Amino acid sequence of the N-terminal region of human hemopexin

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Cyanogen bromide digestion of hemopexin at its 6 methionine residues results in 7 fragments (CB1–CB7) partially connected by disulfide bridges. By sequence studies of fragments CB1–CB4 and peptides prepared by their enzyme cleavage, a continuous amino acid sequence of the N-terminal region of human hemopexin, comprising 220 amino acid residues, was determined. The presence of intramolecular disulfide bonds, connecting half-cystine residues 126/130 and 165/170, was proved in fragments CB2 and CB3. Fragments CB1–CB4 include 5 sites, where hexosamine oligosaccharides are attached (positions 1,41,164, 217 and probably 223). In the sequenced region two sites sensitive to acid hydrolysis – bonds ...Asp–Pro... in positions 20/21 and 187/188 were found. In spite of the fact that pooled material of many donors was studied, no sequence heterogeneity was discovered.

Hemopexin *Amino acid sequence* *Heme transport*

1. INTRODUCTION

Hemopexin is a serum β-glycoprotein with M_r ~60000 [1], 20% of which represents the carbohydrate moiety consisting of galactose, mannose, glucosamine and sialic acid [2]. It is a transport protein which mediates the transfer of heme from methemoglobin to liver parenchymal cells and prevents its excretion from the organism. The physical and chemical properties of hemopexin and its physiological role have been summarized [3,4]. However, there is very little information about its covalent structure necessary for deeper understanding of its binding function. Only the N-terminal amino acid sequence of hemopexin has been published [5,6]; some partial sequential data have been presented in a recent paper characterizing the glycosyl sites and some of the tryptophan sequences [7]. All half-cystine residues are paired in disulfide bonds [2].

The sequence region determined in this paper covers the N-terminal half of the hemopexin molecule.

2. EXPERIMENTAL

2.1. *Materials*

Human hemopexin was isolated as in [8]. Trypsin (treated with L-1-tosylamido-2-phenylethyl chloromethyl ketone) and α-chymotrypsin were obtained from Worthington (Freehold, NJ), soybean trypsin inhibitor and thermolysin from Calbiochem (San Diego, CA), *Staphylococcus aureus* V8 proteinase from Miles (UK). Citraconic anhydride was from Pierce (Rockford, IL), 4-*N,N*-dimethylaminoazobenzene-4-isothiocyanate from Fluka (Buchs, Switzerland), and the chemicals for Edman degradation from Beckman (Berkeley, CA). Sephadex® G-25, G-50 and G-75 were products of Pharmacia (Uppsala, Sweden), Separon SI C-18 for preparative high-performance liquid

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chromatography (HPLC) was supplied by Laboratorní přístroje (Prague, Czechoslovakia).

2.2. *Chemical and enzymatic cleavage*

Hemopexin was digested for 20 h at 37°C in 70% formic acid at a CNBR:protein weight ratio of 1:2. Acid hydrolysis was performed under similar conditions for 90 h. Enzymatic digestion proceeded at pH 8, 37°C, 4 h at a weight ratio of 1:50, in case of staphylococcal proteinase the ratio was 1:30, 20–90 h, in the presence of 2 mM EDTA. For limited tryptic cleavage citraconylated [9] material was used.

2.3. *Isolation and analytical methods*

Cyanogen bromide digest of native hemopexin was fractionated on Sephadex G-50 in 0.1 M acetic acid. The retarded fraction was rechromatographed in 1.0 M acetic acid, the material of higher M_r was *S*-sulfonated and subjected to further separation by reversed phase HPLC with a gradient of 1-propanol in 0.05% trifluoroacetic acid (TFA). The products of acid hydrolysis were fractionated on Sephadex G-75 and by HPLC under similar conditions. The details concerning the isolation and characterization of the fragments as well as determination of their order are included in another paper (to be published). For smaller peptides HPLC with a methanol gradient in 0.05% TFA was used. The determination of amino acid and amino sugar composition was done in a Durrum D-500 analyzer. Automated Edman degradation by a modified quadrol program [10] was performed in a Beckman sequencer 890 C; phenylthiohydantoins were evaluated by HPLC [11]. Smaller peptides were sequenced by a manual technique [12].

3. RESULTS AND DISCUSSION

By gel filtration of the cyanogen bromide digest of hemopexin fragments CB2 and CB3 were obtained. Each contains two half-cystine residues which form intramolecular bridges. After S-sulfonation of the remaining material fragments CB1 and CB4–CB7 were isolated by HPLC. The order of the fragments was derived from the determination of methionine sequences. The determination of the N-terminal sequence of hemopexin is presented in fig.1.

