

Temperature induced complex formation-deformation behavior of collagen model peptides and polyelectrolytes in aqueous solution

Ken Terao ^{a, *}, Ryoko Kanenaga ^a, Tasuku Yoshida ^a, Kazunori Mizuno ^{b, 1},
Hans Peter Bächinger ^{b, c, **}

^a Department of Macromolecular Science, Osaka University, 1-1, Machikaneyama-cho, Toyonaka, Osaka 560-0043, Japan

^b Research Department, Shriners Hospital for Children, Portland, OR 97239, USA

^c Department of Biochemistry and Molecular Biology, Oregon Health & Science University, Portland, OR 97239, USA

ARTICLE INFO

Article history:

Received 2 February 2015

Accepted 5 March 2015

Available online 12 March 2015

Keywords:

Intermolecular interactions

Small-angle X-ray scattering

Sodium carboxymethyl amylose

ABSTRACT

Since the triple-helical collagen model peptides with a free *N*-terminus have three cationic groups at one end, it may have strong interactions with polyelectrolytes. In this study, complex formation behavior was investigated for sodium carboxymethyl amylose (NaCMA) + H-(Pro-Pro-Gly)₁₀-OH (PPG10), a collagen model peptide, in aqueous NaCl with ionic strength of 10 mM and 100 mM by means of small-angle X-ray scattering (SAXS) and circular dichroism at different temperatures. The previously reported [Macromolecules **2012**, *45*, 392–400] sodium polyacrylate (NaPAA) and H-(Gly-Pro-4-(*R*)-Hyp)₉-OH (GPO9) system was also investigated to elucidate complex formation nearby the transition temperature region between triple helix and single coil of the peptide. The complex formed near the melting temperature of the triple helices, confirmed that the triple helical structure is directly related to the complex formation.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Intermolecular interaction between peptide and polyelectrolyte molecules is an important topic since ionic exchange is one of the most useful methods to purify or to analyze peptide molecules [1,2]. Indeed, complex formation behaviors of proteins and polyelectrolytes are investigated by static scattering methods [3–5]. On the one hand, some collagen model peptides [6] (CMP or triple helical peptide, THP [7]) show fully thermo-reversible triple-helix – single chain conformational change in aqueous solution, and thus their detailed structure and thermodynamic properties are extensively studied to clarify the structure and the functionality of collagen *in vivo* [8–11]. Interestingly, three CMP molecules align in parallel and three *N*-termini locate nearby each other [10–12] even in aqueous solution [13,14]. We recently found that the triple helical structure of CMP is stabilized in the presence of polyelectrolyte

* Corresponding author. Tel.: +81 6 6850 5459; fax: +81 6 6850 5461.

** Corresponding author. Research Department, Shriners Hospital for Children, Portland, OR 97239, USA. Tel.: +1 503 221 3433; fax: +1 503 221 3451.

E-mail addresses: kterao@chem.sci.osaka-u.ac.jp (K. Terao), hpb@shcc.org (H.P. Bächinger).

¹ Present address. Nippi Research Institute of Biomatrix, 520-11 Kuwabara, Toride, Ibaraki 302-0017, Japan.

[15], and the complex consisting of H-(Gly-Pro-4-(*R*)-Hyp)₉-OH (GPO9) and sodium polyacrylic acid (NaPAA) of which chemical structures are shown in Fig. 1 are found in saline at low temperature [16]. The obtained particle scattering function data are well explained by the comb-like wormlike-chain model [17] as schematically shown in the graphical abstract and therefore we concluded that positively charged *N*-termini strongly interact attractively with anionic groups of NaPAA. This phenomenon is just observed for the one system and it is preferable to study other systems to confirm that this is due to the electrostatic interactions. Furthermore, it is still unclear if triple helix formation is directly related to complex formation since the complex formation-deformation behavior was just investigated at much higher (75 °C) and much lower (15 °C) temperatures than the conformational transition temperature (~45 °C) of GPO9.

