THE ORDER OF THE CNBr PEPTIDES FROM THE α2 CHAIN OF COLLAGEN

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Summary

The isolation and characterization of peptides containing an uncleaved methionyl residue after CNBr cleavage of the α2 chain of chick bone and rat skin collagen show that α2-CB1 and α2-CB0 are adjacent and come from the NH_2-terminal end in the order 1-0 and that α2-CB3 and α2-CB5 come from the COOH-terminal end in the order 3-5. These results were confirmed and the remaining two peptides, α2-CB2 and α2-CB4, were positioned by pulse-labeling of rat collagen in culture and isolation of the CNBr peptides in a Dintzis-type experiment. The predicted specific activity gradient along the chain is obtained when the peptides are arranged in the order 1-0-4-2-3-5. These results are in agreement with electron microscopy of the renatured larger peptides α2-CB3, 4 and 5 from chick skin collagen (published in an accompanying article) which shows the order x-4-x-3-5 where x indicates spaces large enough for the remaining smaller peptides.

Introduction

Six CNBr peptides have been isolated from the α2 chain of chick bone (1), chick skin (2) and rat skin (3) collagen. The peptides from the two chick collagens probably have identical primary structures and are homologous to the peptides from rat skin collagen. The peptides consist of a tripeptide (α2-CB0), a peptide of 14 (15 in the case of the chick collagens) residues (α2-CB1), a peptide of 30 residues (α2-CB2), and three large peptides with molecular weights of about 30,000 (α2-CB3, 4 and 5) which together account for all of the weight and the amino acid composition of the α2 chain within experimental error. The order of the peptides as they exist in the original α2 chain as determined in part by chemical methods and in part by pulse-labeling studies is reported here. The results are in agreement with the order of the three large peptides estab-

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lished by electron microscopy and reported in an accompanying paper by Igarashi, Kang and Gross. The order of the CNBr peptides from the α1 chain has previously been reported (4).

Chemical Studies

The presence in CNBr digests of the α2 chain of small amounts of peptides containing an uncleaved methionine and therefore representing two adjacent CNBr peptides has permitted the ordering of some of the peptides. A peptide identified as containing the residues in α2-CB1 and α2-CB0 including an uncleaved methionine, and designated α2-CB(1-0) was isolated by molecular sieve chromatography on Bio-Gel P-4 (1) of CNBr digests of the α2 chain from chick bone collagen. Its presence could be seen in some preparations as a noticeable degree of asymmetry on the leading edge of the peak containing α2-CB1 (see Fig. 2, ref. 1) and was purified by rechromatography in the same system. Its amino acid composition (7) together with the values predicted from the sum of the compositions of α2-CB1 and α2-CB0 (1) appear in Table I. Since these two peptides are both small, totaling 18 residues, the identification is unequivocable.

That the order must be 1-0 is known from the previous demonstration (8) that α2-CB1 is derived from the NH_2-terminal end of the α2 chain. The order was confirmed by tryptic cleavage of α2-CB(1-0) at the partially hydroxylated lysyl residue. Chromatography on Bio-Gel P-2 yielded two fragments, the smaller NH_2-terminal fragment having the composition (glu,tyr,asp,pro,ser)-lys (or hyl) and the larger COOH-terminal fragment having the composition (ala_2,gly_3,pro_2, asp,phe,met,leu)hse (hse=homoserine). Since α2-CB0 is (gly,leu)-hse and the COOH-terminal fragment contains all the glycine and the single residues of leucine and homoserine in α2-CB(1-0), the order must be 1-0. NH_2-terminal analysis of α2-CB0 shows glycine (Lane and Miller, unpublished), permitting the COOH-terminal portion of α2-CB(1-0) to be assigned the sequence met-gly-leu-hse. The amino acid sequence of α2-CB1 from chick skin has recently been determined (9); since the peptide from chick bone has the same composition, it probably has the same sequence.

Table I

Amino Acid Composition[a/] of Uncleaved CNBr Peptides from the α2 Chain of Chick Bone Collagen

	α2-CB(3-5)		α2-CB(1-0)	
Amino Acid	Predicted(1)	Found	Predicted	Found
3-Hydroxyproline	1	1(0.9)	0	0
4-Hydroxyproline	62	60	0	0
Aspartic Acid	30	30	2	2(1.9)
Threonine	14	13	0	0
Serine	19	18	1	1(1.1)
Glutamic Acid	46	41	1	1(0.8)
Proline	74	82	3	3(3.0)
Glycine	218	218	3	3(3.2)
Alanine	68	64	2	2(2.0)
Valine	20	21	0	0
Isoleucine	13	13	0	0
Leucine	19	20	1	1(0.7)
Tyrosine	1	1(0.8)	1	1(0.8)
Phenylalanine	9	10(9.9)	1	1(0.8)
Hydroxylysine[b/]	6.1	6.1	0.5	0.5
Lysine[b/]	14.7	14.5	0.5	0.4
Histidine	5	5(5.3)	0	0
Arginine	34	34	0	0
Methionine	1	1(0.6)	1	1(0.5)
Homoserine	0	(0.4)[c/]	1	1(1.0)
Total	655	653	18	18

a/ Residues per peptide rounded off to nearest whole number. Actual values are listed in parentheses where less than 10 residues were found. A value of zero indicates less than 0.2 residues.

b/ The values for lysine and hydroxylysine are not rounded off since there is evidence for partial hydroxylation giving rise to non-integer values (5,6).

c/ Methionine apparently converted to homoserine but uncleaved.

