PRIMARY STRUCTURE OF HUMAN HEPATIC METALLOTHIONEIN

Margrit M. KISSLING and Jeremias H. R. KÄGI
Institute of Biochemistry, University of Zürich, Zürichbergstrasse 4, CH-8028 Zürich, Switzerland

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1. Introduction

Metallothioneins constitute a family of low molecular weight proteins (6000–7000) of unusually high metal and cysteine content which occur in parenchymatous tissues of vertebrates and which are most abundant in liver and kidney [1].

Depending on the source, they contain variable amounts of Zn, Cd and Cu which add up to a stoichiometric ratio of 6 or 7 g-atom metal/mol [2]. In experimental animals the formation of metallothionein can be induced by the administration of a variety of metals (Zn, Cd, Cu, Hg, Ag) and, hence, they have been suggested to play a role in metal detoxication [3]. However, recent studies have shown that the naturally occurring metallothioneins of human liver contain zinc as the only significant metallic component thus suggesting that their primary biological function is related to the metabolism of this essential element [4].

All metallothioneins examined thus far are single chain proteins of about 60 amino acid residues [5]. Besides their high cysteine content they are unusual by a total lack of aromatic amino acids and histidine (see table 1). In general, each metal ion is bound by three cysteinyl residues forming a negatively-charged trimercaptide complex [1].

In this paper we report the first amino acid sequence of a human zinc metallothionein and compare it to the known primary structure of equine metallothionein (MT-1B) [1].

2. Methods

Human hepatic metallothionein, type MT-2, was isolated by the method of Bühler and Kägi [4] and modified by *S*-pyridylethylation following removal of the metal [1]. The N-terminus is blocked but a free end group for sequence analysis could be generated by selective cleavage of the acid-labile bond, between Asp-2 and Pro-3, resulting in the dipeptide AP-I and the fragment AP-II (see fig.1). These fragments were separated by gel filtration on Bio-Gel P-2 (Bio-Rad Laboratories, Richmond, Calif.). Two other large fragments, SP-I and SP-II, were obtained by cleavage of the single glutamyl bond by *Staphylococcus aureus* protease (Miles Laboratories, Slough, England), at pH 4.0, in ammonium acetate buffer [6] and by separation on Sephadex G-25. Sequence determination was carried out on an automatic sequenator (Beckman, Model 890B, updated) using previously applied procedures [1]. The average repetitive yield was 92%. The phenylthiohydantoin derivatives of the amino acids were identified by gas chromatography, by thin-layer chromatography, and by amino acid analysis after their conversion to free amino acids by hydrolysis in $HCl/SnCl_2$ [7].

3. Results

The complete amino acid sequence of human hepatic MT-2 is shown in fig.1. The structure of the N-terminal peptide AP-I was deduced from comparison with the homologous peptide of equine MT-1B and synthetic acetyl-Met–Asp using thin-layer chromatography [8]. Residues 3–34 were determined on fragment AP-II, residues 24–56 on SP-II. The C-terminal sequence from residues 55–61 was established by digestion of the carbamidomethylated derivative of MT-2 with carboxypeptidases A and B (Worthington) [1]. Further confirmation of the