We therefore made small-angle X-ray scattering and circular dichroism measurements for the sodium carboxymethyl amylose (NaCMA, Fig. 1) and H-(Pro-Pro-Gly)₁₀-OH (PPG10, Fig. 1) system in 100 mM and 10 mM aqueous NaCl at various temperatures. To investigate association-dissociation phenomena for the former system, SAXS measurements were also made for NaPAA and GPO9 solution at different temperatures including the transition region between triple helices and random coils.

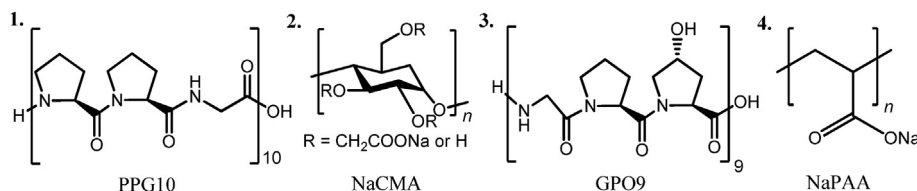


Fig. 1. Chemical structures of investigated samples. **1.** H-(Pro-Pro-Gly)₁₀-OH (PPG10), **2.** Sodium carboxymethyl amylose (NaCMA), **3.** H-(Gly-Pro-4-(R)-Hyp)₉-OH (GPO9), **4.** Sodium polyacrylic acid (NaPAA).

2. Experimental section

2.1. Samples and solvents

A previously investigated [16] sodium carboxymethyl amylose sample NaCMA26K prepared from enzymatically synthesized linear amylose [18,19] and a sodium polyacrylate NaPAA267 were chosen as polyelectrolytes for this study. The weight-average molar mass M_w and the degree of substitution of NaCMA26K were determined to be $2.67 \times 10^4 \text{ g mol}^{-1}$ and 0.64, respectively, and M_w for the latter was reported as $2.51 \times 10^4 \text{ g mol}^{-1}$. The previously investigated GPO9 sample and a PPG10 sample purchased from Peptide Institute Inc. were used for this study.

2.2. Measurements

Synchrotron-radiation small-angle X-ray scattering (SAXS) measurements for mixed solutions of NaCMA26K and PPG10 in 10 mM and 100 mM aqueous NaCl and those of NaPAA267 and GPO9 in 20 mM aqueous NaCl were performed at the BL-10C beamline in KEK-PF (Ibaraki, Japan) or at the BL40B2 beamline in SPring-8 (Hyogo, Japan) in the temperature range from 10 °C to 75 °C. The wavelength, camera length, and accumulation time were chosen to be 0.15 nm, 2000 mm, and 300 s in KEK-PF and 0.1 nm, 3000 mm, and 180 s in SPring-8, respectively. The scattered light was detected by using R-AXIS VII imaging plate detectors (Rigaku, Japan). The beam center and the actual camera length were determined from the powder diffraction pattern of silver behenate and/or lead stearate. The circularly average method was utilized to obtain the scattering intensity $I(q)$ at each magnitude of the scattering vector q . Molar ratio of carboxylic unit of polyelectrolytes to the collagen model peptide α was chosen to be 3, 6, and 10 for NaCMA26K and PPG10 in 10 mM, $\alpha = 6, 10$, and 30 for NaCMA26K and PPG10 in 100 mM, and $\alpha = 10$ for NaPAA267 and GPO9 in 20 mM aqueous NaCl. For each system, the solvent and four solutions with different total mass concentration c of polyelectrolyte and peptide were filled in a quartz capillary cell with the diameter of 2.0 mm. The total concentration c range of the two solutes was set to be 1×10^{-3} – $1 \times 10^{-2} \text{ g cm}^{-3}$ for all systems. The optical constant K was determined from the excess scattering intensity $\Delta I(q)$ of NaCMA26K in saline solution or NaPAA267 + GPO9 at 15 °C assuming full complexation which was determined in our previous study [16] (see Results and Discussion).

