Experimental report

27/04/2022

Proposal: 9-13-815 **Council:** 10/2018

Title: Refolding of the outer membrane PagP from an original surfactant-based method

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: D2O
SDS
NaCl
NaH2PO4
ethanol-d6
2-Methyl-2,4-pentanediol
Methanol-d4
PagP
Na2HPO4
Sodium dodecyl-d25 sulfate
2-Methyl-2,4-pentane-d12-diol

Instrument	Requested days	Allocated days	From	To
D22	2	2	25/09/2019	26/09/2019
			19/02/2020	20/02/2020

Abstract:

Membrane proteins (MPs) are important therapeutic targets and of biotechnological relevance because they can improve the performance of biomaterials. However, studying MPs in vitro is challenging, because it implies their overexpression under a functional form. The development of new efficient refolding techniques is urgently awaited in this field.
In that context, we have first shown that specific amphipathic cosolvents such as 2-Methyl-2,4-PentaneDiol (MPD) is able to protect proteins from Sodium Dodecyle Sulfate (SDS) denaturation, and can refold proteins from the SDS-denatured state. Several types of MPs were already refolded by using this method.
Our general objective is to understand the molecular basis of the particular assembly between SDS and MPD in order to improve its efficiency. In the present project, we would like to investigate the refolding of a model MP, PagP, from the SDS-MPD protocol. First, several d-MPD and d-SDS concentrations will be assayed to follow the refolding of PagP. Then, a similar experiment will be performed but highlighting SDS. It will provide information on the degree of SDS-protein association as a function of d-MPD concentrations.

Research proposal: Refolding of the outer membrane PagP from an original surfactant-based method (experiment 9-13-815)

Context

In the framework of protein refolding, we have studied the reliability of a new method based on the synergistic association of an anionic detergent, Sodium Dodecyl Sulfate (SDS), and a diol-type organic cosolvent, the 2-Methyl-2,4-pentanediol (MPD). Remarkably, both soluble (like hen egg-white lysozyme) and membrane proteins can be refolded by this original protocol. Generally, 1 or 2M MPD is needed to refold the SDS-denatured proteins.

Objective: Understand the molecular basis underlying the particular assembly of SDS and MPD, in order to highlight its role in the refolding process.

Results

Before analysing the refolding of proteins such as lysozyme and finally the membrane protein PagP, we first continued to analyse the effect of cosolvent on the SDS micelles. Our first experiments (see report 9-13-782) were conducted with 7mM h-SDS with various amounts of d-MPD. To evaluate the effect of SDS concentration, we used a smaller amount of SDS, 3.5mM, close to the SDS CMC (Fig.1A). In the same way as for 7mM SDS, it appears that from 0.2 to 2M MPD, the size of the SDS micelle is gradually decreasing. Large size aggregates are also observed at low scattering angles, already with 0.2M d-MPD. This phenomenon is also observed at lower SDS concentration, below the CMC (Fig.1B).

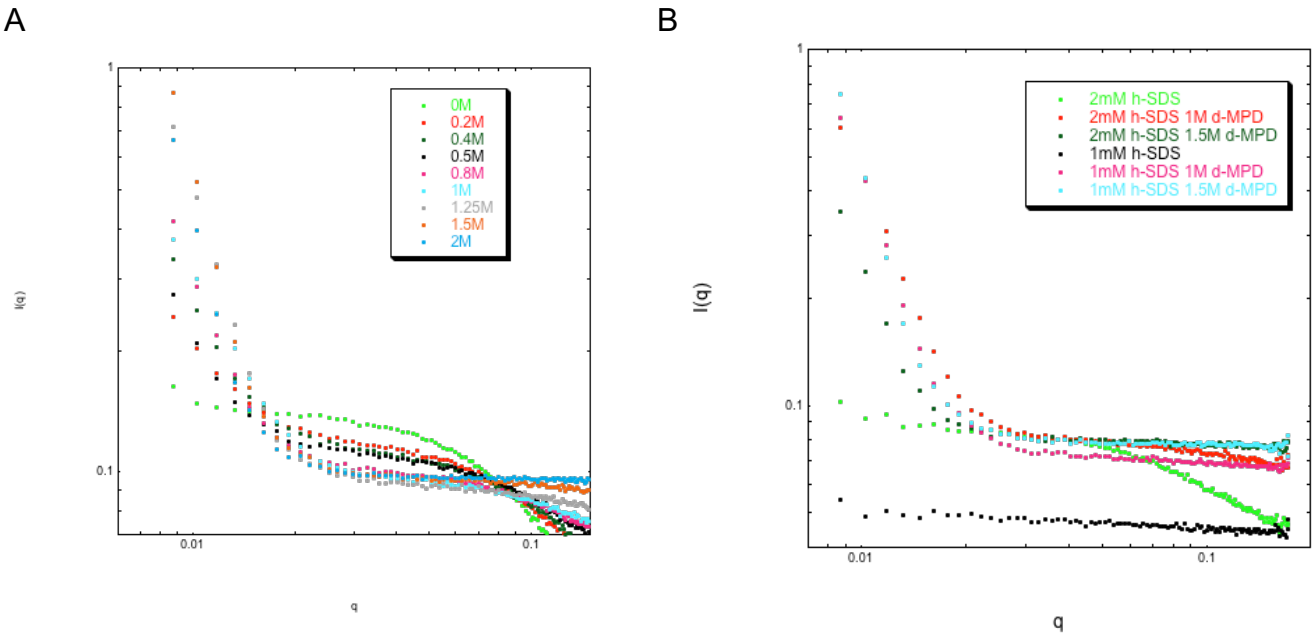
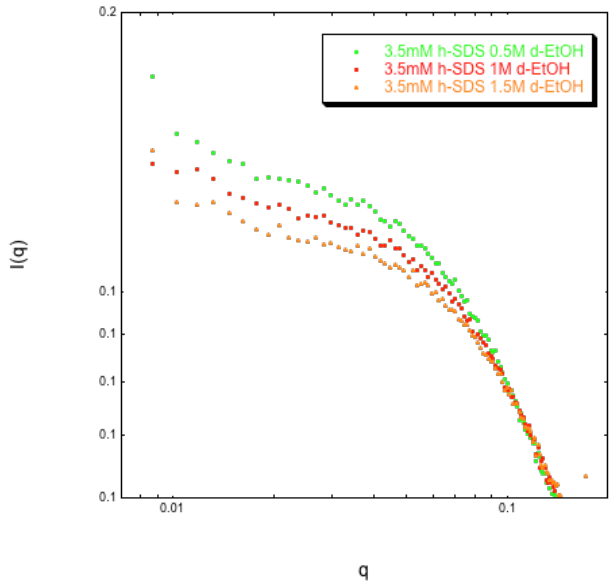


