STRUCTURAL RELATEDNESS BETWEEN HUMAN LACTOTRANSFERRIN AND HUMAN CERULOPLASMIN

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1. Introduction

Lactotransferrin, also called lactoferrin, from human milk is a glycoprotein of M_r = 76 500 constituted of a single polypeptide chain to which two carbohydrate groups are attached (review [1]). We have described 70% of the sequence of human lactotransferrin and pointed out a 6-fold internal homology [2]. In [3], 564 residues of the amino acid sequence of the single polypeptide chain of human ceruloplasmin (Cp, EC 1.16.3.1) of M_r = 130 000 were aligned. The comparison of the sequences allowed us to establish:

(i) A remarkable sequence homology between the two glycoproteins;
(ii) A similarity concerning their internal replication;
(iii) A homology between fragments containing the copper binding site(s) of ceruloplasmin and fragments of lactotransferrin.

Some of these homologies were also found when human serum transferrin (STF) was compared to ceruloplasmin.

2. Methods, results and discussion

2.1. *Sequence homologies between human transferrins (lactotransferrin and serum transferrin) and human ceruloplasmin*

Table 1 indicates some homologies between fragments of LTF [2], STF [4,5] and human ceruloplasmin (Cp 50-kDa, Cp 19-kDa) [3]. Several strong homologies were observed around the structural important half-cystine residues (c), as well as around tryptophan (a), histidine (b) and tyrosine residues (d) (table 1).

Abbreviations: Cp, human ceruloplasmin; LTF, human lactotransferrin; STF, human serum transferrin; kDa, kilodalton(s)

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2.2. *Internal homology of LTF and Cp and evolution of possible precursor genes*

Recent results concerning the alignment of 445 residues of the amino acid sequence of LTF [2] allowed one to suggest a 6-fold internal homology beside the internal homology, resulting from the duplication of an ancestral gene [6]. The occurrence of basic N-terminal sequences and the alignment of half-cystine, tryptophan, histidine and basic amino acid residues should more particularly be pointed out. We suggest that the molecule evolved from a protein of 1/6th its present size, as a result of evolution of an ancestral structural gene. We noted a similar situation in the case of STF.

Human Cp resulted from the alignment of 3 fragments Cp 67-kDa, Cp 50-kDa and Cp 19-kDa which were obtained spontaneously after cleavage by proteolytic enzymes [7]. With the recent completion of the primary structures of the Cp 50-kDa [7] and the Cp 19-kDa [8–10] fragments arising from the C-terminus, a remarkable degree of internal homology in Cp was identified.

Incomplete sequence data obtained for the N-terminal Cp 67-kDa fragment indicated that it has also strong homology with the C-terminal half of Cp. Thus, the model for the evolution of the gene for Cp in [3] is based on the internal homology of this protein and on its homology to other copper-binding proteins. In this model, a primordial gene coding for an azurin-type protein of ~160 amino acids was fused

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to a gene coding for a protein of ~190 amino acids, and a triplication of this ancestral fused gene could give rise to the present day gene for Cp. This model predicts that the complete sequence of human ceruloplasmin will exhibit a 3-fold repeat pattern of two alternating structures.

Table 1
Examples of sequence homology between human lactotransferrin (LTF) [2], serum transferrin (STF) [4,5] and ceruloplasmin (Cp) [3]

(a) Homologies around Trp residues :

LTF	FIT3 (1-7)	Thr	Ala-Gly	Trp	Asn	Val	Pro
	FIV (74-80)	Thr	Ala-Gly	Trp	Asn	Ile-Pro	
STF	(125-131)	Ser	Ala-Gly	Trp	Asx	Ile-Pro	
	(457-463)	Thr	Ala-Gly	Trp	Asn	Ile-Pro	
Cp 50-kDa	(16-22)	Thr	Tyr-Glu	Trp	Thr	Val	Pro
Cp 50-kDa	(356-362)	Thr	Tyr-Val	Trp	Lys	Ile-Pro	

(b) Homologies around His residues :

