AMINO ACID SEQUENCE OF CRAB METALLOTHIONEIN

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1. Introduction

Metallothioneins constitute a group of low molecular weight cysteine-rich proteins, which contain unusually high amounts of zinc, cadmium and/or copper [1–3]. These proteins occur ubiquitously in higher eucaryotic organisms [4] and are synthesized de novo upon exposure of cell cultures or animals to various heavy metal ions [5,6]. The metallothioneins from higher vertebrates have been studied in great detail and the primary structures of thioneins from different species been determined [7–9]. A number of reports have also indicated the presence of metal-binding proteins very similar to metallothioneins in invertebrates, lower eucaryotic organisms [10–14] and recently in a procaryotic species [15,16]. However with the exception of a small Cu-binding metallothionein from the ascomycete *Neurospora crassa*, whose primary structure was determined in [7], little structural information on these proteins is available. Here, we report the first determination of the amino acid sequence of an invertebrate metallothionein from the crab *Scylla serrata* and compare it to the primary structures of human and mouse metallothionein and of the copper metallothionein from *Neurospora crassa.*

2. Methods

The metallothionein isoprotein MT-1 from the crab *Scylla serrata* was isolated as in [11] and modified by *S*-pyridylethylation and *S*-aminoethylation following removal of the metal [7,19]. The aminoethylated derivative was digested with trypsin and the resulting peptides were separated on a Beckman M-72 cationic exchange resin [20] and further purified by gel filtration on Sephadex G-25 in 50% acetic acid and by paper electrophoresis at pH 1.9 or 6.5. To increase the solubility of the pyridylethylated protein at alkaline pH, the derivative was further modified by succinylation and subsequently cleaved with trypsin. The resulting peptide mixture was then subjected directly to automatic sequence analysis. Sequence determinations were carried out with a Beckman sequencer 890 B (updated) or manually as in [7,21]. The phenylthiohydantoin derivatives of the amino acids were identified by high pressure liquid chromatography [22], and by amino acid analysis after conversion to the free amino acids [23]. Amino acid analyses were made by established procedures on a Durrum D-500 amino acid analyzer.

3. Results

The complete amino acid sequence of the *Scylla serrata* metallothionein isoprotein MT-1 is shown in fig.1. Automatic sequence analysis of the pyridylethylated derivatives allowed the unambiguous determination of the first 28 residues. Taking advantage of the presence of a single arginyl residue in position 26, the pyridylethylated sample was succinylated and subsequently cleaved with trypsin. The peptide mixture, now containing a blocked prolyl residue in position 1 and a free pyridylethylated cysteinyl residue in position 27 was again subjected to automatic sequence analysis establishing the sequence of residues 27–54. The primary structure of the C-terminal part was deduced from the tryptic peptides of the aminoethylated derivative (fig.1) using manual Edman degradation and hydrazinolysis. As shown in table 1 the amino acid composition calculated from the sequence data agrees well with the amino acid composition obtained from the aminoethylated derivative.

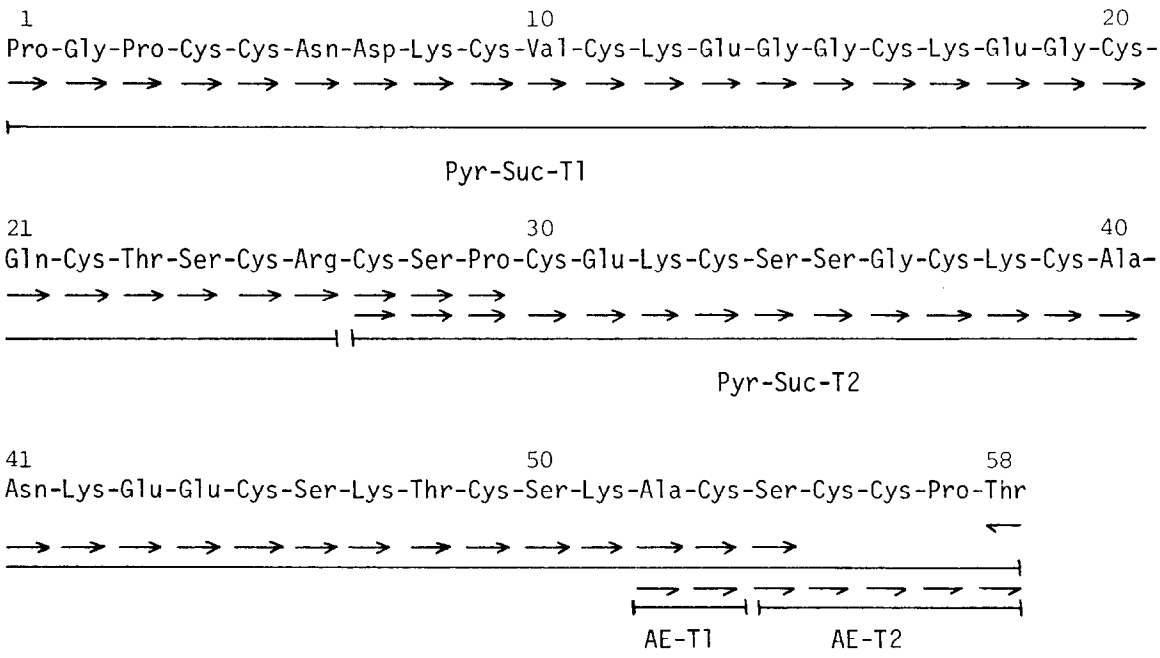

Fig.1. Amino acid sequence of *Scylla serrata* metallothionein MT-1 and a schematic outline of the peptides used to establish the complete primary structure. The peptides were designated according to the protein modification and the cleavage method used as follows: Pyr-Suc-T, tryptic peptides of pyridylethylated and succinylated thionein; AE-T, tryptic peptides of aminoethylated thionein. The method of sequencial degradation is indicated:⟶, automatic Edman degradation; ⟶, manual Edman degradation; ⟵, hydrazinolysis.

