Experimental report

09/03/2024

Proposal: 9-13-1062 **Council:** 10/2022

Title: Solubilising protein nanoconstructs in deep eutectic solvents with tailored hydrophobicity

Research area: Soft condensed matter

This proposal is a new proposal

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Local contacts: Sylvain PREVOST

Samples: choline chloride:glycerol
Myoglobin with polymer surfactant corona
Tetrabutylammonium chloride:decanoic acid
Tetrabutylammonium chloride:glycerol
Choline chloride:acetic acid

Instrument	Requested days	Allocated days	From	To
D33	2	1	31/03/2023	01/04/2023
D22	2	0		

Abstract:

Deep eutectic solvents (DESs) have emerged as promising solvents for the solubilization and preservation of biomolecules, in particular proteins. DESs are obtained through the complexation of simple organic molecules, where the physicochemical properties of the solvent can be tailored through different combinations of precursors. Although their tailorable character expands the potential range of bio-related applications of DESs, we found that some DESs lead to rapid protein denaturation or show very low protein solubility. To overcome these, we began an investigation on the behaviour of protein-polymer-surfactant nanoconstructs in DESs. These nanoconstructs allow stabilising proteins in different DESs with increased stability compared to aqueous buffers and wild-type proteins. Here, we will investigate the conformation of two protein nanoconstructs, modified myoglobin and lipase, in DESs with a wide range of properties, from hydrophilic to hydrophobic. The results from this investigation will be used to elaborate a framework to design biobased systems using DES, where the properties of the solvent can be optimised for new applications.

Solubilising protein nanoconstructs in deep eutectic solvents with tailored hydrophobicity

Introduction

Deep eutectic solvents (DESs) have emerged as promising solvents for the solubilization and preservation of biomolecules, in particular proteins. DESs are obtained through the complexation of simple organic molecules, where the physicochemical properties of the solvent can be tailored through different combinations of precursors [1]. Although their tailorable character expands the potential range of bio-related applications of DESs [2,3], we found that some DESs lead to rapid protein denaturation or show very low protein solubility. To overcome these, we began an investigation on the behaviour of protein-polymer-surfactant nanoconstructs in DESs. These nanoconstructs allow stabilising proteins in different DESs with increased stability compared to aqueous buffers and wild-type proteins. Here, we have investigated the conformation of a model protein nanoconstructs, modified myoglobin, in DESs with varied hydrophobicity.

Experiment

The experiment performed on D33 (ILL) was focused on the study of the conformational landscape of modified myoglobin, [C-Mb][S], in three DESs with systematically varied hydrophobicity: 1:2 d-choline chloride:d-glycerol (aka. 1:2 ChCl:Glyc) – hydrophilic, 1:4 d-tetrabutylammonium chloride:d-glycerol (aka. 1:4 TBAC:Glyc) – partly water soluble, and 1:2 d-tetrabutylammonium chloride:d-octanoic acid (aka. 1:2 TBAC:OctAc) – hydrophobic. In addition, we have performed measurements using the temperature-controlled oil bath to test the conformational resilience of the protein to heat stress (75 and 125 °C) and at room temperature after submitting the samples to high temperatures. Control measurements were performed using wild-type myoglobin (Mb) and [C-Mb][S] in deuterated aqueous buffer.

Results

Figure 1 shows the SANS data of [C-Mb][S] in the different DESs at increasing temperatures. By observing the scattering curves, the nanoconstruct changes conformation with varying the constituents of the solvent. In the hydrophilic 1:2 ChCl:Glyc, the nanoconstruct retains a globular structure and forms a discrete fractal network, whereas the hydrophobic DESs cause the partial unfolding of the nanoconstruct and the stabilisation of a molten globule. Notably, the structure of [C-Mb][S] in 1:2 ChCl:Glyc is very similar to that in aqueous buffer.

