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Thermal stability of AOT w/o microemulsion occluding proteins

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Introduction

Structure and function of water-in-oil (w/o) microemulsions occulding proteins have been attracting significant interests concerning with possible practical applications such as microreactors because proteins entrapped in w/o microemulsions ocassionaly show much higher catalytic activity, so-called super-activity, in comparison with the case of those in usual solutions. We have been studying the relation between the catalytic activity of proteins within the water pool of w/o microemulsion and the w/o microemulsion structure, where we have treated *N*-(2-hydroxyethyl)piperazine-*N*'-(2-ethanesulfonic acid) (Hepes) buffer / sodium bis(2-ethylhexyl)sulfosuccinate (AOT) / 2,2,4-trimethylpentane (isooctane) microemulsion systerm. We have already clarified the following points. 1) Catalytic activity of proteins within water/AOT/isooctane microemulsion is enhanced at low water content in the w_0 (= $[H_2O]/[AOT]$) range of 8-16 [1]. 2) Proteins within the micro-emulsion mostly hold native-like secondary structures and there exists an optimized water pool radius which depends on protein size [2]. 3) The microemulsion takes three different phases, that is, oligomeric phase, transient phase and monomeric phase, successively with increasing w_0 value, and the super-activity apprears at transient phase [3]. 4) The dynamics of the microemulsions is enhanced at transient phase. These previous studies suggest that the presence of the transient phase and the enhancement of the bending fluctuation of the microemulsion would induce the increase of an effective surface area of enzymes for the contact with substrates [4], which would result in the acceleration of the metabolic turnover. Then, we have carried out further SR-SAXS experiments to examine the thermal stability AOT microemulsions entrapping proteins.

Experimental

AOT was purchased from Nacalai Tesque Inc. Apolar solvent was 97+ % n-octane, purchased from Wako Pure Chemical Industries Ltd. The protein was α-chymo-trypsin from bovine pancreas produced by Sigma Chemical Co. The AOT microemulsions were prepared by using an injection method. The w_0 values of the samples were varied from 0 to 30. The AOT molar concentrations were 0.1 M for all samples. α-Chymotrypsin was solubilized in 10 mM Hepes buffer adjusted at pH 8.0. The molar concentration of α-chymotrypsin in the samples was varied from 1.83x10^{-5} M to 8.75x110^{-5} M at each w_0 value SR-SAXS experiments were carried out by using a SAXS equipment installed at the SR source at the High Energy Accelerator Research Organization, Tsukuba, Japan.

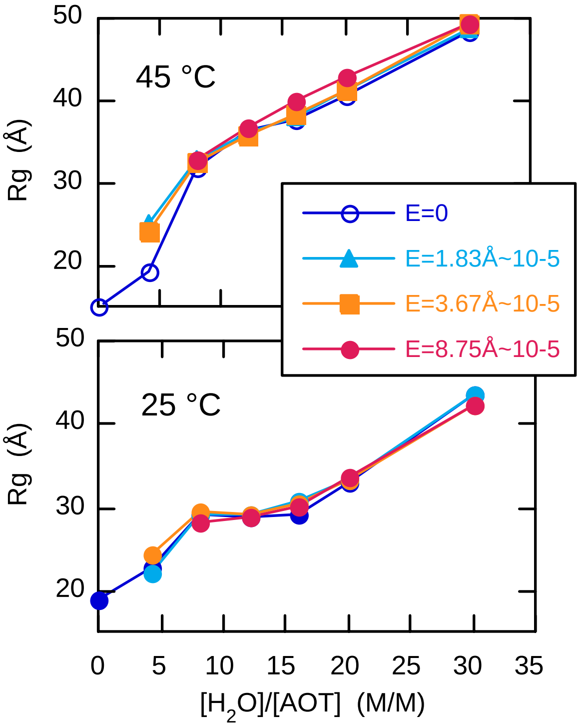

Fig. 1 Protein concentration dependence of $p(r)$ function (a) and $p(r)_{max}$ (b). In (a), water/AOT/*n*-hexane at w_0 = 20.

Results and Didcussions

The thermal stability of AOT microemulsions with proteins (filled micelle) or without proteins (open micelle) has been studied in the temperature range from 25 ˚C to 45 ˚C. With elevating temperature the transient phase region tends to expand to high water-content for both filled and open micelles. The existence of proteins in the water pool weakens the thermal expansion of the microemulsion radius.

References

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