Supporting information to

Interfacial properties of fractal and spherical whey protein aggregates

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SANS models

The scattering from a polydisperse or non-spherical system can be calculated using approximation approaches. The "average structure factor approximation" and the "decoupling approximation" are the most used ones.[1] In the latter, it's assumed that interactions are independent of particle size and orientation. Therefore, the scattering intensity from aggregates formed at 80 °C has been calculated using the decoupling approximation. In addition, it has been found that β-lactoglobulin dimers dissociate into monomers when heated.[2, 3] So, scattering curves are modeled using the form factor of a polydisperse sphere (elementary unit or monomer) and a mass fractal structure factor (Fig. 3):

$$S(q) = 1 + \frac{D}{r_0^D}\int_0^\infty r^{D-3} h(r,\xi)\frac{\sin(qr)}{qr} r^2 dr \qquad 1$$

with the fractal dimension, D, the radius of the monomer, r_0, and the Gaussian cut-off function, $h(r, \xi)$

$$h(r,\xi) = \exp\left[-\left(\frac{r}{\xi}\right)^2\right] \qquad 2$$

with the cut-off length for the fractal correlations, ξ, a characteristic distance above which the mass distribution is no longer described by the fractal law.

The gyration radius can be calculated from the cut-off length, using: [4]

$$\xi^2 = \frac{4}{D} R_g^2 \qquad 3$$

Cryo-TEM images show that aggregates formed at high temperature have a spherical-like shape. SANS scattering curves of aggregates formed at 120 °C are best described using a model of polydisperse sphere, with an internal fractal structure:

$$I(q) = I(q)_{sphere} + c_1 q^{-D} + c_2 \qquad 4$$

with the fractal dimension of the internal structure, D, c_1 and c_2 are fitting parameters, and the scattering from polydisperse spheres, $I(q)_{sphere}$:

$$I(q,R)_{sphere} \approx (\Delta\rho)^2 \Phi V \left(3\frac{\sin(qR) - q.R.\cos(qR)}{(qR)^3} \right)^2 \quad 5$$

with the scattering length density difference between the solvent and the particles, $\Delta\rho$ / cm^{-2}, the volume fraction of particles, ϕ, the particle volume, V / cm^3, and the particle radius, R / nm. For a homogeneous scattering sphere, the radius of gyration is calculated as:

$$R_g = R\left(\frac{3}{5}\right)^{1/2} \quad 6$$

Heat-induced changes in secondary structure

Heating protein solutions induces changes in their secondary structure. To monitor these changes, we used Fourrier Transform Infra-Red (FTIR) spectroscopy. FTIR measurements were recorded using a Magna IR 550 Nicolet spectrometer with a liquid N_2 cooled mercury cadmium tellurium detector and an attenuated total reflection (ATR) cell with 6 internal reflection, 45° fixed angle of incidence ZnSe crystal. Because of the strong water absorption in the amide I band, FTIR experiments were recorded using D_2O. Protein and aggregates were measured in the same conditions as SANS. The spectrometer chamber was continuously purged with dry N_2 to remove water vapour. For each spectrum, 300 scans were coadded at 1 cm^{-1} resolution. Spectra were corrected from a D_2O background spectrum measured at the same conditions and normalized. We have used the second derivative spectra of the FTIR absorption in the amide I region because of its sensitivity to structural changes.

