Available online at www.sciencedirect.com
ScienceDirect
Comparative Biochemistry and Physiology, Part B 146 (2007) 227–233
CBP
www.elsevier.com/locate/cbpb

Identification and distribution of chondroitin sulfate in the three electric organs of the electric eel, *Electrophorus electricus* (L.)

Maisa L.S. Souza [a,b], Cristiano F. Freitas [a,b], Maria-Aparecida O. Domingos [d], Nilson Nunes-Tavares [c,e], Aida Hasson-Voloch [c], Luiz E. Nasciutti [d], Luiz-Claudio F. Silva [a,b,*]

[a] *Laboratório de Tecido Conjuntivo, Hospital Universitário Clementino Fraga Filho, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, 21941-590, Caixa Postal 68041, Rio de Janeiro, Brazil*
[b] *Instituto de Bioquímica Médica, Programa de Glicobiologia, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, 21941-590, Caixa Postal 68041, Rio de Janeiro, Brazil*
[c] *Laboratório de Físico Química-Biológica, Instituto de Biofisica Carlos Chagas Filho, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, 21941-590, Caixa Postal 68041, Rio de Janeiro, Brazil*
[d] *Departamento de Histologia e Embriologia, Instituto de Ciências Biomédicas, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, 21941-590, Caixa Postal 68041, Rio de Janeiro, Brazil*
[e] *Centro Universitário da Zona Oeste (UEZO), Rio de Janeiro, Brazil*

Received 22 August 2006; received in revised form 20 October 2006; accepted 21 October 2006
Available online 1 November 2006

Abstract

The electrogenic tissue of the electric eel *Electrophorus electricus* (L.) is distributed in three well-defined electric organs, the Main electric organ, Sach's organ and Hunter's organ. Sulfated glycosaminoglycan (GAG) composition was characterized in the three electric organs of the electric eel. Sulfated GAGs were analyzed in the electric organs using metachromatic staining, biochemical analysis including electrophoresis before and after specific enzymatic or chemical degradations, and immunostaining with an antibody against chondroitin sulfate (CS). Our results showed in the three electric organs that CS was the main sulfated GAG species detected, accompanied by small and diminutive amounts of CS/dermatan sulfate hybrid chains and heparan sulfate (HS), respectively. However, HS was not detected in the Sach's organ. CS was predominantly detected in the innervated membrane face of the electroplaques in the three electric organs. Our findings extend previous observations on the GAG composition in the electric organs of *E. electricus* and provide new information regarding the tissue distribution and location of CS.

Keywords: Electric eel; Electric organ; Skin; *Electrophorus electricus* (L.); Sulfated glycosaminoglycan; Heparan sulfate; Chondroitin sulfate

1. Introduction

Glycosaminoglycans (GAGs) consist of hexosamine [D-glucosamine or D-galactosamine] and either hexuronic acid [D-glucuronic or L-iduronic acid] or galactose units that are arranged in alternating unbranched sequence, and carry sulfate substitutions in various positions. The sulfated GAG species are composed of chondroitin sulfate (CS), dermatan sulfate (DS), heparan sulfate (HS), heparin and keratan sulfate (KS) (Conrad, 1998; Funderburgh, 2000; Trowbridge and Gallo, 2002; Sugahara et al., 2003; Nader et al., 2004; Coombe and Kett, 2005). Hyaluronic acid (HA) is also a GAG species, but it is not sulfated (Toole, 2004). Owing to the variability in sulfate substitution, all GAGs display considerable sequence heterogeneity, and it is believed that structural differences are responsible for highly specific interactions of GAGs with other macromolecules (Coombe and Kett, 2005). Their strategic location and highly charged nature make them important biological players in cell–cell and cell–matrix interactions that take place during normal and pathological events, including organogenesis in embryonic development, cell recognition, adhesion, migration, regulation of growth factor action, lipid metabolism, neural development

* Corresponding author. Instituto de Bioquímica Médica, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, Caixa Postal 68041, Rio de Janeiro, RJ, 21941-590, Brazil. Fax: +55 21 2562 2090.
E-mail address: lclaudio@hucff.ufrj.br (L.-C.F. Silva).

1096-4959/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpb.2006.10.107

and regeneration, and initiation and modulation of inflammation (Kresse and Schonnherr, 2001; Sugahara et al., 2003; Laabs et al., 2005; Whitelock and Iozzo, 2005; Taylor and Gallo, 2006).

The electric eel *Electrophorus electricus* (L.) is a fresh water teleost showing an electrogenic tissue that produces electric discharges. The electrogenic tissue is distributed in three well-defined electric organs that may be found symmetrically along both sides of the animal. The Main electric organ extends from behind the peritoneal cavity of the viscera down the tail of the animal where it gives rise to Sach's organ, which occupies the remainder of the caudal portion. Hunter's organ is located subjacent to the other electric organs and dorsal to the long swimming fin on the ventral surface of the animal (Gotter et al., 1998; Mermelstein et al., 2000). The Main electric organ generates powerful high voltage discharges, whereas the tonically active Hunter's organ and Sach's organ emit low voltage discharges and are thought to be involved in electrolocation (Gotter et al., 1998).

