Structural relationship between link proteins and proteoglycan monomers

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Structural homologies between link proteins and proteoglycan monomers are demonstrated. A possible redundancy in the proteoglycan monomers structure is discussed and the link proteins domains homologous to other proteins are specified.

Link protein *Proteoglycan monomer* *Structural homology*

1. INTRODUCTION

In the cartilage matrix the proteoglycan monomers (PG) are built up of a central protein core along which various glycosidic side chains are covalently bound; they form stable aggregates by interaction with hyaluronic acid and link proteins (LP) [1]. Despite their heterogeneity, bovine nasal cartilage PG gave rise to cyanogen bromide fragments sufficiently homogeneous for primary structure studies [2,3]. One of them, termed CN-2, after reduction and alkylation gave rise to a fragment which presented an N-terminal amino acid sequence strikingly homologous with two LP peptides [4]; in addition, CN-2 contained mannose-rich structures which could be involved in *N*-linked oligosaccharides [5].

These two observations prompted us to focus our structural studies on this particular CN-2 fragment. The recently reported sequence of LP revealed a tandemly repeated structure [6]: it was of interest to know whether in PG such a structure could be demonstrated in order to increase the structural relationship between PG and LP.

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2. MATERIALS AND METHODS

Cyanogen bromide treatment of PG gave rise to three major fractions (CN-1, CN-2 and CN-3); after reduction and alkylation of CN-2, CN-2 RA/6 B1 was purified by Sepharose CL-6B chromatography [2]. Diphenylcarbamyl chloride-treated trypsin (EC 3.4.21.4) was from Sigma, *Staphylococcus aureus* V8 protease (EC 3.4.21.19) from Miles, and Sephadex G-10, G-25 and G-75 from Pharmacia. Reverse-phase HPLC was performed on an aquapore RP-300 column (Brownlee) in 0.1% trifluoroacetic acid (TFA) using a 0–40% acetonitrile gradient (Gilson chromatograph). Automated Edman degradation was carried out in a Beckman 890 C sequencer by the 0.1 M quadrol single cleavage method; the phenylthiohydantoin-amino acids were characterized as described in [7]. Dansylation was performed according to [8]. Enzymic digestions were performed in a 50 mM ammonium bicarbonate, 2 mM EDTA (pH 7.8) buffer at 37°C for 6 h at an enzyme/substrate ratio of 1:50.

3. RESULTS AND DISCUSSION

The tryptic and V8 protease digests of the CN-2

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RA/6 B1 fraction were desalted by Sephadex G-10 filtration in 30% acetic acid. Preliminary separation of the peptides from both origins was achieved by Sephadex G-25 filtration in 30% acetic acid. The materials recovered in the excluded peaks were applied to Sephadex G-75 in 2 M $CaCl_2$; the recovered fractions were then desalted on Sephadex G-10.

The peptides contained in the Sephadex G-25 or G-75 included fractions were isolated by reverse-phase HPLC. Several were quite pure and could be submitted to amino acid sequence analysis. Table

Table 1

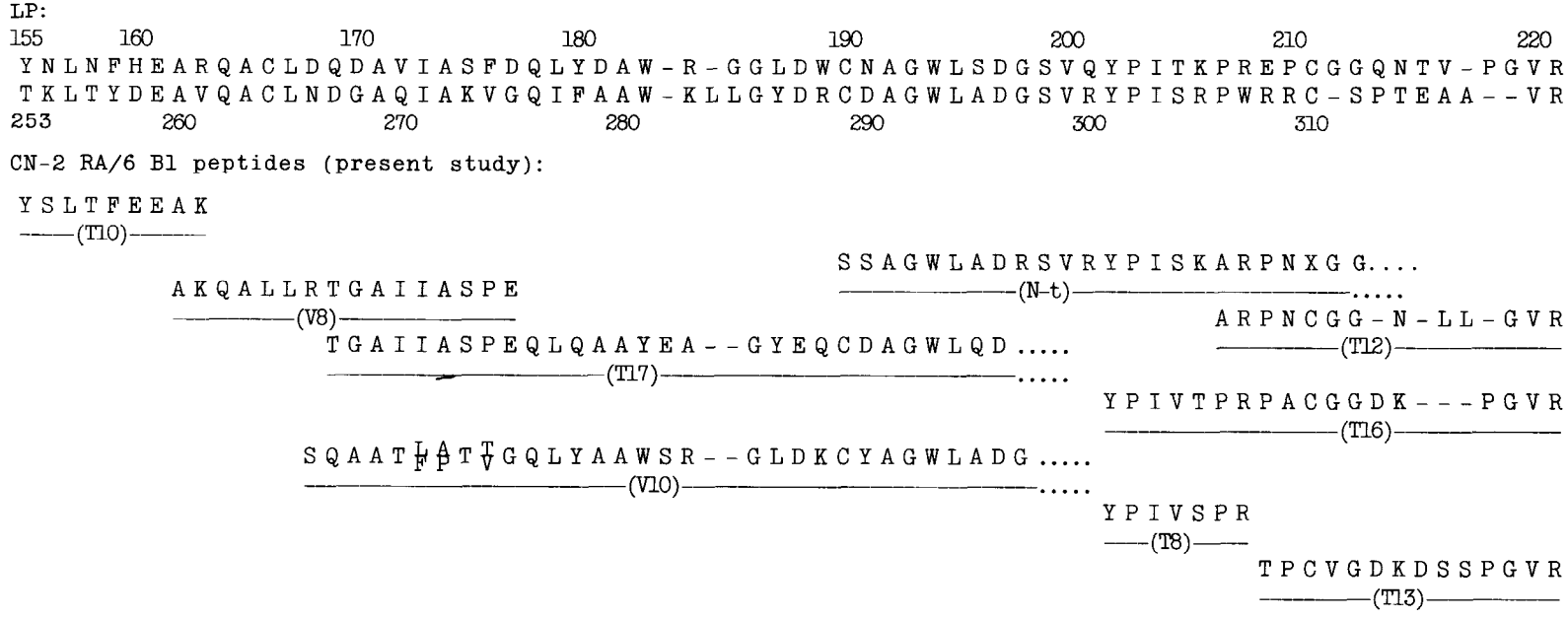
Amino acid sequences of tryptic (T) and *Staphylococcus aureus* V8 protease (V) peptides from CN-2 RA/6B 1