One striking result is the enhancement of the thermal resilience of the protein. While Mb melts at 58 °C in aqueous buffer, the SANS results show an improvement of the denaturation temperature. In the hydrophilic DES at 75 °C, [C-Mb][S] shows a similar monomeric structure to that at room temperature, although the clustering vanishes. At 125 °C, the protein unfolds and

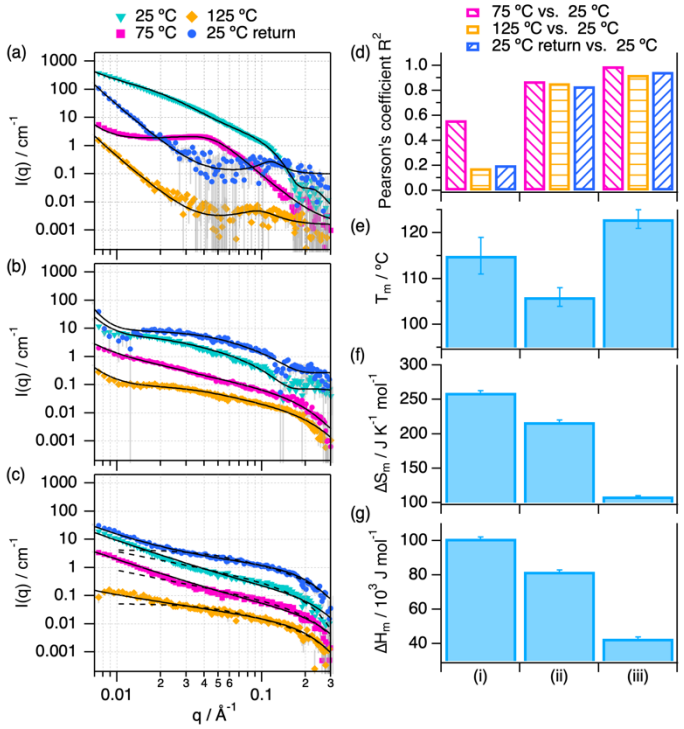

Figure 1 SANS results and best fits for [C-Mb][S] in (a) 1:2 ChCl:Glyc (i), (b) 1:4 TBAC:Glyc (ii), and (c) 1:2 TBAC:OctAc (iii). (d) Pearson's correlation coefficient quantifying the change in the scattering signal at different temperatures, where closer values to 1 indicate a greater similarity. The thermodynamic parameters of unfolding derived from the SANS results and complementary far-UV circular dichroism measurements.

irreversibly aggregates. In contrast, the stabilisation of the molten globule in the hydrophobic DESs is extreme resilient even at 125 °C (Figure 1d), with no further unfolding or aggregation.

Correlating the SANS results and synchrotron radiation far-UV circular dichroism (CD), we derived the thermodynamic parameters of protein unfolding (Figure 1e, f, g). The results confirm the stabilisation of the nanoconstruct by incorporation into the DESs. The results in 1:2 TBAC:OctAc are particularly impressive, with a 2-fold increment in the half-denaturation temperature of the nanoconstruct. The SANS results confirmed that the stabilisation comes from a highly flexible conformation, where the nanoconstruct can adapt to the gradual heating and hinder the thermal degradation and aggregation of the protein.

Finally, we studied how the protein nanoconstruct responds to cooling down to room temperature after remaining at high temperature for at least 2 h (the time required to measure the full sample rack). These experiments showed that the unfolding and aggregation observed in 1:2 ChCl:Glyc was irreversible as the initial conformation was not retrieved. In contrast, the two hydrophobic DESs not only protected the protein against severe denaturation, but also the nanoconstruct recovered the same initial conformation after cooling. This agrees with our CD results, which showed the ability of 1:2 TBAC:OctAc to refold the protein even after heating the sample to 190 °C. These values of thermal stabilisation and refolding are unmatched, to the best of our knowledge.

Conclusion

The outcome of this experiment has been highly positive with a major contribution from our local contact, Sylvain Prevost. Furthermore, the data obtained is of high quality with a good signal-to-noise ratio. Unfortunately, the limitations of the oil-bath sample environment did not allow to collect data at the expected temperatures, i.a., > 150 °C. Hopefully, follow up studies will allow us to explore higher temperatures using the new temperature-controlled Peltier sample stage.

This study is an important part of the systematic investigation of the behaviour of proteins in DESs. A manuscript is in the final steps of preparation and will soon be submitted for publication, likely targeting high impact factor journals (e.g., *Chem, J. Amer. Chem. Soc., Angew. Chem.*).

This project will continue with future investigations on exploring new enzymatically active protein nanoconstructs, aiming to determine the structure-function relationships and open new avenues in the development of biomaterials.

References

[1] Hansen *et al.*, *Chem Rev* **2021,** *121* (3), 1232-1285.
[2] Sanchez-Fernandez *et al.*, *Green Chem.* **2022,** *24* (11), 4437-4442.
[3] Sanchez-Fernandez *et al.*, *J. Am. Chem. Soc.* **2022,** *144* (51), 23657-23667.