CYANOGEN BROMIDE PEPTIDES OF TYPE III COLLAGEN: FIRST SEQUENCE ANALYSIS DEMONSTRATES HOMOLOGY WITH TYPE I COLLAGEN

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1. Introduction

The triple helical collagen molecules consist of three polypeptide chains. The molecules of the most abundant and, therefore, most thoroughly investigated skin collagen (type I) contains two α1(I)- and one α2-chain. The molecules of cartilage collagen (type II), in contrast, are built from three identical α-chains, termed α1(II) [1,2]. The chromatographic properties of α1(I) and α1(II) chains are similar; the chains differ, however, in amino acid composition, carbohydrate content and the peptide pattern obtained upon cyanogen bromide cleavage [3,4].

Two peptides were isolated from the cyanogen bromide digest of insoluble human skin which could be attributed to neither collagen type I nor type II. The existence of an additional type of collagen (type III) was inferred from these data [5]. It was later possible to isolate intact molecules of type III by limited pepsin digestion [6] and to demonstrate the presence of this collagen type in other tissues (leiomyoma, aorta) [6–8]. Isolation and characterization of the cyanogen bromide peptides of type III collagen from human skin have recently been reported [9]; the order of those cyanogen bromide derived peptides within the peptide chain is yet unknown.

All the different α-chains (α1(I), α2, α1(II) and α1(III)) are of similar size and have a similar amino acid composition. Therefore, these proteins may be assumed to be homologous. This notion has been experimentally verified for α1(I), α1(II), and α2-chains by comparing the amino acid sequence of corresponding areas of these chains [10–13].

The present study was initiated to decide whether or not collagen type III is homologous to the other α-chains. We have investigated three cyanogen bromide peptides of collagen type III from calf aorta and determined by automated Edman degradation the sequence of amino acid residues at the N-terminal ends of these peptides. These sequences were highly homologous to certain regions of the α1(I)-chain. The position of these peptides within the α1(III)-chain became thus apparent as well.

2. Experimental

Isolation of cyanogen bromide peptides from aortic collagen will be described elsewhere [14]. Sequence analysis was performed with the aid of an automatic sequencer (model 890, Beckman Instruments, Palo Alto, Calif. USA) using programme 072172 B of Beckman Instruments. Reagents and

chemicals were obtained from Beckman and from Pierce. The phenylthiohydantoin derivatives of the amino acid residues were identified by thin-layer chromatography on silica gel plates (Merck F 254) and by gas-liquid chromatography using a single column system with 10% SP 400 (Beckman) on either a Beckman GC45 instrument or a Hewlett Packard gas chromatography 5700 A, equipped with automatic sample application. Arginine was identified on an amino acid analyzer after hydrolysis of the PTH derivative.

3. Results and discussion

We have determined by automated Edman degradation the sequences of 26, 35 and 31 N-terminal residues of the three cyanogen bromide peptides. The results are presented in figs. 1, 2 and 3. These data represent the first sequences determined of a type III collagen and comprise a total of 92 residues. The α1(III) chains were found to display the same structural features as α1(I) and α2 chains [12,15]. Thus, glycine occupies every third position. Hydroxyl-

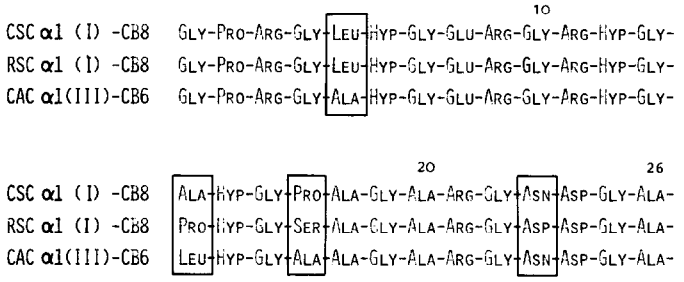

Fig.1. Comparison of the sequence of amino acid residues positions 1–26 of α1(I)-CB8 from calf (CSC) and rat (RSC) skin collagen [20,21] and of α1(III)-CB6 from calf aortic collagen (CAC). Non-identical residues found at the same position are enclosed in boxes.

