Volume 250, number 1, 57-59 FEB 07151 June 1989

N-terminal region of *Proteus mirabilis* glutathione transferase is not homologous to mammalian and plant glutathione transferases

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Received 13 March 1989

The N-terminal amino acid sequence of glutathione transferase, Pm-GST-6.0, purified from *Proteus mirabilis* [(1988) Biochem. J. 255, 971–975] up to residue 38 and a comparative peptide fingerprint are reported. No obvious homology with the sequences of alpha, pi and mu classes of mammalian glutathione transferases as well as with those of plant glutathione transferases has been noted. These results suggest that the classification so far adopted for glutathione transferases cannot be extended to the bacterial enzyme.

Glutathione transferase; N-terminal sequence; Bacteria; Fingerprint

1. INTRODUCTION

Glutathione transferases (GST; EC 2.5.1.18) are a group of related enzymes that facilitate the conjugation of glutathione with a large number of compounds that carry an electrophilic centre [1,2]. Cytosolic glutathione transferases have been most extensively studied from a large number of mammalian tissues, including human, in which multiple isoforms all composed of two subunits are present [2–8]. On the basis of several criteria, including apparent molecular mass, isoelectric point, immunological reactivity, substrate specificity and inhibition characteristics, the considerable number of mammalian glutathione transferases has been grouped into at least three distinct classes, i.e. alpha, mu and pi [9]. The N-terminal amino acid sequences now available for most of the purified glutathione transferases have given definite support for the three classes of mammalian cytosolic glutathione transferases [3]. Isoenzymes of the same class have 70–80% N-terminal sequence identity, whereas identity in the N-terminal region between members of different classes is less than 30% [3]. We have recently purified and characterized from the procaryote *Proteus mirabilis* a glutathione transferase isoenzyme with an acidic isoelectric point (p*I* 6.0) and have termed it Pm-GST-6.0 [10]. This enzyme is composed of two subunits that comigrate with glutathione transferase π on SDS-polyacrylamide gel electrophoresis. Despite this similarity, Pm-GST-6.0 appears to be kinetically, structurally and immunologically different from glutathione transferase π as well as from all the mammalian glutathione transferases so far characterized [10]. Thus, the results reported in the previous paper seem to indicate that Pm-GST-6.0 does not fall within any of the three classes of glutathione transferases identified to date. In order to characterize Pm-GST-6.0 further and to clarify its relationship with the mammalian glutathione transferases, we have now determined the N-terminal amino acid sequence of Pm-GST-6.0 and undertaken a comparative peptide fingerprint with glutathione transferase π. The results show that also by these criteria bacterial glutathione transferase does not fall into any of the above mentioned glutathione transferase classes.

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50 © 1989 Federation of European Biochemical Societies

2. MATERIALS AND METHODS

Pm-GST-6.0 was prepared from *Proteus mirabilis* by the procedure previously described [10]. The enzyme was homogeneous as judged by SDS-polyacrylamide gel electrophoresis. Both Pm-GST-6.0 and glutathione transferase π were subjected to limited proteolysis with chymotrypsin following the procedure described by Frey et al. [11]. A sample of each glutathione transferase (50 μg) was dissolved in 25 μl of 75 mM sodium phosphate buffer (pH 7.5) and an equal volume of 0.5% SDS was added. The mixture was heated for 2 min at 100°C and cooled, and 50 μl of 0.5% Triton X-100 was added. After the addition of 5 μl of chymotrypsin solution (20 μg/ml), the mixture was incubated for 5 min and proteolysis stopped by the addition of 1 μl of 0.1 M phenylmethylsulfonyl fluoride in ethanol and 40 μl PAGE sample buffer. Samples were processed for SDS-PAGE and analyzed on 15% slab gels [12], and peptides were visualized by a silver stain procedure [13]. Automated amino acid sequence analysis was performed on an Applied Biosystem model 470A gas-phase protein sequencer equipped with an Applied Biosystem model 120A PTH analyzer for the on-line detection of phenylthiohydantoin (PTH) amino acids. A sample (~2 nmol) of native or carboxymethylated protein was loaded onto a trifluoroacetic acid (TFA)-treated glass-fiber filter coated with polybrene. Secondary structures of glutathione transferases were predicted according to the method of Garnier et al. [14]. The decision constants were set at zero. Calculations were performed by means of a simple program written in Applesoft Basic on an Apple IIc [15].

3. RESULTS

The fragmentation pattern obtained subjecting both glutathione transferase π and Pm-GST-6.0 to limited proteolysis is presented in fig.1. The pattern of Pm-GST-6.0 differs markedly from that of glutathione transferase π. Although Pm-GST-6.0 and glutathione transferase π show identical electrophoretic mobility none of the fragments obtained appears to be common to glutathione transferase and Pm-GST-6.0. Consequently, this experimental finding suggests that the primary structures of the two enzymes are clearly different. In order to better examine the structural relationship of Pm-GST-6.0 with glutathione transferase π as well as with other glutathione transferases, the N-terminal amino acid sequence of Pm-GST-6.0 was deter-

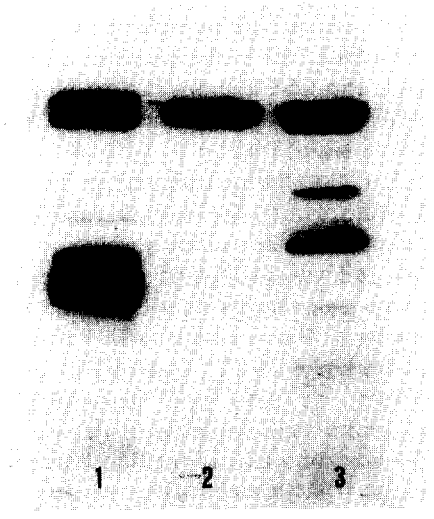

Fig.1. Limited proteolysis of glutathione transferases. Lanes: 1, glutathione transferase π; 2, control sample (glutathione transferase π + Pm-GST-6.0); 3, Pm-GST-6.0. Experimental details are given in the text.

