



Title	Kinetics of denaturation and renaturation processes of double-stranded helical polysaccharide, xanthan, in aqueous sodium chloride
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Kinetics of denaturation and renaturation processes of double-stranded helical polysaccharide, xanthan, in aqueous sodium chloride

Multiple helices are important secondary structures in biological molecules, such as DNA, collagen, and polysaccharides. Since the rodlike conformation of multiple helices makes a solution highly viscous even at quite low concentrations of such molecules, these molecules can be applied as food additives. Xanthan, a double helical polysaccharide (Fig. 1(a)) [1], is one of the most useful rheology control materials because of its high solubility in water. Furthermore, unlike flexible polyelectrolytes in aqueous media, the viscosity of an aqueous xanthan solution is insensitive to changes in salt concentration. This is because the high chain stiffness of a rodlike double helical structure determines the solution viscosity.

The double helical structure of xanthan in aqueous sodium chloride was confirmed by the weight-average molar mass in a solvent, that is, the molar mass of xanthan was twice that in dimethyl sulfoxide. However, this finding of a double helical structure was not accepted at that time because the optical rotation of xanthan solution in the salt-jump experiments showed the first-order reaction [2]. On the other hand, this double helical structure was found by atomic force microscopy techniques, which is widely accepted. There have only been a few research studies to clarify this inconsistency. Another interesting feature of xanthan is its hairpin-like conformation and branched complexes (Figs. 1(b,c)) [3], which may have unique rheological properties. Time-resolved measurement of the chain conformation is desired to clarify the denaturation and renaturation processes and to control the renatured conformation of xanthan.

Small-angle X-ray scattering (SAXS) is a powerful tool to determine the molecular conformation of polymers and macromolecular assemblies in solution. In particular, the conformational change from a single chain to a double helix can be detected as a significant increase in scattering intensity at the magnitude q of the scattering vector between 0.3 and 2 nm⁻¹. We thus conducted time-resolved SAXS measurements of double helical xanthan to observe the conformational change processes using the synchrotron radiation at SPRiNG-8 BL40B2. Time-resolved circular dichroism (CD) measurement was also conducted since CD signals reflect the chiral structure of chromophores; therefore, the temperature change of the CD signal of xanthan reflects the conformational change of side groups. Although this is consistent with the double helix content at the equilibrium state, we found that

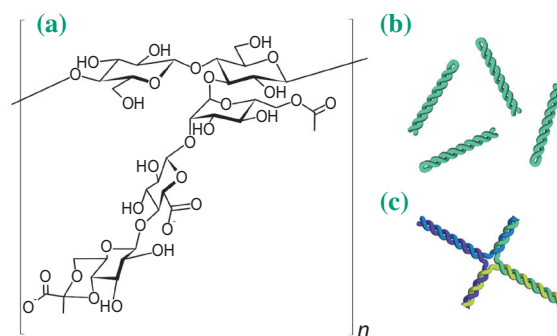


Fig. 1. (a) Chemical structure of xanthan [1]. (b) Hairpin-like conformation. (c) Branched complex.

clear differences were found immediately after the temperature change. The specific ellipticity $[\theta']_{210}$ detected by CD measurement at $\lambda_0 = 210$ nm with λ_0 being the wavelength of incident light in a vacuum and the weight fraction α of double helix determined by SAXS measurement are plotted in Fig. 2.

As shown in the figure, the $[\theta']_{210}$ value immediately reaches the denatured (red line in Fig. 2(a)) for the single chain after rapid heating, whereas the α value reaches zero after more than 1000 s, clearly indicating that after rapid heating, the side chains in xanthan immediately fluctuate markedly as observed by CD, whereas that of the rodlike double helical conformation was maintained for least several minutes. On the other hand, in the rapid cooling process, the α value immediately reaches an asymptotic value, whereas the $[\theta']_{210}$ value gradually increases. This shows that the rodlike double helix forms in a short time range after rapid cooling, whereas it took more than one day to recover the CD signal. This change can be regarded as the first-order process as in the case of the rapid heating process. Another interesting point is that the α values after cooling are however smaller than unity for the renatured samples. This is most likely due to the fact that the complete double helical conformation cannot be formed by materials with the hairpin-like conformation or branching complexes.