If three components, that is, CMP, polyelectrolyte (NaCMA or NaPAA), and their complex exist in solution, the total excess scattering intensity $\Delta I(q)_{c=0}$ at infinite dilution can be expressed as

$$\left[\frac{\Delta I(q)}{Kc} \right]_{c=0} = w_1 \Delta z_1^2 M_1 P_1(q) + w_2 \Delta z_2^2 M_2 P_2(q) + w_3 \Delta z_3^2 M_3 P_3(q) \quad (1)$$

Here, w_i , Δz_i , M_i , and $P_i(q)$ are the weight fraction in the total solute, the excess electron density, molar mass, and the particle

scattering function of the component i , respectively. Now we assume components 1, 2, and 3 to be isolated (single coil or triple helical) PPG10 (or GPO9), molecularly dispersed NaCMA (or NaPAA), and their complex. The excess electron density Δz_i is related to the partial specific volume v_p [20]. The v_p value for NaCMA was determined to be $0.572 \text{ cm}^3 \text{ g}^{-1}$ and $0.586 \text{ cm}^3 \text{ g}^{-1}$ at 25 °C and 55 °C, respectively, for the solution dialyzed by 100 mM aqueous NaCl at 25 °C. In 10 mM aqueous NaCl, this value was found to be $0.591 \text{ cm}^3 \text{ g}^{-1}$ and $0.612 \text{ cm}^3 \text{ g}^{-1}$ at 25 °C and 55 °C, respectively. The v_p value for PPG10 was determined to be $0.711 \text{ cm}^3 \text{ g}^{-1}$ and $0.746 \text{ cm}^3 \text{ g}^{-1}$ in pure water at 15 °C and 75 °C, respectively. These values are used to calculate Δz_2 in saline at each temperature since PPG10 have up to two ionized groups in one peptide molecule, and therefore preferable adsorption effects may be negligible.

Circular dichroism measurements were performed for NaCMA26K and PPG10 in 10 mM NaCl, and NaPAA267 and GPO9 in 20 mM aqueous NaCl both at $\alpha = 10$ with substantially the same concentration as the SAXS measurements by using JASCO J720WO spectropolarimeter with a Peltier thermostated cell holder and a rectangular cell with 1 mm path length. Temperature scans were recorded at a fixed wavelength and $6 \text{ }^\circ\text{C h}^{-1}$ to determine molar ellipticity $[\theta]$. Since the resultant $[\theta]$ obeyed straight lines both at high and low temperature ranges, those for triple helices ($[\theta]_{\text{helix}}$) and single coil ($[\theta]_{\text{coil}}$) were determined from the lines and then the helix content $F(T)$ at transition region was estimated from the equation as a function of temperature.

$$[\theta(T)] = F(T)[\theta(T)]_{\text{helix}} + [1 - F(T)][\theta(T)]_{\text{coil}} \quad (2)$$

3. Results and discussion

3.1. Scattering intensity and complex formation at low temperature

Conventional Berry plots [21] for NaCMA26K + PPG10 in 10 mM aqueous NaCl are illustrated in Fig. 2(a) at three typical temperatures below and above the conformational transition temperature, that is, $\sim 35 \text{ }^\circ\text{C}$ for PPG10, where subscript $c = 0$ means the value at infinite dilution. It should be noted that $[Kc/\Delta I(q)]^{1/2}$ data were irrespective of the total concentration c in the range of investigated c except for low- q region, indicating that complex behavior does not significantly depend on c , and hence the extrapolated values to infinite dilution reflect the molar mass of the complex in the concentration range investigated. While the Berry plots in 100 mM aqueous NaCl were substantially independent of temperature between 10 and 60 °C (not shown here), the $\Delta I(q)$ data in 10 mM aqueous NaCl significantly increase with lower temperatures, indicating complex formation at lower temperatures. It should be noted that the scattering intensity from dispersed peptide molecules is much smaller than that from NaCMA26K and its complex. Dashed lines indicate the initial slope to determine $\Delta I(0)$. The z-average radius of gyration $\langle S^2 \rangle_z$ was determined from the

Download English Version:

<https://daneshyari.com/en/article/5180424>

Download Persian Version:

<https://daneshyari.com/article/5180424>

[Daneshyari.com](https://daneshyari.com)