Experimental report

13/02/2017

Proposal: 9-13-640 **Council:** 4/2016

Title: Effects of pH on the Structure of beta-Lactoglobulin at Air/Water andOil/Water Interfaces

Research area: Chemistry

This proposal is a new proposal

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Samples: beta-lactoglobulin

Instrument	Requested days	Allocated days	From	To
FIGARO User-supplied	4	4	16/09/2016	20/09/2016

Abstract:

The whey protein β-Lactoglobulin (BLG) is one of the most frequently studied biomacromolecules. The strong effects of pH on the interfacial behavior of BLG (and proteins in general) and on the stability of foams and emulsions are well-recognized issues in the literature but still remain not fully understood. Our preliminary work show that variations of the solution pH lead to non-monotonic changes in the dynamical, mechanical and electrical properties of the interfacial layer, which in turn affect foam stability. To deeper understand the unique pH dependent interfacial behavior of proteins, we plan to study the structure of BLG interfacial layers on the molecular scale. This issue can be resolved by using a range of non-invasive techniques among which is the neutron reflectometry (NR). Hence, we propose comparative NR studies of BLG adsorption layers at the water/air interface as related to foam stability. As a second goal in the project, we plan to investigate this effect also at the water/tetradecane interface (as related to emulsion stability) and to make a comparison between the two types of interfaces. Such information is not accessible by any other experimental methods.

The whey protein β-Lactoglobulin **(**BLG) is a major component in milk protein fraction; therefore it is of great interest in e.g. food technology as an efficient stabilizer of foam and emulsions. The strong effects of pH on the interfacial behavior of BLG (and proteins in general) and on the stability of foams are well-recognized issues in the literature but still remain only partially understood. Variation of the solution pH leads to non-monotonic changes in the dynamical, mechanical and electrical properties of the interfacial layer, which in turn affect foam stability.[1,2] Deeper understanding of the unique pH dependent behavior of BLG at the water/air (W/A) interface requires structural information on the molecular scale. However, one can hardly conclude a robust pH-dependent trend in the structure of BLG layers on the basis of literature results[3-7].

During beamtime 9-13-640 we therefore systematically studied BLG adsorption layers at the W/A interface using neutron reflectometry (NR) at pH-values below (pH 3), close to (pH 5) and above (pH 7) the BLG isoelectric point in solution (p*I* ~5.1)[1]. The measurements for the first time also allowed us to follow the dynamics of adsorption.

The surface layers were studied for solutions at two BLG concentrations (C_{BLG} = 10^{-5} and 10^{-7} M) and at constant buffer (Na_2HPO_4/Citric acid) concentration of 10 mM. The 6-position sample changer of FIGARO was used and the samples were measured in both D_2O and air contrast matched water (ACMW – 8.1% v/v D_2O in H_2O) in order to gain unambiguous structural results. For each protein concentration, the six troughs (T1 – T6) were filled with sample solutions (S) in ACMW and D_2O in the way shown in the following table:

C_{BLG}	T1 **ACMW, pH 5**	T2 **ACMW, pH 7**	T3 **ACMW, pH 3**	T4 **D_2O, pH 5**	T5 **D_2O, pH 7**	T6 **D_2O, pH 3**
10^{-7} M	(S1)	(S2)	(S3)	(S4)	(S5)	(S6)
10^{-5} M	(S7)	(S8)	(S9)	(S10)	(S11)	(S12)

Therefore, in two rounds we measured 12 samples in total (S1-6 and S7-12).

The experiments with ACMW allowed for estimation of the adsorbed amount Γ of protein. Initially, therefore, only the first three positions were used at a time for continuous measurements by periodic scans on each of them so that the initial changes in adsorption were resolved. The data for the two protein concentrations are shown in Fig. 1A; Fig 1B shows complementary data on the dynamic surface pressure Π(t) of these solutions for comparison purposes.

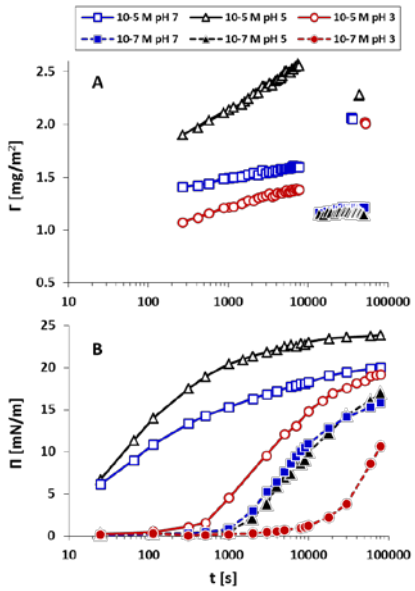

Figure 1. *Kinetic dependences of A: the adsorbed amount Γ(t) and B: the surface pressure Π(t).*

The data for C_{BLG} = 10^{-5} M (S7-9) show continuous increase of the adsorbed amount Γ(t) with time – in agreement with the dynamic surface pressure Π(t) data in Fig. 1B – except the case of pH 5 where Γ was found to decrease after long time (while the values of Π remain steady). Such behaviour has not been reported before, and the adsorption of proteins is commonly thought to proceed as an essentially irreversible process.

