THE ACTION OF TRYPSIN ON PURIFIED LINK PROTEINS FROM BOVINE NASAL CARTILAGE PROTEOGLYCAN COMPLEX

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1. Introduction

Two major proteins, termed link proteins a and b, are present in bovine nasal cartilage complex [1]. A 'T-G 200-3' fraction from a chondroitinase–trypsin digest of a proteoglycan complex fraction which was able to bind hyaluronic acid was isolated [2]. It was postulated that this 'T-G 200-3' protein might be derived from the smaller link protein a. The present note deals with the action of trypsin on the purified a and b link proteins [3].

2. Materials and methods

2.1. *Materials*

Bovine nasal cartilages were obtained immediatly after slaughter, freed of non-cartilagenous tissues and stored at –30°C prior to use. Guanidinium hydrochloride was from Carlo Erba and Sephacryl S-200 from Pharmacia; electrophoretic reagents were purchased from Canalco and Labo-Disc, TPCK-trypsin from Worthington and all other reagents (analytical grade) from Prolabo.

2.2. *Methods*

Sodium dodecylsulfate (SDS)–polyacrylamide gel electrophoreses were performed according to [4] (10% polyacrylamide, pH 8.9) in the absence and in the presence of 2-mercaptoethanol. Protein staining was performed by R.250 Coomassie brillant blue; the presence of sugars was ascertained by the periodic acid–Schiff (PAS) reagent.

The proteoglycan complex (PGC) and protein-rich fractions were isolated as in [3,5] following the methods in [6,7]. The link proteins were further purified by submitting the protein rich fraction to gel filtration on a Sephacryl S-200 column (250 × 1.5 cm) with a 4 M guanidinium hydrochloride, 0.05 M sodium acetate, pH 5.8 solution as eluent (data not shown). Link proteins a and b were separated by preparative SDS–polyacrylamide gel electrophoresis as in [3].

Trypsin digestion was carried out at 37°C in a 0.05 M Tris–HCl, 0.01 M $CaCl_2$, pH 8.0 buffer. The mixture of the two purified link proteins a and b (70–90 μg determined with the Folin reagent employing bovine serum albumin as standard) was stirred for 30 min in 375 μl of the pH 8.0 buffer; 61 mU TPCK-trypsin in 250 μl of the same buffer were then added. At different time intervals 75 μl aliquots were taken off, immediatly frozen and lyophilized. The tryptic digest was characterized by SDS–polyacrylamide gel electrophoresis.

3. Results

Two series of experiments were carried out successively on the mixture of the purified a and b link proteins and on each pure link protein.

3.1. *Tryptic digestion of the a and b link proteins mixture*

By analytical SDS–polyacrylamide gel electro-

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phoresis in the absence of 2-mercaptoethanol, a protein band (X) with a slightly greater electrophoretic mobility than that of the smaller link protein a was observed at different time intervals while the intensity of the bands corresponding to the a and b link proteins decreased (fig.1). Figure 2 presents the electrophoretic characterization of the components obtained after trypsin treatment for short periods when the samples have been reduced with 2-mercaptoethanol prior to electrophoresis. In addition to the unhydrolysed a and b link proteins (the two upper bands), two new bands (X1 and X2) with electrophoretic mobilities greater than that of the link protein a were detected. When the enzymatic digestion was carried out for longer periods no accumulation of any of these degradation products (X, X1 and X2) was observed. The molecular weight of the two

