Sequence data concerning the protein core of the cartilage proteoglycan monomers

Characterization of a sequence allowing the synthesis of an oligonucleotide probe

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The present report develops our previous structural data concerning the cyanogen bromide fragments from the bovine nasal cartilage proteoglycan monomers. Among the reported sequences a Met-Ile-Trp-His sequence was characterized, useful for future studies devoted to the molecular cloning of the proteoglycan monomers.

Sequence *Cartilage* *Proteoglycan* *Probe*

1. INTRODUCTION

Structural studies devoted to the protein core of the bovine nasal cartilage proteoglycan monomers have only recently been developed. The polydispersity of these compounds [1–3], mainly due to their level of glycosylation, appeared to be a major difficulty for such research. However, while heterogeneous, the protein core of the monomers possesses some regions which could give rise to fragments sufficiently homogeneous for primary structure studies. We reported the isolation of homogeneous cyanogen bromide (CNBr) fragments from the hyaluronic acid-binding region of the monomers and from the monomers themselves [4,5]. No sequence among those which have already been determined seemed to constitute a useful tool for future structural studies concerning the gene implicated in the protein core biosynthesis. We report additional data concerning the CNBr fragments obtained from the monomers. One of them is of special interest as the synthesis of an oligonucleotide probe, based on its structure, can now be considered.

2. MATERIALS AND METHODS

2.1. *Reagents*

Guanidine hydrochloride (Gdn-HCl) was from Jannsen Chemica. Benzamidine hydrochloride, cyanogen bromide, suprapur cesium chloride and anisole were from Merck. Aminohexanoic acid, ethylenediaminetetraacetic acid (disodium salt) and chondroitinase ABC (EC 4.2.2.4) were from Sigma and hydrogen fluoride/pyridine from Fluka. Sepharose CL-6B was from Pharmacia. All other reagents (analytical grade) were from Prolabo, Labo Disc or Canalco.

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2.2. *Analytical procedures*

Sodium dodecyl sulfate–polyacrylamide gel electrophoreses (SDS–PAGE) were performed according to [6] (12 and 6% polyacrylamide, pH 8.9); protein bands were stained with Coomassie brilliant blue R-250. Automated Edman degradation was carried out 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.

2.3. *Preparative procedures*

Proteoglycan monomers were sequentially extracted as in [7]; they were obtained from the proteoglycan complex fraction (A1) from the Gdn-HCl extract and isolated after CsCl density gradient centrifugation under dissociative conditions. 3.4 g (dry weight) of monomers were submitted to CNBr treatment as in [5] and the reaction mixture, after concentration, was subjected to CsCl density gradient centrifugation as in [5]. The lower half of the gradient (bottom fraction) contained the totality of the uronates of the starting material: it corresponded to the CN-1 fraction [5]; the upper half (top fraction) contained the CN-2 and CN-3 fractions [5]. Chondroitinase treatment was carried out as in [5]. Hydrogen fluoride/pyridine treatment in presence of anisole was performed according to [8] for 5 h at room temperature. Sepharose CL-6B gel filtration was performed with a 0.05 M CH_3COONa, 4 M Gdn-HCl, pH 5.8, buffer as eluent. Preparative SDS–PAGE was performed as in [4].

3. RESULTS AND DISCUSSION

3.1. *Study of the fragments contained in the top fraction (CN-2 and CN-3)*

The CN-2 and CN-3 fractions were separated by Sepharose CL-6B gel filtration (not shown).

One major component, embedded in a diffuse stained zone, was characterized in the CN-3 fraction by 12% analytical SDS–PAGE; its relative molecular mass was 19.5 kDa (fig.1, gel 4).

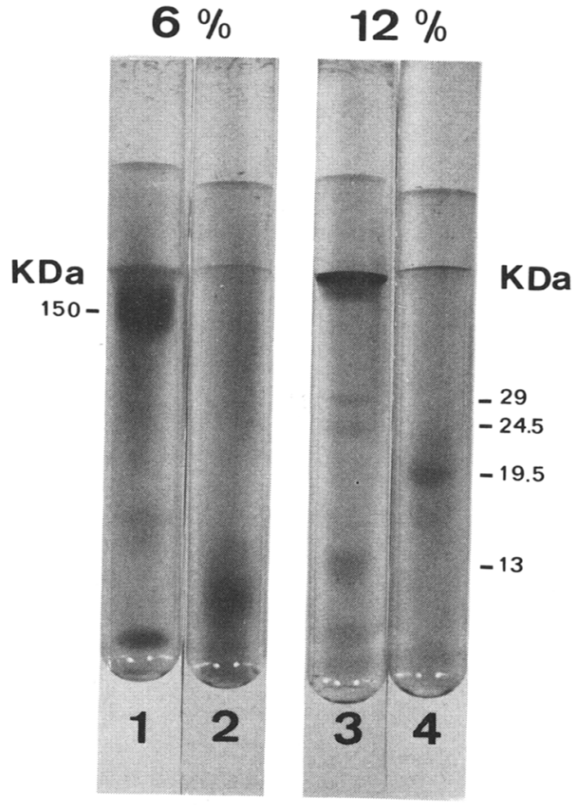

Fig.1. SDS–PAGE of the reduced and alkylated CN-2 and CN-3 fractions on 6 and 12% gels. CN-2 fraction: gels 1 and 3; CN-3 fraction: gels 2 and 4.

