FEBS Letters 584 (2010) 1721–1727

FEBS Letters

journal homepage: www.FEBSLetters.org

Review

Membrane protein assembly into Nanodiscs

Timothy H. Bayburt, Stephen G. Sligar *

Department of Biochemistry, School of Molecular and Cellular Biology, University of Illinois, Urbana-Champaign, Urbana, IL 61801, United States

ARTICLE INFO

Article history:
Received 1 September 2009
Accepted 9 October 2009
Available online 16 October 2009

Edited by Wilhelm Just

Keywords:
Nanodisc
Membrane protein
Self-assembly

ABSTRACT

Nanodiscs are soluble nanoscale phospholipid bilayers which can self-assemble integral membrane proteins for biophysical, enzymatic or structural investigations. This means for rendering membrane proteins soluble at the single molecule level offers advantages over liposomes or detergent micelles in terms of size, stability, ability to add genetically modifiable features to the Nanodisc structure and ready access to both sides of the phospholipid bilayer domain. Thus the Nanodisc system provides a novel platform for understanding membrane protein function. We provide an overview of the Nanodisc approach and document through several examples many of the applications to the study of the structure and function of integral membrane proteins.
© 2009 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

1. Introduction

Membrane proteins have been difficult to study from the mechanistic perspective as many of the biophysical and chemical techniques applicable to soluble enzymes fail to deal with insoluble aggregates. Ideally, one would prefer to have a membrane protein of interest in a solubilized state for ease in purification, functional biochemical assay, application of various biophysical methods and spectroscopies, crystallization for structure determination and biochemical manipulations that maintain the target protein in a stable state. Historically, membrane protein solubilization utilized detergents to form mixed detergent–protein–lipid micelles. However, detergent poses a hazard to membrane protein stability and the excess micellar phase can interfere with many assay techniques and often has non-ideal optical properties (absorbance and light scattering) as well as undesired partitioning of substrates and products into the excess detergent micelle. Detergent also presents technical obstacles during the manipulation of membrane proteins as they often co-concentrate with the protein target and can lead to inactive or denatured entities. Furthermore, many membrane protein systems require specific types of phospholipids to maintain active function, a requirement which is not mimicked by detergent micelles. Liposome preparations have been used to incorporate membrane proteins and this approach has been found to be useful when compartmentalization of each side of the bilayer is needed, as for example in the assay of ion channels. However liposomes are large, unstable and difficult to prepare with precisely controlled size and stoichiometry.

Nanodisc technology offers a solution to some of these challenges. In this approach, the membrane protein target is transiently solubilized with a detergent in the presence of phospholipids and an encircling amphipathic helical protein belt, termed a membrane scaffold protein (MSP) [1]. When the detergent is removed, by dialysis or adsorbtion to hydrophobic beads, the target membrane protein simultaneously assembles with phospholipids into a discoidal bilayer with the size controlled by the length of the MSP. The resultant Nanodiscs thus keep membrane proteins in solution, provide a native-like phospholipid bilayer environment that provides stability and functional requirements of the incorporated target and also allow control of the oligomeric state of the target membrane protein. Nanodiscs thus provide a cassette, rendering membrane proteins soluble at the single molecule level, and opening up structural–functional investigations that were heretofore limited to the class of soluble proteins and enzymes. Membrane proteins having many different topologies have been introduced into Nanodiscs (Table 1). In addition, the provision of a soluble membrane surface with defined phospholipid composition has provided a means to investigate the mechanism of molecular recognition between protein and membranes. In the ensuing sections we highlight the utility of the Nanodisc platform through several specific examples and suggest future applications.

Abbreviations: DDM, dodecylmaltoside; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; MSP, membrane scaffold protein; OG, octylglucoside; PC, phosphatidylcholine; PL, phospholipid; POPC, 1-palmitoyl-2-oleoyl phosphatidylcholine; PS, phosphatidylserine; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; rHDL, reconstituted high density lipoprotein; SAXS, small angle X-ray scattering; SEC, size exclusion chromatography

* Corresponding author. Address: 116 Morrill Hall, 505 S, Goodwin Ave., University of Illinois at Urbana-Champaign, Urbana, IL 61801, United States. Fax: +1 217 265 4073.
E-mail address: s-sligar@uiuc.edu (S.G. Sligar).

0014-5793/$36.00 © 2009 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2009.10.024

Table 1
Proteins, detergents and phospholipids used for Nanodisc formation.

