Volume 319, number 3, 249–252 FEBS 12240 March 1993

Decorin is specifically solubilized by heparin from the extracellular matrix of rat skeletal muscles

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Received 2 February 1993

We have previously communicated that heparin co-solubilizes the asymmetric form of acetylcholinesterase (AChE) and a dermatan sulfate proteoglycan from the extracellular matrix (ECM) of rat skeletal muscles. In this report we unequivocally demonstrate by biochemical and immunological analyses that the proteoglycan that is solubilized by heparin from rat skeletal muscle ECM corresponds to decorin. These results support the concept for the role of decorin in the ECM organization.

Decorin; Proteoglycan; Glycosaminoglycan; Neuromuscular junction: Extracellular matrix

1. INTRODUCTION

The extracellular matrix (ECM) is composed mainly of collagens, proteoglycans, and various non-collagenous glycoproteins. The assembly of these macromolecules into ECMs is thought to be dependent on specific high affinity interactions between individual components. We have shown that in the ECM of rat skeletal muscle, the glycosaminoglycan heparin co-solubilizes the asymmetric form of acetylcholinesterase (AChE) and a dermatan sulfate proteoglycan [1] suggesting some kind of interaction between these ECM constituents. On the other hand, the asymmetric form of AChE have been shown to be anchored to the skeletal muscle ECM through specific interactions with proteoglycans [2–3] and that these interactions are mediated via the collagen tail present in the AChE [2,11].

It has been shown that the level of expression of AChE [4] and the small dermatan sulfate proteoglycan decorin [5,12] are regulated by motor nerve activity. Recently it has been demonstrated that decorin binds to the collagen type VI [6] and the amount of decorin increases during myogenesis [7]. In this study we show that the proteoglycan that is specifically co-solubilized with AChE from the rat skeletal muscle ECM correspond to decorin.

2. MATERIALS AND METHODS

The following chemicals were used: heparin from bovine lung and chondroitinase ABC (Sigma); $Na_2{}^{35}SO_4$ (carrier free) from New England Nuclear. Polyclonal rabbit antiserum against decorin purified from rat skin fibroblast [8] was a generous gift of Prof. Hans Kresse (Institute of Physiological Chemistry and Pathobiochemistry, University of Münster, Germany). This antiserum was produced by injecting decorin core protein purified from the secretion of rat skin fibroblast as described [8]. Other reagents were obtained from commercial sources. Male Sprague–Dawley rats (220 g body wt.) were used. The rats were injected with 5–10 mCi of radioactive sulfate in saline. Animals were sacrificed after 18 h of the injection.

2.1. *Preparation of the extracellular matrix material*

A detergent-insoluble fraction enriched in ECM components from rat skeletal muscle was prepared as described previously [1].

2.2. *Characterization of proteoglycans*

The fraction containing the heparin solubilized proteoglycan was diluted with 8 M urea, 0.1 M NaCl and 50 mM sodium acetate, pH 5.8, containing protease inhibitors. The sample was applied to a DEAE-Sephacel column. The bound material was eluted with a pulse of the same above solution but containing 1.0 M NaCl. Analytical Sepharose CL-4B chromatography was carried out exactly as explained [5]. Solubilized proteoglycans were determined by binding to CPC filters as explained previously [18].

2.3. *Enzymatic treatment*

Chondroitinase ABC treatment was carried out as described previously [5].

2.4. *SDS-PAGE analysis of proteoglycans and immunodetection*

The heparin solubilized material was electrophoresed on a 3–10% polyacrylamide gel as described by Carlson and Wight [9]. After electrophoresis proteins were transferred to nitrocellulose, incubated in Blotto (20 mM Tris-HCl, pH 7.4, 0.1 M NaCl, 5% nonfat dry milk) at room temperature for 1 h to block nonspecific binding, and incubated with rabbit anti decorin antiserum (1/100 dilution in Blotto). The bound antibody was visualized with affinity-purified phosphatase alkaline conjugated goat anti-rabbit IgG (Sigma, St. Louis, MO, USA).

Immunoprecipitation of decorin was done exactly as described previously by us [7].

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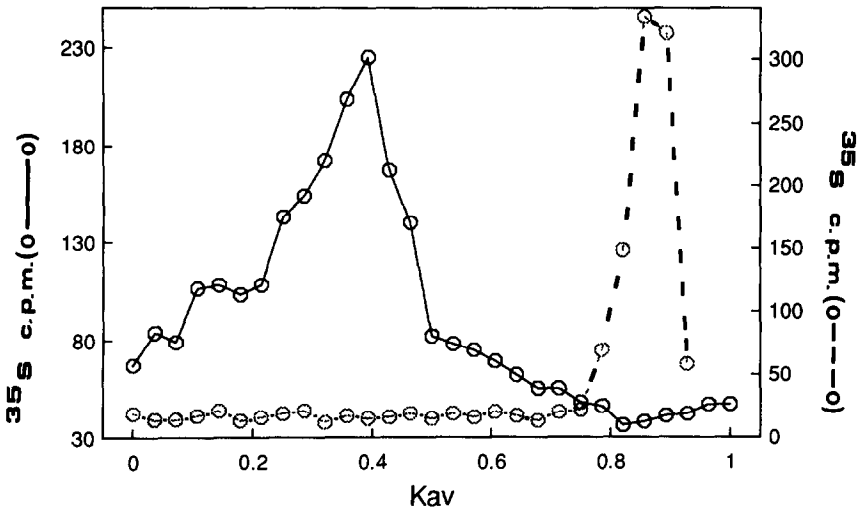