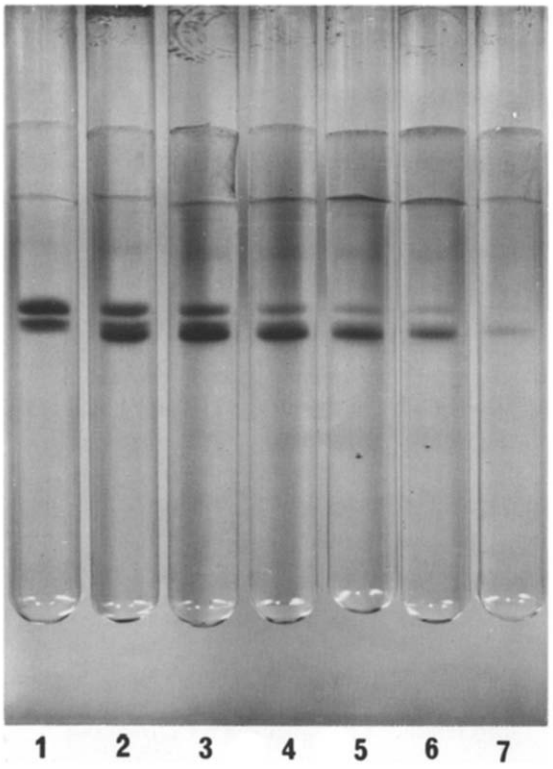


Fig.1. Analytical SDS–polyacrylamide gel electrophoreses in the absence of 2-mercaptoethanol of the products obtained after trypsin treatment of the a and b link proteins. Gels 1–7: products obtained after 0, 0.25, 0.5, 1, 2, 4 and 6 h of trypsin action, respectively.

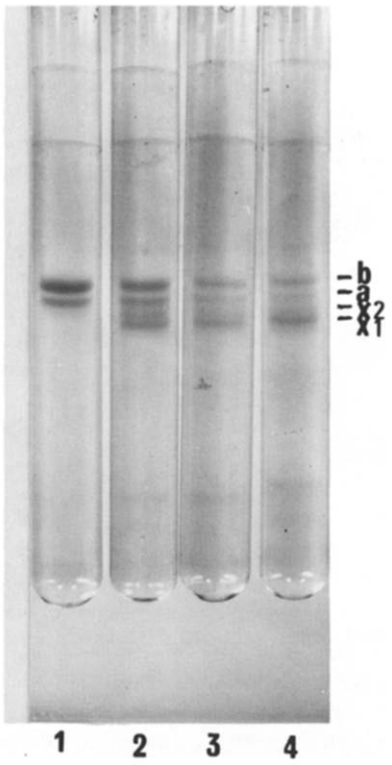


Fig.2. Analytical SDS–polyacrylamide gel electrophoreses in the presence of 5% 2-mercaptoethanol of the products obtained after trypsin treatment of the a and b link proteins. Gels 1–4: products obtained after 0, 0.25, 0.5 and 1 h of trypsin action, respectively.

protein bands corresponding to X1 and X2 was estimated to be ~40 000; both were PAS-positive as the two a and b link proteins.

3.2. *Tryptic digestion of the a and b link proteins after their separation*

Experiments similar to those above were carried out with pure link protein a or link protein b prepared by SDS–polyacrylamide gel electrophoresis. When the electrophoreses were performed in the absence of 2-mercaptoethanol, both a and b link proteins gave rise to a protein band corresponding to a degradation product whose electrophoretic mobility was identical to that of the X component observed with the mixture of the link proteins (fig.3). When the electrophoreses were carried out on samples reduced

