STRUCTURAL DATA CONCERNING THE LINK PROTEINS FROM BOVINE NASAL CARTILAGE PROTEOGLYCAN COMPLEX

J.-P. PÉRIN, F. BONNET, V. PIZON, J. JOLLÈS and P. JOLLÈS
Laboratoire des Protéines, Université de Paris V, 45 rue des Saints-Pères, F 75270 Paris Cedex 06, France

Received 31 July 1980

1. Introduction

The current model for bovine nasal cartilage complex consists of hyaluronic acid, proteoglycan monomers and link proteins which are firmly bound together by non-covalent linkages [1]. Since Keiser et al. [2] detected the presence of two link proteins by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis, three important dates can be considered in their structural study. In 1974, Heinegard and Hascall [3] showed that upon trypsin treatment of the proteoglycan complex a 'T-G200-3 fraction' deriving from one or from the two link proteins could be obtained. In 1977, Baker and Caterson [4] indicated that cyanogen bromide (CNBr) treatment of the two link proteins yielded one prominent peptide. In 1978, Bonnet et al. [5] reported the first preparative separation of the two link proteins by SDS–polyacrylamide gel electrophoresis: their amino acid compositions were almost identical; this result was corroborated later [6]. All these observations present good evidence for a possible structural relationship between the two link proteins. However, no structural study has so far been devoted to these proteins. This letter deals with some sequence data of fragments obtained from the link proteins.

2. Methods

2.1. *Preparative procedures*

Proteoglycan complex components were sequentially extracted from the cartilage powder as in [7]; the dissociative extract (0.05 M sodium acetate, 4 M guanidinium–HCl (pH 5.8) was used. The T-G200-3 fraction was prepared following the method in [8]. The fraction containing the link proteins recovered after a density gradient centrifugation under dissociative conditions (density $\leqslant$1.45 g/ml) was submitted to two successive Sephacryl S-300 gel filtrations (250 × 1.5 cm column; 0.05 M sodium acetate, 4 M guanidinium–HCl (pH 5.8) buffer). After reduction and alkylation the link proteins were further purified but not separated during preparative SDS–polyacrylamide gel electrophoresis by the method in [5]. This material was submitted to CNBr treatment. The separation of the two link proteins was obtained following [5].

2.2. *Analytical procedures*

Analytical electrophoreses were done on SDS-polyacrylamide gels according to [9]. Apparent M_r values of reduced and alkylated samples were determined as a function of relative electrophoretic mobilities on SDS–polyacrylamide gels with bovine serum albumin, rabbit aldolase, bovine trypsinogen, horse myoglobin and hen egg-white lysozyme as standard markers (in their reduced form). Automated Edman degradation was done in a 890 C Beckman-Sequencer by the 1 M quadrol single cleavage method; the phenylthiohydantoin-amino acids were characterized by thin-layer chromatography (chloroform–methanol, 90:10 (v/v); pure chloroform) and by high performance liquid chromatography (Waters chromatograph, model ALC/GPC-204) on a 30 cm Waters μ-Bondapak C18 column as in [10].

3. Results

3.1. *Link proteins fragments obtained by CNBr treatment*

3.1.1. CNBr treatment

Reduced and alkylated link proteins (32 mg) were

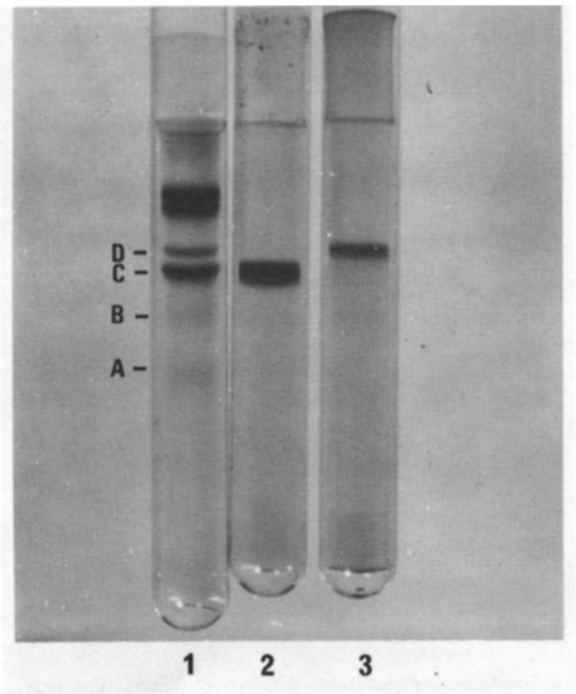

Fig.1. Analytical SDS–polyacrylamide gel electrophoreses of the CNBr-treated reduced and alkylated link proteins. Gels: (1) CNBr-treated reduced and alkylated link proteins; (2) fragment C isolated in peak 2 (fig.2); (3) fragment D isolated in peak 3 (fig.2).