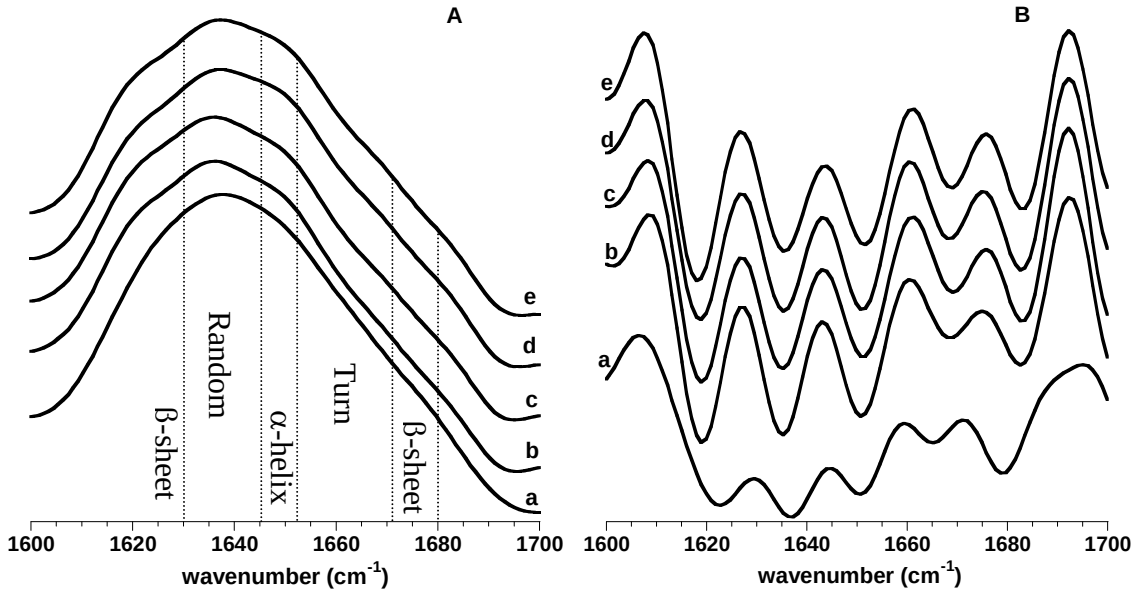

Fig. S1 (A) FTIR spectra (1600-1700 cm^{-1}, amide I band) of native WPI 0.1 M (a), WPI heat-treated at pH 7 for 145 s at 80 °C 0.1 M (b), 80 °C 0.003 M (c), 120 °C 0.1 M (d) and 120 °C 0.003 M (e). (B) The corresponding second-derivative spectra. The curves are shifted for clarity.

Insight into the heat induced conformational changes was obtained by the analysis of the amide I region (1600-1700 cm^{-1}) of the infrared (IR) spectra. Fig. S1 shows the original IR spectra and the corresponding second derivative spectra of native and heated solutions of whey proteins at 80 and 120 °C at two ionic strengths. Heating whey protein solutions has almost no effect on the position of the amide I band maximum (Fig. S1.A), which is found at 1636 cm^{-1} at 80 °C and 1637 cm^{-1} at 120 °C compared to 1638 cm^{-1} for the native WPI. However, one can see the formation of two shoulders at 1615 to 1620 cm^{-1} and 1645 to 1652 cm^{-1}. The first shoulder corresponds to the formation of intermolecular β-sheets and the second to changes in the overlapping bands corresponding to random and helical conformations.[5] Analysis of second derivative spectra allowed extracting more details concerning the changes of secondary structure upon heating (Fig. S1.B). For native WPI solution, the five following frequencies were obtained for the band components in the amide region I: 1623 and 1679 cm^{-1} (intermolecular β-sheet), 1637 cm^{-1} (intramolecular β-sheet), 1651 cm^{-1} (α-helix) and 1665 cm^{-1} (turns).[6-8] We were unable to cleanly resolve unordered (1643 cm^{-1}) and helical bands (1647-1654 cm^{-1}).[9] Contrary to the residual fraction and the hydrodynamic radius, the secondary structure seems to be independent of the heating temperature and the ionic strength since almost identical IR spectra were obtained for all the heated systems investigated. Heating WPI solutions induces principally the formation of a doublet, with a strong component at 1618-1619 cm^{-1} and a weaker one at 1683 cm^{-1}. The appearance of these components has been associated with the formation of antiparallel intermolecular β-sheets and the onset of the aggregation.[9, 10] Heating WPI solutions also resulted in a downshift of the band attributed to the intramolecular β-sheet to 1635 cm^{-1} and an upshift of the band attributed to turn structures to 1669 cm^{-1}, while the band attributed to the α-helices remains at the same frequency (1651-1652 cm^{-1}). In addition to the frequency shifts, the intensity of all the bands increase, especially of those attributed to intermolecular β-sheet (1618-1619 cm^{-1}) and α-helices. Nevertheless, the IR spectra of heated solutions are the sum of bands that correspond to secondary structures of a mixture of residual non-aggregated proteins and protein aggregates. Whether the residual proteins undergo secondary conformation changes upon thermal treatment or not cannot be concluded from the IR spectra.

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