A C-terminal fragment of hemoglobin β-chains in extracts of porcine upper intestine

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A 32-residue peptide has been isolated from extracts of porcine upper intestine. Amino acid sequence determination showed that the peptide is a fragment of hemoglobin, corresponding to the C-terminal part of the β-chain. The region in the β-chain which precedes the isolated fragment is hydrophobic (8 Leu/Val/Ile) and has no charges at 10 preceding positions. It therefore, to some extent, resembles the structure of 'signal sequences' which may suggest a specific cleavage site in hemoglobin β-chains.

Hemoglobin β-chain fragment *Native protein fragment* *Cleavage site* *Signal sequence resemblance* *Intestinal extract* *Peptide isolation*

1. INTRODUCTION

It has been reported that some peptide fragments of hemoglobin can give rise to biological activities. Thus, the tetradecapeptide corresponding to residues 33–46 of the α-chain of porcine hemoglobin, has an in vitro ACTH-releasing activity [1], and neo-kyotorphin is an analgesic peptide corresponding to the C-terminal pentapeptide of human or bovine hemoglobin α-chains [2].

During attempts to improve the isolation procedure [3] for the vasoactive intestinal polypeptide (VIP), the VIP fraction was obtained contaminated with an additional polypeptide. We report here the characterization of this peptide. It is separated from VIP by one further step of ion-exchange chromatography. In spite of co-purification with VIP through 8 steps, the polypeptide was found not to be VIP-related. Instead, the results show it to be a C-terminal fragment of hemoglobin β-chains. Data on the purification and amino acid sequence of this novel fragment, obtained as a side-fraction from the last purification step of porcine VIP, are given. The identification shows the presence of a special segment in hemoglobin with some properties resembling signal peptide cleavage sites.

2. MATERIALS AND METHODS

2.1. *Isolation of the polypeptide*

The material was obtained from the VIP-containing fraction after the first CM–cellulose chromatography (step 6, table 1) during VIP purification (cf. [3,4]). This fraction was chromatographed (step 7, table 1) on a second CM–cellulose column in 0.02 M Na–phosphate buffer (pH 6.4) with a gradient of NaCl (0–0.3 M). The fraction containing VIP, detected with the secretin bioassay [5], was further purified (step 8, table 1) by counter-current distribution [3]. Finally, the material containing both VIP and the additional polypeptide was chromatographed on a third CM–cellulose column in 0.1 M ammonium bicarbonate (step 9, table 1).

2.2. *Structural analysis*

Total compositions were determined with a Beckman 121 M amino acid analyzer after hydrolysis for 22 h at 110°C in evacuated tubes with 6 M HCl containing 0.5% phenol. The amino acid sequence was determined by the manual dimethylaminoazobenzene isothiocyanate method [6] utilizing by-products to assist identification [7] and by liquid-phase sequencer degradations in a

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Table 1

Isolation from intestinal extracts of the C-terminal fragment of hemoglobin β-chain

Step no.		Weight
	Upper part of porcine intestine (boiled, frozen and minced)	36000 kg
1	Extraction with 0.5 M acetic acid; peptides adsorbed on alginic acid, eluted with 0.2 M HCl, and precipitated with NaCl	36 kg
2	Fractionation in 66% ethanol; soluble material adsorbed to alginic acid, eluted with 0.2 M HCl and precipitated with NaCl	2150 g
3	Exclusion chromatography on Sephadex G-25 (fine) in 0.2 M acetic acid	556 g
4	Extraction into methanol, soluble peptides precipitated with ether	82 g
5	Exclusion chromatography on Sephadex G-25 (fine) in 0.2 M acetic acid	24 g
6	Ion-exchange chromatography on CM–cellulose in 0.01 M Na–phosphate buffer (pH 8.0)	7 g
7	Ion-exchange chromatography on CM–cellulose in 0.02 M Na–phosphate buffer (pH 6.4) with a salt gradient (0–0.3 M NaCl)	1.5 g
8	Counter-current distribution in 0.1 M NH_4HCO_3:1-butanol	93.4 mg
9	Ion-exchange chromatography on CM–cellulose in 0.1 M NH_4HCO_3	1.3 mg

Beckman 890 D sequencer using a peptide program in the presence of glycine-precycled polybrene [8]. The phenylthiohydantoin derivatives were identified by high-performance liquid chromatography [9] and thin-layer chromatography [10].