Target protein class	Phospholipids	Detergents
Single TM, seven TM, multi-TM, cytochrome P450s, multi-protein complex, peripheral, tethered	DPPC, DMPC, POPC, phosphatidylcholine (PC)/PS, PC/PE, *E. coli* lipids, Sf9 membrane, PC/PG, PC/DOTAP, soy PC, egg PC, soy asolectin	CHAPS, cholate, cymal, deoxycholate, digitonin, dihexanoyl PC, dodecylmaltoside (DDM), Emulgen 911, FOS-choline, octylglucoside (OG), sodium dodecylsulfate, Triton X-100, Tween 20

2. What is a Nanodisc?

The Nanodisc is a non-covalent assembly of phospholipid and a genetically engineered "membrane scaffold protein" (MSP) which itself is based upon the sequence of human serum apolipoprotein AI. The phospholipid associates as a bilayer domain while two molecules of MSP wrap around the edges of the discoidal structure in a belt-like configuration, one MSP covering the hydrophobic alkyl chains of each leaflet (Fig. 1). A detailed picture of the Nanodisc self-assembly process has emerged from a combination of theoretical simulations using coarse grain and whole-atom molecular dynamics and solution X-ray scattering [2,3]. A critical component, the MSP, is related to the serum apolipoproteins that are the primary component of high density lipoproteins (rHDL). The latest MSP sequences were engineered into a synthetic gene optimized for expression in *Escherichia coli* and include various affinity tags (6xHis, FLAG, Cys, etc.) and of varying lengths which control the overall Nanodisc size (see Table 2). Although a relatively new technology, we have spent considerable effort over the past few years to characterize Nanodiscs and their assemblies with integral membrane protein targets. For instance, the phospholipid bilayer and structural organization of the Nanodisc has been probed by atomic force microscopy and analyzed using small angle X-ray scattering (SAXS), confirming that the Nanodisc contains a phospholipid bilayer with MSP associated at the edge [1,4,5]. The belt organization was directly proven by solid state magic angle spinning NMR of a uniformly labeled ^{13}C,^{15}N-labeled MSP [6].

Some simple rules and relationships arise from the belt-disk organization, providing a check for self-consistency. The diameter of the Nanodisc is dictated by the length of the MSP belt at the optimum lipid content. This relationship is supported by experimental SAXS and size exclusion chromatography (SEC) on data obtained using MSPs of different lengths [4,5]. MSP and apolipoprotein AI consist of 22-mer helical repeats punctuated by

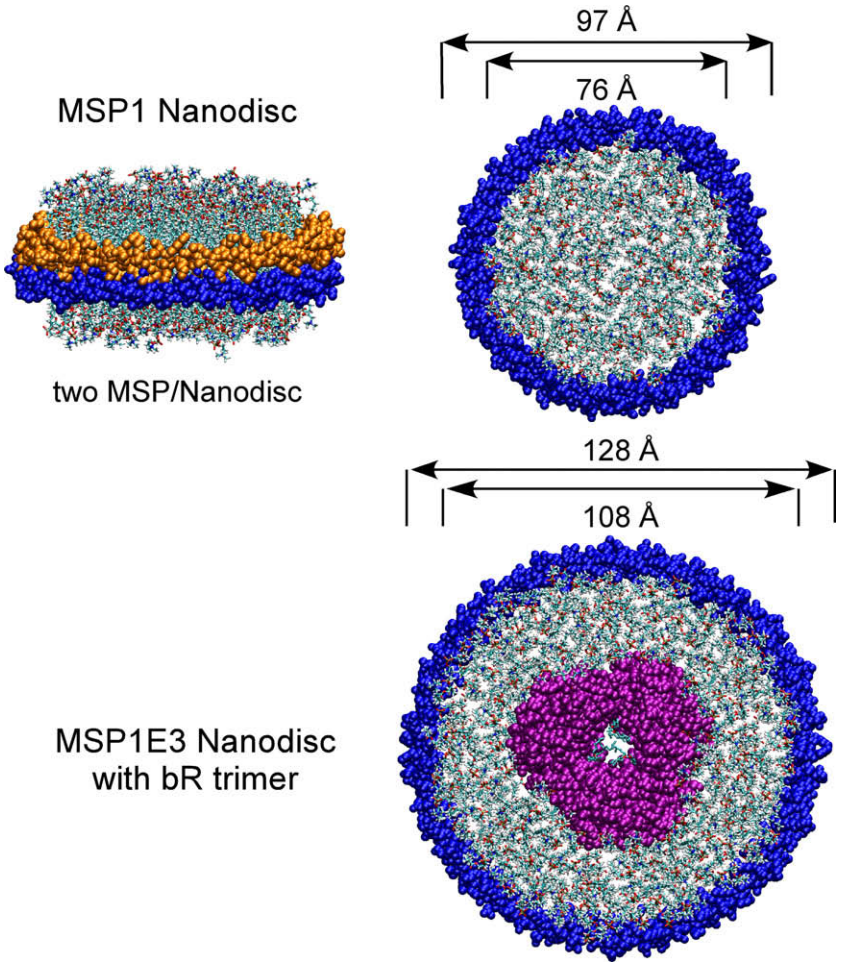

Fig. 1. Illustrations of Nanodisc structures. Top panel: Nanodiscs composed of MSP1D1 and phospholipid shown in side view and top view. The two MSPs are colored orange and blue. Bottom panel: Nanodisc composed of MSP1E3D1, phospholipid and bR trimer (21 transmembrane helices). The MSP1D1 and MSP1E3D1 structures are drawn to the same scale for comparison.

Download English Version:

https://daneshyari.com/en/article/2049295

Download Persian Version:

https://daneshyari.com/article/2049295

Daneshyari.com