HUMAN AND RABBIT HEMOPEXINS: TRYPTIC PEPTIDES AND N-TERMINAL SEQUENCES

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1. Introduction

Hemopexin, a β-glycoprotein present in the plasma of mammals [1], plays an important role in the disposal of plasma heme and has been implicated in the metabolism of other porphyrins [2]. The total number of residues in rabbit and human hemopexin (mol. wt approx. 57 500) is about 410 [3,4] and both proteins contain 20% carbohydrate [2,3]. Despite previous reports on the physicochemical properties of hemopexin and its interaction with porphyrins and heme [2,3,5,6], knowledge of its primary structure will be required to more fully appreciate these interactions and to compare hemopexins derived from different species.

This communication compares the peptide maps obtained from tryptic digests of human and rabbit hemopexin and their N-terminal amino acid sequences. The results indicate that a high degree of homology exists in the primary structure of the two hemopexin molecules. Moreover, preliminary evidence suggests the existence of genetic polymorphism in human hemopexin.

2. Materials and methods

Hemopexin was prepared from human and rabbit sera and its purity was estimated as previously described [3]. A limited amount of human hemerich hemopexin (Lot #689) was kindly donated by the Behringwerke A.G., Marburg, West Germany. Concentrations of hemopexin solutions were determined employing an $E^{1\%}_{280\ mm}$ of 19.7 for the human and the rabbit protein [4].

Samples of hemopexin were reduced and carboxymethylated [7] prior to digestion and in most cases prior to sequence analysis. Tryptic digests of 10–30 mg hemopexin were performed at 1:50 (w/w) ratios of trypsin (Worthington Lot #TCCA 6303) to hemopexin at 37°C in 2–4 ml water. The pH was maintained between 7 and 9 by adding 0.3 N NH_4 OH, and after 90 min the solution was frozen and lyophilized. The dried material was dissolved in water to a concentration of 50 mg/ml, and 50 μl was applied to Whatman No. 3 mm paper. Descending chromatography was performed in a mixture of sec-butanol, formic acid, water (70:9:21) for 15 hr. After drying, electrophoresis was carried out in the second dimension at 2000 V in acetic acid:pyridine:H_2O (10:1:589) at pH 3.8 in a Savant apparatus. The peptide maps were stained with ninhydrin to locate peptides and with specific reagents to locate arginine- and histidine-containing peptides [8], and examined with ultraviolet light for tryptophan-containing fluorescent peptides.

N-terminal sequences were determined with an updated Beckman Model 890A automatic sequencer using a dimethylallylamine buffer as previously described [9–11]. Identification of the phenylthiohydantoin–amino acid derivatives (PTH) was accomplished on a Beckman gas chromatograph [12] and in some cases by amino acid analysis after acid hydrolysis (6 N HCl, 150°C, 16 hr or 57% HI, 110°C, 20 hr) of the PTH derivative.

