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Human pre-α-inhibitor: isolation from a by-product of industrial scale plasma fractionation and structural analysis of its H3 heavy chain

Charlotte Mizon[a], Antoine Héron[b], Callioppe Capon[a,c], Pierre Sautière[d], Catherine Michalski[e], Richard Sesboüé[b], Jacques Mizon[a,*]

[a]*Laboratoire de Biochimie (DRED E.A. 1052), Faculté de Pharmacie, Avenue du Professeur Laguesse, B.P. 83, F-59006 Lille, France*
[b]*INSERM Unité 295, Faculté de Médecine-Pharmacie de Rouen, B.P. 97, Avenue de l'Université, F-76803 St. Etienne-Rouvray, France*
[c]*Laboratoire de Chimie Biologique, UMR 111 CNRS, Université des Sciences et Techniques de Lille, F-59655 Villeneuve d'Ascq, France*
[d]*Service de Chimie des Biomolécules, URA 1309 CNRS, Institut Pasteur, 1, Rue du Professeur Calmette, B.P. 245, F-59019 Lille, France*
[e]*Laboratoire Français du Fractionnement et des Biotechnologies (L.F.B.), Etablissement de Lille, 59, Rue de Trévise, B.P. 2006, F-59011 Lille, France*

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Abstract

Pre-α-inhibitor (PαI) is a serine proteinase inhibitor from human plasma. It comprises bikunin (BK) responsible for antiprotease activity, covalently linked to a heavy chain H3. Here we describe its isolation from a side fraction of an industrial preparation of plasma clotting factors. By using a highly specific polyclonal antiserum prepared from rabbit immunized with a H3P polypeptide obtained in a bacterial expression system, we were able to identify the fractions containing PαI. Then, taking advantage of the differential affinity of the members of the inter-α-inhibitor family (IαI) for heparin-Sepharose and blue-Sepharose, we isolated PαI. Its specific antitryptic activity was 580 IU/g, higher than that of IαI: 420 IU/g. Its M_r, determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis, with or without prior reduction, was 130 000. Its peptide chains were identified by N-terminal sequencing. The H3 heavy chain was isolated from PαI by alkaline dissociation and anion-exchange chromatography. Its electrophoretic mobility was compared to that of the H1 and H2 heavy chains of IαI. In reducing conditions, it was quite similar to that of H2 (M_r 85 000) but clearly different from that of H1 (M_r 78 000). Thus, the so-determined apparent M_r of H3 was overestimated since its molecular mass determined by MALDI–TOF was 74 100. This result agrees with the proposed structure for H3. Indeed, by carbohydrate analysis and PNGase F digestion, we demonstrate that the two potential *N*-glycosylation sites present in the core-protein (theoretical mass: 69 454) are really occupied by two *N*-glycans, probably of biantennary type.

Keywords: Pre-α-inhibitor; H3 heavy chain

*Corresponding author.

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1. Introduction

Among the Kunitz-type proteinase inhibitors of human plasma, a family of components is characterized by the presence of human bikunin (BK) covalently linked by a glycosaminoglycan (GAG) chain, to one or two among three homologous heavy chains called H1, H2, H3 [1,2]. Human inter-α-inhibitor (IαI) (M_r 220 000) which is the major component of this family of multichain proteins is made up of BK linked together with one H1 and one H2 chain [3,4]. The other BK-containing proteins are present in human plasma in much lower concentrations than that of IαI. They are heterodimers (M_r near 125 000) made up of BK linked to one of the three heavy chains. The H3–BK combination corresponds to human pre-α-inhibitor (PαI) [4].

Daveau et al. [5] recently reported that PαI is the only BK-protein, the synthesis of which increases in inflammatory conditions. Moreover H3, as well as H1 and H2, were recently characterized in pathological synovial fluid from human arthritis patients [6]. As a pre-requisite to investigate the physiological role and metabolism of PαI and H3, we developed an original procedure allowing the isolation of these proteins from human plasma. We took advantage of (i) the presence of BK-proteins in side-fractions of a process designed for extracting coagulation factors from the prothrombin complex concentrate (PCC), as recently described for IαI [7], (ii) the use of a highly specific polyclonal antiserum prepared from rabbit immunized with a H3P[1] polypeptide obtained in a bacterial expression system [8]. Thus, for the first time, the H3 heavy chain was obtained in sufficient amount to allow its structural characterization.

2. Experimental

2.1. Reagents

Blue-Sepharose and Resource-Q columns (6 ml) were from Pharmacia-Biotech (Saclay, France). Chondroitin AC lyase (EC 4.2.2.5) from *Arthrobacter aurescens* was obtained from Seikagaku Kogyo (Tokyo, Japan). Recombinant peptide-*N*-glycosidase F (PNGase F) (EC 3.2.2.18) from *Flavobacterium meningosepticum* was purchased from Oxford Glycosystems (Coger, Paris, France). Anti-H3P serum and specific anti-H1 and H2 immunoglobulins were those recently described [8,9]. Rabbit anti-IαI immunoglobulins were obtained from Dakopatts (Dako, Trappes, France). Molecular mass standards for SDS–PAGE were from Bio-Rad (Ivry sur Seine, France). All the chemicals used were of analytical grade.