3. RESULTS

Table 2 shows the amino acid composition of the polypeptide. It lacks several amino acid residues: serine, threonine, methionine, isoleucine, tryptophan and cysteine.

Fig.1 shows the complete amino acid sequence. This structure is identical to the C-terminal part (115–146) of the β-chain of porcine hemoglobin [11].

4. DISCUSSION

Significantly, in size and some chemical properties the new peptide is similar to the secretin group of peptides [12]. The extensive co-purification between VIP and the new peptide is compatible with the structural results. The final separation in the third cation-exchange step (step 9, table 1) is also consistent with the structure determined and appears to be explained by the less basic properties of the new peptide in relation to VIP (lower content of lysine and lack of C-terminal amide).

The present detection of a hemoglobin fragment is one further illustration that such fragments exist in different biological extracts. Notably, an ACTH-releasing activity from an α-chain fragment has been detected [1], as well as an analgesic pentapeptide [2] also from α-chains, and now the C-terminal β-chain fragment. Similarly, it has been shown that peptides with opioid activity, exorphins, are formed after partial enzymatic digestion of gluten and casein [13,14]. Although no biological activity has been ascribed to the present hemoglobin fragment, its consistent presence may indicate it to have some role(s), and suggests a specific cleavage in the parent molecule. The cleavage site is preceded by a highly hydrophobic segment and produces an N-terminal alanine (fig.2). In both these respects, the preceding structure resembles sites for signal peptide cleavages [15,16]. In fact, this region of hemoglobin β-chains has close to 10 hydrophobic branched-chain residues without interruption by charged residues, and with positive charges at both ends (positions -11 and $+2$ in fig.2). Differential proteolysis to produce multiple forms of peptides has also been shown for molecules with established biological functions, like gastrin and cholecystokinin (CCK) [17], gastric inhibitory polypeptide (GIP) [18], adrenocorticotropic hormone (ACTH) [19] and insulin-like growth factor-2 (IGF-2) [20,21]. In addition, specific fragments of proteins, demon-

Table 2
Amino acid compositions of the new hemoglobin fragment and its tryptic peptides isolated (T_2–T_4)

Amino acid	Intact peptide		T_2	T_3	T_4
	Acid hydrolysis	Sum of sequence determination			
Asp	4.2	4	3.0 (3)	1.2 (1)	–
Glx	2.1	2	2.0 (2)	–	–
Pro	1.2	1	1.0 (1)	–	–
Gly	2.3	2	1.2 (1)	1.5 (1)	–
Ala	6.1	7	1.9 (2)	3.7 (4)	–
Val	3.1	4	1.1 (1)	2.4 (3)	–
Leu	2.4	2	1.1 (1)	1.1 (1)	–
Tyr	0.8	1	–	–	1.0 (1)
Phe	1.9	2	1.9 (2)	–	–
Lys	2.1	2	1.0 (1)	1.2 (1)	–
His	2.6	3	0.9 (1)	0.9 (1)	1.0 (1)
Arg	1.9	2	–	–	–
Total		32	15	12	2

Values within parentheses represent the integers deduced from sequence determination