LTF	FIT2 (1-7)	Ser	Cys	His	Thr-Gly-	θ	- θ	-Leu	Arg
	FIV (66-73)	Ser	Cys	His	Thr-Ala-	θ	Val-Asp-Arg		
STF	(117-124)	Ser	Cys	His	Thr-Gly-Leu-	θ	-Gly	Arg	
	(449-456)	Ser	Cys	His	Thr-Ala-	θ	Val	Thr	Arg
Cp 50-kDa	(101-109)	Thr	Thr-Ala-Pro-Asp-Gln				Val-Asp-Lys		
Cp 19-kDa	(33-41)	Ser	Asp	His	Pro-Glu-Lys		Val	Asn	Lys

(c) Homologies around Cys residues :

LTF FIV	(24-38)	Lys-Ser	Gln-Gln	Ser-Ser	Asp-Pro	Asp-Pro	Asn	Cys	Val-Asp	Arg				
Cp 50-kDa	(24-37)	-Glu-Val-Gly-Pro	Thr	Asn-Ala-	θ	Asp-Pro	Val	Cys	Leu-Ala	Lys				
Cp 50-kDa	(364-377)	Arg-Ser	Gly-Ala-Gly	Thr	Glu-	θ	Asp	Ser-Ala	Cys	Ile-Pro-Trp-				

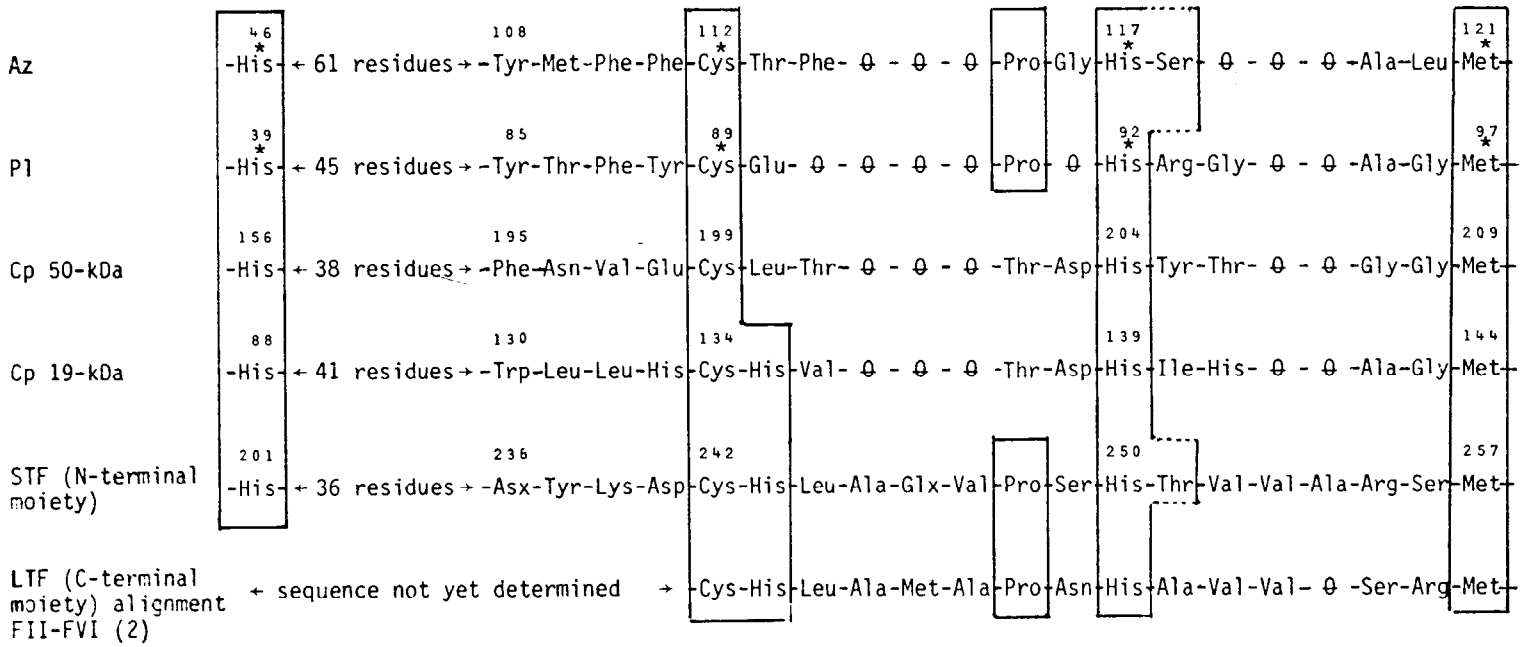
(d) Homologies around Tyr residues :

