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A versatile viscometric method for the study of dissolved proteins, exemplified for casein micelles in ammoniacal solutions

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ABSTRACT

Viscosities of casein solutions were measured within the dilute range in ammoniacal water and in saline solvents containing different amounts and different kinds of salt. All these data are modeled quantitatively by means of an approach accounting for the polyelectrolyte character of casein micelles. Two parameters are required: The intrinsic viscosity $[\eta]$ and a viscometric interaction parameter β. The behavior of casein is compared with that of chain-like polyelectrolytes. For both polymers one observes a pronounced reduction of $[\eta]$ with increasing salt concentration. However, for casein the decline of $[\eta]$ is less pronounced by more than an order of magnitude and depends on the chemical nature of the salt. In the case of low solvent salinities, the β values are in both cases positive (*less* than exponential increase of the viscosity with rising solute concentration). However, for casein β changes from positive to negative (*more* than exponential increase) with rising salt concentration. Reasons for the dissimilarities between the two types of polyelectrolytes are discussed.

1. Introduction

The interaction of biomacromolecules in solution with other components, particularly with the ions of saline solvents, is of central importance for their performance. Nevertheless, reliable viscometric studies for protein solutions containing no or only little extra salt are to the best of the authors' knowledge missing. In order to demonstrate the capabilities of the present type of investigation, we have selected casein as a typical globular biopolymer.

As one of the most important proteins in commercial dairying species, caseins have been extensively studied (Fox & Brodkorb, 2008; Holt, Carver, Ecroyd, & Thorn, 2013). Virtually all established research methods (like light, neutron, X-ray scattering, and Electron Microscopy Field Emission Scanning Electron Microscopy and rheology) have been applied to investigate casein micelles, as documented by numerous review articles (Broyard & Gaucheron, 2015; Dahbi, Alexander, Trappe, Dhont, & Schurtenberger, 2010; Dickinson, 2015, 2016; Kethireddipalli & Hill, 2015; Kimpel & Schmitt, 2015; Lenton, Nylander, Teixeira, & Holt, 2015; McGrath, Fox, McSweeney, & Kelly, 2016; Nobel, Weidendorfer, & Hinrichs, 2012; Pan & Zhong, 2016; Pitkowski, Nicolai, & Durand, 2008; Redwan, Xue, Almehdar, & Uversky, 2015; Tan & McGrath, 2010). There seems to be general consensus on the following items: A typical casein micelle contains thousands of casein molecules, most of which form thermodynamically stable complexes with nanoclusters of amorphous calcium phosphate (Holt et al., 2013). Out of the many proposals (Horne, 2006) that have been made for the structure of casein micelles, the submicelle model is probably the most wide spread. Hydrophilic amino acid residues are considered to stretch outwardly on the surface of the casein micelles where the internal structure is stabilized by linked calcium phosphate nanoclusters and protein hydrophobic bonding (Ingham et al., 2015; Trejo, Dokland, Jurat-Fuentes, & Harte, 2011; de Kruif, Huppertz, Urban, & Petukhov, 2012). Due to the observation that casein under processing or under specific conditions can re-assemble (Dalgleish & Corredig, 2012; Liu & Guo, 2008) casein has been applied to prepare drug delivery vehicles (Elzoghby, El-Fotoh, & Elgindy, 2011).

By means of the present contribution, we want to shed some new light on the solution behavior of casein. A viscometric approach, describing the solution behavior of chain like

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polyelectrolytes quantitatively, is for the first time applied to a protein. The tools used for that purpose are briefly recalled in the next section.

2. Theory

The viscometric behavior of linear polyelectrolytes in dilute solutions is by now well studied and reasonably understood: The coil dimensions increase with the degree of charging and decrease as the solvent contains rising amounts of extra salt. The following relation (Wolf, 2007, 2015, 2016) holds true for the quantitative modeling of viscosities as a function of polymer concentration

$$\ln \eta_{rel} = \tilde{c} + (\alpha - \beta)\tilde{c}^2 + \left(\beta^2 - \alpha\beta - \gamma\right)\tilde{c}^3 - \left(\beta^3 - \alpha\,\beta^2 - 2\,\gamma\,\beta + \alpha\,\gamma\right)\tilde{c}^4 \ldots. \qquad (6)$$

$$\ln \eta_{rel} = \tilde{c} + M_2\,\tilde{c}^2 + M_3\,\tilde{c}^3 - M_4\,\tilde{c}^4 \ldots.$$

$$\ln \eta_{rel} = \frac{\tilde{c} + \alpha\tilde{c}^2}{1 + \beta\,\tilde{c} + \gamma\,\tilde{c}^2} \qquad (1)$$

where η_{rel} is the relative viscosity of the solution ($\eta_{solution}/\eta_{solvent}$) and $\tilde{c}$ stands for the reduced polymer concentration

$$\tilde{c} = c[\eta] \qquad (2)$$

The intrinsic viscosities [η] are accessible from the initial slop of $\ln \eta_{rel}$ as a function of c. Which of the parameters α, β or γ are necessary for the modeling of a given system varies widely; the typical case requires the leading parameter β in combination with either α or γ.

The introduction of a generalized intrinsic viscosity (Wolf, 2007) as the derivative of the viscosity at a given solute concentration at constant temperature, pressure and shear rate according to

$$\{\eta\} = \left(\frac{\partial \ln \eta}{\partial c}\right)_{T,\,p,\,\dot{\gamma}} \qquad (3)$$

has turned out to help the understanding. The idea of studying the concentration dependence of the relative viscosity, i.e. of $\ln \eta$ instead of η itself, has a long history (Kraemer, 1938; Baranov et al., 1987). It is the only reliable way to determine intrinsic viscosities of polyelectrolytes in pure water (Pavlov, Gubarev, Zaitseva, & Sibileva, 2006; Wolf, 2007). Differentiation of Eq. (1) according to Eq. (3) yields the following relation

$$\{\eta\} = \frac{[\eta](1 + 2\alpha\tilde{c} + (\alpha\beta - \gamma)\tilde{c}^2)}{(1 + \beta\,\tilde{c} + \gamma\,\tilde{c}^2)^2} \qquad (4)$$

The present approach permits an unequivocal determination of intrinsic viscosities for any charged or uncharged high molecular weight compound from the initial slope of $\ln \eta$ as a function of solute concentration. It turned out to be particularly helpful for the modeling of the effects of extra salt on the flow behavior of polyelectrolytes. How the intrinsic viscosities depend on the salt concentration c_{salt} is well described by means of the following Boltzmann sigmoid

$$\log[\eta] = \log[\eta]_\infty + \frac{\log[\eta]_0 - \log[\eta]_\infty}{1 + \exp\left(H \log\left(c_{salt}/c_{salt}^{pi}\right)\right)} \qquad (5)$$

The index zero of the intrinsic viscosity denotes the [η] value in the absence of extra salt, the index ∞ signifies the limiting value for a large surplus of extra salt. H characterizes the steepness of the transition and c_{salt}^{pi} signifies the salt concentration at the point of inflection.

The present study was performed to investigate the ability of Eq. (1) - so far applied to charged chain molecules only - to model the behavior of protein solutions. The fact that Eq. (1) contains some of the system specific parameters in the denominator impedes their direct molecular interpretation. For that reason we perform a series expansion with respect to the reduced concentration up to the forth power in view of the dilute nature of the present solution. This procedure yields to the following relation

The leading term of the expansion is unity and stands for isolated solute molecules, the factors M_2, M_3 and M_4 quantify the contributions of binary, ternary and quaternary interactions; their numerical value can be calculated from the system specific parameters as specified below

$$M_2 = \alpha - \beta; M_3 = \beta^2 - \alpha\beta - \gamma;\quad M_4 = -\beta^3 + \alpha\,\beta^2 + 2\,\gamma\,\beta - \alpha\,\gamma \qquad (7)$$

3. Materials and experimental procedures

Casein from bovine milk with a nitrogen content of over 13.5 wt % was purchased from Sigma-Aldrich and from Guangdong Huankai Microbial Sci. & Tech. Co., LTD (Guangzhou, China). The supplier Sigma-Aldrich indicates their casein contains α-s1, α-s2, β; and κ caseins. Meanwhile, literature [P.F. Fox, A. Brodkor, The casein micelle: Historical aspects, current concepts and significance, International Dairy Journal, 2008, 18: 677–684; D. J. McMahon, B. S. Oommen, Supramolecular structure of the casein micelle, Journal of Dairy Science, 2008, 91: 1709–1721; C. Holt, J.A. Carver, H. Ecroyd, D.C. Thorn, Journal of Dairy Science, 2013, 96: 6127–6146.] reports that normal casein from bovine milk is a mixture of α, β and κ caseins, their mass ratio being 5:4:1.

Aqueous ammonia solutions (28–30 wt% NH_3), sodium chloride (NaCl), $Zn(NO_3)_2 \cdot 6H_2O$ and all other reagents were reagent grade and were supplied by Sigma-Aldrich or Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

Ammonia solutions were diluted by Millipore-Q water by a factor of 5, and the salts were dissolved in the diluted ammonia solutions to prepare mixed solvents. Casein was weighted and then dissolved in the above obtained solvent using a volumetric flask to prepare casein solutions with desired concentration. The viscosities of the casein solutions were measured at 25 °C by means of an Ubbelohde capillary viscometer (0a, capillary diameter of 0.53 ± 0.01 mm) in combination with an automatic viscosity measurement system (Schott Instruments, Mainz, Germany).

The complete dissolution of casein in the different solvents takes typically some 45 min. After adding solutions of extra salts to them, some 2 min are required to establish constant running times.

4. Orienting experiments

Before starting a comprehensive study we wanted to obtain