[C3. K. Yoshida] Hydration water in dynamics of a hydrated beta-lactoglobulin

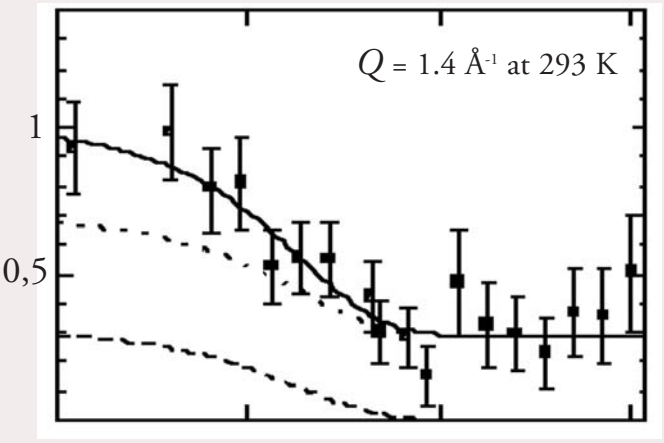

Figure 1. Typical intermediate scattering functions I(Q, t) / I(Q, 0) at Q = 1.4 Å^{-1} at 293 K. The solid line indicates the fitting results by the KWW equation. The upper and lower dashed lines are contributions from the protein and the surface water, respectively

It is well-known that water plays an important role in protein folding and function of proteins. Although computer simulation is a powerful tool to investigate the dynamics of protein and hydrated water, its result depends on the interatomic potential used in the calculation. Therefore, it is important to verify simulation results by comparing the calculated intermediate scattering function I(Q,t) with that obtained from experiment. In the present study, incoherent spin echo signals of a hydrated β-lactoglobulin protein were measured at 275 and 293 K. In the measured protein, ~69 % of water exists on the surface of the protein and the rest remains as the bulk. The intermediate scattering functions were divided into two contributions from surface water and protein, respectively, as shown in Figure 1. On one hand, the dynamics of the surface water follows a Kohlrausch-Williams-Watt (KWW) stretched exponential function (the exponent is ~0.5), on the other hand, that of the protein follows a single exponential. The behavior of elastic incoherent structure factor (EISF) as a function of Q shows the feature of the confined diffusion. The present results are consistent with our previous results of hydrated C-phycocyanin combining elastic and quasielastic neutron scattering and by molecular dynamics simulation. Moreover, the behavior of surface water is similar to that of water confined in hydrophilic porous materials. We can stress that water confined in hydrophilic porous materials is an adequate model to investigate water in biomolecules.

[Collaboration : K. Yoshida, T. Yamaguchi, Fukuoka Univ. , Japan; M.-C. Bellissent-Funel, S. Longeville, LLB]

[C4. G. Gibrat] Thermal denaturation of apo-calmodulin

Calmodulin is a small (16.7 kDa) calci-protein (a protein that can fix calcium ions) that is well adapted to neutron scattering experiments. Indeed, it allows reaching concentrations of about 100 g/L in physiological-like conditions (pH 7.5 and [KCl] ~ 100mM) without any aggregation. Moreover calmodulin is made of two N- and C-terminal domains, with a 70% sequence homology, linked by a central _-helix. Despite the high sequence homology, these two domains show significantly different stabilities (about 10°C difference in thermal denaturation temperatures). It is so an interesting system to study the sequence-folding relationship.

From fluorescence, circular dichroïsm and UV absorption spectroscopy experiments, it is quite clear that for apo-calmodulin (calmodulin without calcium) thermal denaturation occurs at least in two steps, corresponding to the successive unfolding of the two N- and C-terminal domains (respectively Tm=63°C and Tm=51°C). Holo-calmodulin (calmodulin with calcium) is stable up to 100°C. From SANS measurements, it appears that apo-calmodulin looses progressively its structure between 40°C and 80°C. At high temperature, apo-calmodulin adopts a "polymer-like" conformation (SANS spectrum follows a Debye law for QRg<3), with a radius of gyration of 32Å. However, the high Q exponent of 2.3 suggests the existence of residual secondary structures, also seen by circular dichroïsm. Indeed the 2.3 value is between polymer chain values (1.7 or 2) and the compact chain value (4).

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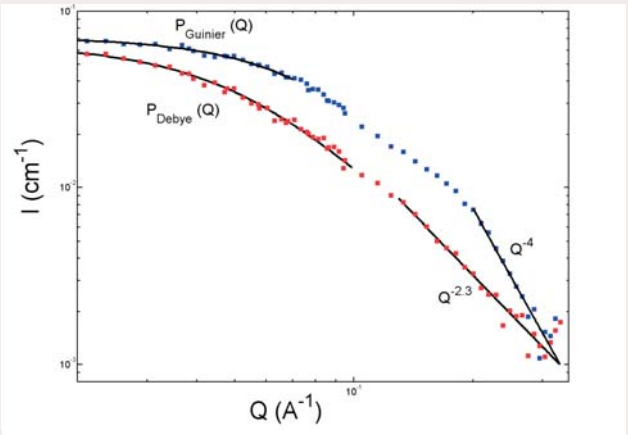

Figure 1. SANS spectra of native apo-calmodulin (in blue) and of calmodulin at 80°C (in red)

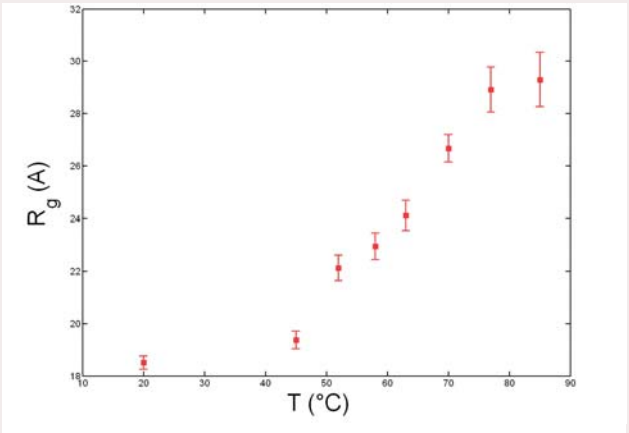

Figure 2. Radius of gyration of apo-calmodulin at 5g/L as a function of temperature