2.2. Preparation of the PαI source

Source material was a side-fraction obtained from a factor IX purification process which involves different chromatographic steps, as described previously [7]. Briefly, cryo-poor plasma (1500 l/batch) was first absorbed in batch on DEAE-Sephadex A-50 (Fig. 1). The resin was washed with 20 m*M* sodium citrate buffer, pH 7.0, containing 0.23 *M* NaCl and prothrombin complex concentrate (PCC) was then eluted by increasing the NaCl concentration up to 0.5 *M*. After concentration, PCC was subjected to solvent–detergent (SD) treatment with 0.3% tri-*n*-butyl phosphate and 1% Tween-80 at 25°C for 8 h, in order to deactivate lipid-enveloped viruses.

The SD–PCC mixture was then applied onto a DEAE-Sepharose fast-flow column equilibrated with a 6 m*M* phosphate buffer, pH 6.0. After three stepwise washings with 0.16, 0.23 and 0.28 *M* NaCl in the same buffer, factor IX was recovered in fraction 4 by further increasing the NaCl concentration to 0.36 *M* [10]. Fraction 4 was then injected onto a 10-l T250/30 BioProcess column (Pharmacia-Biotech) containing heparin-Sepharose CL-6B equilibrated in a 20 m*M* sodium citrate, pH 7.5 buffer. Elution was performed by using different NaCl concentrations in the equilibration buffer.

2.3. PαI isolation

Fraction A (unbound material obtained by washing the heparin-Sepharose column) was equilibrated by dialysis in a 20 m*M* Tris buffer, pH 7.6, containing 4 m*M* EDTA and 0.05% sodium azide and a 40 ml volume (near 130 mg protein) was loaded at 40 ml/h onto a 90 ml Blue-Sepharose column (C 26/40;

[1]H3P: Heavy chain H3 precursor, denomination in agreement with the recently proposed nomenclature by Salier et al. [2].

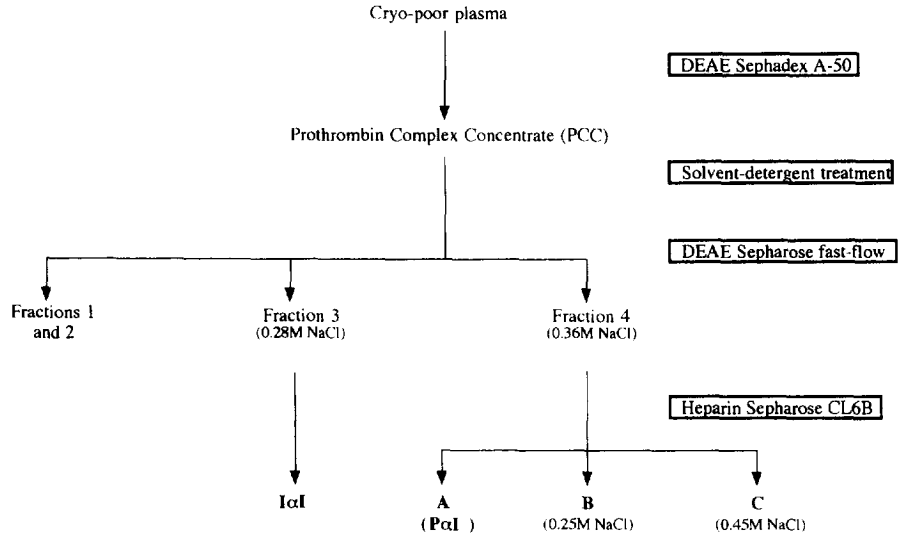

Fig. 1. Starting material for PαI isolation.

Pharmacia-Biotech) equilibrated in the same buffer. The unbound fraction contained PαI. After dialysis in a 20 m*M* Tris buffer, pH 5.5, containing 0.2 *M* NaCl, 4 m*M* EDTA, 10 ml (near 20 mg protein) were loaded onto a Resource-Q column equilibrated in the same buffer and connected to a fast protein liquid chromatography system (FPLC, Pharmacia Biotech). After washing the column for 8 min at a flow-rate of 2.5 ml/min with the same buffer, a linear gradient from 0.2 to 0.45 *M* NaCl was applied for 50 min and then from 0.45 to 1 *M* for 10 min. The absorbance of the eluted fractions was measured at 280 nm, antitryptic activity and further analyzed by 7.5% SDS–PAGE.

