ISOLATION, CHARACTERIZATION AND COMPARATIVE STUDIES OF THE N-TERMINAL PEPTIDES FROM SOLUBLE PIG SKIN COLLAGEN

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1. Introduction

Collagen is an ubiquitous structural protein, which constitutes about 25–30% by weight of all proteins in the mammalian body. It functions as an important supporting element in vertebrate tissues. Using the method of cleaving the protein chains at the methionyl residues with cyanogen bromide (CNBr), many species of collagen from different sources have been isolated and characterized, e.g. rat skin [1,2], chick skin [3], calf skin [4,5] and human skin collagen [6]. Pig skin collagen seems to be interesting from the point of view, that is, its applicability in the medical sector [7,11,12], due to its poor or non-antigenic reactivity. We reported previously about the isolation and characterization of the large CB-peptides derived from both α1- and α2-chain of the pig skin collagen [8]. This report deals with the isolation, characterization and comparison of the N-terminal CB-peptides of soluble pig skin collagen with the corresponding peptides derived from rat skin, calf skin and human skin collagen.

2. Materials and methods

Soluble pig skin collagen and its α-components were prepared as described earlier [8]. The CNBr-cleavage was carried out in 70% formic acid using a 200-fold of CNBr for 24 hr at 15°C, and for 3 hr at 30°C. The small CB-peptides were separated by chromatography on phosphocellulose under the conditions described by Fietzek [5] and Bornstein et al. [1]. After desalting on Biogel P2 the different peptides were purified by rechromatography using the same system. Amino acid analysis was performed on a Biochrom Automatic Amino Acid Analyzer (Biocal, Munich, W. Germany).

3. Results and discussion

The crude neutral salt- and acid soluble collagen extracts, centrifuged at 50 000 *g* for 5 hr were strongly opalescent and exhibited a remarkable high transition temperature of 40°C. This might be attributed to the presence of fatty matters, which possibly stabilize the structure of pig skin collagen. Only the centrifugation at 250 000 *g* brought about clear collagen solution, whose transition temperature was lowered to 36°C. The preparation of collagen solutions deprived of fatty matters (causing strong turbidity) was not easy. The conventional purification by precipitation with NaCl was completely inefficient to remove the turbidity of collagen solutions.

It was only possible to get a clear pig skin collagen solution in two ways:

a) After denaturation for 10 min at 60°C, fine powdered silica gel was throughly stirred in and centrifuged at 50 000 *g* for 40 min. Such solutions were clear enough and could be applied directly to the CM-cellulose column.

b) The freeze dried collagen was washed with cold ethanol and filtered. After dissolution in 0.1 M acetic acid and dialysis against water, the collagen solutions were completely clear.

The disc-electrophoretic pattern, as well as the CM-cellulose elution diagram of pig skin collagen were

Table 1
Amino acid composition of the N-terminal peptides α1-CB 0.1, α1-CB2, α2-CB1 and α2-CB2 derived from pig skin collagen compared with the corresponding peptides derived from rat, calf and human skin collagen

	α1-CB 01				α1-CB 2				α2-CB1				α2-CB2			
	RSC	CSC	HSC	PSC	RSC	CSC	HSC	PSC	RSC	CSC	HSC	PSC	RSC	CSC	HSC	PSC
4-Hyp	–	–	–	–	6	5	5,5	5,6	–	–	–	–	3	2	2,7	3
Asp	1	1	1	1	–	–	–	–	1	1	1	1	3	2	2	2
Thr	–	1	1	–	–	–	–	–	–	–	–	–	1	1		1
Ser	2	3	2,9	2,7	2	2	1,8	2	2	–	–	–	1	2	1,9	2
Hse	0,9	1	0,9	1	1	1	1	1	1	1	0,9	1	1	1	0,9	1
Glu	1	2	2,1	2	4	4	3,9	4	1	1	1	1	1	1	1,2	1
Pro	1,9	2	2,1	2	6	7	6	6	2	2	2	2	3	3	3	4
Gly	3,1	3	4,2	4	12	12	12	12	3	4	5	4	10	10	10	10
Ala	1	–	–	1	2	2	2,1	2	1	1	–	1	2	3	3,2	1
Val	2	1	1,2	1	–	–	–	–	1	–	1	1	1	1	1	1
Ileu	–	1	1,1	2	–	–	–	–	–	–	–	–	1	–	–	1
Leu	–	1	1,1	1	1	1	1	1	–	–	1	–	1	1	1	1
Tyr	1,9	2	1,8	1	–	–	–	–	1	–	0,9	1	–	–	–	–
Phe	–	–	–	–	1	1	1	1	–	1	–	–	–	–	–	–
Lys	1	1	0,9	1	–	–	–	–	1	1	0,3	1	–	–	–	–
Arg	–	–	–	–	1	1	1	1	–	–	–	–	3	3	2,8	3
Total	15	19	20	20	36	36	36	36	14	12	14	13	30	30	30	30