T1	F A T R
T2	G L D K
T3	Q A C L R
T4	A A W S R
T5	Y P I S K
T6	L P G G V F
T7	H P R V G D K
T8	Y P I V S P R
T9	T P C G V D K
T10	Y S L T F E E A K
T11	P A C G G D K P G V R
T12	A R P N C G G N L L G V R
T13	T P C G V D K D S S P G V R
T14	T V Y L H A X Q T G Y P D P S S R
T15	T V Y L Y P X Q T G L L D P L S R
T16	Y P I V T P R P A C G G D K P G V R
T17	T G A I I A S P E Q L Q A A Y E A G Y E Q C D A G W L Q D
V1	A Q E F C E
V2	A G Y E Q C D
V3	V F F A T R L E
V4	V Y C Y V D R L E
V5	T Y D V Y C Y V D R L E
V6	A I C Y T G E D F V D I P E
V7	T Y D V Y C Y V D R L E G E
V8	A K Q A C L R T G A I I A S P E
V9	A G Y E Q C D A G W L Q D Q T
V10	S Q A A T (L/F) (A/P) T (T/V) G Q L Y A A W S R G L D K C Y A G W L A D G

For peptides T17, V9 and V10, only the N-terminal sequences are indicated

Table 2

Sequence homologies between link proteins (LP) [6] and some tryptic (T) and *Staphylococcus aureus* V8 protease (V) peptides from proteoglycan monomers (fraction CN-2 RA/6 B1): the latter quoted here present at least 46% identical amino acids situated in homologous positions in one or the other tandemly repeated LP sequence

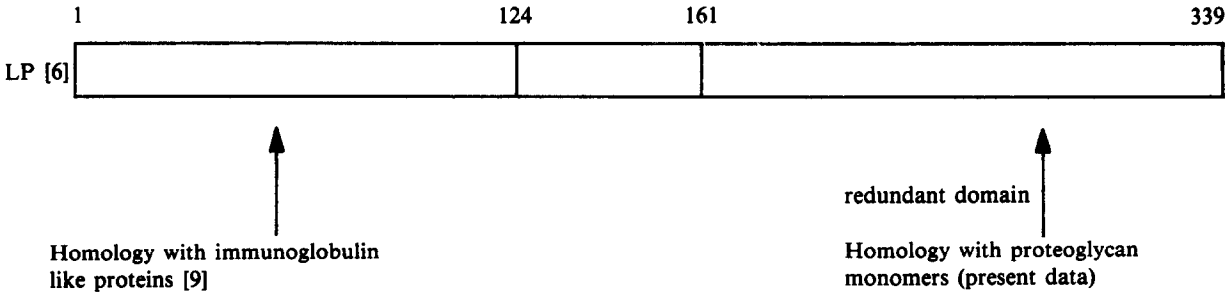


1 presents the sequences determined so far; the peptides are listed as a function of their size: some of them are redundant. Table 2 is an attempt at alignment of some tryptic and staphylococcal peptides (table 1) from CN-2 RA/6 B1 with the tandemly repeated sequence in LP. It is obvious that CN-2 RA/6 B1 presents high homology with this LP region and its different tryptic and *S. aureus* V8 protease peptides have an average of about 50% identical amino acids encountered in homologous positions in one or the other tandemly repeated LP sequence (44% identical residues between the two compared LP sequences). In addition a redundancy in CN-2 RA/6 B1 can be postulated: the latter might explain the slight heterogeneity observed at positions 6, 7 and 9 of peptide V-10. The previously determined N-terminal sequence of CN-2 RA/6 B1 [3] has been lengthened and is included in this table (N-t).

No amino acid could be characterized at step 7 in peptides T-14 and T-15; glucosamine was the only amino sugar characterized in both of them. These peptides were submitted to six Edman degradation cycles and the resulting products were dansylated. Dansyl-aspartic acid was then characterized at position 7 in peptides T-14 and T-15 indicating that *N*-glycosylation occurred at an identical position in both, the classical Asn-X-Thr code sequence being implicated. The two peptides are homologous: they could not be aligned with the LP sequence [6].

T-14	T	V	Y	L	H	A	N	Q	T	G	Y	P	D	P	S	S	R				
T-15	T	V	Y	L	Y	P	N	Q	T	G	L	L	D	P	L	S	R				

Our results did not rule out the existence of different PG populations. Nevertheless the existence of a structural homology between LP and PG might reflect the involvement of these structures in a particular biological function: we suggest that they might be involved in their hyaluronic acid-binding capacity. Previously we noted that the N-terminal sequence of LP presents homologies with immunoglobulin-like proteins [9]: the present study extends our knowledge concerning the domains organization of LP as their C-terminal redundant part is homologous to proteoglycan monomers (scheme 1).



Scheme 1. Structural homologies of link proteins (LP) with other proteins.

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