3. Results and Discussion

The tryptic peptide maps of human and rabbit

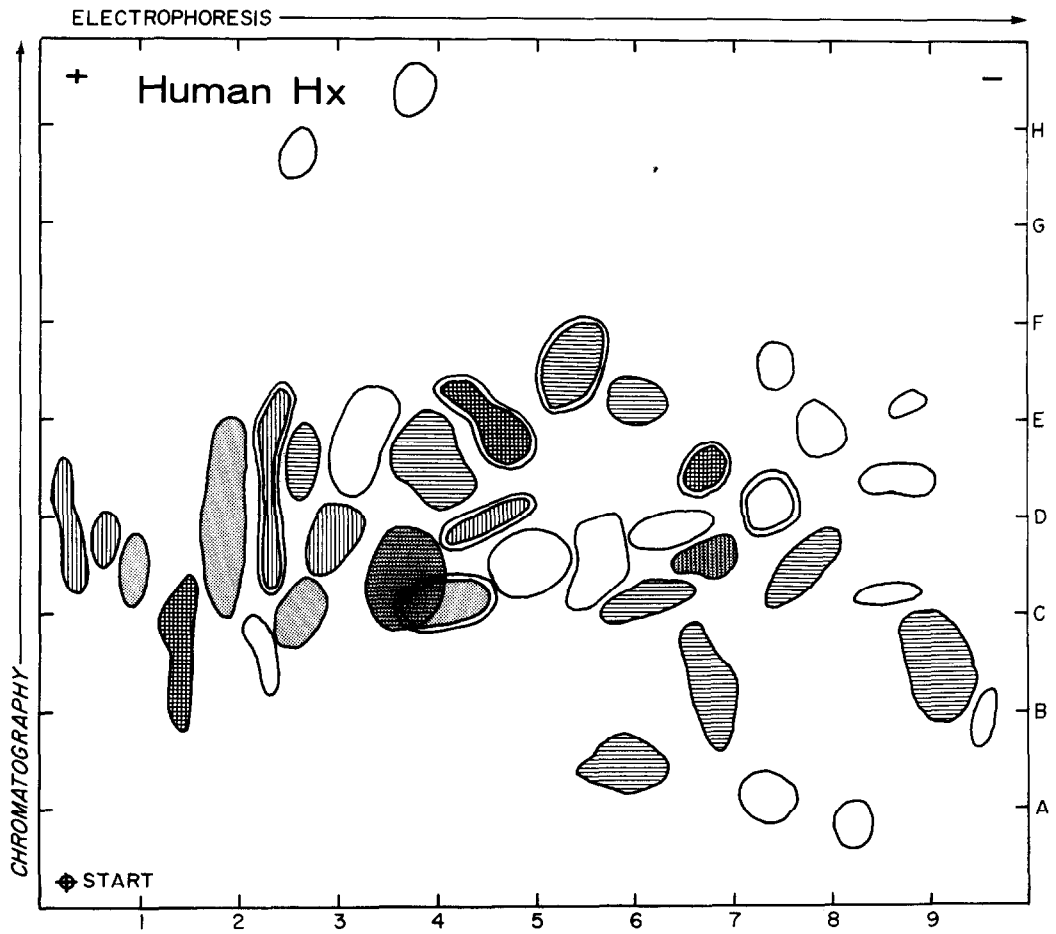

Fig. 1. Tryptic peptide pattern of human hemopexin. Human hemopexin (Hx) was digested with trypsin and 2.5 mg applied to the chromatogram sheet as described in Materials and methods. The chromatogram was stained for peptides with ninhydrin (○) and examined for peptides which contained arginine (⊖), histidine (⊛), tyrosine (⊕), and tryptophan (●).

hemopexin are shown in figures 1 and 2. These patterns were repeatedly observed. As expected from the extreme sensitivity of tryptic peptide mapping to amino acid substitutions, few peptides seem identical to both proteins. However, similar peptides like those at B-9, C-4, D-2, D-7, E-5 appear in both proteins. These peptides may be derived from highly conserved areas of the protein presumably involved in the function of hemopexin.

The N-terminal sequences of human and rabbit hemopexin are aligned in table 1. The homology between the two proteins in this small portion of the total primary structure is evident. Preliminary results on the amino terminal sequence of rat hemopexin indicate that it more closely resembles rabbit hemopexin. Deletion of residues 5 and 6 was observed, but no N-terminal residue was identified. Sufficient amounts of highly purified rat hemopexin are presently not available for further analysis.

The N-terminal residue of human hemopexin could not be unequivocally identified. Hydrolysis in HI of the PTH–amino acid released in the first step from human hemopexin resulted in a large alanine peak on amino acid analysis. In HI hydrolysis this could be either alanine, cysteine or serine. Our results would point to an N-terminal serine since neither PTH–