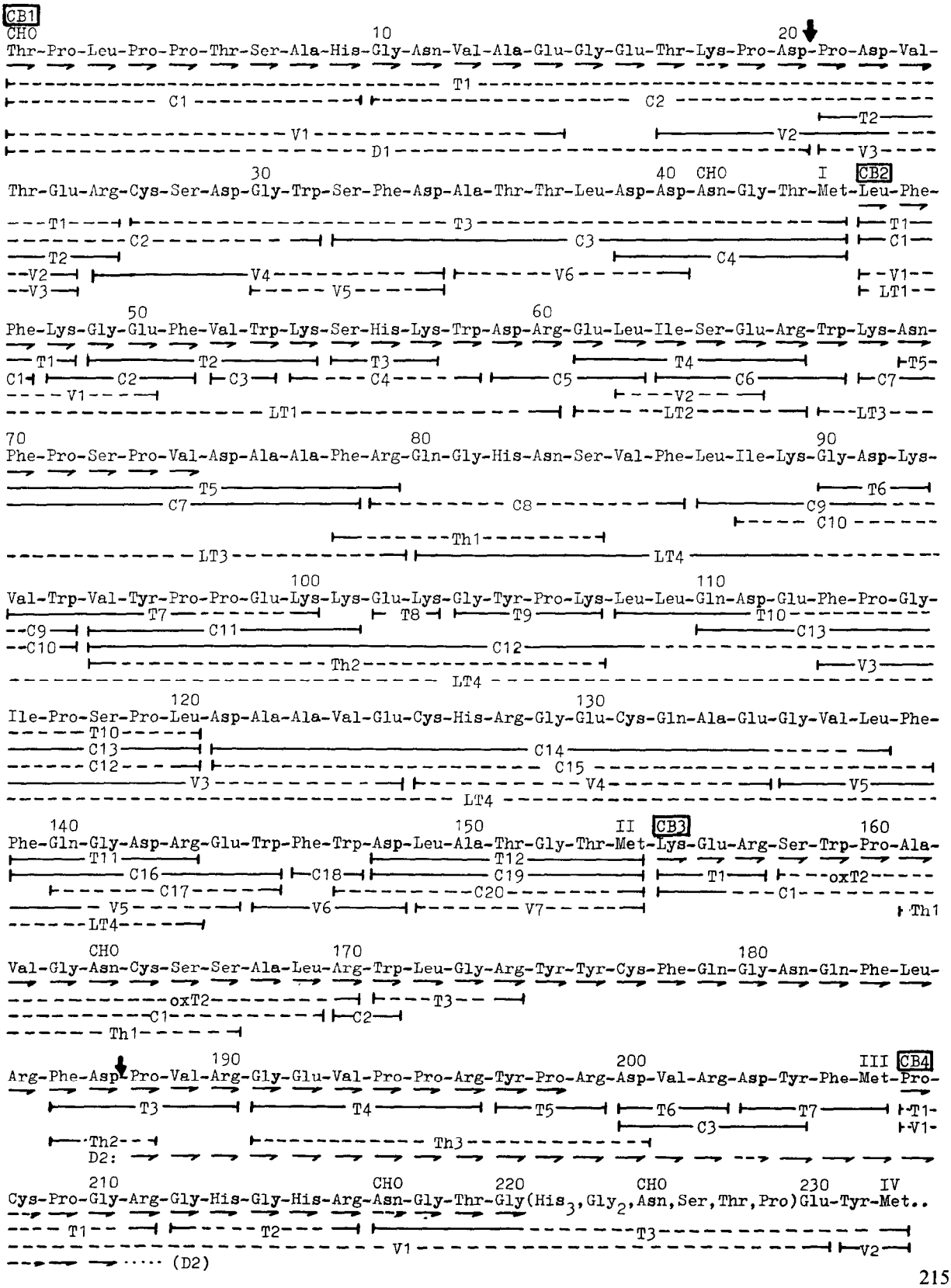
As observed in [5,6] Edman degradation of hemopexin releases an anomalous N-terminal product. With the use of the dansyl-Edman technique [13] N-terminal threonine was determined [6]. Authors in [7] have found that a galactosamine oligosaccharide is linked to this threonine. Repeated Edman degradation of hemopexin together with peptides from enzymatic hydrolysates enabled us to complete the sequence of fragment CB1. Residue Asn(41) was found to be the second site of glycosidation linking a glucosamine oligosaccharide in the usual signal sequence CHO
Asn-X-Ser/Thr [14]. With respect to disulfide bonding in CB2 and CB3 the nearest possible partner to Cys(27) can be Cys(208). Peptide D1 determines one of the bonds sensitive to acid cleavage; also the N-terminus of peptide T2 results from acid cleavage.

The largest of the fragments in this sequence CB2 with a relatively high content of hydrophobic residues (especially of tryptophan) is not glycosylated. This implies for its localization inside the molecule. Limited tryptic digest of CB2 provided among others a long fragment LT4 suitable for automated Edman degradation which gave a close continuation of the N-terminal sequence of the fragment.

