

RHEOLOGICAL PROPERTIES OF WHEAT GLIADINS IN AQUEOUS PROPANOL

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Abstract Rheological properties of wheat gliadins in 50% (*V/V*) aqueous propanol were carried out as a function of gliadin concentration *c* and temperature. The solutions at 20 g L⁻¹ to 200 g L⁻¹ behave as Newtonian fluids with an flow activation energy of 23.5–27.3 kJ mol⁻¹. Intrinsic viscosity [η] and Huggins constant k_H are determined according to Huggins plot at *c* ≤ 120 g L⁻¹. The results reveal that gliadins are not spherical shaped and the molecular size tends to increase with temperature due to improved solvation.

Keywords: Gliadin; Solution; Rheological properties; Intrinsic viscosity.

INTRODUCTION

Gliadins and glutenins are two kinds of major wheat storage proteins with a ratio of about 1:1 in wheat gluten, constituting about 80%–85% of the total grain proteins. Gliadins with a molecular weight of 30–80 kDa are divided into α -, β -, γ - and ω -types based on their mobilities^[1] and exhibit structural heterogeneity at high degrees of hydration^[2], which provides viscous character to wheat flour or gluten. Gliadins are formed by a nonrepetitive domain rich in α -helix structure and by a heterogeneous repetitive domain rich in β -reverse turns^[3]. The unique physical and biochemical properties of gliadin make it suitable for numerous nonfood applications such as biodegradable plastics^[4, 5] and edible films^[6–9].

Rheological test has been employed to study hydrated gliadins^[10] and gliadin suspensions^[11], and to probe the function of gliadins in wheat flour and gluten doughs^[12]. Intrinsic viscosity [η] measuring the hydrodynamic volume occupied by a polymer chain has been used to detect the conformational changes of gliadins in solution. Gliadins in 3 mol L⁻¹ urea plus 0.11 mol L⁻¹ KCl appear to contain a mixture of α -helix and random-coil structure^[13]. Gliadin molecules form a constrained structure with relatively high axial ratio. [η] at pH = 10 is higher than that at pH = 4 due to an increase in asymmetry of the protein molecule^[14]. A-gliadins, a group of α -gliadins purified by ultracentrifugation of a 0.01 mol L⁻¹ acetic acid extract of flour^[15], undergo aggregation at 0.01 mol L⁻¹ NaCl at pH = 4, giving rise to a compact structure and a low intrinsic viscosity (4.0 mL g⁻¹). [η] of A-gliadins increases significantly from 9.5 mL g⁻¹ in 1 mol/L guanidine hydrochloride (GuHCl) to 18.5 mL g⁻¹ in 5.9 mol/L GuHCl^[16].

Gliadin films are usually made using cast method from aqueous alcohol solutions. Understanding the molecular conformation and intermolecular interaction in solution are thus important for tailoring the structure and properties of the final film. Gliadins in 70% (*V/V*) aqueous ethanol appear as prolate ellipsoids with an axial

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ratio of 4.7–5.0^[17]. In this article, we took 50% (*V/V*) aqueous propanol as a solvent to elevate the solubility of gliadins extracted from gluten using 70% (*V/V*) aqueous ethanol. We attempt to examine the rheological behaviors of gliadin solutions with different concentrations in 50% (*V/V*) aqueous propanol. $[\eta]$ data are reported and are discussed as a function of temperature.

EXPERIMENTAL

Materials

Wheat gluten with a protein content $\geq 75\%$, a moisture content $\leq 9\%$ and an ash content $\leq 0.95\%$ was supplied by Shanghai Wangwei Food Co. Ltd. All chemicals were analytical grade reagents.

Sample Preparation

100 g of crude wheat gluten was dispersed in 500 mL of 70% (*V/V*) aqueous ethanol, stirred overnight at 25 °C, and centrifuged at 4000 r/min for 15 min at room temperature. The resulting supernatant containing the gliadin-rich fraction was dried at room temperature and further freeze-dried. The dried gliadin-rich fraction was ground to pass through 100 mesh sieve. The molecular weight of the resulted gliadins ranged from 30 kDa to 100 kDa as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis^[18], suggesting the extract contained some high molecular weight and low molecular weight glutenin subunits. Xu *et al.* found that the tiny amount of glutenins do not affect the rheological properties of gliadin suspensions^[11]. The dried gliadin powers were dissolved in 50% (*V/V*) aqueous propanol mixture to get solutions with concentrations C ranging from 20 g L⁻¹ to 200 g L⁻¹.

Rheological Experiments

Steady shear measurements were conducted using a rotational controlled stress rheometer (AR-G2, TA instruments, USA) with a 40 mm and 2° cone plate geometry. A small amount of 50% (*V/V*) aqueous propanol was placed into the solvent trap. The solvent trap cover had a lip that sat in the solvent, allowing the free space around the sample to become saturated with the solvent vapor, which prevented evaporation.

Steady shear measurements were conducted to determine apparent viscosity over shear rate ranging from 1.585 s⁻¹ to 1000 s⁻¹ at 15–30 °C. The maximum point time was set to 1 min, which was long enough for all samples to get equilibrium at all shear rates. Specific viscosity η_{sp} was calculate according to $\eta_{sp} = (\eta_0 - \eta_s)/\eta_s$. Here, η_0 and η_s are zero shear viscosities of the solution and the solvent.

