Review article

The role of βII spectrin in cardiac health and disease

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A B S T R A C T

Spectrins are large, flexible proteins comprised of α-β dimers that are connected head-to-head to form the canonical heterotetrameric spectrin structure. Spectrins were initially believed to be exclusively found in human erythrocytic membrane and are highly conserved among different species. βII spectrin, the most common isoform of non-erythrocytic spectrin, is found in all nucleated cells and forms larger macromolecular complexes with ankyrins and actins. Not only is βII spectrin a central cytoskeletal scaffolding protein involved in preserving cell structure but it has also emerged as a critical protein required for distinct physiologic functions such as posttranslational localization of crucial membrane proteins and signal transduction. In the heart, βII spectrin plays a vital role in maintaining normal cardiac membrane excitability and proper cardiac development during embryogenesis. Mutations in βII spectrin genes have been strongly linked with the development of serious cardiac disorders such as congenital arrhythmias, heart failure, and possibly sudden cardiac death. This review focuses on our current knowledge of the role βII spectrin plays in the cardiovascular system in health and disease and the potential future clinical implications.

1. Introduction

The spectrin family of proteins was first discovered in 1968 by Marchesi and Steers [1] as components of the human erythrocytic membrane [2], and was initially thought to be exclusively present in red blood cells [3,4]. While many subsequent experimental attempts failed to demonstrate the presence of spectrin in various non-erythroid cells [3,4], notable discoveries in brain did identify new peptides with calmodulin and actin binding properties comprised of 2 subunits (≈ 240 and 235 kDa) and were described as 'Fodrin' [5], 'brain actin-binding protein' [6] and 'Calspectin' [7]. No formal relationship was established between spectrin and these newly discovered brain peptides, yet these new proteins (yet to be termed spectrin) consistently had the same immunological, structural and functional degree of similarity to erythrocyte spectrin [8]. In 1981, Goodman and colleagues discovered that proteins with spectrin-like properties were potentially present in different cells and tissues such as brain, kidney, skeletal muscle, lens, small and large intestines and cardiac muscle [5,8–12]. Non-erythroid spectrins emerged as novel large actin-associated cytoskeletal proteins [2] that maintained cell shape and integrity and formed larger molecular complexes with ankyrins [13].

βII spectrin (the most common member of non-erythroid spectrins) [14] has emerged as a key cytoskeletal protein that is part of a larger macromolecular complex involved in diverse physiologic functions. βII spectrin is critical in posttranslational targeting and localization of essential membrane proteins [15–17], plays a prominent role in signal transduction [18,19], and notably serves as a major scaffolding protein [2]. Defects in βII spectrin have been associated with serious cardiac pathologies such as congenital arrhythmia, acquired and congenital forms of heart failure, and possibly sudden cardiac death [20–23]. This review focuses on the recent advances in defining the role of βII spectrin in the cardiovascular system in physiologic and pathologic states.

2. The spectrin genes and nomenclature

The nomenclature of spectrins has undergone several iterations. Winkelmann and Forget [24] classified spectrins by Roman numerals in the order of their characterization while subtypes were denoted by the Greek symbol sigma (Σ: capital letter, σ: small letter) followed by Arabic numbers.

Spectrin proteins are expressed from numerous genes in metazoans. There are seven genes that code for spectrins in mammalian organisms compared to only three genes in invertebrates. Unlike the three spectrins in invertebrates, the mammalian genome contains two α and five β spectrin genes named in the order of their discovery. SPTA1 gene [25] codes for αI that is expressed in erythroid cells, while SPTAN1 [26]

Table 1
The physiologic roles of βII spectrin in different organ systems.

Organ system	Physiologic roles of βII spectrin	Ref.
Pulmonary system	De novo synthesis and stabilization of lateral membrane of bronchial epithelial cells.	[17]
	Maintaining polarity of E-cadherin and Na^+-K^+-ATPase.	[16,63,64]
Nervous system	Binding to small synaptic vesicles via synapsin-I involved in neurotransmission.	[65,66]
	Important component of paranodal junctions involved in saltatory conduction.	[67,68]
	Maintaining structural integrity of neurons.	[68,69]
	Molecular partner to α-synuclein that regulate neurite growth during synaptogenesis.	[70]
Hepatic system	Hepatocellular carcinoma suppression mainly via serving as an adaptor protein for Smad3 and Smad4 involved in TGF-β signaling pathway.	[19,71]
	Involved in the regenerative process following partial hepatectomy.	[72–74]
	A mediator and effector protein in acetaminophen-induced liver injury.	[14]
	ELF-3 has a role in intrahepatic bile duct formation and hepatic cells differentiation and polarization	[52]
Renal system	Maintaining polarity of Na^+-K^+-ATPase in renal tubular cells	[75,76]

codes for at least four and possibly up to eight different αII isotypes that are present in all non-erythroid cells [27]. The conventional βI-IV-spectrins are encoded by *SPTB*, *SPTBN1*, *SPTBN2* and *SPTBN4*, respectively and *SPTBN5* encodes a heavy βV-spectrin [2,28]. βI spectrin is the only form expressed in erythrocytes. The spectrin product of these genes can be modified via extensive alternative processing of pre-mRNA giving rise to a wide diversity of spectrin spliceoforms. This is particularly important with respect to regulating the interactive and modulatory characteristics of spectrins [2,26–29].