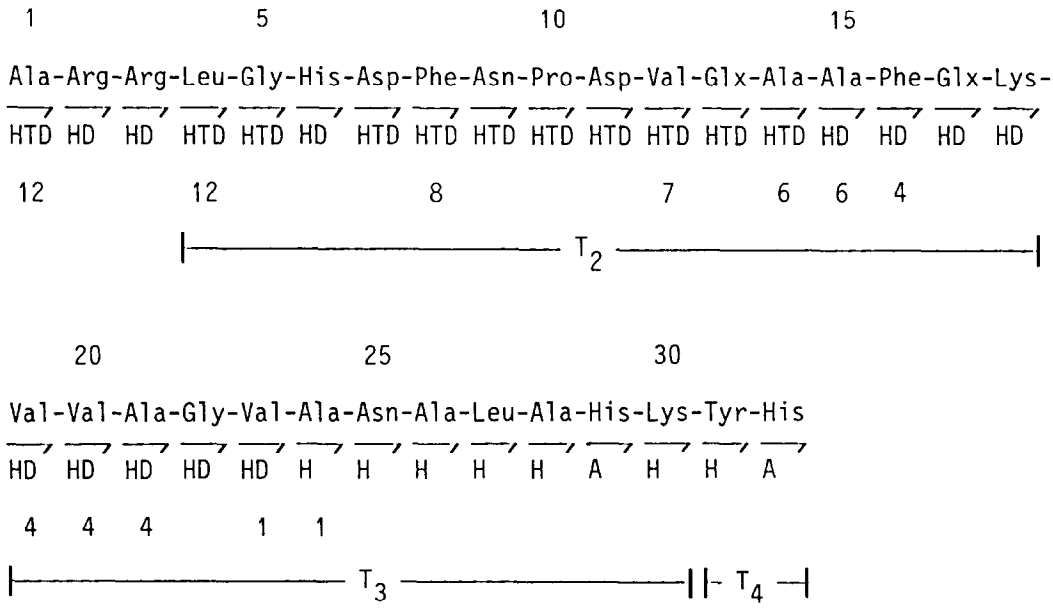

Fig.1. Results of amino acid sequence analysis of the new peptide. Liquid-phase sequencer-assisted analysis is shown by ⟶. Residue identification: high-performance liquid chromatography, H; thin-layer chromatography, T. Figures below the letters denote nmol stable residues recovered from degradation of 20 nmol peptide. Repetitive yield calculated on alanine 1–21 is 95%. In addition, results of manual sequence analysis of the intact peptide (9 nmol) with the dimethylaminoazobenzene isothiocyanate method are shown by D and identifications from total compositions by A.

Tryptic fragments T_2–T_4, purified by reverse-phase high-performance liquid chromatography, are also shown.

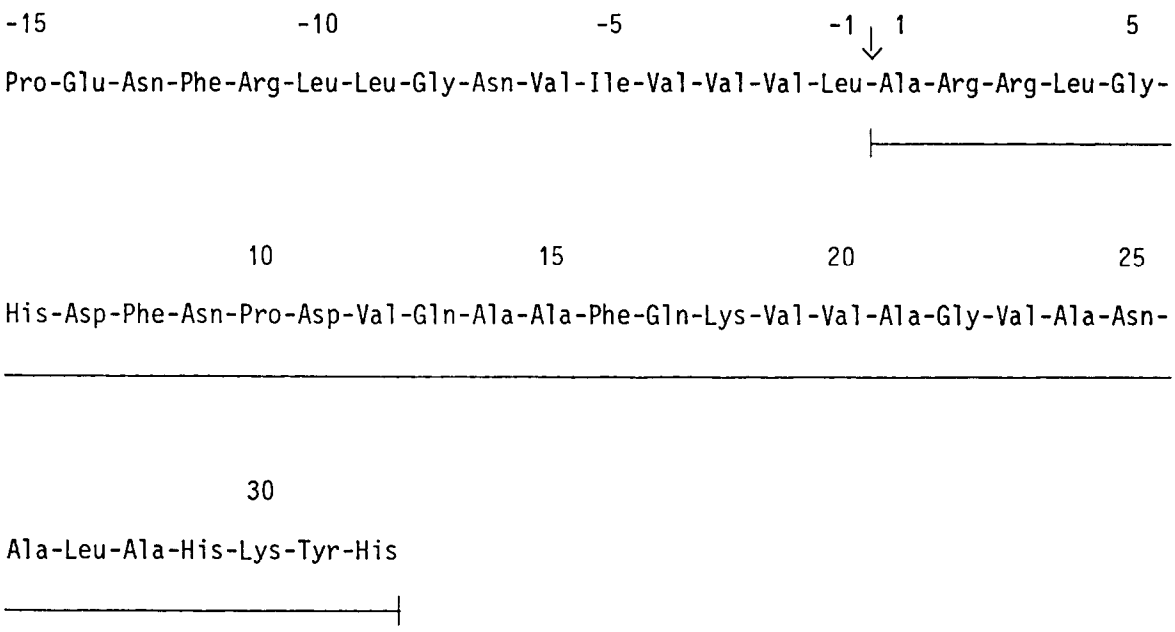

Fig.2. The amino acid sequence of the C-terminal part of hemoglobin β-chains, demonstrating the structure around the site where cleavage (arrow) liberates the isolated peptide (underlined).

strating cleavages in the parent molecules, have been described for several large polypeptide chains including fibrinogen [22] and β_2-microglobulin [23]. These examples indicate that cleavages in proteins may be specific and more frequent than often assumed, raising the question as to whether the cleavage products may have biological functions.

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