A peptide identified as α2-CB(3-5) was isolated from the peak following α2-CB4 in the effluent of a CM-cellulose column (see Fig. 3, ref. 1) by chromatography on agarose (see Fig. 5, ref. 1). Its molecular weight (10) was found to be about 60,000. Its amino acid composition together with the predicted composition derived from the sum of α2-CB3 and α2-CB5 (1) is shown in Table I. The predicted composition of other possible peptide pairs that might arise from α2-CB3, 4 and 5 did not agree with the observed composition. A peptide that is similar and undoubtedly homologous to α2-CB(3-5) from chick bone collagen was also isolated from rat skin collagen.

Since α2-CB5 has no homoserine (1), it must be COOH-terminal and the order of the peptide pair in α2-CB(3-5) must be 3-5. The unassigned peptides

α2-CB2 and α2-CB4 must then be derived from the region between 1-0 and 3-5, but, from the chemical data, in unknown order.

Pulse-labeling studies

The classic experiments of Dintzis (11) and Naughton and Dintzis (12) established that the biosynthesis of polypeptide chains occurs by sequential addition of amino acids beginning at the NH_2-terminal end. This was demonstrated by the finding of a specific activity gradient along the chain when tryptic peptides from pulse-labeled hemoglobin were placed in their proper order. It was pointed out at the time (11) that if sequential synthesis were assumed, the order of specifically derived peptides could be established from their relative specific activities after pulse-labeling. We have been able to apply the principle to the determination of the order of the CNBr peptides from the α2 chain of rat collagen. Detailed data for both the α1 and α2 chains will be reported separately (Vuust and Piez, in preparation). The data for the α1 chain are consistent with the order established for the CNBr peptides by other methods (4) showing that the α chains of collagen are synthesized as a single chain by the sequential addition of amino acids beginning at the NH_2-terminal end in the same manner as other polypeptide chains.

For each experiment 40-50 calvaria from new-born rats were incubated in media (Eagles NEM) in the presence of β-aminopropionitrile to prevent cross-linking and increase the amount of soluble collagen. The cultures were labeled with glycine-U-^{14}C (>100mc/mM) for 3, 7.5 or 15 minutes at 37°C and the newly synthesized collagen was extracted by stirring the calvaria with cold 1M NaCl for 3 days. Carrier collagen, 40-50 mg, from the skin of lathyritic rats was added and collagen was precipitated at 5°C with 20% NaCl. The α1 and α2 chains were isolated as described (13). Chains uniformly labeled with glycine-2-^{3}H were obtained in the same way only incubation with labeled amino acid was continued for 24 hours. The ^{3}H- and ^{14}C-labeled α2 chains were combined and additional unlabeled α2 chain was added. The mixture was cleaved with CNBr and the peptides were isolated by procedures that have been described

(1,3,14,15). The peptides were counted in a scintillation counter with appropriate double-label settings. The $^{14}C/^{3}H$ ratios were normalized with a value of 1.0 assigned to α2-CB5, the peptide with the largest ratio. The tripeptide α2-CB0 was not included in these experiments since it contains too little glycine to give statistically significant counting data.

The results are shown in Fig. 1. Although there is some scatter in the points, the only order that provides a continuous gradient at all three times is 1-0-4-2-3-5, 0 being assigned its position on the basis of the chemical data alone. These results then confirm the positions of α2-CB1, 3 and 5 and allow α2-CB4 and 2 to be assigned positions. The order is summarized in Fig. 1 where the length of each peptide is proportional to its size (1,2,3).

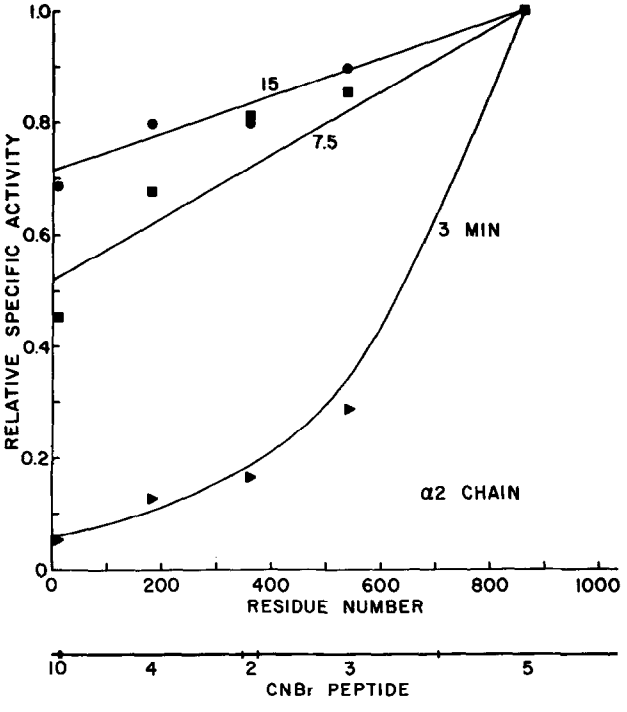

Fig. 1. The relative specific activities of the CNBr peptides from the α2 chain of rat collagen after pulse labeling for 3 (▶), 7.5 (■) and 15 (●) min. The values were plotted at the residue number calculated from the known size of the peptides (1,2,3), to be the midpoint. The peptides were arranged in the order which gives the best specific activity gradient for all three times. The position of α2-CB0 is assigned on chemical data alone.

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