ESRF	**Experiment title:** The formation of particulate gels by bovine β-lactoglobulin	**Experiment number**: SC1127
Beamline:	**Date of experiment**: from: 02/12/02 to: 9/12/02	**Date of report**: 25/02/04
Shifts: 12	**Local contact(s)**: Dr Wim Bras and Dr Igor Dolbyna	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

E.H.C. Bromley*

M.R.H. Krebs*

A.M. Donald

A.J. Ryan

P&C Group, Cavendish Lab., University of Cambridge, CB3 0HE, Cambridge, UK

Department of Chemistry, University of Sheffield, Sheffield, South Yorkshire, S3, 7HF UK

Report:

β-lactoglobulin (BLG) is of great interest to the milk industry, as it is an abundant component of milk and is thought to be responsible for the fouling of milk when milk is heat-treated during its processing. As BLG is easily purified in large quantities, it is has been studied in great detail [1].

Heating of BLG solutions results in the formation of gels that fall into two categories: fine stranded gels at low pH and particulate gels at pH 4-6.5. In this work, the structure and mechanism of formation of the particles in the particulate gels was studied. The particles vary in size and polydispersity depending on the exact conditions of formation. Figure 1 shows a representative electron micrograph. The experimental setup during our time at the ESRF proved ideal to investigate their internal structure. The size-range accessible (10-70 nm) was on the order of 10's of protein molecules, providing insight into how individual protein molecules are arranged relative to each other, without being influenced significantly by the internal (secondary and tertiary) structure of the proteins themselves.

Some of the plots of scattering intensity vs. the magnitude of the scattering vector q are shown in Figure 2. When plotted on a log-log scale, the curves could be fit with power law functions, yielding exponents between -3.6 and -3.9. Power law exponents in this range are generally associated with compact objects with fractally rough surfaces [2]. This suggests the particles have fairly compact interiors with either fractal surfaces or pore structures. This also rules out Diffusion Limited Association (DLA) as an aggregation mechanism, as particles formed by this mechanism would have power law exponents of around -2 [3].

As the temperature is increased beyond that of the onset of aggregation at 74C, the exponent of the power law increases (Figure 3). This corresponds to a decrease in the surface fractal dimension, most likely by a process of infilling: 'decoration' of already existing structures formed at lower temperatures [4].

Together with other data, obtained using ESEM, we therefore propose the particles form by a nucleation and growth-based mechanism. The nucleating species is likely to be the unfolded protein, whose concentrations increases as the temperature is raised.

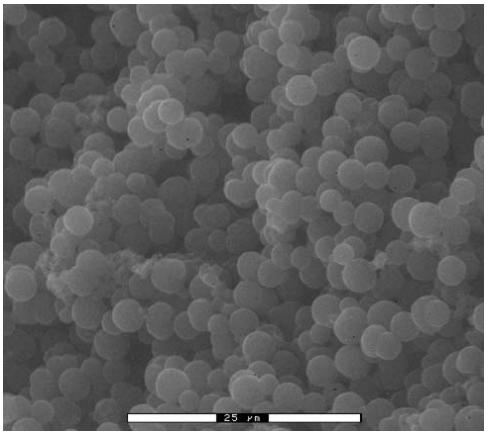

Figure 1
An environmental scanning electron micrograph showing a 2% (w/w) BLG gel which has been set at 75ºC. The particles appear relatively monodisperse in size. Their internal structure cannot be discerned. Scale bar represents 25 µm.

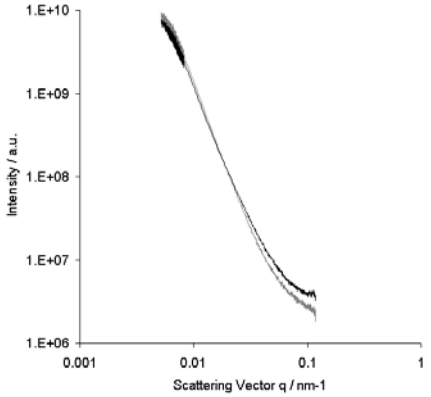

Figure 2
Scattering intensity as a function of scattering vector q for particulate gels formed at pH 5.12 (■) and pH 5.25 (■). The lower q region is limited by the position of the beam stop and has been removed. The straight portion of the graph can be fitted with a power law.

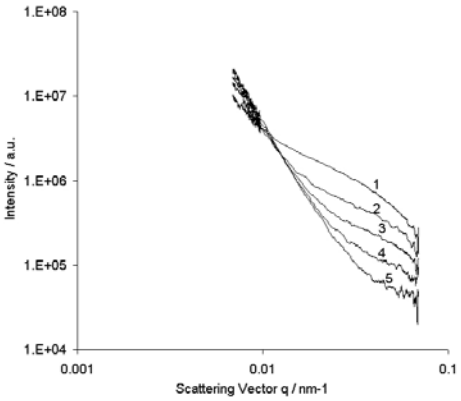

Figure 3
Scattering intensity as a function of scattering vector q at various temperatures for a pH 5.3, 7% (w/w) solution heated *in situ*. Temperatures shown are 74ºC (1), 80ºC (2), 81ºC (3), 82ºC (4) and 84ºC (5). With increasing temperature, scattering from long length scale correlations increases, while that from small lengthscales decreases. The change in gradient indicates a change in the structure of the particles present in solution.

References

[1] Sawyer, L. and Kontopidis, G. (2000) *Biochim. Biophys. Acta*, **1482**, 136
[2] Keefer, F.D. and Schaefer, D.W. (1986) *Phys. Rev. Lett.*, **56**, 2376
[3] Verheul, M. and Roefs, S.P. (1998) *J. Agr. Food. Chem.*, **46**, 4909
[4] Vicsek, T (1992), "Fractal Growth Phenomena", 2nd Edition, World Scientific, Singapore