RESULTS AND DISCUSSION

Figure 1 shows apparent viscosity η as a function of shear rate $\dot{\gamma}$ for the solutions at 25 °C. The solutions behave as Newtonian liquid because η does not vary with $\dot{\gamma}$. η increases from 6.2 mPa s to 80.6 mPa s as increasing c from 20 g L⁻¹ to 200 g L⁻¹. Gliadin solutions also show Newtonian behavior at 15, 20 and 30 °C (Figures not shown).

Figure 2 shows η against reciprocal temperature $1/T$ for the gliadin solutions. The temperature dependency of η was evaluated by applying the Arrhenius law

$$\eta = A \exp(E_a / RT) \quad (1)$$

where A , E_a , and R are frequency factor, flow activation energy and gas constant, respectively. No obvious differences were observed in E_a for the solutions with different concentrations. E_a was determined as 23.5–27.3 kJ mol⁻¹, being a little higher than those of wheat gliadins (18.8–22.0 kJ mol⁻¹)^[19] or zein (17.8–22.4 kJ mol⁻¹)^[20] in 70% (*V/V*) aqueous ethanol.

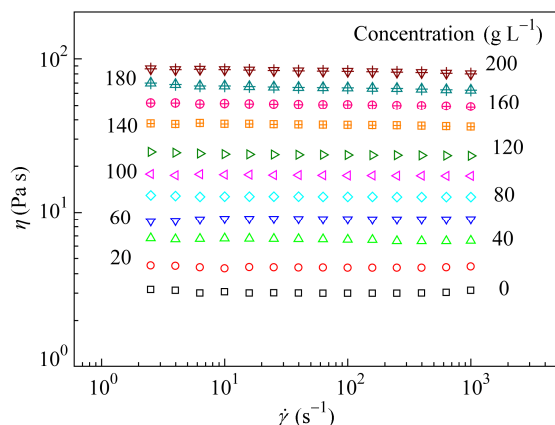


Fig. 1 Apparent viscosity η versus shear rate $\dot{\gamma}$ for gliadin solutions with different protein concentrations c at 25 °C

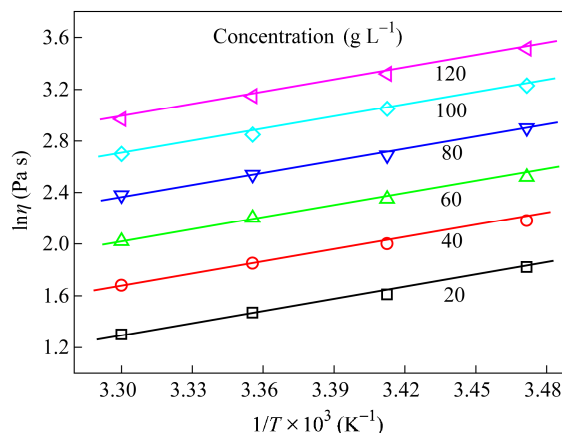


Fig. 2 Apparent viscosity η as a function of reciprocal temperature $1/T$ for gliadin solutions. Hollow symbols represent the experimental data and solid lines are drawn according to data fitting by the Arrhenius equation.

Figure 3 shows double-logarithmic plot of specific viscosity $\eta_{sp} = (\eta_0 - \eta_s)/\eta_s$ as a function of c for the solution at 25 °C. The data in Fig. 3 fall into two linear regions characterized by

$$\eta_{sp} \sim c^x \quad (2)$$

with $x = 1.45$ and $x = 2.64$, respectively. The overlapping concentration c^* is evaluated as 90 g L^{-1} at the crossover of the two regions^[21]. The exponent x is predicted as 1, $5/4$, and $15/4$, respectively, in the dilute, the semi-dilute unentangled and the semi-dilute entangled regions of uncharged polymers in good solvent or polyelectrolytes in salt excess^[22]. $x \approx 1.1$ has been observed in globular proteins such as lysozyme^[23] and ovalbumin^[24] in the dilute region. Gliadins show a rather high x value in the dilute region. In the semi-dilute region, the x value of gliadin solution is larger than that of semi-dilute unentangled solution but smaller than that of semi-dilute entangled solution. These high x exponent values in both the dilute and the semi-dilute regions may be considered as the expression of a high number of contacts between macromolecular chains related to the presence of interchain interactions. Gliadins behave as associating polyelectrolyte that probably forms hydrophobic domains bridging multiple polymer chains so that the solution viscosity rises significantly with increasing concentration.

