The Effect of Sugar Stereochemistry on Protein Self-Assembly: The Case of β-Casein Micellization in Sugar Solutions

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Supporting Information

Appendix A – Calculating molar concentrations of the samples

The molar concentration of each sample was calculated by Eq. (1):

$$C = \frac{\frac{m_c}{MW_c}}{V_{total}} \cdot 1000 \, ml/l \qquad (1)$$

C = final co-solute concentration $[Molar]$; m_c = co-solute mass in a sample $[gr]$ see Eq. (2); MW_c = co-solute molecular mass $[gr/mole]$; V_{total} = Total sample volume $[ml]$

Solute mass in sample was evaluated by Eq. (2):

$$m_c = \rho \cdot v \cdot f_C = \rho \cdot v \cdot \frac{m_{c0}}{m_{c0} + m_{PBS0}} = \rho \cdot v \cdot \frac{m_{c0}}{m_{c0} + v_{PBS0} \cdot \rho_{PBS0}{}^{*}} \quad (2)$$

ρ = co-solute stock solution density $\left[{}^{gr}\!/\!_{ml} \right]$; v = volume of co-solute stock solution in sample $[ml]$; f_C = mass fraction of co-solute in co-solute stock solution $\left[{}^{gr}\!/\!_{gr} \right]$; m_{c0}= mass of co-solute in co-solute stock solution $[gr]$; m_{PBS0} = PBS mass in co-solute stock solution $[gr]$; v_{PBS0} = PBS volume in co-solute stock solution $[ml]$; ρ_{PBS0} = PBS density $\left[{}^{gr}\!/\!_{ml} \right]$ *.

* Approximated as 1 gr/ml according to average of 6 analytical weight measurements of carefully pipetted 1ml of PBS (1.005±0.003 ${}^{gr}\!/\!_{ml}$)

Appendix B – Modeling of I1/I3 Vs. β-Cas concentration Sigmoid

We defined the fraction of pyrene which entered the micelles formed as f . The protein concentration was expressed as $C\left[{}^{mg}\!/\!_{ml} \right]$. As both the processes of micellization and the partition of pyrene inside and out of the micelles, are reversible equilibrium processes, the CMC was defined as the concentration at which $f = 0.5$. We further expressed the cooperativity of the micellization by a parameter (K) which expresses the sigmoidal slope steepness. By combining these requirements and the expected sigmoidal behavior of the function we obtained Eq (3).

$$f = \frac{1}{1+\left(\frac{CMC}{C}\right)^{K}} \tag{3}$$

I3/I1 ratio is limited between two finite extreme cases: I3/I1 ratio for $C = 0$ ($\left(\frac{I_3}{I_1}\right)_{C=0}$), when its value should resemble that of pyrene in pure PBS, and I3/I1 ratio for $C = \infty$ ($\left(\frac{I_3}{I_1}\right)_{C=\infty}$), when all of the pyrene is thought to be confined inside micellar hydrophobic cores and the expected value should be the maximal value of I3/I1 ratio. The relation between I3/I1 ratio and f is thus:

$$\frac{I_3}{I_1} = \left(\frac{I_3}{I_1}\right)_{C=\infty} \cdot f + \left(\frac{I_3}{I_1}\right)_{C=0} \cdot (1-f) \tag{4}$$

Combining Eq. (3) and Eq. (4) we get the complete sigmoidal model equation:

$$\frac{I_3}{I_1} = \left(\frac{I_3}{I_1}\right)_{C=\infty} \cdot \left(\frac{1}{1+\left(\frac{CMC}{C}\right)^{K}}\right) + \left(\frac{I_3}{I_1}\right)_{C=0} \cdot \left(1 - \frac{1}{1+\left(\frac{CMC}{C}\right)^{K}}\right) \tag{5}$$