The observed decrease of Γ(t) for S7 may be related to (bulk and/or surface) aggregation leading to protein desorption and deserves further detailed investigations. To this end we suggest monitoring the kinetic dependence Γ(t) at pH 5 systematically for several BLG concentrations, thus obtaining the onset and the magnitude of the decrease of Γ that seem to be concentration dependent. In addition, experiments with pre-formed BLG aggregates with well-defined shape and size could be very informative in this regard, revealing the surface behaviour of such aggregates, which can be compared with the current results.

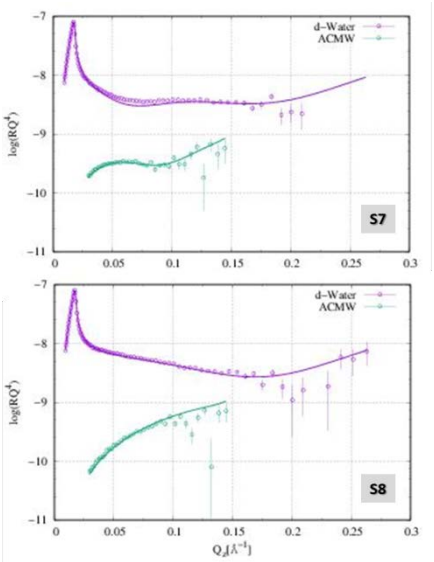

Figure 2. *Exemplary reflectivity curves (symbols) and best fits (lines).*

After at least 18 hours of adsorption we measured all six samples (see the table above) in the full accessible q_z-range and with higher statistics, in order to resolve structural details of the interfacial layers – so for the two protein concentrations we obtained 12 reflectivity curves. For a given pH and C_{BLG}, we simultaneously fitted the reflectivity curves obtained both in ACMW and in D_2O (Fig. 2 shows two examples); note that optimization of the fits is still under progress but we do not expect qualitative changes. Fig. 3 shows the results in terms of protein volume fraction Φ distribution in direction z normal to the plane of the interface $z = 0$. Almost all the reflectivity data could be well fitted with a one-slab model describing a rather thin and dense layer of adsorbed proteins. However, for the case of S7 it was necessary to introduce a second slab, representing an additional, thicker but more dilute protein layer adjacent to the dense layer (Fig. 3, bottom right). Such a multilayer structure was revealed earlier by ellipsometry measurements[1].

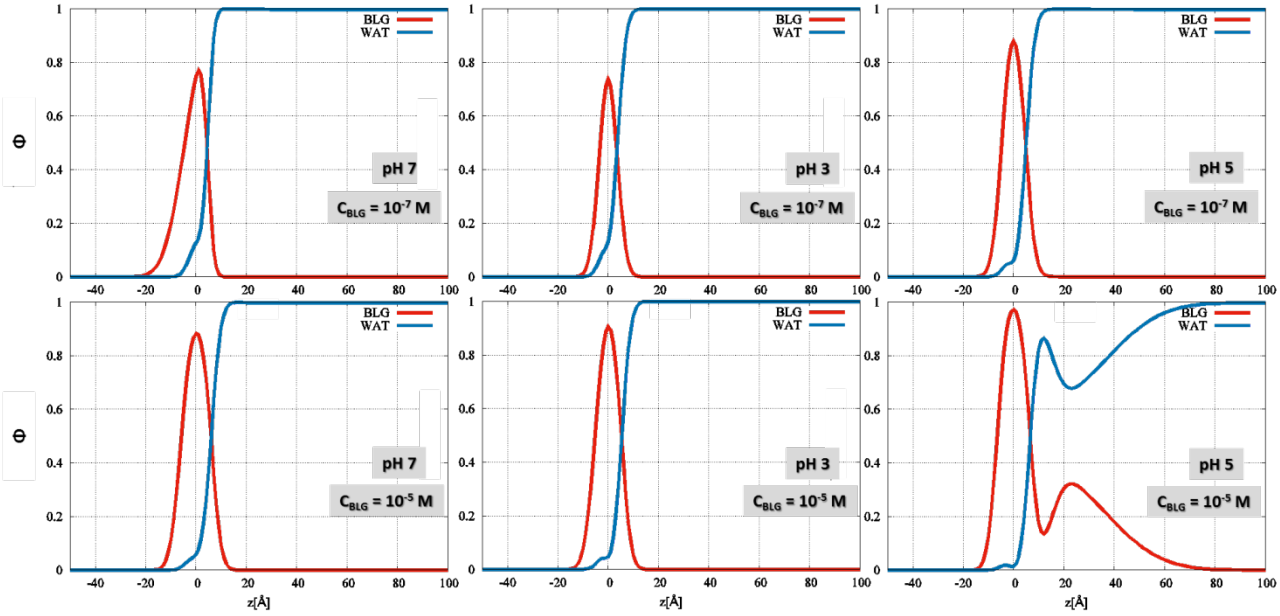

Figure 3. *Volume fraction Φ(z) profiles of BLG at the water/air surface as affected by the protein concentration and pH of the sample solutions (time of adsorption > 18 hours).*

Interestingly, the reflectivity curves for S7 could not be fitted via a "uniform two-slab model" but were well fitted only via a model allowing for a water gap between the primary monolayer and the secondary protein layer. Correspondingly the Φ(z) profile for BLG exhibit a dip which matches a peak in the profile for water (solution) both suggesting that the "two-slab" surface protein structure comprises certain amount of solution in between.

Refrences

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