2.4. Alkaline dissociation and isolation of the H3 heavy chain

PαI (10 mg in 10 ml) was mixed with an equal volume of 0.1 *M* Na_2CO_3. The pH was then adjusted to 12.5 with 10 *M* NaOH and the reaction mixture was stirred for 10 min at room temperature before neutralization with 2 *M* HCl. After dialysis overnight against a 20 m*M* Tris buffer, pH 7.6, containing 4 m*M* EDTA, the dissociated PαI was loaded on a Resource-Q column connected to the FPLC system and equilibrated in the same buffer. After washing the column for 8 min at a flow-rate of 2.5 ml/min, a linear gradient from 0 to 0.4 *M* NaCl was applied for 25 min and then from 0.4 to 1 *M* for 30 min. The absorbance of the eluted fractions was measured at 280 nm and further analyzed by 10% SDS–PAGE.

2.5. Analytical procedures

Determination of protein concentration was performed as already described [11] using a Coomassie brilliant blue reagent containing sodium dodecyl sulfate (SDS) and bovine serum albumin as the reference. Trypsin inhibitory capacity (TIC) was measured by the automated method previously described, using L-benzoylarginin-*p*-nitroanilide as the substrate [12]. One inhibitory unit (IU) is defined as the amount of inhibitor which decreases the enzymically catalyzed hydrolysis of the substrate by 1 μmol/min. Factor II was measured by coagulant methods using the corresponding deficient plasma (Stago, Asnière, France).

SDS–PAGE on 7.5 or 10% (w/v) polyacrylamide slab gels (and subsequent transfer onto nitrocellulose with immunological detection) were carried out as previously described [3]. The antitryptic revelation was performed according to Uriel and Berges [13].

The molecular mass of H3 was measured by

matrix-assisted laser desorption (MALDI–TOF) on a "Vision 2000" time of flight instrument (Finnigan Mat, Hemel-Hampstead, UK) equipped with a 237 nm UV laser. The mass spectra were acquired in linear mode under 30 kV accelerating voltage and positive detection. The sample was dissolved in water–acetonitrile (70:30, v/v) at a concentration of 10 pmol/μl. A 2 μl volume of the analyte solution were mixed with an equal volume of the matrix solution (sinapinic acid 10 mg/ml dissolved in water–ethanol (80:20)). External calibration was performed using a serotransferrin standard (M_r 79 553±10) purchased from Sigma (L'Isle d'Abeau Chesnes, France).

N-terminal sequencing of PαI and H3 was carried out directly on the native proteins on a gas-phase sequencer (Applied Biosystems 470A) using the 03 RPth program. Phenylthiohydantoin derivatives of amino acids were identified on-line on a 120 A amino acid analyser (Applied Biosystems, Foster City, CA, USA).

After desalting by extensive dialysis against a 20 m*M* HCO_3NH_4 buffer, pH 7.4 and lyophilization, carbohydrate analysis of the H3 heavy chain was performed using gas chromatography with a silicone OV 101 capillary column (25×0.32 mm I.D.). The sample was analyzed after methanolysis (0.5 *M* HCl in methanol for 24 h at 80°C) followed by *N*-reacetylation and trimethylsilylation, according to Kamerling et al. [14] modified by Montreuil et al. [15].

For chondroitinase digestion, an aliquot of pure PαI (0.3 mg) was mixed with 120 mU enzyme in 2 ml of a 0.4 *M* acetate buffer, pH 6.0. Digestion was carried out overnight at 37°C. After denaturation, the sample was analyzed by 10% SDS–PAGE and Coomassie blue staining.

For PNGase F digestion, H3 (60 μg) in 60 μl of a 10 m*M* phosphate buffer, pH 7.5, containing 25 m*M* EDTA, 0.5% SDS and 5% mercaptoethanol was heated for 2 min at 100°C. After cooling the mixture, 20 μl of 10% Triton X-100 then 10 μl PNGase F (5 U) were successively added. The reaction mixture was incubated at 25°C in a water-bath. Aliquots were taken after 6 and 24 h incubation time, respectively and immediately denatured before SDS–PAGE analysis.

3. Results

3.1. Characterization of IαI reactivity in side fractions during plasma clotting factors preparation

As previously reported [7], PCC contains IαI and lower molecular mass derivatives (M_r near 125 000) which are immunologically related to IαI and exhibit an antitryptic activity. For production process (Fig. 1), PCC was fractionated by ion-exchange chromatography on a DEAE-Sepharose fast flow column [16]. Four fractions were successively eluted by increasing the NaCl concentration. IαI was mainly eluted by 0.28 *M* NaCl in fraction 3. It was also present in lower amounts in fraction 4, eluted by 0.36 *M* NaCl. Fractions 3 and 4 in addition to PCC were analyzed by SDS–PAGE without prior reduction where BK-containing-proteins, thus PαI, could be visualized by their antitryptic activity as well as by Coomassie blue staining and immunoblot analysis after transfer onto nitrocellulose (Fig. 2). In fractions 3 and 4 as well as in PCC, anti-IαI immunoglobulins allowed to detect two bands moving faster than IαI. The fastest of these two bands was visualized by its antitryptic activity; in PCC and fraction 4, it exhibited an anti-H3P immunoreactivity. Further immunoanalysis (data not shown) showed that it was also detected by specific anti-H2 immunoglobulins whereas the slower band was revealed by specific anti-H1 immunoglobulins. Overall, the immunoblot analysis with anti-H3P serum showed that PCC contained PαI which was eluted from DEAE-Sepharose in fraction 4 and moved close to the H2–BK complex in SDS–PAGE under non-reducing conditions. Besides PαI, fraction 4 contained all the bikunin-proteins (IαI, H1–BK, H2–BK) and factor II (M_r 72 000) as revealed by Coomassie blue staining and esterolytic activity (Fig. 2).