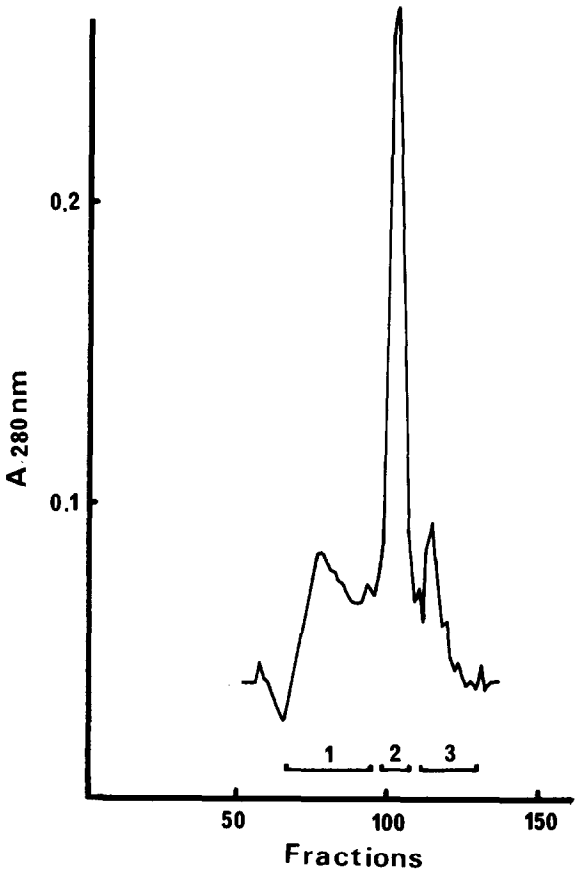

Fig.2. Preparative SDS–polyacrylamide gel electrophoresis applied to the CNBr-treated reduced and alkylated link proteins: experiment done on a Canalco apparatus, column PD; stacking and separating (12% polyacrylamide) gels were 2.5 and 8.5 cm high; electrode buffer, 0.192 M glycine, 0.025 M Tris–HCl (pH 8.3) containing 1% SDS; Elution buffer, 0.3 M Tris–HCl (pH 8.3) containing 0.2% SDS; applied current, 6 mA, 120 V; 1.8 ml fractions were collected every 20 min; loading buffer, see section 3.

solubilized in 70% formic acid (5 ml); 200 mg CNBr were added to the solution and the reaction was allowed to proceed for 24 h at room temperature in the dark with continuous stirring. The reaction was stopped by adding 4 vol. 25% acetic acid and the solution was dried under vacuum at 30°C.

3.1.2. Isolation of the fragments

On analytical SDS–polyacrylamide gel, in addition to the two upper bands corresponding to the undegraded link proteins, one major fragment (C) was characterized with est. M_r 27 500 ± 1000; a second protein band (D) with M_r 30 000 ± 1000 was present (fig.1(1)). The ratio of fragments D/C was 1/5 as determined by scanning densitometry of the gels and the ratio of the two non-degraded link proteins was identical to that of the nonCNBr-treated link proteins. Two other faint bands (A,B) with greater electrophoretic mobilities were also detected. Preparative SDS–polyacrylamide gel electrophoresis applied to the CNBr treated material allowed the isolation of 3 peaks (fig.2). The first eluted one contained the mixture of the two protein bands (A,B) with lower mobilities, the second large one contained fragment C (fig.1(2)) and the third one fragment D (fig.1(3)). The undegraded link proteins were eluted later. During the preparative electrophoresis special attention was given to the sample buffer: it was 0.0625 M Tris–HCl (pH 8.3) containing 2% SDS and 10% glycerol instead of the pH 6.8 buffer in [9] because a large amount of material was insoluble in the latter.

