Micron 56 (2014) 37–43

Contents lists available at ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

X-ray solution structure of the native neuronal porosome-synaptic vesicle complex: Implication in neurotransmitter release

Ladislau C. Kovari[a,1], Joseph S. Brunzelle[b,1], Kenneth T. Lewis[c,d], Won Jin Cho[c], Jin-Sook Lee[c], Douglas J. Taatjes[e], Bhanu P. Jena[c,*]

[a] Wayne State University School of Medicine, Department of Biochemistry and Molecular Biology, Detroit, MI, USA
[b] Life Sciences Collaborative Access Team, Synchrotron Research Center, Northwestern University, Argonne, IL 60439, USA
[c] Department of Physiology, Wayne State University School of Medicine, Detroit, MI, USA
[d] Department of Biochemistry and Molecular Biology, Wayne State University School of Medicine, Detroit, MI, USA
[e] Department of Pathology, Microscopy Imaging Center, University of Vermont College of Medicine, Burlington, VT 05405, USA

ARTICLE INFO

Article history:
Received 19 September 2013
Received in revised form
29 September 2013
Accepted 2 October 2013

Keywords:
Neuronal porosome complex
X-ray solution scattering
Photon correlation spectroscopy
Electron microscopy

ABSTRACT

Nanoportals at the cell plasma membrane called porosomes, mediate secretion from cells. In neurons porosomes are 15 nm cup-shaped lipoprotein structure composed of nearly 40 proteins. The size and complexity of the porosome has precluded determination of its atomic structure. Here we report at nanometer resolution the native 3D structure of the neuronal porosome-synaptic vesicle complex within isolated nerve terminals using small-angle X-ray solution scattering. In addition to furthering our understanding of the porosome structure, results from the study suggests the molecular mechanism involved in neurotransmitter release at the nerve terminal.

1. Introduction

The chemistry of life processes is governed at the molecular level. Hence a major challenge is the determination of atomic structure of cellular organelles and macromolecules required for understand their function in cells. Determination of the atomic structure of cellular organelles and macromolecules is required for understand their cellular function. It is especially challenging, when such organelles involve membrane proteins. Cup-shaped lipoprotein structures called porosomes (Schneider et al., 1997; Cho et al., 2002a, 2004, 2008; Jena et al., 2003; Jena, 2009; Jeremic et al., 2003; Lee et al., 2012; Anderson et al., 2004; Anderson and Scanes, 2012) are the universal secretory nanoportals at the cell plasma membrane. Membrane-bound secretory vesicles transiently dock and fuse at the base of the porosome to deliver intravesicular contents outside the cell. Fusion of membrane-bound secretory vesicles at the porosome base is mediated by calcium and a specialized set of three soluble *N*-ethylmaleimide-sensitive factor (*NSF*)-attachment protein receptors called SNAREs (Jeremic et al., 2004a; Malhotra et al., 1988; Trimble et al., 1988; Oyler et al., 1989). In neurons, target membrane proteins SNAP-25 and syntaxin called t-SNAREs present at the base of neuronal porosomes, and a synaptic vesicle-associated membrane protein (VAMP) or v-SNARE, are part of the conserved protein complex involved in membrane fusion and neurotransmission. Neuronal porosomes measure approximately 15 nm in diameter, and are present at the presynaptic membrane of nerve terminals called synaptosomes. Nearly 40 proteins (Cho et al., 2004; Lee et al., 2012) including SNARE and lipids that include cholesterol (Cho et al., 2007), compose the neuronal porosome complex. The overall morphology (Figs. 1 and 2) (Cho et al., 2004, 2008) and dynamics (Cho et al., 2010) of the neuronal porosome complex which has previously been studied and reported using both atomic force microscopy (AFM) and electron microscopy (EM), is presented (Figs. 1 and 2) for clarity and to introduce the reader to the subject. Additionally, the neuronal porosome has been functionally reconstituted into artificial lipid membrane (Cho et al., 2004), and the 3D contour map of its protein backbone at nanometer scale established (Cho et al., 2008). A set of eight protein units lining the neuronal porosome cup is present (Cho et al., 2004, 2008), each connected via spoke-like elements to a central plug, suggested to be involved in the rapid opening and closing of the structure to the outside during neurotransmission. AFM micrographs of the presynaptic membrane of isolated synaptosome preparations demonstrates the presence of the neuronal porosome plug at various conformations (Cho et al., 2010). The central plug

* Corresponding author. Tel.: +1 313 577 1532.
E-mail address: bjena@med.wayne.edu (B.P. Jena).
[1] These authors contributed equally to the study.