In a large-scale production of therapeutic concentrates, factor IX is purified by affinity chromatography on heparin-Sepharose. Three fractions are separated, named A, B and C (Fig. 1). Fractions B and C, successively eluted by 0.25 and 0.45 *M* NaCl in the equilibration buffer, respectively contain factors X and IX. Only a weak antitryptic activity due to IαI was found in fraction B. Indeed, as already

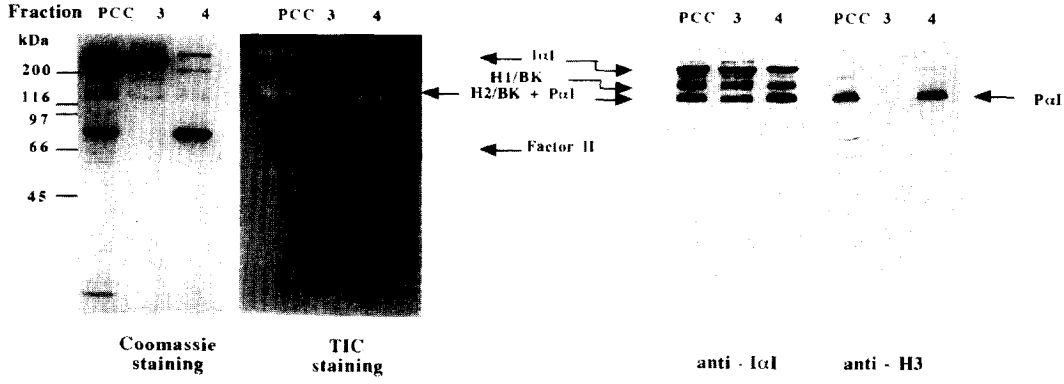

Fig. 2. Analysis of PCC and fractions 3 and 4, both eluted from the DEAE-Sepharose fast-flow column. 10% SDS–PAGE was performed under non-reducing conditions. Each lane was loaded respectively with 2 μg protein for Coomassie blue staining, 1.5 mU TIC for antitryptic revelation, 0.5 μg and 1.5 μg protein for immunodetection by anti-IαI immunoglobulins and anti-H3P serum, respectively.

described [7], IαI exhibited low affinity for heparin and was mainly eluted in this fraction. Fraction A corresponded to the dropthrough material and contained the main body of the antitryptic activity. By immunoblot analysis using anti-H3P as detection antibody, PαI was characterized in this fraction besides low amounts of residual IαI and heterodimers BK-proteins as detected by anti-IαI immunoglobulins (data not shown). This fraction was therefore chosen as starting material for PαI isolation.

3.2. Isolation and characterization of PαI

Because factor II is the main component of the latter fraction and presents a strong affinity for blue-Sepharose, this matrix was used for the first purification step of our procedure. Factor II was actually retained as well as IαI, H1–BK and H2–BK, while PαI was obtained in the dropthrough material. Further purification was achieved by anion-exchange chromatography at pH 5.5 (Fig. 3). PαI eluted as a broad heterogeneous peak between 0.3 and 0.4 *M* NaCl in the equilibration buffer. Only fractions eluted later were directly obtained in a pure state. The other ones were pooled and again fractionated in the same conditions. SDS–PAGE analysis of samples along the whole process demonstrated the progressive purification of PαI (Fig. 4). Factor II, a major component of the starting material, was absent from the final product as further confirmed by coagulation determination in the samples (Table 1). Because there is presently no specific procedure to measure PαI levels, the improvement brought up at each step of our process was assessed by the increase in antitryptic activity (IU/g protein), thus estimating all the BK-proteins. The purity of PαI was definitively established by identification of BK and H3 by *N*-terminal sequencing carried out directly on the native protein (Table 2). PαI migrated as a single band (M_r 130 000) in 7.5% SDS–PAGE, with or without prior reduction. The mean specific antitryptic activity of our preparation was 580±25 IU/g, (n=5). Its specific absorbance $A^{1\%,1\ cm}$ at 280 nm was 7. Immunoblot analysis, prior to or after chondroitinase digestion, showed that PαI released bikunin, detected by anti-IαI immunoglobulins and the H3 heavy chain, revealed by specific anti-H3P antibody (data not shown).