3.2. *N-terminal sequences of the tryptic fragment T-G200-3 and of the two major CNBr fragments*

Table 1
N-terminal sequences of the 'T-G200-3 fraction' and of fragments C and D recovered after CNBr-treatment of the reduced and alkylated link proteins

	1 10 20	
T-G200-3	Leu-Leu-Val-Glu-Ala-Glu-Gln-Ala-Lys-Val-Phe-Ser-Arg-Arg-Gly-Gly- X -Val-Thr-Leu-	
	21 30	
	Pro- X -Lys-Phe-Tyr-Tyr-Asp-Pro-Thr-Ala-Phe-Gly-	
	1	
Fragment D	Pro-Thr-Ala-Phe-Gly- X -Gly-Thr-	
	1 10 20	
Fragment C	Gly-Tyr- X -Lys-Lys-Thr-Tyr-Gly-Gly-Tyr- X -Gly- X -Val-Phe-Leu-Lys-Gly- X -Gly-	
	21 30 40	
	Ser-Asp-Asn-Asp-Ala-Ser-Leu-Val-Ile-Thr-Asp-Leu-Thr-Leu-Glu-Asp-Tyr-Gly- X -Tyr-Lys-	

(X) Amino acid not yet characterized; (□) the identical residues between the T-G200-3 fraction and fragment D are boxed.

from the link proteins

The N-terminal sequences of the T-G200-3 fraction and of fragments C and D are presented in table 1 and corroborate their purity. It appears that the N-terminal sequence of fragment D is identical to sequence 28–32 of the T-G200-3 fraction.

All the attempts for sequencing after separation the two purified reduced and alkylated link proteins have remained unsuccessful: it seems thus very likely that the fragments in table 1 arise from their C-terminal region.

4. Discussion

According to the amino acid compositions of the two isolated link proteins established by two different laboratories [5,6] they contain only 1 Met residue/molecule taking into account M_r 45 000–50 000. Consequently, if the two link proteins possess different primary structures, a maximum of 4 fragments can be expected after CNBr treatment. In fact we characterized two major fragments (C,D) and two minor ones (A,B). The ratio of the two link proteins before and after CNBr treatment remained the same while the ratio of fragments C/D was quite different from the former; this suggests that fragments C and D arise from both link proteins. Thus one of the fragments might be the result of a side effect during the CNBr treatment, the other representing the main C-terminal CNBr fragment. The two faint bands with greater mobilities (A,B) might arise from the N-terminal region of the link proteins. This conclusion seems in accordance with our structural data: indeed from the structural identity observed between fragment D and sequence 28–32 of the T-G200-3 fraction it appeared that this peptide results from an Asp–Pro cleavage due to the acidic medium; this result was corroborated by the characterization of a peptide whose mobility was identical to that of fragment D when the T-G200-3 fraction was treated only with 70% formic acid (unpublished). However the relative M_r values of the T-G200-3 component and of fragment D did not fit exactly with a difference of 27 amino acids which can perhaps be explained by the presence of sugars. The possibility of a redundancy of an Asp–Pro sequence, although unlikely, cannot be excluded yet.

Summarizing, the following data are in favour of a possible structural identity (or at least high homology) of the two link proteins: identical amino acid compositions [5,6]; obtaining one peptide after tryptic treatment of the proteoglycan complex [3]; similar susceptibility of the purified proteins to trypsin [11]; similar susceptibility to the V8 *Staphyllococcus aureus* protease [6]; obtaining a single main CNBr fragment ([4], this work), inaccessibility of the N-terminal sequences to the Edman reagent (this work), structural identity between a part of the T-G200-3 component and the N-terminal sequence of fragment D arising from both link proteins (this work).

Acknowledgements

The authors thank Miss M. Rougeot and Mr Ly Quan Le for skillful technical assistance. This research was supported by the INSERM (group U-116), the CNRS (E. R. no. 102) and the Fondation pour la Recherche Médicale française.

References

[1] Hascall, V. C. (1977) J. Supramol. Struct. 7, 101–120.
[2] Keiser, H., Shulman, H. J. and Sandson, J. I. (1972) Biochem. J. 126, 163–169.
[3] Heinegard, D. and Hascall, V. C. (1974) J. Biol. Chem. 249, 4250–4256.
[4] Baker, J. and Caterson, B. (1977) Biochem. Biophys. Res. Commun. 1, 1–10.
[5] Bonnet, F., Périn, J.-P. and Jollès, P. (1978) Biochim. Biophys. Acta 532, 242–248.
[6] Baker, J. R. and Caterson, B. (1979) J. Biol. Chem. 254, 2387–2393.
[7] Bonnet, F., Périn, J.-P. and Jollès, P. (1980) Biochim. Biophys. Acta 623, 57–68.
[8] Heinegard, D. and Axelsson, I. (1977) J. Biol. Chem. 252, 1971–1977.
[9] Laemmli, U. K. (1970) Nature 227, 680–685.
[10] Zeeuws, R. and Strosberg, A. D. (1978) FEBS Lett. 85, 68–72.
[11] Périn, J.-P., Bonnet, F. and Jollès, P. (1978) FEBS Lett. 94, 257–260.