Fig1. SANS profiles of A) SDS particles (3.5mM) as a function of d-MPD concentration; B) 1 and 2mM h-SDS as a function of d-MPD

Interestingly, high concentrations of d-EtOH are not able to decrease the size of the SDS micelles and no aggregates are observed at low scattering angles. Unlike MPD, EtOH is not able to refold SDS-denatured proteins (Fig.2A). Concerning d-butan-1-ol, it decreases the size of the micelles but this effect is less pronounced than in the case of d-MPD. From 0.5M d-butanol, a scattering is also observed at low angles (Fig.2B).

A



B

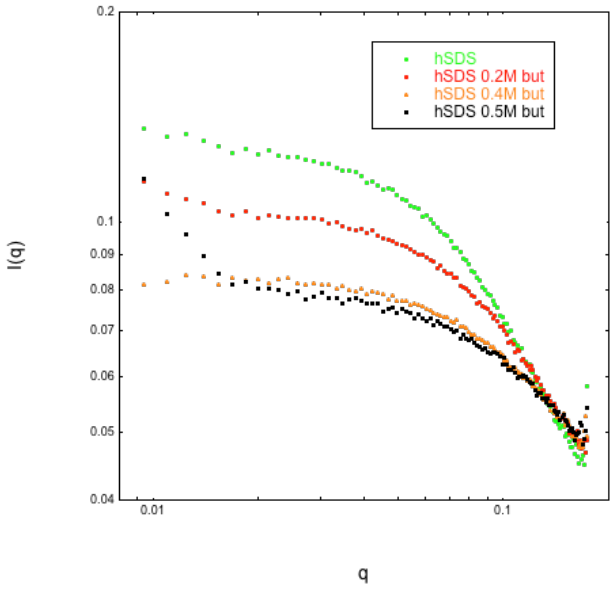
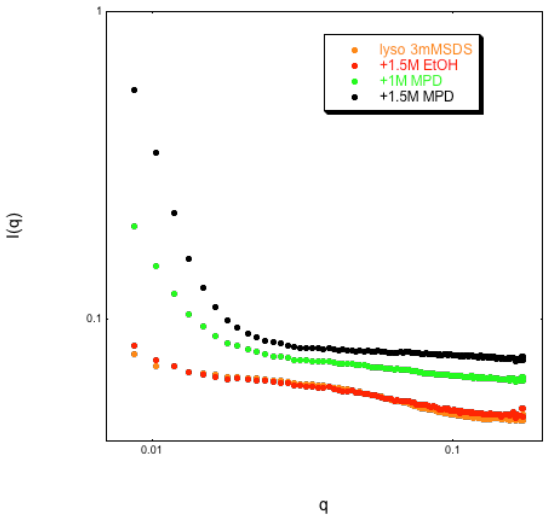


Fig.2 SANS profiles of 3.5mM h-SDS with various concentrations of A) d-EtOH; B) d-butane-1-ol.

In a second part, we have reproduced (see report 9-13-782) the SANS experiment showing the refolding of the d-SDS-denatured hen egg-white lysozyme by d-MPD. We have also shown that d-EtOH is not able to refold the protein (Fig.3A). High scattering is again observed at low angles with d-MPD. Finally, first experiments were conducted with the membrane protein PagP (Fig.3B).

A



B

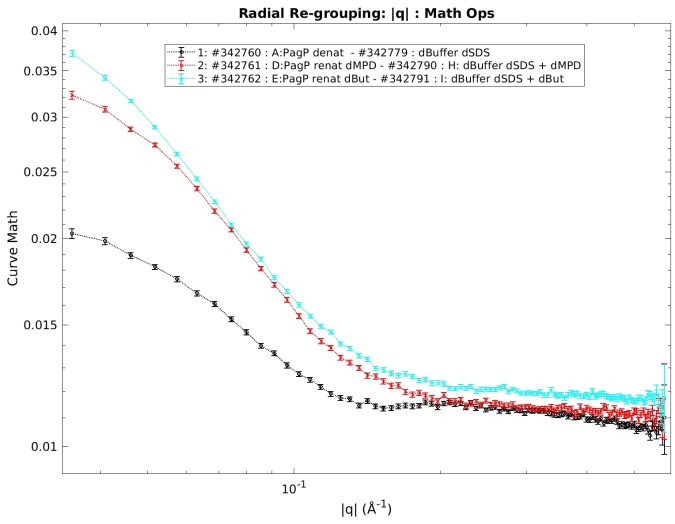


Fig.3 SANS profiles of A) 3mM SDS-unfolded Lysozyme as a function of d-MPD or d-EtOH concentration; B) 3mM SDS-unfolded PagP as a function of d-MPD or d-butanol concentration