3.3. Isolation and characterization of the H3 heavy chain

PαI was dissociated by alkaline treatment and fractionated by ion-exchange chromatography on the Resource-Q column equilibrated in a pH 7.6 buffer (Fig. 5). Fractions eluted by increasing the NaCl concentration in the equilibration buffer were analyzed by SDS–PAGE with Coomassie blue staining.

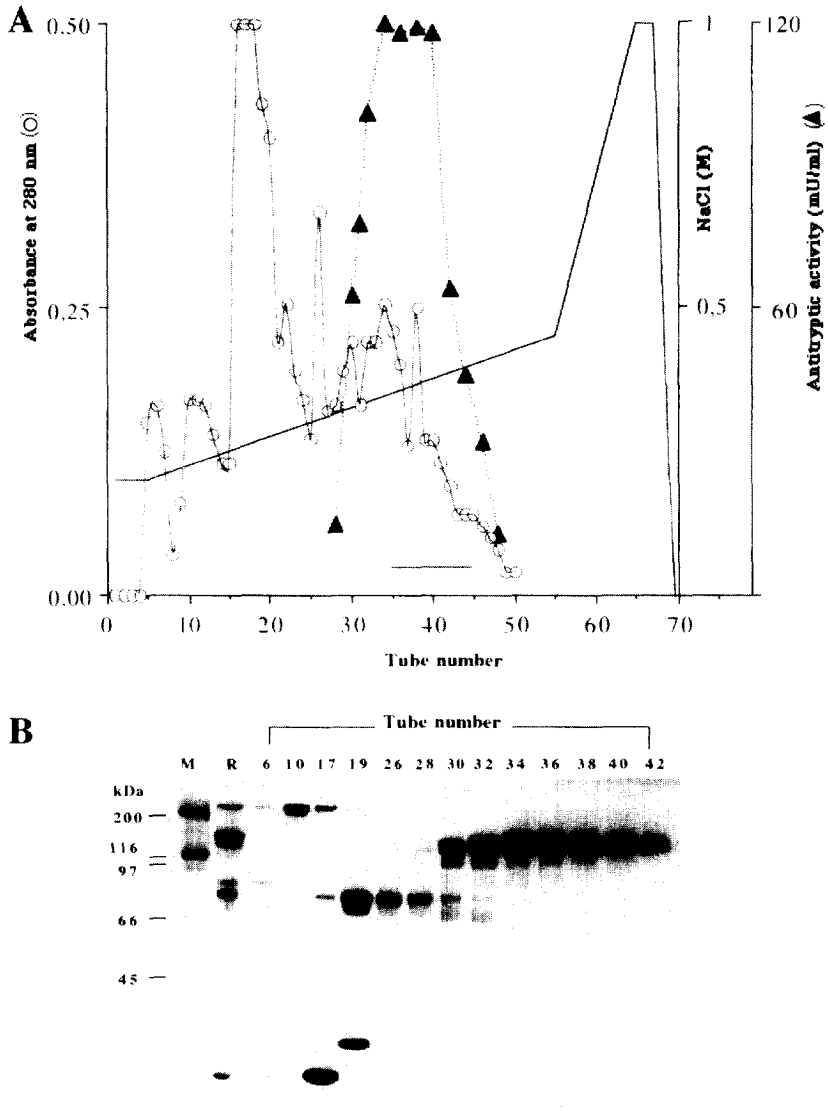

Fig. 3. Isolation of PαI by anion-exchange chromatography. (A) The dropthrough fraction obtained after blue-Sepharose chromatography was loaded on a Resource-Q column equilibrated with a 20 m*M* Tris buffer, pH 5.5, containing 0.2 *M* NaCl and 4 m*M* EDTA. Elution was performed by a linear gradient 0.2–0.45 *M* NaCl in the same buffer for 50 min and then from 0.45 to 1 *M* for 10 min at a flow-rate of 2.5 ml/min. The absorbance of the eluate was monitored at 280 nm and antitryptic activity was determined. Tubes were pooled as indicated by the horizontal bar. (B) The collected fractions were analyzed by 10% SDS–PAGE with Coomassie blue staining. M: high molecular mass markers from Bio-Rad: skeletal muscle myosin 200 000; β-galactosidase 116 250; rabbit muscle phosphorylase β 97 400; bovine serum albumin 66 200; egg white albumin 45 000. R: unbound fraction after blue-Sepharose chromatography.

H3 was recovered in pool 2. Pools 1 and 3 exhibited an antitryptic activity, corresponding to BK without GAG eluted early from the column and more anionic components corresponding to residual undissociated PαI and BK with GAG, respectively.