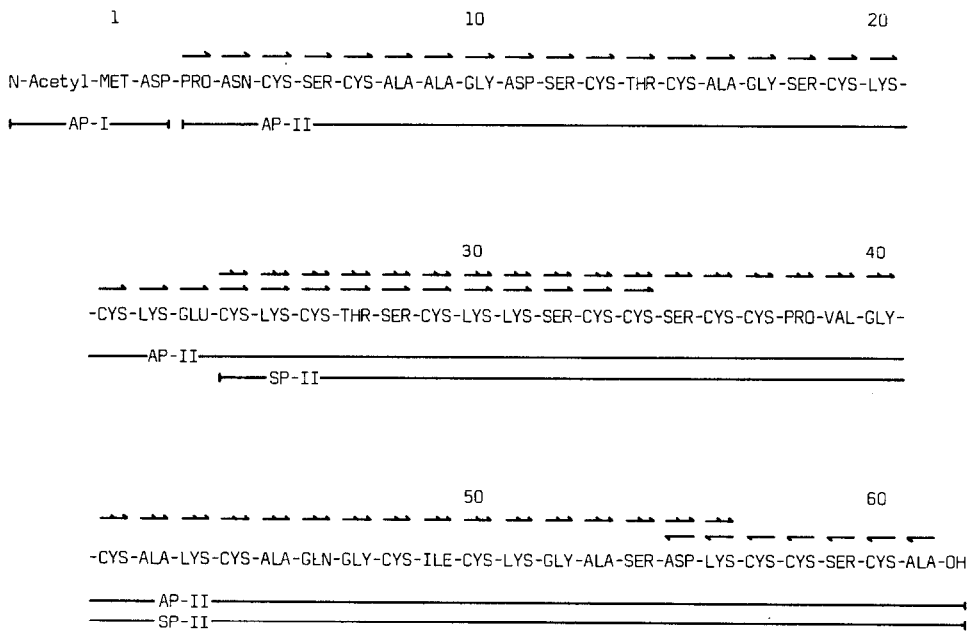

Fig.1. Amino acid sequence determination of human hepatic metallothionein MT-2, AP-I and AP-II, fragments from acid cleavage; SP-II, fragment from *Staphylococcus aureus* protease digestion. Arrows to the right, automated sequence analysis; arrows to the left, carboxypeptidase A and B digestion.

sequence was obtained from supplementary analyses of tryptic peptides prepared from the carboxymethylated derivative of the protein [9] and from fragments obtained by cleavage of a second acid-labile bond occurring between Asp-11 and Ser-12 on prolonged incubation with 70% formic acid. The sequence accounts for the amino acid composition reported previously (table 1).

4. Discussion

The primary structure of the human protein is similar to that of equine MT-1B (fig.2). Their most characteristic common feature is the identical distribution of the 20 cysteinyl residues along the chain which provide the principal ligands for metal binding. Based on stoichiometric considerations it has been suggested that the seven Cys–*X*–Cys sequences constitute the primary chelation sites for the seven metal ions bound [1]. Through appropriate folding of the polypeptide chain most of these initial dimercaptide complexes are thought to interact with one additional cysteinyl side chain to form the negatively-charged trimercaptide complexes indicated by spectroscopic data [2,10,11]. With the one exception of Lys-25 which corresponds to Arg-25 in equine MT-1B, the positions of Ser and Lys, the next most abundant amino acid residues, are also maintained. These residues are located predominantly in juxtaposition to cysteine and have been suggested to participate also directly or indirectly in metal binding [1]. The differences between human MT-2 and equine MT-1B are limited to six amino acid substitutions which can be accounted for by single-base changes. With the exception of the Gln–Glu replacement in position 23 they can be considered as conservative substitutions [12]. The degree of identity emerging from these data (91.2%) can be compared with the corresponding value for cytochrome *c* (88.5%) [13] and indicates thus a similarly low rate of molecular evolution. The very striking conservation of the metal-binding residues in these two orders of mammals suggests that the competence of the protein

Table 1
Amino acid compositions of human and equine metallothioneins

Residue	Human hepatic metallothionein (MT-2)		Equine renal metallothionein (MT-1B)	
	Number per molecule			
	Analysis[4]	Sequence	Analysis[1]	Sequence[1]
Cys	20.55	20	20.70	20
Asx	4.06		3.05	
Asp		3		2
Asn		1		1
Thr	2.08	2	1.09	1
Ser	8.30	8	7.29	8
Glx	2.39		2.87	
Glu		1		1
Gln		1		2
Pro	2.07	2	1.80	2
Gly	5.14	5	5.60	5
Ala	6.81	7	7.11	7
Val	1.12	1	2.74	3
Met	0.87	1	0.94	1
Ile	0.95	1	–	–
Lys	7.78	8	6.79	7
Arg	–	–	1.16	1
Total		61		61
Metal[a]				
Zinc	7.12		2.55	
Cadmium	0.10		4.61	
Total	7.22		7.16	

[a] The content of copper was less than 0.1 atoms per molecule in both proteins The molecular weights of the chains (61 residues including the amino-terminal acetyl group) calculated from sequence data are 6085 for human MT-2 and 6110 for equine MT-1B. The corresponding values for the molecular weights including 7 metal ions are 6548 and 6780, respectively

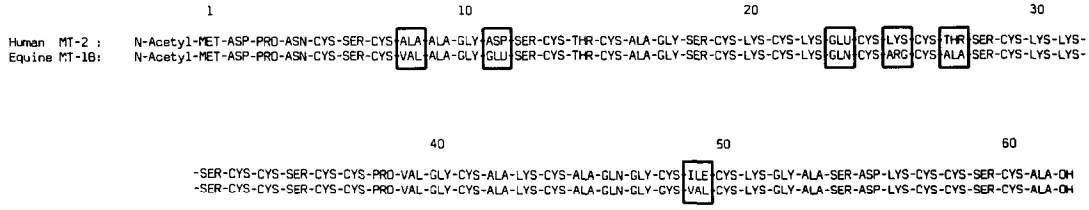

Fig.2. Primary structure of human and equine metallothioneins. Boxes indicate positions of nonidentical amino acid residues.

for metal binding is the feature selected for in these molecules.

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