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Original article

Activation of the cardiac Na^+–Ca^{2+} exchanger by sorcin via the interaction of the respective Ca^{2+}-binding domains

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ABSTRACT

Sorcin is a penta-EF-hand protein that interacts with intracellular target proteins after Ca^{2+} binding. The sarcolemmal Na^+/Ca^{2+} exchanger (NCX1) may be an important sorcin target in cardiac muscle. In this study, RNAi knockdown of sorcin, purified sorcin or sorcin variants was employed in parallel measurements of: (i) NCX activity in isolated rabbit cardiomyocytes using electrophysiological techniques and (ii) sorcin binding to the NCX1 calcium binding domains (CBD1 and (iii) using surface plasmon resonance and gel overlay techniques. Sorcin is activated by Ca^{2+} binding to the EF3 and EF2 regions, which are connected by the D helix. To investigate the importance of this region in the interaction with NCX1, three variants were examined: W105G and W99G, mutated respectively near EF3 and EF2, and E124A that does not bind Ca^{2+} due to a mutation at EF3. Downregulation of sorcin decreased and supplementation with wt sorcin (3 μM) increased NCX activity in isolated cardiomyocytes. The relative stimulatory effects of the sorcin variants were: W105G>wt sorcin>Sorcin Calcium Binding Domain (SCBD)>W99G>E124A. Sorcin binding to both CBD1 and 2 was observed. In the presence of 50 μM Ca^{2+}, the interaction with CBD1 followed the order W105G>SCBD>wt sorcin>W99G>E124A. In sorcin, the interacting surface can be mapped on the C-terminal Ca^{2+}-binding domain in the D helix region comprising W99. The fast association/dissociation rates that characterize the interaction of sorcin with CBD1 and 2 may permit complex formation/dissociation during an excitation/contraction cycle.

1. Introduction

Sorcin (*so*luble *r*esistance-related *c*alcium binding prote*in*) is a 21.6 kDa protein identified in the cytosol of multidrug resistant cells [1,2] that belongs to the penta-EF-hand (PEF) family, a small group of regulatory calcium binding proteins comprising calpain, ALG-2, grancalcin, peflin and PEF1 [3–8]. Sorcin shares the typical structural and functional features of all PEF family members. It has a two-domain architecture, characterized by a flexible and hydrophobic Gly/Pro-rich N-terminal domain and a C-terminal calcium binding domain containing the five EF-hand motifs (Fig. 1), and dimerizes through the unpaired EF5 hand. Like the other PEF proteins, sorcin undergoes a Ca^{2+}-dependent activation that promotes translocation to membranes where interaction with several molecular targets occurs [9]. In turn, these features render sorcin an effective participant in a number of Ca^{2+}-mediated processes. Sorcin activation is induced by Ca^{2+} binding to the two functionally relevant EF3 and EF2 motifs, that are not paired structurally as in most EF-hand proteins, but are connected by the long and rigid D helix (Fig. 1). An essential step of sorcin activation therefore consists in the transfer of information concerning Ca^{2+} binding from the site with the highest affinity for the metal, EF3, through the D helix to EF2, and from there to the rest of the molecule. The ensuing conformational change is believed to loosen the hydrophobic and hydrophilic interactions that bring the N- and C-terminal domains together. This renders both domains available for target protein recognition, in particular the D helix residues [10]. It follows that the EF3-D helix-EF2 region should be considered as a tightly coupled functional unit.

Sorcin is expressed in most human tissues including the heart where Ca^{2+}-bound sorcin interacts with and regulates several ionic channels, such as the L-type voltage-dependent channel, the ryanodine receptor, RyR2, and the sarcoplasmic reticulum Ca^{2+}-ATPase, SERCA2a, and thus plays an important role in the regulation of the excitation–contraction–relaxation processes [11–18]. Discordant effects of sorcin overexpression on cardiac function have been reported. According to Seidler et al. [13] and Meyers et al. [19] cardiac-specific overexpression of sorcin in rabbit myocytes and transgenic mice leads to a significant reduction in contractility, while

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A

N-terminal domain

MAYPGHPGAGGGYY**PGGYGGAPGGP**SFPGQTQ 1-32

C-terminal domain (SCBD)