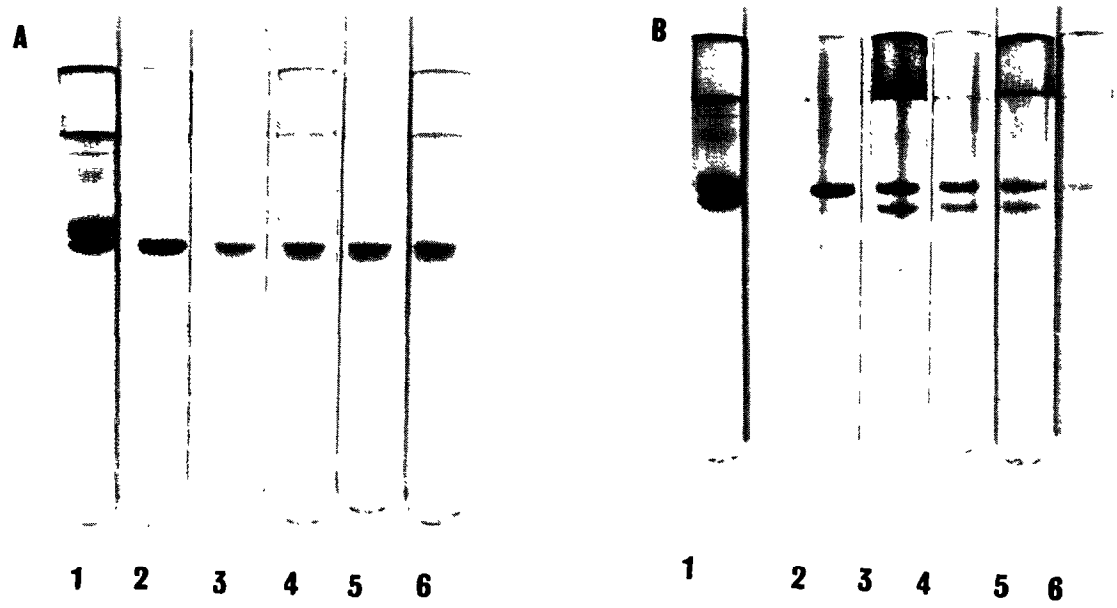
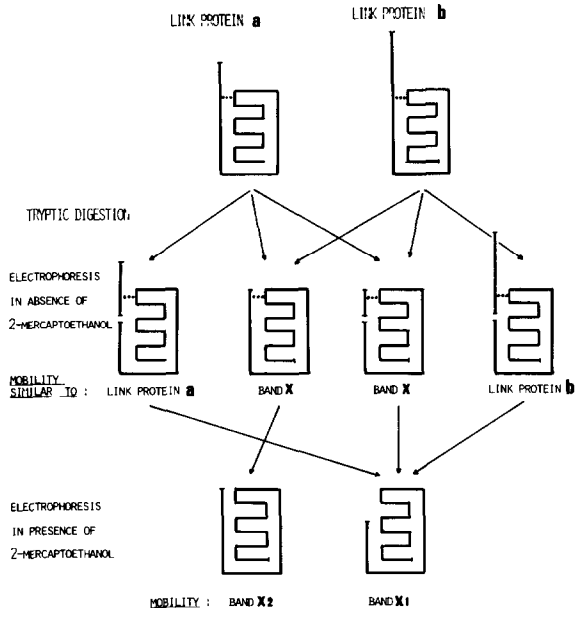


Fig.3. Analytical SDS–polyacrylamide gel electrophoreses in the absence of 2-mercaptoethanol of the components obtained after trypsin treatment of (A): isolated link protein a and (B) isolated link protein b. Gel 1: a and b link proteins (reference sample); Gels 2–6, components obtained after 0, 0.25, 0.5, 1 and 4 h of trypsin action, respectively.

with 2-mercaptoethanol, the faint protein bands X1 and X2 above were characterized again.

4. Discussion

The characterization of two protein bands X1 and X2 after reduction of the tryptic digest instead of the X band characterized in the absence of 2-mercaptoethanol might be due to the cleavage of at least two peptide fragments, one leaving a small peptide directly and the other giving rise to a peptide still attached by some disulfide bond(s) to the core of the molecule (scheme 1). As the isolated a and b link proteins behaved identically to the purified but unseparated link proteins during these experiments, boiling in presence of SDS before preparative SDS–polyacrylamide gel separation did not affect the susceptibility of the link proteins to trypsin. Our results indicate that the degradation products of the link proteins after trypsin treatment are not accumulated but are further digested; no degradation product could be detected after digestion of the totality of the link proteins. This observation differs somewhat from



Scheme 1. Tryptic digestion of the a and b link proteins; disulfide bond (· · ·)

experiments in [2] where it is shown that, when involved in proteoglycan complex, the 'T-G 200-3' fraction deriving from the link proteins was in part protected from extensive trypsin degradation. The similar electrophoretic behaviour of the digestion products of the a and b link proteins suggests a close relationship between these molecules in accordance with recent data [8,9]. Recently the existence of a small amount of a third link protein with a lower molecular weight than the a and b link proteins has been reported [9]. In [3] we noticed that this third smaller link protein was not present in all our preparations. From the results presented in this note we assume that this third link protein is probably a degradation product of the two former link proteins.

Acknowledgements

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