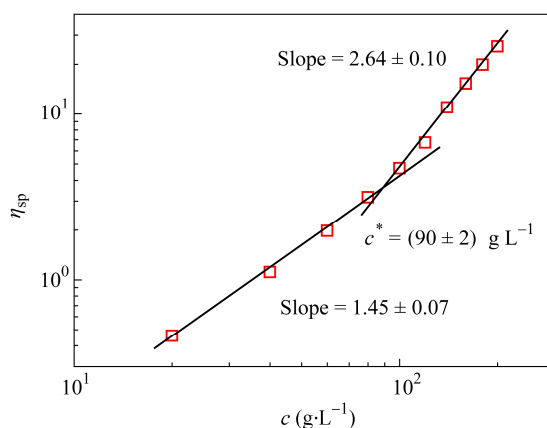


Fig. 3 Concentration dependence of the specific viscosity η_{sp} for gliadin solutions at 25 °C

Figure 4 shows η_{sp}/c as a function of c for the gliadin solutions with $c \leq 120 \text{ g L}^{-1}$ at four different temperatures. The c dependence of η_{sp} is usually expressed in the form of Huggins equation^[25]

$$\eta_{sp} = \frac{\eta(c) - \eta_s}{\eta_s} = [\eta]c + k_H([\eta]c)^2 + \dots \quad (3)$$

where k_H is the Huggins constant. The high order terms could be omitted in the dilute solution region. $[\eta]$ was determined by extrapolating η_{sp}/c to $c = 0$ and k_H was determined from the slope of η_{sp}/c against c . The resultant $[\eta]$ and k_H values are listed in Table 1. The overlapping concentration c^* was conveniently determined according to $c^*[\eta] = 1$ ^[26] and is also listed in Table 1. At 25 °C, the value of $c^* = 72 \text{ g L}^{-1}$ determined from Huggins plot is a little lower than that of 90 g L^{-1} determined from the crossover of two linear regions in plot of η_{sp} against c .

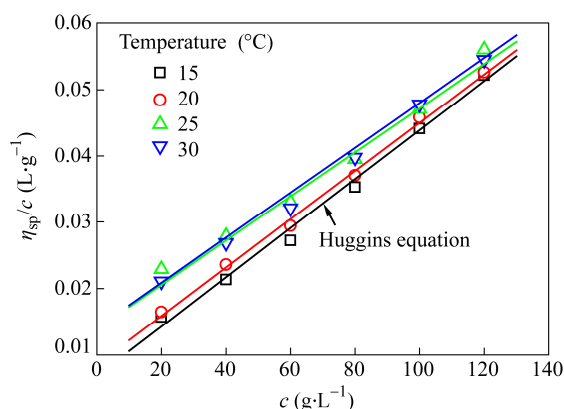


Fig. 4 η_{sp}/c as a function of concentration c for gliadin solutions

Hollow symbols represent the experimental data and solid lines are drawn according to the Huggins's representation for determination of intrinsic viscosity $[\eta]$.

Table 1. Huggins constant k_H , intrinsic viscosity $[\eta]$ and overlapping concentration c^* for gliadin solutions at four temperatures

Temperature (°C)	k_H	$[\eta] (\text{mL g}^{-1})$	$c^* (\text{g L}^{-1})$
15	7.5	6.9	145
20	4.9	8.6	116
25	1.7	13.9	72
30	1.7	14.1	71

A theoretical value of $[\eta] = 3.4 \text{ mL g}^{-1}$ is known for solid spheres which do not interact with the solvent^[27]. Most native globular proteins have $[\eta]$ values in the range of $2.5\text{--}6.0 \text{ mL g}^{-1}$ ^[24, 28–36]. However, salt addition, pH adjustment and denaturation could increase the $[\eta]$ value, by altering shape or charge of proteins^[37–41], or inducing the formation of asymmetric aggregation^[37, 42] or random coil-like structure^[43]. Furthermore, proteins unfold and behave as linear random coils^[40, 44–46] in the presence of denaturant and/or reducing agent.

As shown in Table 1, the $[\eta]$ values of $6.9\text{--}14.1 \text{ mL g}^{-1}$ are higher than the typical values of native globular proteins, suggesting a nonspherical but rather extended protein shape^[17]. Alcohols are known to destabilize the native structure of proteins^[47]. Propanol may promote the formation of α -helix structure^[48], which is less dense than the secondary structures present in globular proteins. k_H represents a quantitative measure of intermolecular interactions. The numerical values of k_H published in the literature vary widely for different protein solutions^[24, 31–33, 49]. Usually, low k_H values ranging from 0.25 to 0.5 are assigned to good solvation. The high k_H values of 1.7–7.5 in Table 1 reveal that 50% (V/V) aqueous propanol is a poor solvent for gliadins.

Theoretically, $[\eta]$ of rigid macromolecules is independent of temperature and the nature of the solvent, as long as the extent of solvation does not change markedly. For actual globular proteins, increasing temperature usually causes a decrease in $[\eta]$ and a monotonic increase in k_H ^[24, 30–32, 50–52]. For linear randomly coiled proteins,

increasing temperature facilitates backbone rotations, resulting in a reduction in $[\eta]$ ^[44]. However, an $[\eta]$ raise with respect to temperature has been observed at 35–40 °C where the prevailing nonbonded repulsive force tends to expand the coil^[45]. As shown in Table 1, $[\eta]$ increases while k_H decreases with increasing temperature. Gliadins become more extended with increasing temperature^[53, 54] because quality of the solvent becomes better as depicted by the reduction of k_H . