAYHH GNTDP.RGFG HICVSVPDVV AACERFE...

      151                                                200
SpoA  SKGVSFKKKL SDGKMKHIAF ALDPDNYWIE LVS...QSET KPKANISNFR
SpoB  AEGLPFKKKL TDGRMKDIAF LLDPDNYWVE VIE...QK.. ..........
SceA  SQGVKFKKRL SEGRQKDIAF ALGPDGYWIE LITYSREGQE YPKGSVGNK.
SceB  GDKIQWSPKF NQGRMKNIAF LKDPDGYSIE VVPHGLIA.. ..........
Hsa   ELGVKFVKKP DDGKMKGLAF IQDPDGYWIE ILNPNKMATL M.........
Ppu   ALQVPFQKRL SDGRMNHLAF IKDPDGYWVE VIQPTPL... ..........
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Figure 1 Alignment of the primary structures of human (*Homo sapiens*, Hsa) [6,7] and *Pseudomonas putida* (Ppu) [5] glyoxalase I with segments A (residues 1–182) and B (residues 183–326) of the corresponding *Saccharomyces cerevisiae* (Sce) [19] sequence as well as similar segments A (residues 1–176) and B (residues 177–302) from *Schizosaccharomyces pombe* (Spo) [20]

Identities of amino acids in corresponding positions in the yeast segments, as well as concurring residues in the Hsa and Ppu sequences, are indicated in **bold** and similarities in *italics*. Similarities between the SceA/B, Hsa and Ppu sequences were evaluated pairwise, using the program Gap (Genetics Computer Group, 575 Science Drive, Madison, WI 53711, U.S.A.). The values are expressed as percentage identities of the compared sequences, with values including similar amino acid residues in parentheses: SceA/SceB, 47 (71); SceA/Hsa, 42 (61); SceA/Ppu, 42 (61); SceB/Hsa, 45 (66); SceB/Ppu, 47 (69); Hsa/Ppu, 55 (67).

the enzyme from *P. putida* has previously been noted [6,7]. This similarity would appear to stand in sharp contrast with the difference between the mammalian enzyme and glyoxalase I from other microbial sources [16]. The enzyme from *S. cerevisiae*, *Escherichia coli* and *Rhodospirillum rubrum* is monomeric, with an M_r approx. 50% higher than the subunit M_r of mammalian glyoxalase I; the enzyme from *P. putida* appears to be a dimer like the mammalian counterparts. The finding that the primary structure from yeast is composed of two similar segments, each resembling a subunit of dimeric glyoxalase I, strongly suggests that the yeast polypeptide is folded into two covalently linked similar units to give a protein mimicking the enzyme composed of two subunits. Thus the available evidence indicates that the different glyoxalase I structures have arisen by divergent evolution from a common ancestor rather than by convergent evolution of distinct structures to a common function. Two protein subunits may be required for glyoxalase I activity, and in some biological species these structural entities are genetically linked by a tandem repeat at the DNA level.

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