0968-4328/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.micron.2013.10.002

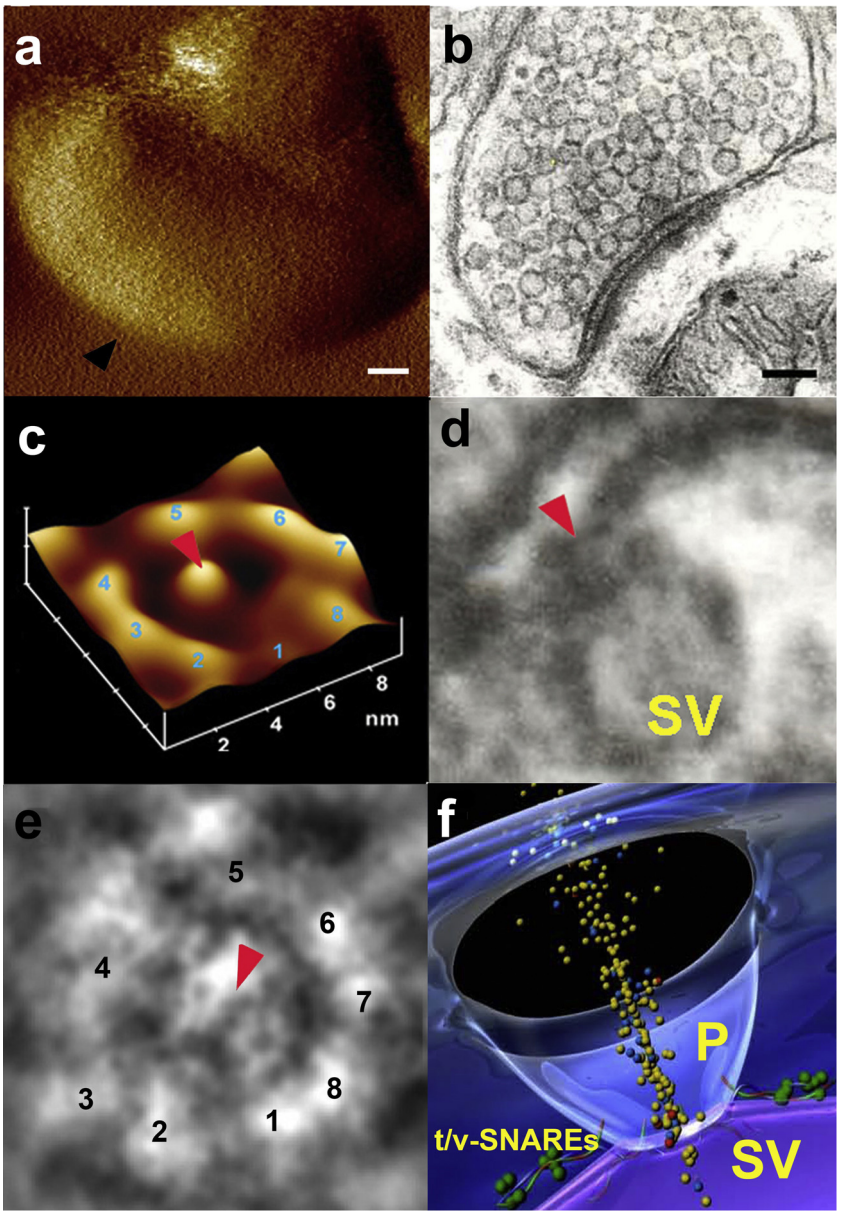

Fig. 1. Neuronal porosomes at the presynaptic membrane of nerve terminals determined in previous (Cho et al., 2004; Jena et al., 2003; Lee et al., 2012; Jeremic et al., 2004a) studies. (a) Atomic force micrograph (AFM) amplitude image Bar = 100 nm (black arrowhead points to presynaptic membrane), and (b) electron micrograph (EM) of isolated synaptosome Bar = 100 nm. (c) High resolution AFM micrograph of isolated neuronal porosome from synaptosomes followed by reconstitution into a lipid membrane. Note the near 10 nm neuronal porosome complex with a central plug (red arrowhead) and eight peripheral densities. (d) EM micrograph of the cup-shaped neuronal porosome complex with its central plug (red arrowhead) and a docked synaptic vesicle at its base. (e) Negative staining EM of an isolated neuronal porosome complex. Note the nano scale structure and assembly of proteins within the complex, and a schematic drawing (f) of the cup-shaped neuronal porosome (P) associated with a docked synaptic vesicle (SV). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

when fully retracted is in the closed conformation, when partially retracted, it is in its semi-open state, and when completely inserted into the porosome cup, in its open conformation (Cho et al., 2010). The presence of such a mechanism in the neuronal porosome complex makes sense since it would allow the rapid open and close states of the organelle during neurotransmission at the nerve terminal. This is opposed to a slow secretory cell such as the exocrine pancreas (Schneider et al., 1997) or the neuroendocrine growth hormone secreting cell (Cho et al., 2002a) where no such plug is present at the porosome opening; instead the porosome opening dilates during secretion and returns to its resting size following completion of the process (Schneider et al., 1997; Cho et al., 2002a).

The size and complexity of the membrane-associated porosome has precluded determination of its atomic structure. For example, solution NMR has not been possible primarily due to the large molecular size of the porosome complex, which is beyond the operating limits of current NMR's. Similarly, X-ray crystallography is impractical, due in part to the solubility problems of this membrane-associated structure composed of neatly 40 proteins. Although these limitations have been partially overcome by the use of AFM and EM in furthering our understanding of the fine structure and nano-arrangement of proteins within the native porosome complex (Cho et al., 2008), its 3D structure in association with a docked secretory vesicle in intact synaptosome had not been observed. In recent years, with major advances in instrumentation and computational power, small angle X-ray scattering (SAXS) has become a powerful method to study biological material at nanometer to sub-nanometer resolution in solution (Round