RSC, rat skin collagen; CSC, calf skin collagen; HSC, human skin collagen; PSC, pig skin collagen

identical with those obtained by other collagen species [8]. Table 1 shows the amino acid composition of the N-terminal peptides from α1- and α2-chain derived from soluble pig skin collagen compared with the corresponding peptides derived from rat skin, calf skin and human skin collagen. It displays the great similarity between the CNBr N-terminal peptides derived from pig skin collagen to the corresponding peptides of human skin collagen. The α1-CB 0.1 peptide seems to be interesting from the point of view, that one Tyr and one Thr residues are missing in the peptide derived from pig skin. These might be substituted by Ileu and Ala as can be deduced from the amino acid composition. The α1-CB2-peptide has exactly the same amino acid composition in both species, human and pig. The α2-CB O-peptide (not presented in table 1) is a tripeptide having the same composition as in the case of human and calf, i.e. Gly–Leu–Met. The α2-CB1-peptide of pig skin exhibits a similar amino acid composition to the corresponding human skin peptide. It is one residue shorter, having only 13 residues. One Ala resudie in case of the pig peptide seems to substitute a Leu residue in the human peptide. The α2-CB2-peptide of pig skin exhibits more or less the same amino acid composition as the corresponding peptide of human. The fact that both peptides are made up to 30 amino acids, and that the pig skin peptide possesses one Pro and one Thr more, and two Ala residues less than the human peptide, some substitutions seem to have been taken place in this region of the molecule.

Fig. 1 illustrates the sequence of the peptide α1-CB 0.1 and the size of the antigenic determinants of different collagen species as estimated by Timpl et al. [10]. It shows our proposed sequence for the pig peptide, which fits the amino acid composition and analogy in the sequence to the other species. The substitution of Tyr and Thr by Ileu and Ala in α1-CB 0.1 together with the fact, that the human and the pig peptide possess one Gly residue more than calf peptide does, may be used to discuss the poor or non-antigenic reactivity of pig skin collagen towards human. This has been stated by Struck [11] and Song et al. [12] and is also shown in its applicability in the medical sector for the preparation of wound healing plasters and some medical protheses [7].

Fig. 2 illustrates the sequence of the peptide α2-CB1 and α2-CB O [13]. By analogy we can suppose a sequence for the obtained amino acid composition

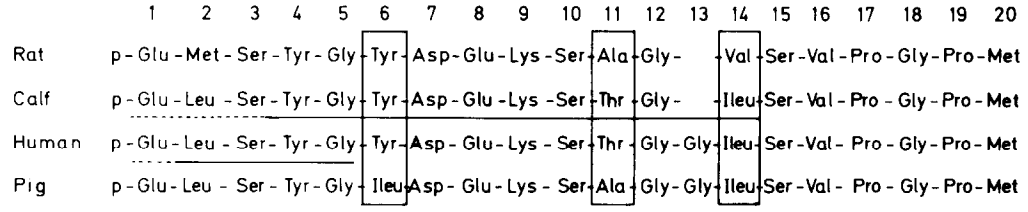

Fig. 1. Sequence of the peptide α1-CB 0.1 from rat, calf, human and our proposed pig skin collagen. The solid underline indicates the antigenic determinants, see Timpl et al. [10]; the solid boxes indicate the sites of heterogeneity. The amino acid values for CSC has been quite recently corrected by a personal communication from P.P. Fietzek.

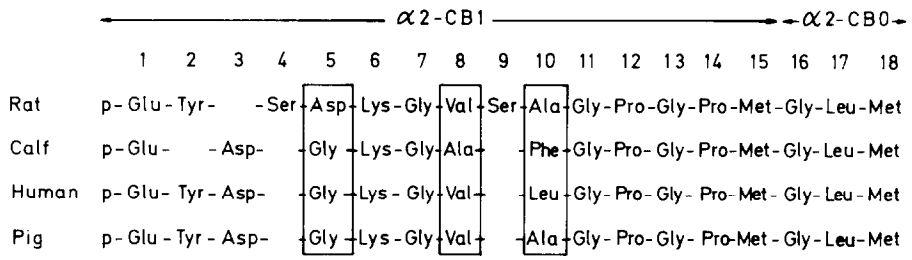

Fig. 2. Sequence of the peptide α2-CB1 and α2-CB O including the proposed sequence for the pig peptides. For more details see Piez and Traub [13].

of the N-terminal peptide derived from pig skin collagen. Our proposed sequence exhibits a great similarity between the pig and human peptides.

However, the microheterogeneity is obvious as indicated by the positions enclosed in boxes. This remains, however, to be confirmed experimentally.

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