Fig. 1. The heparin solubilized proteoglycan correspond to a chondroitin/dermatan sulfate. The heparin solubilized radioactive material was chromatographed on a Sepharose CL-4B in the presence of 50 mM Tris, pH 7.4, 1.0% sodium dodecyl sulfate, 0.1 M NaCl at a flow rate of 5.0 ml/h. Solid line corresponds to the control; dashed line=after chondroitinase ABC treatment. V_0 and V_t were calculated using Dextran blue and Phenol red, respectively.

2.5. *AChE activity*

AChE activity was determined by the method of Ellman as explained previously [13]. AChE was assayed in the presence of 0.1 mM ethopropazine, a specific inhibitor of butyrylcholinesterases.

3. RESULTS

Incubation of isolated sulfate-labeled skeletal muscle ECM fraction with heparin resulted in an important solubilization of sulfated proteoglycans. Table I shows that over 10-fold of sulfated material was solubilized with heparin from the ECM fraction compared with the controls (buffer alone). The extent of solubilized material correspond approximately to 40% of the total sulfated proteoglycans present in the rat skeletal muscle ECM. Table I also shown that heparin co-solubilizes 85% of the total AChE activity solubilized by sodium chloride. Other glycosaminoglycans such chondroitin sulfate and hyaluronic acid solubilized low levels of proteoglycans and AChE (data not shown). Fractionation of the solubilized sulfated material on a Sepharose CL-4B column indicates that the proteoglycan released by heparin eluted with a Kav of approximately 0.4 (Fig. 1, closed circles). Incubation of the solubilized proteoglycan with chondroitinase ABC lyase followed by fractionation in the same column indicates that the proteoglycan solubilized with heparin correspond to a chondroitin/dermatan sulfate proteoglycan (Fig. 1, open circles). In order to visualize the heparin solubilized proteoglycan, an aliquot of this material was fractionated on SDS-PAGE followed by fluorography. Fig. 2, lane 1 shows that the solubilized proteoglycan has 75–90 kDa and was totally degraded after chondroitinase ABC treatment (Fig. 2, lane 2). The molecular weight and glycosaminoglycan composition of the heparin solubilized proteoglycan are very similar with the proteoglycan decorin previously characterized by us in the same tissue [10].

Table I

Heparin solubilizes sulfate labeled material and AChE activity from skeletal muscle extracellular matrix

	Sulfated material		AChE	
Treatment	CPM/g of tissue	%	Units/g of tissue	%
Control	2,910	9.3	0.002	5.7
Heparin	31,340	100.0	0.030	85.7

Aliquots from sulfate labeled rat skeletal ECM were incubated with buffer alone (control) or with heparin 2.0 mg/ml at 4°C for 15 min. At the end of the incubation the mixture was centrifuged and sulfated material and AChE activity was determined. The values correspond to the average of three determinations. Total solubilized counts by heparin represent about 40% of the total radioactive material present in the skeletal muscle ECM. 100% AChE activity correspond to the activity solubilized by 1.0 M NaCl.

In order to unequivocally confirm that the proteoglycan solubilized by heparin indeed correspond to decorin the solubilized sulfated material was incubated with antiserum against rat decorin. Fig. 2, lane 3 indicates that a 80 to 90 kDa proteoglycan was specifically immunoprecipitated with this antiserum. No significant precipitation was observed when a nonimmune rabbit antiserum was used (Fig. 2, lane 4). To confirm that the immunoprecipitated proteoglycan is decorin we decided to determine the core protein size of the solubilized proteoglycan. To this, western blot analysis was performed. Fig. 3, lane 1 shows that after electrophoresis followed by transfer to nitrocellulose membrane, the