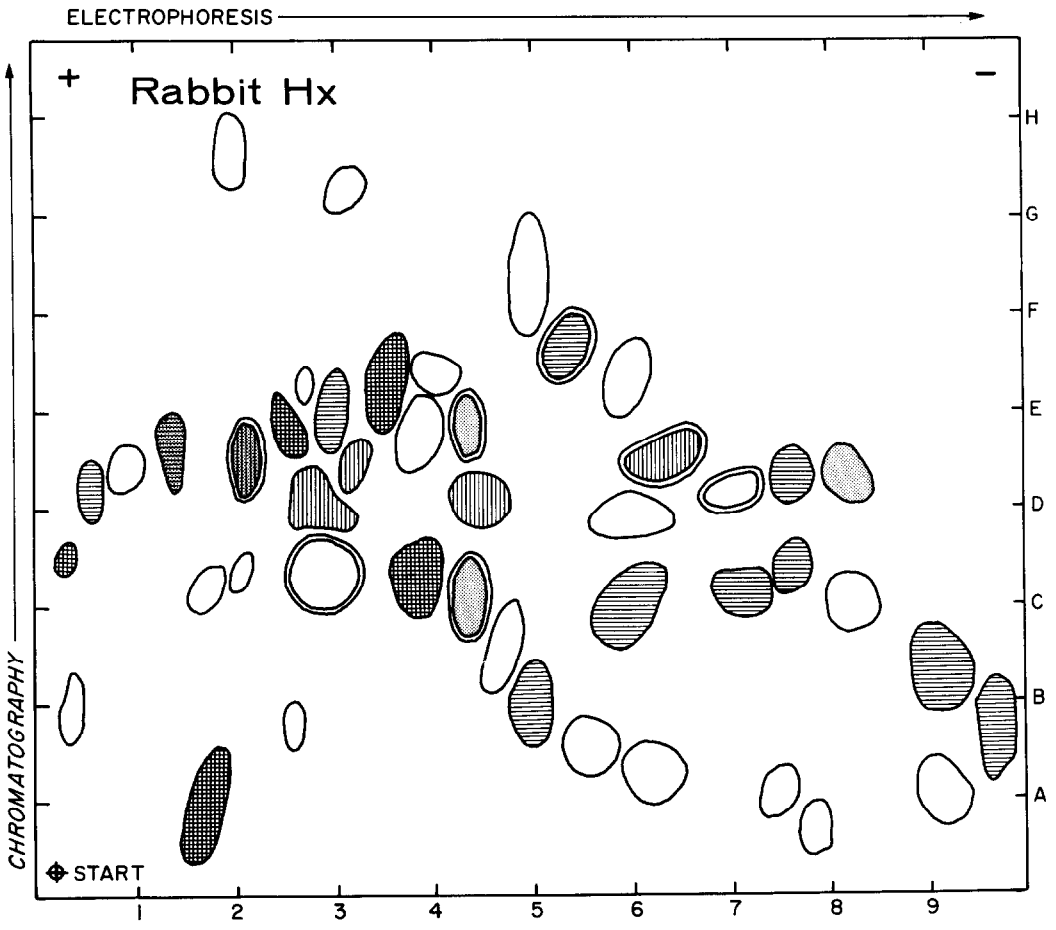

Fig. 2. Tryptic peptide pattern of rabbit hemopexin. Rabbit hemopexin (Hx) was digested with trypsin and 2.5 mg applied to the chromatogram sheet as described in Materials and methods. The chromatogram was stained for peptides with ninhydrin (○) and examined for peptides which contained arginine (⊖), histidine (⊖), tyrosine (●), and tryptophan (⊛).

alanine nor PTH–carboxymethylcysteine could be detected by gas chromatography or amino acid analysis, respectively. In a previous report, threonine was stated to be the N-terminal residue of human hemopexin (13). Hydrolysis in HI of PTH–threonine should yield α-aminobutyric acid; this was not found by us. An explanation for this discrepancy must await further study of the primary structure.

Evidence for genetic polymorphism in human hemopexin is shown in table 1 where two residues, leucine and phenylalanine, occur at position 3 of human hemopexin. The results obtained for the Behringwerke human hemopexin, which was isolated from pooled human sera, yielded nearly equal amounts of these amino acids. When human hemopexin isolated from separate donors was used, only leucine was obtained. No differences in the results were noted at any other position. A previous attempt by Stewart and Lovrein [14] to detect genetic polymorphism in human hemopexin by electrophoretic means was unsuccessful although polymorphism in rabbit and swine hemopexins have been reported [15,16]. It is to be expected that many, perhaps most, genetic variations are electrophoretically undetectable as in the case of human hemopexin and α_1-acid glycoprotein [17].

Table 1
N-Terminal amino acid sequences of human and rabbit hemopexins[a]

Position		1				5				10				15				20			
Human Hemopexin	NH_2–	?	Leu[b] –Pro–	Phe–	Pro–	Arg–	Gly–	Ser–	Ala–	His–	Gly–	?	–Val–	Ala–	Glu–	Gly–	Glu–	Thr–	?	–Thr–	Asn–	Pro–	Asp–Val ...
Rabbit Hemopexin	NH_2–Val	———	———	———	[	] [c]	———	———	———	———	His	———	Thr	———	———	Ser–	Gly	———	———	Lys	———	Glu–	Ala ...

a The question marks indicate residues that were not unambiguously assignable.
b This does not represent uncertainty in assignment but rather presumed genetic polymorphism. See text for details.
c This represents a deletion of residues 5 and 6. A similar deletion was also observed in rat hemopexin. Alignment of the rabbit protein with the human incorporating this deletion shows the highest degree of homology.

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