Indirect studies have provided isolated information concerning the GAG composition in the Main electric organ of *E. electricus*. Bon et al. (1978) isolated GAGs composed of CS and HA from the Main electric organ of *Electrophorus*. Granafei and Hasson-Voloch (1980) studied chemical properties of HA from the Main electric organ, which was identified in this organ with histochemical methods by Couceiro and Freire (1951). In 1983, curiously, Vigny and colleagues analyzing an acetylcholinesterase enzyme preparation from the Main electric organ of *Electrophorus* have provided indirect evidences that HS GAG chains were associated with the enzyme. So far, only these three GAG species (CS, HS and HA) have been identified in the Main electric organ of *Electrophorus*. There is no information on the presence of GAGs in the other two electric organs. Here, we examined the sulfated GAG composition and tissue distribution in the three electric organs of *E. electricus*, using biochemical and immunohistochemical analysis, and demonstrated that CS was the predominant sulfated GAG species present, accompanied by small and diminutive amounts of CS/DS hybrid chains and HS, respectively.

2. Materials and methods

2.1. Electric organs from Electrophorus electricus (L.)

All experiments were performed with adult specimens of *E. electricus* (L.) supplied by Museu Paraense Emílio Goeldi (Belém do Pará, Brazil) and kept in an aquarium filled with fresh filtered water. Each animal was anesthetized in ice-cold water containing 2.0% urethane before being killed by decapitation. Slices of electric organ were obtained from the *E. electricus* Main electric organ and from Hunter's and Sach's electric organs.

2.2. Material

CS, DS, HS, twice-crystallized papain (15 U/mg protein), chondroitin B lyase (EC 4.2.2) from *Flavobacterium heparinum*

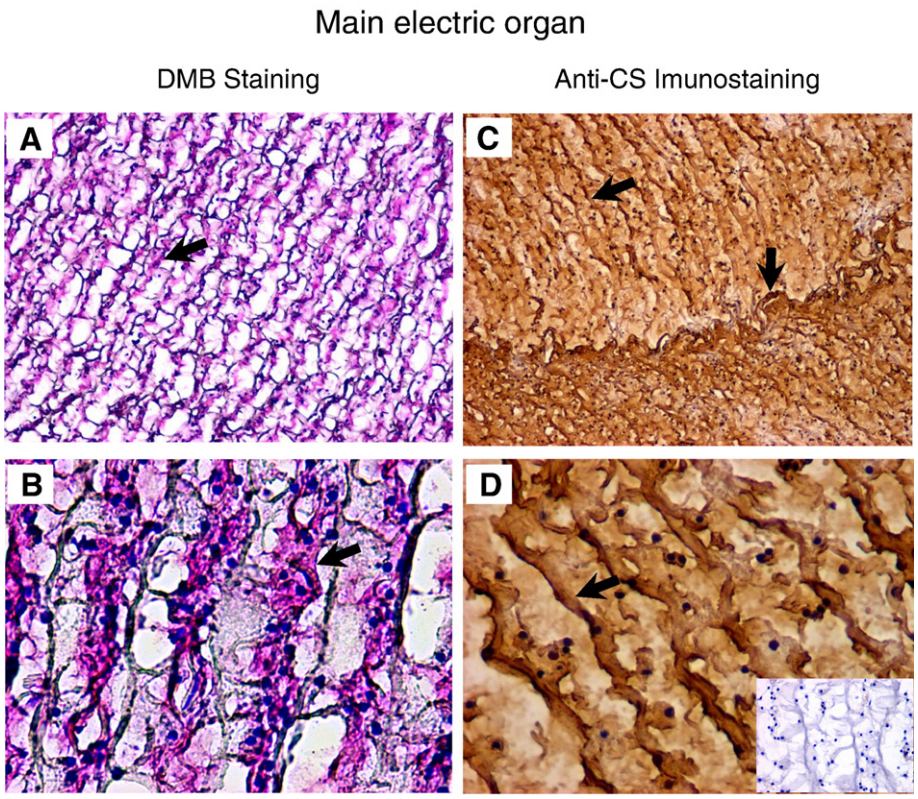

Fig. 1. Photomicrographs of the Main electric organ stained with DMB (A, B) or immunostained with an antibody against CS (C, D). Observe abundant purple metachromatic material (A, B) and peroxidase product (C, D) distributed all over the longitudinal and transverse connective septa and concentrated in the innervated face of the electroplaques (→). Insert in D: negative control. (A, C) 100×; (B, D) 400×; insert 400×.