The H3 chain thus obtained (M_r 85 000) moved

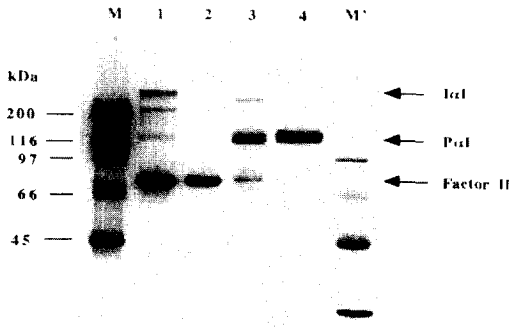

Fig. 4. Purification process of PαI. Lane 1: fraction 4 after DEAE-Sepharose fast-flow, lane 2: fraction A after heparin-Sepharose, lane 3: dropthrough fraction after blue-Sepharose chromatography, lane 4: PαI obtained after anion-exchange chromatography. M: High molecular mass markers from Bio-Rad; M': low molecular mass markers from Bio-Rad: phosphorylase β 97 400; bovine serum albumin 66 200; egg white albumin 45 000; carbonic anhydrase 31 000; soybean trypsin inhibitor 21 500. 10% SDS–PAGE was performed under non-reducing conditions with Coomassie blue staining. 1.5 μg protein per lane was loaded.

Table 2
Amino-terminal sequence of native PαI

Cycle No.	Amino acid residues	Amounts (pmol)
1	A–S	96.2–53.0
2	V–L	42.9–28.1
3	L–P	33.7–19.1
4	P–E	31.5–8.8
5	Q–G	10.1–9.0
6	E–V	9.4–6.4
7	E–A	9.1–4.6
8	N–E	8.2–2.4
9	G	5.6
10	S[a]–I	0–1.8

Amino-terminal sequencing of PαI (500 pmol) was carried out directly on the native protein. The amino acid residues detected at each cycle are indicated. Only amino acids which do not overlap are quantified in pmol, except for Ser in cycle 10. The sequence for each protein is:

Chain	Residue No.
Bikunin	AVLPQEEEGS
Heavy chain H3	SLPEGVANGI

[a] Residue not quantified.

close to H2 in 10% SDS–PAGE under reducing conditions (Fig. 6A) but was clearly differentiated from H1 (M_r 78 000). Mass measurement by MALDI–TOF gave a molecular mass for H3 of 74 100±50 Da (Fig. 7).

N-terminal sequence analysis confirmed the previously reported results (data not shown). Carbohydrate composition (Table 3) was in agreement with the presence of *N*-linked glycans, mainly of biantennary type. These data were confirmed by peptide-*N*-glycosidase F digestion of pure H3 and subsequent SDS–PAGE analysis (Fig. 6B). Indeed two bands with higher mobility (M_r values 83 000 and 81 000) were visualized; thus two *N*-glycans per mol H3 would be present. No GalNAc was detected which agreed with the absence of *O*-linked glycans.

4. Discussion

Little is known about the physiological role and metabolism of PαI since it has been only recently described [4]. Further progress in our knowledge about this protein depends upon the improvement of procedures allowing its isolation and characterization. However, their development was hampered because PαI is only a minor component of human plasma and it exhibits strong structural homologies with the other members of the IαI family, which makes its purification more difficult.

The presence of BK-proteins has been reported in several fractions obtained on a large-scale when purifying factor IX from PCC [7]. By using a

Table 1
Purification chart of PαI from human plasma

	Protein (mg)	Trypsin inhibitory capacity (IU)	Specific antitryptic activity (IU/g)	factor II (mU)
Starting source (fraction A)	1560	39	25	576
After blue-Sepharose	138	39	285	4.1
After Resource-Q	24	14	576	<2.7