Table 1

Comparison of the N-terminal region sequences of Pm-GST-6.0 with other mammalian and plant glutathione transferases

	Bacterial glutathione transferase
Pm-GST-6.0	MKLYYTPGSCSLSPHIVLRQTGLDFSIERIDLRRLLTE
	Class Alpha glutathione transferase
r1a	SGKPVLHYFIARGRMECIRVLLAAAGVEFEEKLIQSPE
r1b	SGKPVLHYFIARGRMECIRWLLAAAGVEFDEKFIQSPE
r2	PGKPVLHYFDGRGRMEPIRWLLAAAGVDFEEQFLKTRD
hH1	AEKPKLHYFNARGRMESTRWLLAAAGVEFEEKFIKSAE
hH2	AEKPKLHYSNIRGRMESIRWLLAAAGVEFEEKFIKSAE
h9.9	PGKPVLHYFDGRGRME
	Class Mu glutathione transferase
r3a	--PMILGYWNVRGLTHPIRLLLEYTDSSYEEKRYAMGDAP
r3b	--PMILGYWNVRGLTHPIRLLLEYTDSSYEEKRYAMGDAP
r4	--PMTLGYWDIRGLAHAIRLFLEYTDTSYEDKKYSMGDAP
hu	--PMILGYWDIRGLAHAIRLLLEYT
mN1	--PMILGYWNVRGLTHPIRMLLQYT
M8.7	--PMILGYXNVRGLXHPIRMLLEYXDXXYNEKXYXMGDAP
m9.3	--PMTLGYWNTRGLTHSIRLLLEWTDSSYEEKRYVMGDAP
bov	--PMILGYWDIRGLAHAISLLL
Si	TKLPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGD
	Class Pi glutathione transferase
r7	-PPYTIVYFPVRGRCEATRMLLADQGQSWKEEVVTIDVW
hπ	-PPYTVVYFPVRGRCAALRMLLADQGQSWKEEVVTVETW
mN3	-PPYTIVYFPVVDGCEAM
	Plant glutathione transferase
mz1	-APMKLYGAVMSWNVTRCATALEEAGSDYEIVPINFATA
mz3	-APLKLYGMPLSPNVVRVATVLMEKGLDFEIVPVDLTTG

The nomenclature and the amino acid sequences of mammalian and plant glutathione transferases were taken from [3]

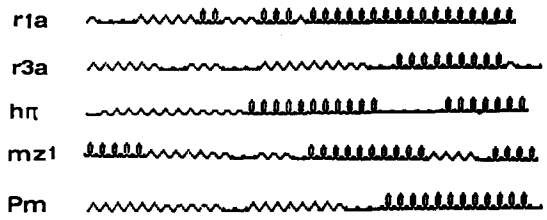

Fig.2. Comparison of the predicted secondary structure of Pm-GST-6.0 with those of glutathione transferases of different classes (see table 1) according to the method of Garnier et al. [14]. (∧) Extended conformation; (⌒) turn; (▲▲) random coil; (▲) α-helix.

mined up to residue 38, and compared with those of other reported [3] glutathione transferase isoenzymes (table 1). Methionine is the N-terminal amino acid of the Pm-GST-6.0. The finding that only a single sequence could be detected during automated Edman degradation suggests that the two subunits of Pm-GST-6.0 [10] are identical. As can be seen in table 1, the N-terminal region of bacterial glutathione transferase displays no obvious homology with the sequence of the isoenzymes belonging to the three classes of mammalian glutathione transferases. There is less than 10% identity between the Pm-GST-6.0 and the glutathione transferases of alpha and pi classes, and about 20% identity between Pm-GST-6.0 and class mu glutathione transferases. In addition, there is less than 20% identity in the N-terminal region between Pm-GST-6.0 and the maize glutathione transferases mz1 and mz2. The same results are obtained from comparison of the predicted secondary structures (fig.2).

4. DISCUSSION

This study reports the N-terminal sequence analysis of Pm-GST-6.0 isolated from *Proteus mirabilis* cytosol as well as a comparative peptide fingerprint of bacterial glutathione transferase and the most widely distributed mammalian glutathione transferase π. Limited proteolysis of both enzymes generates a number of different fragments clearly showing differences in the primary structure. On the other hand, when the N-terminal sequence of Pm-GST-6.0 is compared with the amino acid sequence of reported [3] mammalian glutathione transferases, no obvious homology between them can be noted. In addition, the differences in the N-terminal region between mammalian and bacterial glutathione transferases are also reflected in their secondary structure. It has been previously demonstrated that Pm-GST-6.0 is kinetically and immunologically distinct from all mammalian glutathione transferases so far isolated [10]. In particular, none of the antibodies raised against a number of isoenzymes of alpha, mu and pi class glutathione transferases cross-reacted with Pm-GST-6.0 in the immunodiffusion study [10]. The structural, kinetical and immunological data now available on Pm-GST-6.0, especially the sequence information, suggest that the classification adopted for the mammalian enzymes cannot be extended to bacteria. Moreover, the plant glutathione transferases mz1 and mz3, isolated from maize, share no apparent sequence homology with the sequence of bacterial glutathione transferase.

Acknowledgement: This work was in part supported by grant 87.01427.04 from the Consiglio Nazionale delle Ricerche.

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