When submitted to 12% analytical SDS–PAGE, the unreduced CN-2 fraction did not give rise to any gel penetrating component (not shown). Upon reduction, 3 fragments with molecular masses of 13, 24.5 and 29 kDa, respectively, were characterized on a 12% gel (fig.1, gel 3); 150 kDa was assigned to the broad 6% gel penetrating band (fig.1, gel 1). The 13, 24.5 and 29 kDa fragments appeared to be linked by disulfide bonds to the 150 kDa component. The latter corresponds to the previously described CN-2 RA/6B-1 fraction [5]. The separation of the 4 fragments was achieved, after reduction and alkylation, by Sepharose CL-6B gel filtration and preparative SDS–PAGE (not shown). The N-terminal amino acid sequences of these different fragments are reported in table 1. The 150 kDa fragment was studied before and after deglycosylation. According to previous

Table 1

N-terminal sequences of CNBr-fragments isolated from the CN-2 fraction

Fragment	
	10 20
13 kDa	Glu-Gly-Glu-Val-Phe-Tyr-Ala-Thr-Ser-Pro-Glu-Lys-Phe-Thr-Phe-Gln-Glu-Ala-Ala-Asn-
	30
	Glu-Cys-Arg-Arg-Leu-Gly-Ala-Arg-Leu-Ala-Thr-Thr-Gly-Gln-Leu-Tyr-
	10 20
24.5 kDa	Ser-Gly-Ile-Glu-Asp-Ser-Gln-Ala-Thr-Leu-Glu-Val-Val-Val-Lys-Gly-Ile-Val-Phe-His-
	30
	Tyr-Arg-Ala-Ile-Ser-Cys-Arg-Tyr-Thr-Leu-
	10
29 kDa	Ser-Gly-Ile-Glu-Asp-Ser-Gln-Ala-Thr-Leu-Glu-Val-Val-Val-Lys-Gly-
	10
150 kDa	Ser-Ser-Ala-Gly-Trp-Leu-Ala-Asp-Arg-Ser-Val-Arg-Tyr-Pro-Ile-Ser-

results [4,9] the 13, 24.5 and 29 kDa fragments arise from the hyaluronic acid-binding region.

3.2. *Study of the fragments contained in the bottom fraction (CN-1)*

No gel penetrating band could be detected by 12 or 6% SDS–PAGE with either unreduced or reduced samples of the CN-1 fraction (fig.2, gels 2). Upon chondroitinase treatment, a gel penetrating band was characterized on 12 and 6% gels (fig.2, gels 3 not reduced); a large amount of material still did not penetrate into the gel. After subsequent reduction and alkylation, an additional band was characterized on a 12% gel (fig.2, gel 3, reduced); its molecular mass was 15 kDa. The molecular mass of the diffuse 6% gel penetrating

