CHEMICAL PROPERTIES OF THE PEPTIDE EXTENSION IN THE pα1 CHAIN OF DERMATOSPARACTIC SKIN PROCOLLAGEN

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1. Introduction

The biosynthetic precursor of collagen, procollagen, is known to be larger in size than the collagen molecules building up the extracellular fibrils [1–4]. The predominant peculiarity is a cysteine-containing peptide extension linked to the N-terminal end of the collagen α-chains [3–6]. Collagen displaying such features accumulates in the skin of dermatosparactic calves due to a genetic defect in their proteolytic system [7]. Chemical [5] as well as immunological studies [8] suggested a close relationship between procollagen and dermatosparactic collagen. Significant functions as control of collagen helix formation, facilitation of transport across the cell membrane and regulation of fibrillogenesis have been proposed for the additional peptide of procollagen [2, 4, 6, 9]. In the present study it was attempted to isolate and characterize this peptide extension in order to provide an advanced basis for elucidating these functions.

2. Methods

Collagen was extracted from skin of dermatosparactic calves by neutral salt as described [5]. The extended pα1-chains were obtained from urea extracts [10] of such skin by chromatography on CM-cellulose [1] and were further purified by chromatography on agarose [11]. The collagen α1-chains were prepared from skin of normal calves using the same methods. Dermatosparactic collagen or the isolated chains were digested in 0.2 M ammonium bicarbonate pH 7.8 (2 mg/ml) with purified collagenase (CLSPA, Worthington, Freehold, N.J.) at an enzyme–substrate ratio of 1:100 for 4 hr at 37°. Prior to digestion the substrates were denatured (30 min, 50°). The proteolytic treatment was terminated by addition of acetic acid followed by lyophilization. Initial chromatography of 30 mg digest was performed at 38° on a Bio-Gel P-10 column (115 × 1.5 cm) equilibrated with 0.5 M sodium acetate pH 4.5. Stoichiometric proportions between the additional peptide and the more degraded part of the pα1-chain were established after this fractionation by amino acid analysis. Phosphocellulose chromatography (column 1 × 6 cm) was performed at 40° in 0.001 M sodium acetate pH 3.6 using a concave gradient (100/100 ml) from 0 to 0.2 M NaCl. Reduction with 0.02 M dithioerythritol in 6 M guanidine HCl, 0.1 M Tris pH 8 was carried out for 4 hr at 37°. After addition of solid sodium iodoacetate to a final concentration of 0.08 M the solution was incubated for a further 1 hr period at room temp.

The amino acid composition was determined as described elsewhere [12]. Analysis for half cystine was done after performic acid oxidation [13] or after reduction and alkylation. Tryptophan was estimated by the method of Edelhoch [14]. Hexoses [15] and sialic acids [16] were determined by colorimetric procedures. Determination of hexosamine was carried out on the amino acid analyser after hydrolysis with 2 M HCl (4 hr, 110°).

Molecular weights were calculated from acrylamide

gel electrophoresis [17] performed in the presence of sodium dodecylsulfate (SDS). Immunoglobulin G, serum albumin, ovalbumin, chymotrypsinogen, myoglobin and insulin served as calibrating substances. For studies with the reduced and alkylated peptide the marker proteins were applied after being treated by the same procedure.

3. Results

Collagenase digestion of the pα1-chain rendered a large peptide which could be separated from the other degradation products on Bio-Gel P-10 (fig. 1A). It could not be demonstrated in similarly prepared digests of normal α1-chain. Assuming the molecular weight given below the molar recovery of this additional peptide was about 60%. After purification on phosphocellulose (fig. 1B) the peptide exhibited a distinct amino acid composition characterized by high cystine content and the absence of hydroxyproline and hydroxylysine (table 1). Methionine could not be detected but an atypical peak appeared on the amino acid analyser very close to the position expected for methionine. Carbohydrate analyses revealed 2–3% hexoses and less than 0.2% sialic acids and hexosamines, respectively. The same additional peptide could be obtained from digests of the entire salt-extracted dermatosparactic collagen but the molar yield was reduced to about 30%.

After reduction and alkylation of the additional peptide a single, homogeneous peak with traces of obviously aggregated material was observed on Bio-Gel P-10. This peak emerged in a position somewhat ahead of that observed for the native peptide. Amino acid analysis (table 1) revealed within the limits of analytical error identity to the untreated material and an almost complete conversion of cystine into carboxymethyl-cysteine. No carboxymethyl-cysteine could be detected after alkylation in 6 M guanidine without prior reduction.

A single band was found for the native and reduced additional peptide, respectively, in SDS-acrylamide gel electrophoresis. However, determinatior of the molecular weight by this method revealed a striking dependence on the gel porosity (fig. 2). At and above 10% acrylamide a constant value of 19,500 was observed for both preparations. An anomalous behaviour was also revealed for the native peptide in molecular sieve chromatography on calibrated Bio-Gel columns. On Bio-Gel P-150 in 0.2 M ammonium bicarbonate pH 8.5 a molecular weight of 30,000 and on Bio-Gel P-10 in 0.05 M sodium acetate pH 4.5, a molecular weight of 18,000 was found.