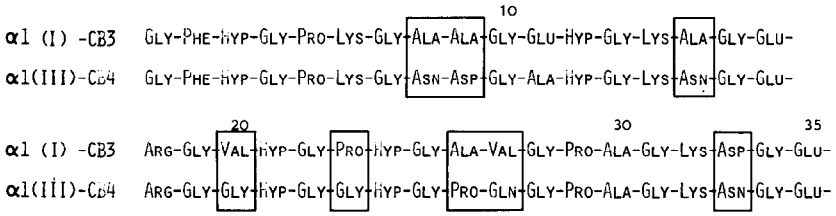

Fig.2. Comparison of the sequence of amino acid residues positions 1–35 of α1(I)-CB3 from calf skin and α1(III)-CB4 from calf aortic collagen [22]. Non-identical residues found at the same position are enclosed in boxes.

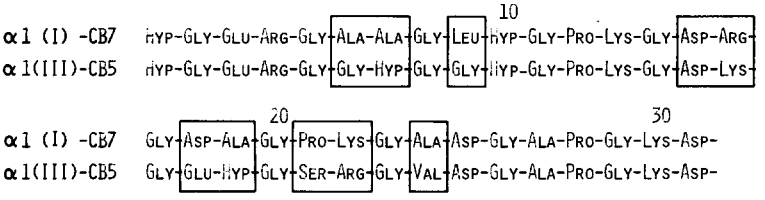

Fig.3. Comparison of the sequence of amino acid residues positions 1–31 of α1(I)-CB7 from calf skin and α1(III)-CB5 from calf aortic collagen [23]. Non-identical residues found at the same position are enclosed in boxes.

ation of proline to hydroxyproline was found only in position Y of the tripeptide unit Gly–X–Y and appears to be almost complete. Hydroxylation of lysine to hydroxylysine was not encountered within the areas studied. Almost all arginine residues are present in position Y, again as in α1(I)- and α2-chains. Likewise, residues arginine and lysine occupied positions in the immediate vicinity of the negatively charged amino acids aspartic acid and glutamic acid. The areas so far studied are too short to justify statements on the distribution of residues carrying large hydrophobic side chains such as phenylalanine and leucine, which play an important role during aggregation of the molecules into fibrils [16]. Remarkable is the occurrence within two peptides (peptide 4 and 5) of the sequence Gly–Gly–Hyp–Gly–Gly–Hyp which has never been encountered before. These cyanogen bromide peptides have been shown by amino acid analysis to contain slightly more glycine than the usual one third of the residues [9]. This is now confirmed by these sequence data.

Following successful fragmentation of a peptide chain, the order of the peptides within the chain must be determined. Each of the types of α-chains displays a characteristic peptide pattern. The order of cyanogen bromide peptides of the α1- and the α2-chain was determined by renaturation of peptides to yield SLS-fragments by chemical methods, and from pulse labelling experiments [17–19]. It was later possible to demonstrate in various areas of the molecule the existence of homology of corresponding sections of the α1- and the α2-chain [10–13]. Since extensive areas of the sequence of collagen type I are known and since no large repetitive areas within a chain have been encountered, it may be attempted to establish the order of the cyanogen bromide peptides from collagen type III on the basis of identity or homology with respect to sequences of collagen type I.

The sequences determined of peptides 6, 4 and 5 were, therefore, compared to the sequences of the α1-chain. Each of the newly determined sequences was found to have a counterpart within the α1(I)-chains. The peptide α1(III)-CB6 which has been described by Miller et al. [5] as being homologous to α1(I)-CB4.5 appears to be homologous to α1(I)-CB8 as judged from the present sequence data. The N-terminal area of peptide 6 is compared in fig.1 to the N-terminal area of α1-CB8 from rat skin [20] and calf skin [21] collagen. Homology is strikingly apparent as it is in the two other peptides 4 and 5 (fig.2 and 3). Any other correlation would drastically reduce the degree of homology. The homology of peptide 5 from type III collagen to α1(I)-CB7 was confirmed by electronmicroscope studies of the renatured peptide [8]. The elucidated sequence (fig.3) is derived from an area where the cross-striation pattern is identical. Although there are several substitutions found, the charge pattern in this area of the molecule is the same in both types.

It had already been observed earlier that the number of positions at which substitutions occur appears to be rather small within the N-terminal third of the molecule (peptide 6 from collagen type III). A relatively large number of positions in which substitutions occur is observed within the central third of the molecule (peptides 4 and 5). More detailed statements must be based on longer sequences of residues which have yet to be established.

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