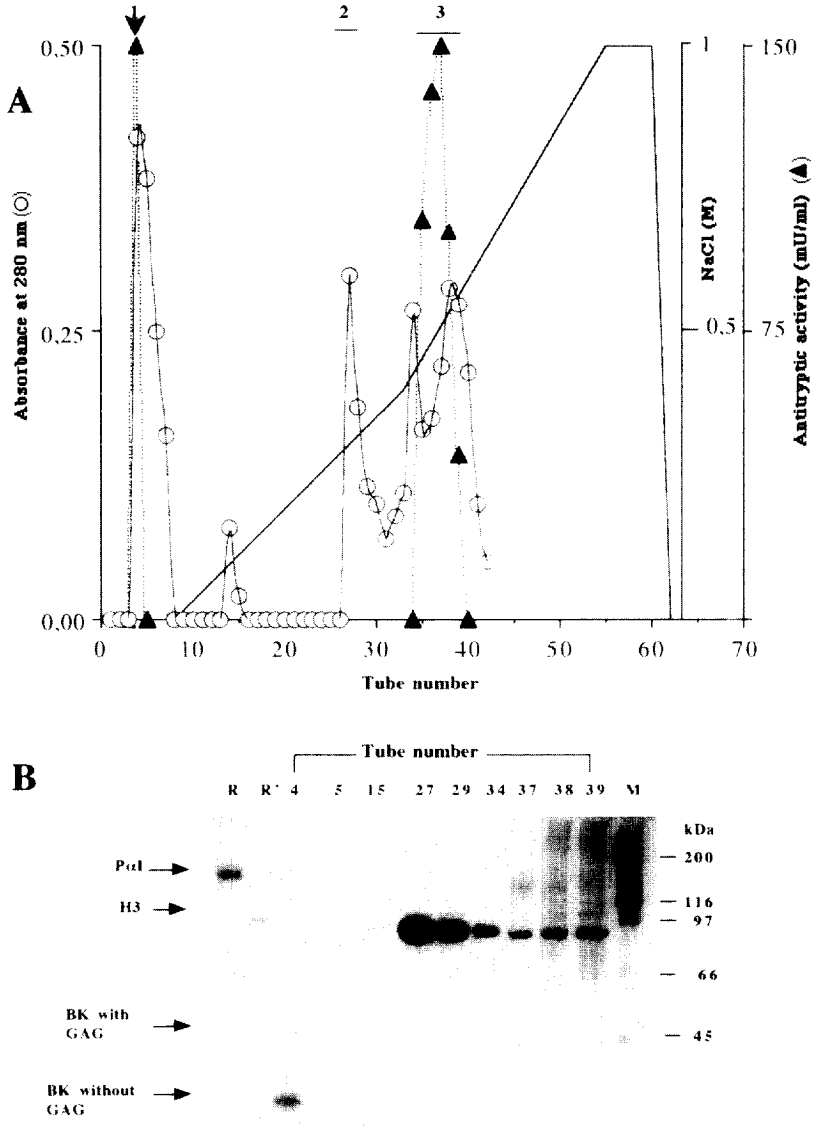

Fig. 5. Isolation of H3 by anion-exchange chromatography. (A) The mixture obtained after PαI dissociation by alkaline treatment was loaded on a Resource-Q column equilibrated with a 20 m*M* Tris buffer, pH 7.6, containing 4 m*M* EDTA. Elution was performed by a linear gradient 0–0.4 *M* NaCl in the same buffer for 25 min and then from 0.4 to 1 *M* for 30 min at a flow-rate of 2.5 ml/min. The absorbance of the eluate was monitored at 280 nm and antitryptic activity was determined. The arrow indicates BK without GAG; 2: H3; the horizontal bar shows the pooled tubes. (B) The collected fractions were analyzed by 10% SDS–PAGE with Coomassie blue staining. M: High molecular mass markers from Bio-Rad. R: Reference PαI before alkaline dissociation, R′: reference PαI after alkaline dissociation.

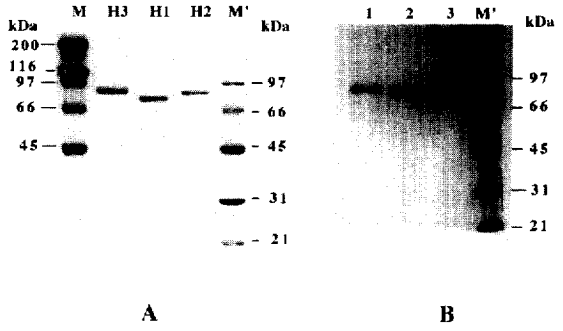

Fig. 6. (A) Relative electrophoretic mobility of the three heavy chains. Pure proteins (0.5 μg per lane) were analyzed by 10% SDS–PAGE under reducing conditions with Coomassie blue staining. M: High molecular mass markers; M′: low molecular mass markers from Bio-Rad. (B) PNGase F digestion of the H3 heavy chain. Samples of H3 (0.7 μg protein) prior to (lane 1) and after PNGase F digestion (6 h: lane 2; 24 h: lane 3) were analyzed by 10% SDS–PAGE under reducing conditions with Coomassie blue staining. The PNGase F digestion was carried out as described in Section 2.5.

Table 3
Carbohydrate composition of the H3 heavy chain

	Molar ratios[a]				
	Gal	Man	GalNAc	GlcNAc	NeuAc
H3	2	3	–	3	1

[a] The molar ratios were calculated on the basis of three Man residues. Since the GlcNAc–Asn linkage is only partially cleaved by methanolysis (14, 15), the number of GlcNAc residues is underestimated (about 1 residue for 3 Man).

polyclonal antibody obtained from recombinant proteins whose specificity towards H3P has been established [8], we were able to characterize PαI inside the PCC fraction. It was noticeably purified during the next two steps of the industrial fractionation procedure. Indeed, PαI was partially separated from the less anionic IαI by ion-exchange chromatography on DEAE-Sepharose. The residual IαI which exhibits a low affinity for heparin-Sepharose [7] was mainly retained by this matrix. In contrast, PαI and the other heterodimers (namely H1–BK and H2–BK), containing only one heavy chain, were eluted in the dropthrough fraction. Factor II is the main component of this fraction and was completely removed because of its well-known binding to blue-Sepharose [17]. PαI was eluted in the dropthrough fraction whereas, as already reported [4], IαI and H2–BK remained tightly bound to the support. We found that H1–BK was also adsorbed by blue-Sepharose. The isolation of PαI was achieved by ion-exchange chromatography performed at pH 5.5, in order to take advantage of its strong anionic charac-

