Effect of protein occlusion on w/o microemulsion structure

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Introduction

Enhancement of catalytic activity, so-called super-activity, of enzymes entrapped in w/o microemulsions has attracted significant interest concerning not only with future practical applications such as microreactors but also with biophysical catalytic mechanisms of enzymes at a limiting condition such as a water pool of w/o microemulsion. By using SR-SAXS and enzymatic activity measurements we clarified that the catalytic activity of α-chymotrypsin entrapped in the water/AOT /isooctane microemulsion is enhanced at low w_0 (= [H_2O]/[AOT]) range of 8-16 and that the three different phases (oligomeric phase, transient phase and monomeric phase) appear successively with increasing w_0 value [1]. As shown in other SR-SAXS study [2], there exists the penetration limit of apolar solvent depending on the linear hydrocarbon chain length, which results in the shift of the w_0 value of the above phase boundaries. 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, which would result in the acceleration of the metabolic turnover [3]. Then, we have carried out further SR-SAXS experiments to examine more precisely the structural features of the various AOT microemulsions entrapping enzymes.

Experimental

AOT was purchased from Nacalai Tesque Inc. Apolar solvents used were 96.5+ % *n*-hexane, 99.9+ % *n*-heptane and 97+ % *n*-octane, which were purchased from Wako Pure Chemical Industries Ltd. The enzyme used was α-chymotrypsin from bovine pancreas, type II produced by Sigma Chemical Co. Water was purified by a Millipore system. The AOT microemulsions were prepared by using an injection method. The w_0 values of the samples were varied from 0 to 50. The AOT molar concentrations were 0.1 M for all samples. *a*-Chymotrypsin was solubilized in 10 mM Hepes buffer adjusted at pH 8.0. The molar concentrations of *a*-chymotrypsin in the samples were varied from 2.4×10^{-5} M to 2.8×10^{-4} M depending on w_0.

SR-SAXS experiments were carried out by using a small-angle X-ray scattering equipment installed at the synchrotron radiation source (PF) at the High Energy Accelerator Research Organization (KEK), Tsukuba, Japan. We carried out the standard analyses for the obtained scattering data as given elsewhere. To estimate structural changes of the AOT microemulsion depending on both α-chymotrypsin concentration and apolar solvent, we calculated the distance distribution functions $p(r)$ by the Fourier transform of the scattering intensity $I(q)$.

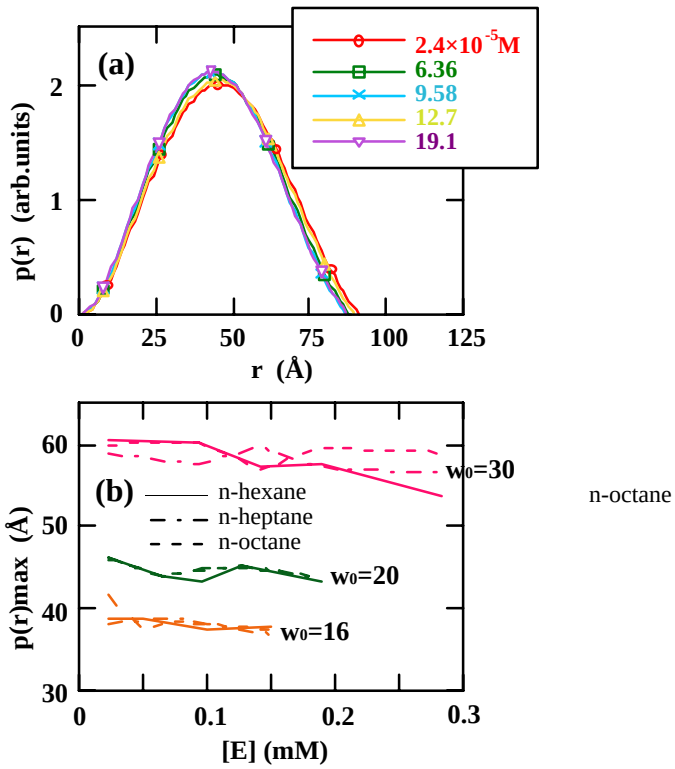

Fig. 1 Protein concentration dependence of $p(r)$ function (a) and $p(r)_{max}$ (b). In (a), water/AOT/*n*-hexane at w_0 = 20.

Results and Didcussions

In Fig. 1 the protein concentration dependence of the $p(r)$ function and $p(r)_{max}$ shows that the occlusion of the proteins tends to decrease the microemulsion radius, which is more clearly seen with increasing water content or with shortening the hydrocarbon chain length. This would result from the attractive electrostatic interaction between the polar head of AOT and the basic residues of the protein surface.

References

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