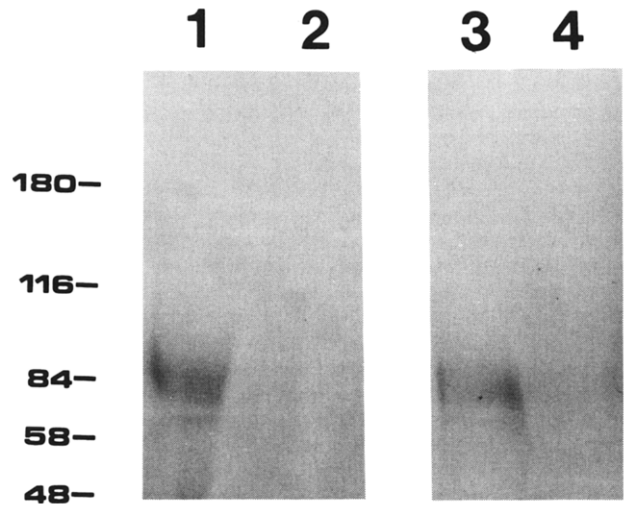

Fig. 2. Antibodies against decorin specifically immunoprecipitated the proteoglycan solubilized by heparin. Lane 1=the heparin solubilized proteoglycan was separated on a 3–10% polyacrilamide gel; lane 2=same a lane 1 but preincubated with chondroitinase ABC; lane 3=the solubilized material was incubated with antiserum against rat decorin and the immunoprecipitate separated on the gel; lane 4=same as lane 3, but using a nonimmune antiserum. On the left, the molecular weight standards are shown (kDa).

antiserum against rat decorin recognized a proteoglycan of 80–100 kDa in the heparin solubilized material. Correspondingly, digestion of the heparin solubilized material with chondroitinase ABC lyase before the electrophoresis made possible the visualization by the antibodies of a single core protein of 45 kDa characteristic of decorin (Fig. 3, lane 2).

4. DISCUSSION

The results presented in this paper unequivocally shows that the glycosaminoglycan heparin solubilized from rat skeletal muscle ECM the proteoglycan decorin. The hydrodynamic characteristics, the sensitivity to chondroitinase ABC treatment, the molecular weight observed on SDS-PAGE and the recognition by specific antibodies indicate that the proteoglycan solubilized correspond to rat decorin. This result is particularly relevant because heparin also solubilizes from the muscle skeletal ECM the synaptic form of AChE [1]. This asymmetric molecular form of AChE contains 12 catalytic globular subunits together to a collagen tail [11], being this latter responsible of the anchorage to the synaptic ECM [2]. Recently it has been shown that decorin binds specifically to collagen type VI [6] suggesting the presence of specific binding sites for decorin in this collagen structure. We are now investigating if the collagen tail present in the asymmetric AChE indeed has binding domains for decorin. Interestingly, the expression in skeletal muscles of both types of molecules, asymmetric AChE [4] and decorin [5,12] is strongly influenced by the presence of the motor nerve.

Decorin is found in the ECM of several tissues, including blood vessel walls, tendon, sclera as well as other interstitial tissues [13–14]. In rat skeletal muscle, we have shown that decorin localizes at the perymisium as well as in the endomysium [5]. Besides collagen type VI, decorin binds to a variety of other proteins, including fibronectin [15], transforming growth factor-beta [16], as well as high affinity receptor on the surface of fibroblasts [17]. These observations suggest that this proteoglycan in a key molecule for the ECM organization and for the binding and/or presentation of soluble factors to the cell.

The fact that heparin specifically solubilizes decorin and AChE from the muscle ECM might be an indication of the mode of association of the asymmetric form of AChE to the neuromuscular junction. We have shown before that heparitinase but not chondroitinase ABC treatment of electric organ ECM also released AChE, probably by degrading a heparan sulfate proteoglycan [2]. These antecedent together with the results presented in this paper might be an indication of a complex association between different proteoglycans (decorin and heparan sulfate), collagen containing mol-

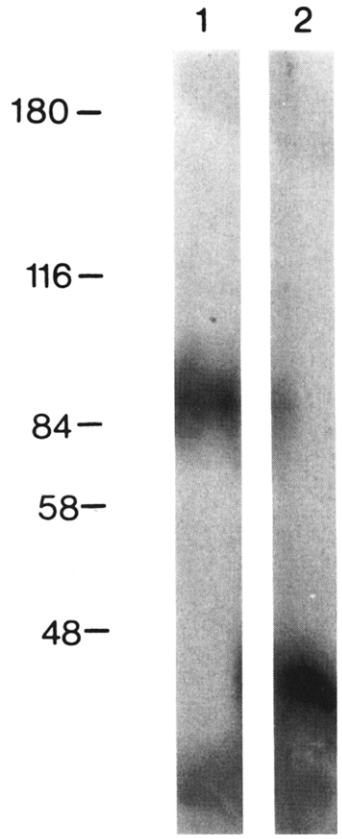

Fig. 3. The proteoglycan solubilized by heparin has a core protein with a mass of 45 kDa. An alicuot of the heparin solubilized material was separated in a 3–10% SDS-PAGE and transferred to nitrocellulose. This was incubated with antibodies against rat decorin and developed with a second phosphatase alkaline conjugated antibodies. (1) Untreated sample, (2) chondroitinase ABC treated sample. On the left the position of molecular weight markers are indicated.

ecules (AChE) and glycoproteins (fibronectin) constituents of the rat skeletal ECM.

Acknowledgements: We thank Dr. N.C. Inestrosa for carefully reading the manuscript. This work was supported by grants from FONDECYT 565-93 and International Foundation for Science 1407/2.

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