Probing the structure of the deuterated protein adsorption layer on modified surfaces with neutron reflection

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The structure of adsorbed globular protein layer on modified surfaces was probed by using neutron reflection at ILL (France) and ellipsometry techniques. Two proteins were used: a fully deuterated protein, myoglobin (Mb), and a hydrogenated protein Human Serum Albumin. The fully deuteration of the myoglobin allows to increase the contrast between the phases and, in turn, a better resolution of the structure for the neutron reflectivity experiment. Different surfaces have been investigated from hydrophobic surfaces (obtained by grafting self-assembled monolayers of octadecyltrichlorosilane (OTS) and polystyrene) to poly(ethylene glycol) (PEG) of different grafting density. For some systems, different protein concentrations were employed (from 1 mg/ml to 0.01 mg/m). We observe on the hydrophobic interface a change of the conformation of the protein layer depending on the hydrophobicity of the substrate. A two-layer structure for the adsorbed layer is also observed for the higher concentrations.

Onto PEG brushes different adsorption modes would be possible: onto the surface, onto the brush surface and in the brush chains. NR combined with deuteration of the protein allows the investigation of the different adsorption modes. Primary adsorption (onto the surface) of Mb was clearly observed. A two-layer structure was also observed for the lower PEG grafting density. While the total adsorbed amount depends on the PEG grafting density, the protein outer-layer is both connected to PEG density and N. The kinetics of the adsorption measured with ellipsometry showed that while the adsorption kinetics is very fast on the polystyrene substrate, on PEG surfaces the adsorption is slower depending on the grafting density of the brush. The rate of adsorption decreases also exponentially with the adsorbed protein.