The next fragment CB3 contains the second acid-sensitive bond. Automatic degradation of peptide D2 formed by acid cleavage at this bond has made it possible to arrange small peptides in the C-terminal section of CB3 and also supplied a link to the following fragment CB4. Peptide oxT2

→

Fig.1. N-terminal amino acid sequence of human hemopexin. The peptides are denoted by lines, completely determined sequences by full lines, incomplete sequences by broken lines. T, C, Th, V, LT, peptides obtained from trypsin, chymotrypsin, thermolysin, staphylococcal proteinase V8 and limited trypsin hydrolysates, respectively; separate numbering is used within each cyanogen bromide fragment. CB, D, fragments produced by cyanogen bromide cleavage and acid digestion, respectively. Degradation steps in sequencer (—→), undefined products of degradation (- - →). CHO, glycosylation sites; (↓) sites of acid cleavage.


CB1
CHO
10
20
Thr-Pro-Leu-Pro-Pro-Thr-Ser-Ala-His-Gly-Asn-Val-Ala-Glu-Gly-Glu-Thr-Lys-Pro-Asp-Pro-Asp-Val-
T1
C1
C2
T2
V1
V2
D1
V3
30
40
CHO
I
CB2
Thr-Glu-Arg-Cys-Ser-Asp-Gly-Trp-Ser-Phe-Asp-Ala-Thr-Thr-Leu-Asp-Asp-Asn-Gly-Thr-Met-Leu-Phe-
T1
T3
C2
C3
C1
T2
C4
V2
V4
V6
V1
V3
V5
LT1
50
60
Phe-Lys-Gly-Glu-Phe-Val-Trp-Lys-Ser-His-Lys-Trp-Asp-Arg-Glu-Leu-Ile-Ser-Glu-Arg-Trp-Lys-Asn-
T1
T2
T3
T4
T5
C1
C2
C3
C4
C5
C6
C7
V1
V2
LT1
LT2
LT3
70
80
90
Phe-Pro-Ser-Pro-Val-Asp-Ala-Ala-Phe-Arg-Gln-Gly-His-Asn-Ser-Val-Phe-Leu-Ile-Lys-Gly-Asp-Lys-
T5
T6
C7
C8
C9
C10
Th1
LT3
LT4
100
110
Val-Trp-Val-Tyr-Pro-Pro-Glu-Lys-Lys-Glu-Lys-Gly-Tyr-Pro-Lys-Leu-Leu-Gln-Asp-Glu-Phe-Pro-Gly-
T7
T8
T9
T10
C9
C11
C13
C10
C12
Th2
V3
LT4
120
130
Ile-Pro-Ser-Pro-Leu-Asp-Ala-Ala-Val-Glu-Cys-His-Arg-Gly-Glu-Cys-Gln-Ala-Glu-Gly-Val-Leu-Phe-
T10
C13
C14
C12
C15
V3
V4
V5
LT4
140
150
II
CB3
160
Phe-Gln-Gly-Asp-Arg-Glu-Trp-Phe-Trp-Asp-Leu-Ala-Thr-Gly-Thr-Met-Lys-Glu-Arg-Ser-Trp-Pro-Ala-
T11
T12
C16
C18
C19
T1
oxT2
C17
C20
C1
V5
V6
V7
Th1
LT4
CHO
170
180
Val-Gly-Asn-Cys-Ser-Ser-Ala-Leu-Arg-Trp-Leu-Gly-Arg-Tyr-Tyr-Cys-Phe-Gln-Gly-Asn-Gln-Phe-Leu-
oxT2
T3
C1
C2
Th1
190
200
III
CB4
Arg-Phe-Asp-Pro-Val-Arg-Gly-Glu-Val-Pro-Pro-Arg-Tyr-Pro-Arg-Asp-Val-Arg-Asp-Tyr-Phe-Met-Pro-
T3
T4
T5
T6
T7
T1
C3
V1
Th2
Th3
D2:
210
CHO
220
CHO
230
IV
Cys-Pro-Gly-Arg-Gly-His-Gly-His-Arg-Asn-Gly-Thr-Gly(His3,Gly2,Asn,Ser,Thr,Pro)Glu-Tyr-Met..
T1
T2
T3
V1
V2
(D2)

was isolated after oxidation of a tryptophan positive disulfide-connected peptide, including residues 158–170 and 175–185. Asn(104) is the third site, where a glucosamine oligosaccharide is linked.

The whole region occupied by fragment CB4 is markedly hydrophilic which is characteristic for the surface of the molecule. Also, the presence of two glycosylation sites places this sequence region at the surface of hemopexin. The Edman degradation of the fragment seems to be negatively affected by the glycosylation sites. Also the high content of the histidine and glycine residues complicates the interpretation of results. Positions 221–229 remain sequentially undetermined. The alignment of the partial sequence characterizing glycosylation sites III and IV by authors in [7] with positions 213–228 of our sequence would make it possible to formulate the C-terminus of the structure as

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                220          CHO
..Gly-Thr-Gly-His-Gly-Asn -Ser-Thr-His-His-
                230
    Gly-Pro-Glu-Tyr-Met.
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The sequence of the remaining C-terminal part of hemopexin is also under investigation.

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