4. Discussion

Because of the high yield, the characteristic amino acid composition and the specific antigenic activity [8] the additional peptide can be considered as representative for the extension of the pα1-chain. The cystine content in this peptide is even higher than

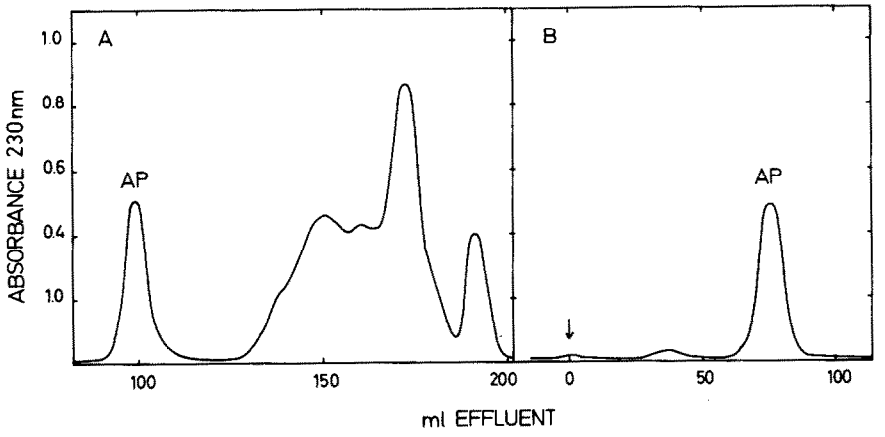

Fig. 1. Separation of the additional peptide (AP) from a collagenase digest of the pα1-chain on Bio-Gel P-10 (A) and purification on phosphocellulose (B).

Table 1
Amino acid composition of the pα1-chain and of the additional peptide (AP)[a].

	pα1 found	pα1 calc.[b]	AP	AP[c]	AP R,A[d]
Half cystine	10	16	16	16	16
4-Hydroxyproline	100	90	–	–	–
Aspartic acid	68	74	27	27	26
Threonine	27	27	10	11	10
Serine	41	38	2.9	3.3	3.0
Glutamic acid	111	117	37	34	35
Proline	156	163	22	20	20
Glycine	369	357	16	16	17
Alanine	132	128	2.8	2.8	3.0
Valine	27	36	17	18	17
Methionine	6.3	7	–	–	–
Isoleucine	14	13	4.4	4.7	5.2
Leucine	29	27	5.4	5.8	6.2
Tyrosine	5.1	6	2.0	1.8	1.9
Phenylalanine	15	14	0.9	0.7	0.8
Hydroxylysine	6.1	5	–	–	–
Histidine	6.1	5	1.7	1.9	1.8
Lysine	37	39	6.2	7.0	7.0
Arginine	58	56	4.2	5.2	5.1
Tryptophan	n.d.	n.d.	n.d.	2.9	n.d.
Total	1217	1218	175	179	175

[a] Given as residues per peptide assuming molecular weights of 115,000 for pα1 and 19,500 for AP. Values are rounded off to the nearest whole number except where less than 10 residues are found. Totals are calculated from rounded off values. A dash indicates less than 0.1 residues. n.d. = not determined.

[b] Calculated from data for AP and for the sum of the CNBr peptides of the calf collagen α1-chain [12].

[c] Isolated from entire soluble dermatosparactic collagen.

[d] Reduced and alkylated, half cystine determined as carboxymethyl-cysteine.

expected from analysis of the pα1-chain (cf. table 1). This finding agrees with the less than stoichiometric recovery of that peptide and suggests heterogeneity of the pα1-chain in terms of varying length or nature of the peptide extension. Unspecific proteolytic degradation before or during extraction might be the reason for this observation as already demonstrated for the C-terminal region of the calf skin collagen α1-chain [10]. The discrepancy can as well be explained if the molecular weight of the additional peptide is smaller than assumed.

The reduction and alkylation treatments suggest a single chain structure for the additional peptide and

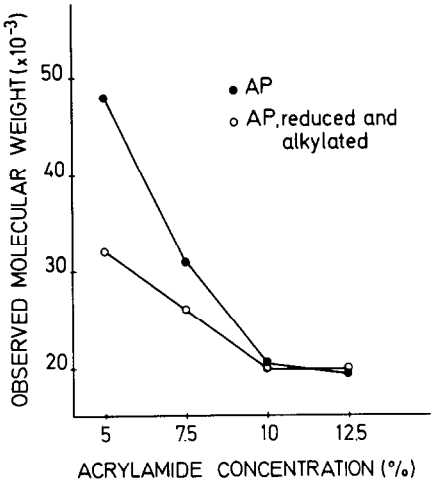

Fig. 2. Dependence on the acrylamide concentration of the molecular weight of the additional peptide (AP) determined by gel electrophoresis in the presence of SDS.

the involvement of all the cysteine residues in disulfide bridges. This indicates a rather compact spatial structure in good agreement with the proposed molecular weight and a shorter than expected length of the extension as seen in the electron microscope [6]. Such a structure might be required to provide the information needed for the supposed functions of the extension. It does not explain the anomalous behaviour in SDS-electrophoresis. Analogous findings have been reported for a membrane glycoprotein [18] and could be traced back to a diminished capacity for SDS-binding. Notwithstanding the uncertainty still remaining on the actual molecular weight, the value assumed is at least in the range proposed from studies with the pα1-chain [2–5].

It remains further to be clarified if the collagenase-derived peptide completely and only covers the extension of the pα1-chain. A short N-terminal sequence of the calf collagen α 1-chain as comprised by the CNBr peptide α1-CB (0.1) and including one methionine (cf. [12]) should not be cleaved by collagenase (unpublished) and therefore be contained in the isolated peptide extension. However, from studies on chicken bone procollagen, Bornstein et al. [4] have suggested that at least a small region of the peptide extension is sensitive to collagenase. Other predictions from this study like a high serine content and the absence of proline in a collagenase-derived peptide are not supported by the present work.

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