Table 1
Amino acid composition of crab metallothionein (MT-1)

Residue	Residues per molecule	
	Analysis	Sequence
Cys	17.1[a]	18
Asx	3.2	
Asp		1
Asn		2
Thr	3.0	3
Ser	6.7	7
Glx	5.8	
Glu		5
Gln		1
Pro	4.2	4
Gly	5.0	5
Ala	2.2	2
Val	1.0	1
Lys	7.5	8
Arg	1.0	1
Total		58
Polypeptide chain M_r[b]		6000

[a] Determined as *S*-aminoethylcysteine
[b] Calculated from the sequence data

4. Discussion

The invertebrate isoprotein amino acid sequence reported here reveals a remarkable structural similarity to the mammalian metallothioneins (fig.2). A comparison of aligned residues of isoprotein MT-1 with human MT-2 showed 46% identity in sequence and 48% homology on considering arginine as a conservative replacement for lysine [24]. The total number of 58 amino acids of the crab metallothionein MT-1 is only slightly smaller than the value of 61 typically found for sequenced mammalian metallothioneins [7–9]. In contrast to the vertebrate metallothioneins sequenced so far, the crab metallothionein displayed a free amino terminus. The lack of *N*-acetylmethionine in the invertebrate metallothionein could be related to the fact that the amino acid sequence does not start with a charged residue thus possibly allowing the complete proteolytic removal of the initiator methionine residue [25].

Despite the smaller number of cysteinyl residues found in the crab metallothionein (18 *vs* 20 in the mammalian forms), the sequence homology shown in fig.2 points out clearly the importance of the spacial

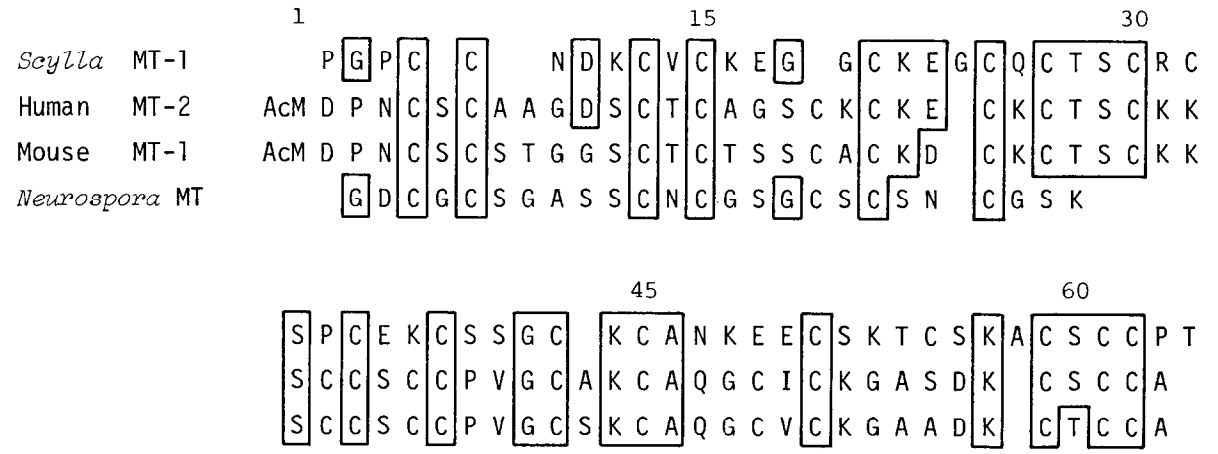

Fig.2. Comparison of the amino acid sequence of *Scylla serrata* MT-1 [this study] with human MT-2 [8], mouse MT-1 [9] and *Neurospora crassa* MT [18]. Identical residues are indicated by boxes. The different amino acid sequences were aligned so as to obtain maximal sequence homology. Residues 58 and 59 of human MT-2 [8] were found to be inversed (personal communication from M. Kimura to J. H. R. Kägi). One-letter notation is used for amino acid residues [24].

distribution of these residues – the principal metal-binding ligands in metallothioneins. The prominent clustering of the cysteinyl residues in the form of 7 Cys–X–Cys sequences found for the mammalian metallothioneins [7–9,18] has been also preserved in the crab metallothionein (5 Cys–X–Cys sequences). Allowing gaps to obtain maximal sequence homology, 16 cysteinyl residues are common to both invertebrate and vertebrate metallothioneins. It is interesting to note that the amino acid sequence homology between invertebrate and mammalian metallothioneins is more pronounced in the middle region and the C-terminal part of the molecule than in the aminoterminal part. Especially striking is the rigid sequence conservation between residues 21–31 (fig.2) suggesting a fundamental structure–function importance to this stretch of the molecule. In contrast, the amino acid sequence of the metallothionein from *Neurospora crassa* is highly altered in this region with the exception of two cysteinyl residues. This could be related to the fact that *Neurospora* metallothionein binds copper. In addition to the highly conserved cysteinyl residues involved in metal binding it was earlier hypothesized that lysyl residues which are frequently juxtaposed to cysteinyl residues might play an important structural role [7]. This conjecture receives strong support from the finding that 6 out of 8 lysyl residues and all of the chemically similar arginine residues are also juxtaposed to cysteinyl residues in the crab metallothionein. In this context it should be mentioned that the copper-binding *Neurospora* metallothionein contains only a single lysyl residue, again suggesting an important structural function of these abundant and conserved residues in the binding and stabilization of specific metal complexes especially in the zinc and cadmium-binding metallothioneins from higher eucaryotic organisms.

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