Available online at www.sciencedirect.com
ScienceDirect
Fungal Genetics and Biology 45 (2008) 1008–1015
FUNGAL GENETICS AND BIOLOGY
www.elsevier.com/locate/yfgbi

Specificity of commercial anti-spectrin antibody in the study of fungal and Oomycete spectrin: Cross-reaction with proteins other than spectrin

Marta Cotado-Sampayo [a], Pilar Okenve Ramos [a], Rubén Ortega Perez [a], Mukti Ojha [b], François Barja [a,*]

[a] *Laboratory of Bioenergetics and Microbiology, University of Geneva, ch. des Embrouchis 10, CH 1254 Jussy-Geneva, Switzerland*
[b] *Department of Biochemistry, University of Geneva, Quai Ernest Ansermet 30, CH 1211 Geneva4, Switzerland*

Received 18 December 2007; accepted 13 February 2008
Available online 21 February 2008

Abstract

Spectrin was first described in erythrocytes where it forms a filamentous network in the cytoplasmic face of the plasma membrane and participates in the membrane's structural integrity in addition to controlling the lateral mobility of integral membrane proteins. In fungi, spectrin-like proteins have been described in the plasma membrane, concentrated mainly in the region of maximum apical expansion. This localization led to the idea of a spectrin based membrane skeleton in fungi participating in mechanical integrity of the plasma membrane, generating and maintaining cell polarity. The occurrence of spectrin-like proteins in filamentous fungi, yeasts and Oomycetes, however, is questionable since the presence of such proteins has only been demonstrated with immunochemical methods using antibodies whose specificity is unclear. There is no evidence of a gene coding for the high molecular weight αβ-spectrin in the genome of these organisms. Mass spectrometric analysis of the anti αβ-spectrin immunoreacting peptides from *Neurospora crassa* and *Phytophthora infestans* identified them as elongation factor 2 (NCU07700.4) and Hsp70 (PITG_13237.1), respectively. An attempt was made to correlate the reactivity of anti-spectrin antibody to a common feature of these three proteins i.e., spectrin, elongation factor 2 and heat shock protein 70, in that they all have a hydrophobic region implicated in chaperon activity.

Keywords: Spectrin; *Neurospora crassa*; Elongation factor 2; Heat shock protein 70; Polyclonal antibody

1. Introduction

Spectrin is a major constituent protein of the metazoans cell membrane, first purified to homogeneity from red blood cells. It is an elongated heterodimer composed of non-identical α and β subunits (about 30% identity) with molecular weight of 240 and 220 kDa estimated, respectively, from electrophoretic mobility and shown to localize at the cytoplasmic face of the plasma membrane. It also interacts with a wide variety of proteins creating a cellular network (Bennett and Gilligan, 1993; Winkelmann and Forget, 1993). These interactions with cytosolic and membrane proteins control the elasticity of the lipid bilayer and therefore the cell shape. (Steck, 1989; Lee et al., 1993; Viel and Branton, 1996; Bennett and Baines, 2001).

The presence of spectrin in non-animal cells has been demonstrated mainly through studies employing immunochemical techniques (Western blot, immunofluorescence and/or immunogold) using commercial anti-spectrin antibodies (Table 1). The proteins recognized have been reported as *spectrin-like proteins* in protists (Hemphill et al., 1991; Ghazali et al., 1995; Holzinger et al., 1999), plants (Michaud et al., 1991; Faraday and Spanswick, 1993; Bisikirska and Sikorski, 1997; De Ruijter et al., 1998, 2000), Oomycetes (Kaminskyj and Heath, 1995) and fungi (Degousée et al., 2000; Slaninová et al., 2003; Cotado-Sampayo et al., 2006). However, our search for the bona fide spectrin in fungal-specific and general databases (Broad Institute Database, MIPS and NCBI) using

* Corresponding author. Fax: +41 22 37 93756.
E-mail address: Francois.barja@bioveg.unige.ch (F. Barja).