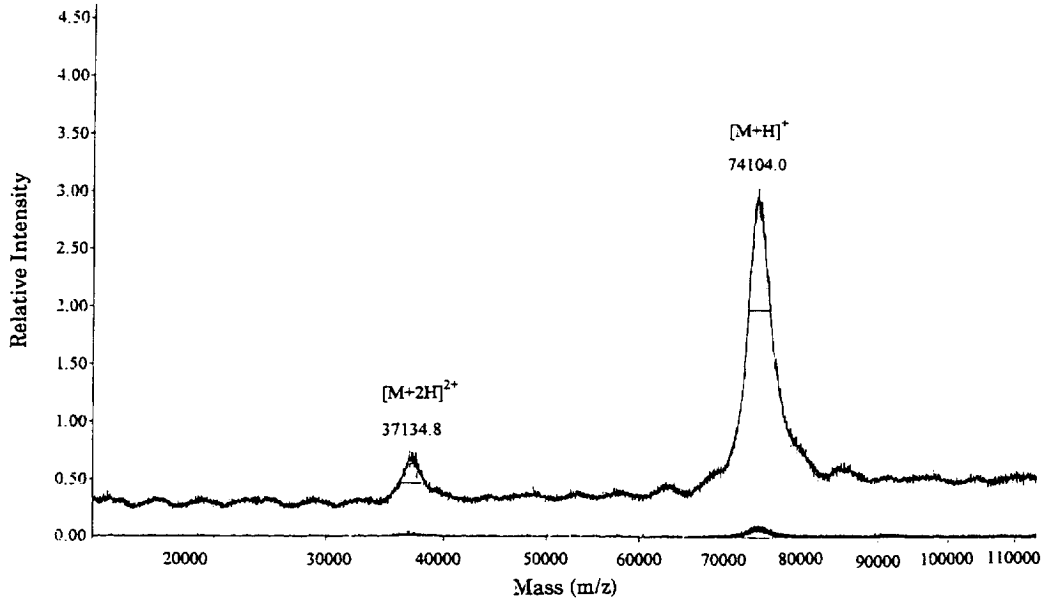

Fig. 7. MALDI–TOF mass spectrum for the H3 heavy chain from PαI.

ter resulting from the presence of the chondroitin-sulfate chain. PαI thus obtained was clearly identified as comprising BK and H3, as assessed by *N*-terminal sequence determination. The H3P antibody used revealed highly specific since it detected neither H1, nor H2, nor IαI by immunoblot analysis.

PαI moved, in SDS–PAGE under reducing conditions, as a band M_r 130 000, close to H1–BK and H2–BK. Such a value, clearly different from that previously reported by Daveau et al. (155 000) [5], was in good agreement with the data of Thogersen and Enghild [1] and Héron et al. [8]. PαI exhibited a specific antitryptic activity of 580 IU/g, obviously higher than that we determined for IαI (420 IU/g) [7]. This result agreed with the presence, in the PαI structure, of one mol BK carrying the antiprotease activity for one mole heavy chain whereas, IαI is made up by one mol BK linked to 2 mol heavy chains (one H1 and one H2). As described by Enghild et al. [4], the protein–glycosaminoglycan–protein cross-link which binds bikunin to H3 is selectively cleaved by chondroitinase digestion or alkaline treatment. We have reported [19] that after alkaline dissociation, IαI releases bikunin under two forms: with and without its GAG chain. Here, we demonstrate that the same result was obtained by dissociating PαI under identical conditions. H3 was then easily recovered by anion-exchange chromatography.

After reduction, H3 moved in SDS–PAGE with a mobility quite identical to that of H2. Its M_r (85 000) was in good agreement with the data reported by Enghild et al. [20] and Héron et al. [8], but noticeably differed from the M_r 101 000, estimated by Daveau et al. [5]. Since the estimated values of molecular masses by SDS–PAGE are diversely affected by the charge and composition of the analyzed proteins, that of H3 was measured by using MALDI–TOF. The result obtained (74 100) agreed with the proposed structure for H3. It was made up by a core-protein comprising 618 amino acids of 69 454 Da with two potential sites for *N*-glycosylation [18]. As demonstrated in this report, those two sites were actually glycosylated and the weight of two *N*-glycans of biantennary type leads up the mass of H3 to the value estimated by mass spectrometry.

The present data, as well as previous results, allow us to suggest that, in spite of their close structural homologies, the three heavy chains H1, H2 and H3 exhibit large differences in their own reactivity: interaction with blue-Sepharose (Ref. [4] and this report), Concanavalin A [19]. Thus the nature of each type of heavy chain may govern the organ distribution of BK-proteins. Heavy chains would serve as specific carriers to deliver BK in various tissues where it acts as an antiprotease [21], an antiinflammatory agent [22] or an endothelial cell growth factor [23]. Furthermore, the biosynthetic pathway of each type of heavy chains is specifically regulated [5]. Thus each member of the IαI family seems to be able to exert a specific role whose study should be facilitated by using the procedures and results reported in this work.

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