LTF	FIT16 (3-10)	Tyr-Tyr	θ	Ala-Val	Ala-Val	Val-Lys		
	FIV (43-50)	Tyr	Leu-	θ	Ala-Val	Ala-Val	Val-Arg	
STF	(95-102)	Tyr-Tyr	θ	Ala-Val	Ala-Val	Val-Lys		
	(426-433)	Tyr	Phe-	θ	Ala-Val	Ala-Val	Val-Lys	
Cp 50-kDa	(39-47)	Tyr-Tyr	Ser	Ala-Val	Asp-Pro-Thr	Lys		
Cp 50-kDa	(379-387)	Tyr-Tyr	Ser-Thr	Val	Asp-Gln	Val-Lys		

The homologous residues (identical residues ☐ and conservative changes, ⋯) are boxed; θ, deletion

Table 2

Homology of the type 1 copper binding site of *Pseudomonas aeruginosa* azurin (Az) [11], *Anabaena variabilis* plastocyanin (Pl) [12] and Cp 19-kDa and Cp 50-kDa fragments [3] with fragments of STF [4,5] and LTF [2]



The homologous residues (identical residues, ☐ and conservative changes, ⬚) are boxed; θ, deletion; *, amino acid residue implicated in metal binding site

The similarity concerning the internal homology of LTF and Cp should be pointed out. However we suggest a somewhat different model of evolution of a possible precursor gene of human LTF. Indeed the latter contains 2 prosthetic sugar groups situated in quite homologous areas. Thus, the ancestral gene seems to have been submitted to a triplication which was followed by a duplication.

2.3. *Homology between fragments containing copper binding sites of human Cp and human LTF sequences*

Cp is a large multicopper oxidase and contains six copper ions generally given as two type 1 Cu^{2+}, one type 2 Cu^{2+} and three type 3 Cu^{2+} [3].

Crystallographic structures and amino acid sequences are established for some of the small blue proteins, such as azurin [11] and plastocyanin [12] which contain 1 copper atom/molecule coordinated to cysteines (residues 112 and 89, respectively), histidines (residues 46, 117 and 39, 92, respectively) and methionines (residues 121 and 97, respectively).

The Cp 50-kDa and Cp 19-kDa fragments were proposed [3] to have 3 amino acids (residues Cys 199, His 204, Met 209 and Cys 134, His 139, Met 144, respectively, see table 2) in positions homologous to 3 out of the 4 residues involved in the type 1 copper binding site of azurin [11] and plastocyanin [12]. The comparison of ceruloplasmin and plastocyanin allowed us to suggest that the fourth Cp residue involved in a type 1 copper binding site could be His 156 and His 88 of the Cp 50-kDa and Cp 19-kDa fragments, respectively.

Table 2 presents some points of homology between a type 1 copper-binding site of azurin and plastocyanin and fragments of ceroluplasmin (Cp 19-kDa and 50-kDa) as well as of human transferrins. These homologies allow to suggest that the latter might be implicated in metal binding.

3. Conclusion

The comparison of the sequences of 2 ceruloplasmin fragments (Cp 19-kDa and Cp 19-kDa corresponding to a total of 564 amino acid residues) and of 70% of the lactotransferrin molecule (445 amino acid residues) allowed us to determine 10 homologous areas including 133 amino acids; 53 out of these 133 residues were identical.

Acknowledgements

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