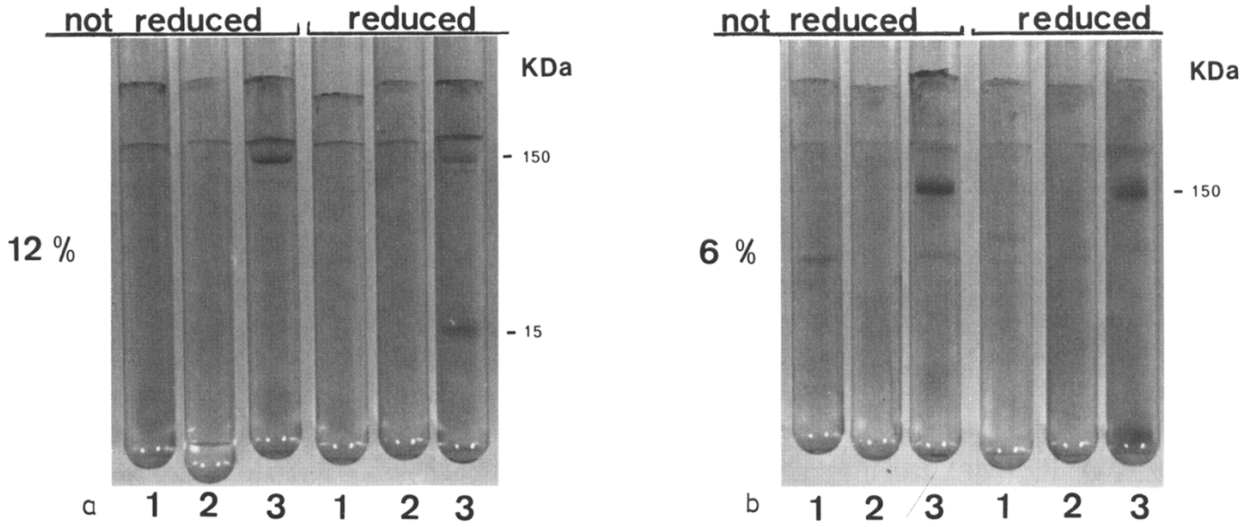

Fig.2. SDS–PAGE of the CN-1 fraction on 6 and 12% gels. Chondroitinase ABC alone: gels 1; CN-1 fraction: gels 2; chondroitinase-treated CN-1 fraction: gels 3.

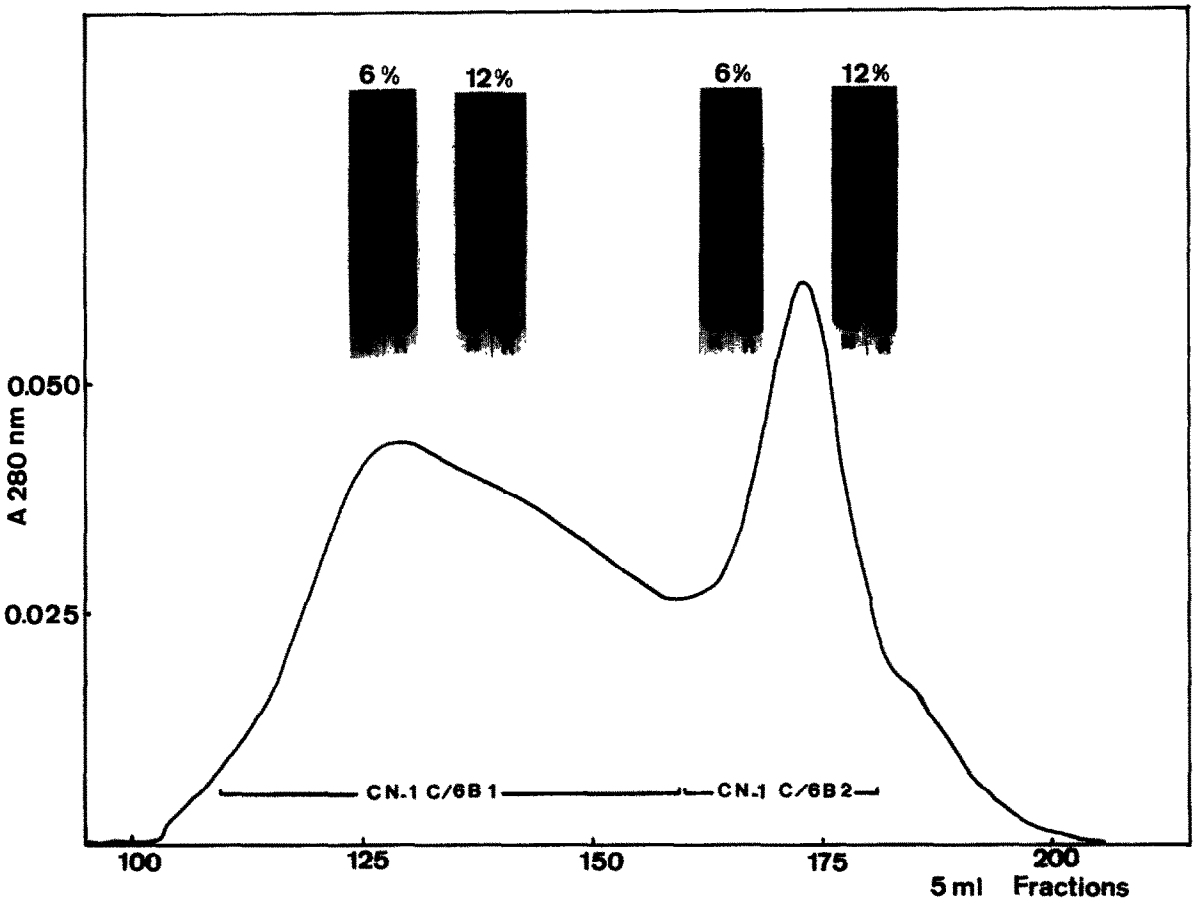

Fig.3. Sepharose CL-6B gel filtration of the chondroitinase-treated CN-1 fraction. The SDS–PAGE were performed on 6 and 12% gels with unreduced (N) or reduced (R) samples of the two isolated peaks.