1087-1845/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.fgb.2008.02.003

Table 1
Reports of spectrin-like proteins in fungi, Oomycetes, plants and low eukaryotes organisms

Organism	Antibodies	Molecular weight (kDa)	Localization	Reference
Neurospora crassa	Anti αβ-Spectrin Sigma S1390	220, 240, 100	Plasma membrane Apex	Degousée et al. (2000)
	Anti αβ-Spectrin Sigma S1390	100	Plasma membrane Apex	Cotado-Sampayo et al. (2006)
Geotrichum candidum	Anti αβ-Spectrin Sigma S1390		Plasma membrane Patches in the apex	Heath et al. (2003)
Saccharomyces cerevisiae	Anti αβ-Spectrin Sigma S1390	220, 240, 60, several low molecular weight bands	Plasma membrane Vacuolar membrane Cytoplasmic	Slaninová et al. (2003)
Schizosaccharomyces japonicus	Anti αβ-Spectrin Sigma S1390	220, 240, 60, several low molecular weight bands	Plasma membrane Cytoplasmic Septum	Slaninová et al. (2003)
Saprolegnia ferax	Anti αβ-Spectrin Sigma S1390 Anti-Spectrin (ICN, St. Laurent)	246, several low molecular weight bands	Plasma membrane	Kaminskyj and Heath (1995)
Phytophtora infestans	Anti αβ-Spectrin Sigma S1390	240, 220, 100, 70, 50, 30	Plasma membrane	Toquin et al. (2006)
Tomato plants	Anti β-Spectrin	240, 220	Plasma membrane	Michaud et al. (1991)
Vivia sativa	Anti αβ-Spectrin Sigma S1390 Anti-Spectrin Sigma S1515		Root hair tips	De Ruijter et al. (1998)
Pisum sativum	Anti αβ-Spectrin (Lorenz et al., 1995)	Native: 800, 280, 170, 110, 70		Bisikirska and Sikorski (1997)
Onion cells	Anti αβ-Spectrin S3396 Anti αβ-Spectrin S1515	Native: 210/230 zone, 580, 380 and smaller bands 100, 130, 170	Associated to plasma membrane. Endoplasmic reticulum and endomembrane system	Reuzeau et al. (1997)
Green algae, Desmidiaceae	Anti αβ-Spectrin Sigma S1390 Anti-Spectrin Sigma S1515 Anti-Spectrin Sigma S3396	220, 120, 70	Plasma membrane Endoplasmatic reticulum Vesicles	Holzinger et al. (1999)
Chara globularis	Anti αβ-Spectrin Sigma S1390	195, 170	Endoplasmatic reticulum (ER) agregates Tip of rhizoids	Braun (2001)
	Anti-Spectrin Sigma S1515	195, 170, 110		

conserved sequence of CH-homology domain did not show any gene coding for spectrin. A protein with features of the spectrin superfamily has been proposed to exist in the Ascomycete *Neurospora crassa* (NCU06429.4) and the Oomycete *Phytophtora infestans* (PITG_13237.1), since an immunoreacting peptide was revealed with anti-spectrin antibodies (Degousée et al., 2000; Cotado-Sampayo et al., 2006; Toquin et al., 2006). This protein, however, is smaller in molecular weight than the fungal spectrins reported earlier (Kaminskyj and Heath, 1995; Degousée et al., 2000). In more recent studies this protein has been considered to be an α-actinin related protein (Cotado-Sampayo et al., 2006; Virel and Backman, 2004 and in MIPS and Broad Institute data bases).

In this report, using the filamentous fungi *N. crassa* and *Magnaporthe grisea* and the Oomycete, *P. infestans*, we demonstrate that the polyclonal anti-chicken αβ-spectrin antibody does not react with α-actinin (NCU06429.4), the spectrin superfamily protein present in these organisms. Further, we clarify the identity of the protein that reacts with anti- chicken spectrin antibody and demonstrate that the reacting protein is EF2 in *Neurospora* and Hsp70 in *Phytophthora.*

2. Materials and methods

2.1. Strains and culture conditions

Wild-type strain *N. crassa* (FGSC 262, strain St. Lawrence STA4) was used in this study. Production of conidial inocula and culture conditions were as described previously (Ortega Perez et al., 1994). *M. grisea* was kindly given by Dr. M.-H. Lebrun (Unité Mixte de Recherche, Centre National de la Recherche Scientifique/BayerCropScience, Lyon). The fungus was inoculated on solid rice medium and allowed to grow in the dark at 27 °C for a few days until white mycelia appeared. In sterile conditions, small pieces were cut and inoculated in 200 ml of Tanaka minimal medium (Ou, 1985) with 0.2% yeast extract and 1% sucrose. The culture was grown for 48 h with agitation (150 rpm) in the dark. Mycelia were collected by filtration and weighed.

The *P. infestans* strain was kindly provided by Dr. R. Beffa (Unité Mixte de Recherche, Centre National de la Recherche Scientifique/BayerCropScience, Lyon) and grown on pea-agar medium. Sporangial inoculum was prepared from a 8–12 days culture, detached from the mycelia