Helix A *EF1* *Helix B* *Helix C* *EF2*
DPLYGYFASVAGQDGQIDADELQRCLTQSGIAGGYKPFNLETCRLMVSMLDRDMSGTMG 33-91

Helix D *EF3* *Helix E* *Helix F*
FNEFKELWA**V**LN**GW**RQ**H**FISFDSDRSGTVDPQELQKALTT**MGFR**LNPQTVNSIAKR 92-147

EF4 *Helix G* *EF5* *Helix H*
YSTSGKITFDDYIACCVKLRALTDSF**R**RRDSAQQ**G**MVNFSYDDFIQCVMTV 148-198

B

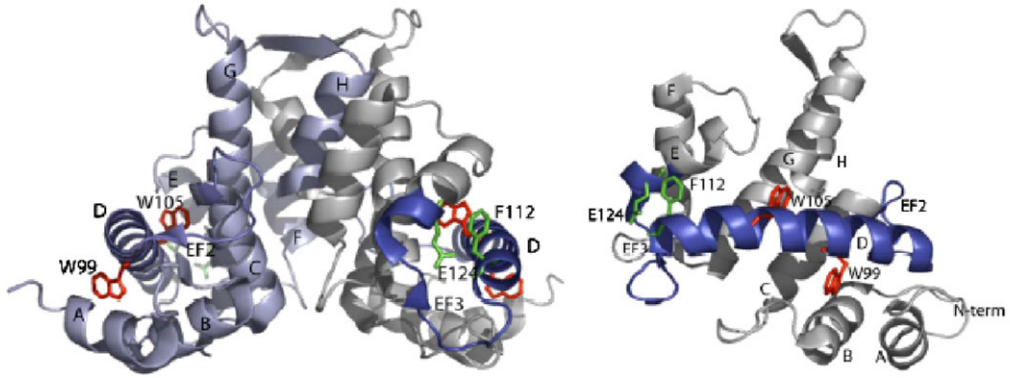

Fig. 1. Sequence of Chinese hamster sorcin and structure of the Ca^{2+}-binding domain, SCBD. (A) The amino acids of the N-terminal domain and of SCBD, involved in the interdomain contact according to Ilari et al. [10], are in boldface. The residues mutated in the variants used (W99, W105, F112 and E124) are underlined. (B) left panel. The two monomers in the SCBD dimer are depicted in different colours (light blue, gray). The D helix and the physiologically relevant EF2 and EF3 hands are coloured dark blue; the two tryptophan residues, W99 and W105, are coloured red, Phe112 and Glu124 belonging to EF3 are coloured green. (B) right panel. The gray SCBD monomer is rotated 90° with respect to the dimer presented on the left. The figure was created with PYMOL [42].

Suarez et al. [20] and Frank et al. [21] reported that transfection of rat or mouse heart or isolated cardiac cells with sorcin-expressing vectors significantly enhanced cardiac function.

The overexpression of sorcin in cardiomyocytes has also been associated with increased activity of the Na^+–Ca^{2+} exchanger, NCX [13,22]. The mammalian NCX family includes three genes (*Ncx1*, *Ncx2* and *Ncx3*) with very similar functional properties. NCX1, the main isoform expressed in the heart, catalyzes the electrogenic exchange of Ca^{2+} and Na^+ across the plasma membrane in both the Ca^{2+} influx and Ca^{2+} efflux mode and is one of the crucial regulators of Ca^{2+} homeostasis within cardiomyocytes and of cardiac contractility. NCX1 consists of nine transmembrane helices with an extracellular N-terminus and a cytosolic C-terminus that is organized in four domains [23,24]. There are two adjacent homologous Ca^{2+}-binding domains, CBD1 and CBD2, arranged in an antiparallel fashion, that are connected via a regulatory catenin-like domain (CLD) to the membrane part of the exchanger (Fig. 2). A rise in cytosolic Na^+ stimulates rapidly and then inactivates the exchanger; in contrast, cytosolic Ca^{2+} activates the exchanger and relieves the Na^+-dependent inactivation [25–28]. Regulation by cytosolic Na^+ and Ca^{2+} involves sites that do not participate directly in the ion translocation process. Thus, an amphipathic sequence, the XIP region (eXchanger *I*nhibitory *P*eptide), takes part in regulation by Na^+, whereas CBD1 and CBD2 are responsible for the Ca^{2+}-dependent activation of the exchanger. Both CBD1 and CBD2 have an Ig-like fold with the Ca^{2+}-binding sites in the distal loops [24,28,29]. They contain also an unstructured loop, the FG loop, which in CBD2 is

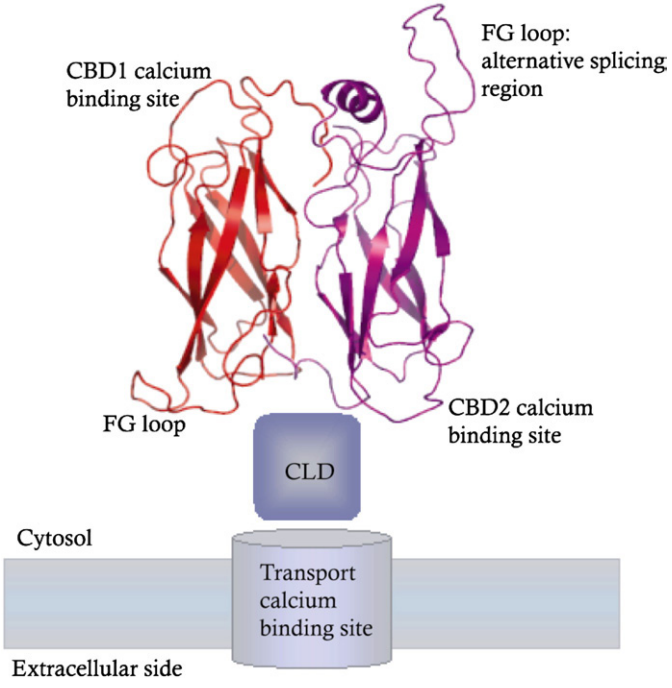

Fig. 2. Model of NCX1, according to Hilge et al. [24]. Structural details are given in the Introduction. CBD1 in red and CBD2 in magenta.