The above-mentioned results of the time-resolved CD and SAXS measurements indicate that both the denaturation and renaturation processes consist of at least two steps, and hence the existence of some intermediate species, as shown in Fig. 3. The initial change after the rapid heating is recognized as

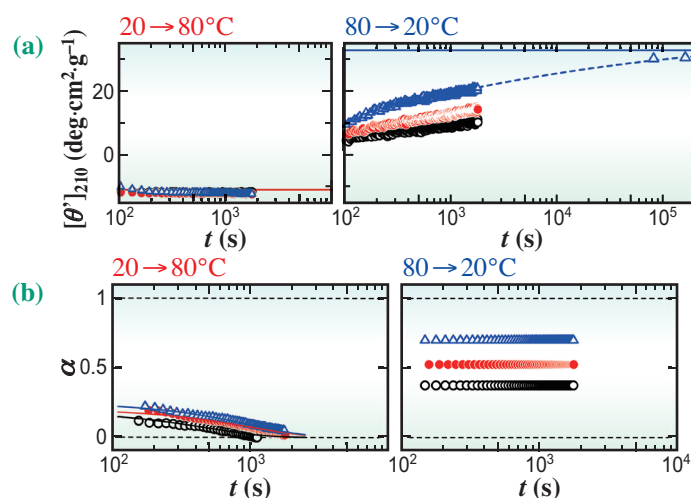


Fig. 2. Time course of (a) $[\theta']_{210}$ and (b) α for X341k in aqueous NaCl. Left, abruptly heated from 20 to 80°C. Right, abruptly cooled from 80 to 20°C. Black unfilled circles, red filled circles, and blue unfilled triangles denote $C_S = 5$ mM and $c = 3$ mg·mL⁻¹, $C_S = 5$ mM and $c = 6$ mg·mL⁻¹, and $C_S = 10$ mM and $c = 6$ mg·mL⁻¹, respectively. The solid blue line indicates the $[\theta']_{210}$ value for the as-prepared solution at 20°C. [1]

the melting of the side chain. The resulting double helix can be looser than that at low temperatures. In contrast, the formation of the loose double helical structure is quite rapid, whereas the reaction leading to the formation of the native double helix takes more than 24 h. These kinetic characteristics of xanthan in the solution can be related to the formation of a higher-order structure shown in Fig. 1, showing that not only static properties but also kinetic properties play important roles in realizing the higher-order structure and rheological properties of renatured xanthan. The current procedure to determine the helical structure can be utilized for various multiple

helical polysaccharides [4] and the complex formation behavior of polysaccharides with other materials [5].

In this study, we clearly showed that the SAXS profile is a direct measure of the helical structure of multiple helical biopolymers. Not only spectroscopic methods but also elastic scattering measurements including SAXS, light scattering, and neutron scattering play a decisive role in elucidating the conformational feature of biopolymers in solution. Since time-resolved SAXS measurements are available using a synchrotron light source, it is an indispensable method to clarify the kinetics of conformational change of multiple helical polymers in solution.

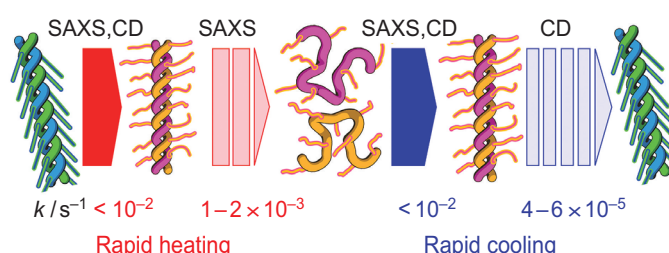


Fig. 3. Schematic representation of denaturation and renaturation processes of xanthan in aqueous NaCl. [1]

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