band characterized on a reduced sample was 150 kDa. The separation of these fragments was achieved by a two-step Sepharose CL-6B gel filtration. During the first, the unreduced chondroitinase-treated CN-1 fraction gave rise to two fractions (fig.3). In the first eluted peak (CN-1 C/6B-1) no gel penetrating material could be detected; the second peak (CN-1 C/6B-2) contained the 150 kDa fragment; upon reduction and alkylation of the latter the 15 kDa fragment was liberated and its separation was achieved by a second gel filtration (fig.4).

The occurrence of the 15 kDa fragment has already been observed upon CNBr treatment of chondroitinase-treated monomers; it was not detected in the hyaluronic acid-binding region [9]. Its N-terminal sequence is reported in table 2. This CNBr-fragment is of special interest, as from its N-terminal area the occurrence of a Met-Ile-Trp-His sequence was deduced, convenient for the synthesis of an oligonucleotide probe.

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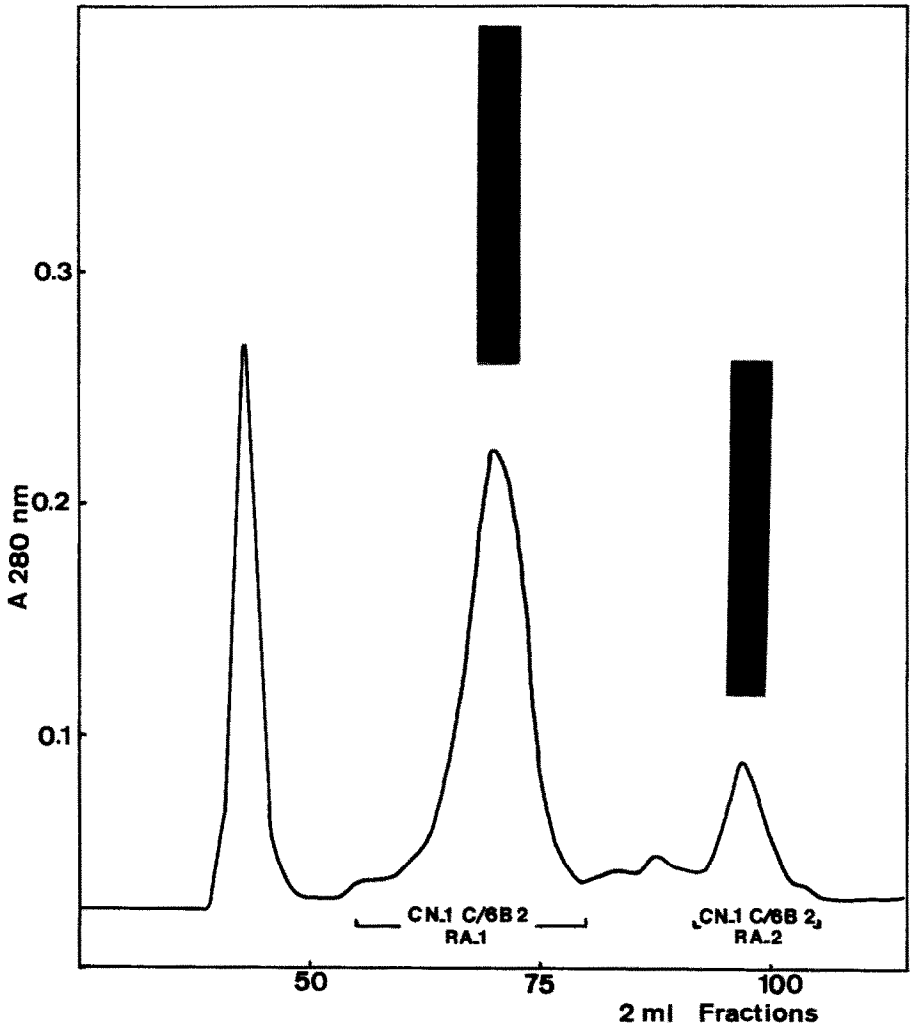

Fig.4. Sepharose CL-6B gel filtration of the reduced and alkylated CN-1 C/6B-2 fraction. The SDS–PAGE were performed on 6% gels.

Table 2

N-terminal amino acid sequence of the 15 kDa fragment isolated from the CN-1 fraction

1	10	20
Ile-Trp-His-Glu-Lys-Gly-Glu-Trp-Asn-Asp-Val-Pro-Cys-Asn-Tyr-Gln-Leu-Pro-Phe-Thr-		

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