3. Structural domains of spectrin

Spectrins, believed to have evolved from α-actinin [30–33], are formed of two large, similar but non-identical subunits, termed α and β [2,30–36]. Spectrins are flexible rods that have a contour length of approximately 200–260 nm with an actin-binding domain (ABD) on each end [2,37–39]. The α and β subunits are connected side-by-side in an antiparallel fashion via hydrophobic interactions supplemented by electrostatic forces of attraction [40,41] to form a heterodimer [2,28,31,39–41]. This involves an interaction between two repeats near the NH₂-terminus of one α spectrin chain and the COOH-terminal region of the antiparallel β subunit. Each of the 2 corresponding dimers is then assembled head-to-head via partial repeats in both α and β subunits to form the final heterotetramer structure of spectrin [2,28,31,42,43]. Owing to the high affinity between α and β chains, spectrins primarily exist as heterotetramers rather than autonomous α or β subunits.

The canonical spectrin subunit is highly conserved among species [2,28], and is comprised of successive repeats of 106 amino acid residues termed spectrin repeats that are folded in a triple α-helical coiled structure. This structural form and interconnection of spectrin repeats are believed to play a role in the flexibility of spectrins [44]. The α and β subunits are comprised of 21 and 17 repeats, respectively [31]. The only exception is βV spectrin which has 30 repeats [45]. The last repeat in each spectrin is an incomplete repeat that mediates end-to-end association between one helix of α spectrin and two helices of β spectrin to form a triple helical bundle [46].

βII spectrin, like all conventional β spectrins, contains 2 tandem calponin homology (CH₁ & CH₂) domains which both comprise an actin-binding domain (ABD) at the amino-terminal [2,28,31,47,48]. Linked to the ABD domain of βII subunit are 17 successive triple helical motifs and terminates with a carboxyl region. The COOH-terminal region of the 14th repeat and the entire 15th repeat are a prerequisite for ankyrin binding [46,49]. The carboxyl-terminal of βII spectrin is differentially spliced giving origin to long and short βII isoforms (βIIΣ1 and βIIΣ2 respectively) [31,50,51]. The long carboxyl terminus of the last partial repeat of βII spectrin is linked to a pleckstrin homology (PH) domain [50]. The spliced βII isoforms with short C-terminal regions lack this PH domain [52]. The PH domain, a seven stranded antiparallel

β-sheet, is comprised of approximately 100–120 amino acids and is located approximately 50–60 amino acid residues before the end of the C-terminus of β chain. This domain serves as a ligand binding site for many phospholipids involved in signal transduction [28,50,53–56]. Immunofluorescence staining shows that βII spectrin is localized in a striated pattern in isolated mouse myocytes [20].

The alternatively spliced short variants of βII spectrin called *ELF* (embryonic liver fodrin) share some similarities with the long βII isoform. *ELF-3*, a 200 kDa β spectrin and the longest form among other *ELFs*, is a short βII isoform with an ABD, a 17 repeat domain and COOH terminal lacking the PH domain [52]. On the contrary, *ELF-1*, a 27 kDa β spectrin, shares no degree of homology in domain 2 of the long βII isoform [57], but has a sole CH₁ domain similar to that of the other β spectrins and a C-terminus similar to the short βII isoform [2,52,57].

4. Role of βII spectrin in the heart

The cardiac cytoskeleton has recently emerged as a crucial player for maintaining the cardiac membrane integrity with respect to structure and function in physiologic and pathologic states. Disorders in cardiac cytoskeletal components have been strongly associated with cardiac myopathies, dystrophies, aortopathies and electrical conduction abnormalities [20,58–61]. While βII spectrin has a prominent role in many organ systems (Table 1), it has also emerged as a pivotal protein in maintaining normal cardiac membrane excitability, mechanical function [20] and proper embryogenesis [62].

5. Role in embryonic heart development

Emerging data suggests that βII spectrin plays an important role in embryonic heart development [62]. Data in mice demonstrate that complete deletion of βII spectrin results in intrauterine death with multiple defects including hepatic, neural, gastrointestinal and angiogenesis abnormalities [77]. Notably loss of βII spectrin is a possible cause for the development of congenital heart defects [62,78,79]. Furthermore, a study performed on homozygous mutant embryos for βII spectrin gene demonstrated that there was a significant difference in heart size between the wild-type embryos and the homozygous mutant embryos with the latter having smaller heart size at embryonic day 15.5 (E15.5). Further histologic studies revealed failure of ventricular wall thickening and blood vessel formation in the homozygous mutant group [62]. Moreover, the embryonic cardiomyocytes of βII spectrin conditional knockout mice displayed an aberrant distribution of tropomyosin and a significant down-regulation in the expression of α-smooth muscle actin (α-SMA), cardiac homeobox protein (NKx2.5) and dystrophin [80] which are muscle differentiation markers [62]. These defects subsequently have an s adverse effect on the contractile ability of cardiomyocytes in vivo. In addition, loss of βII spectrin in homozygous mutant embryos interferes with cardiac cell differentiation and induces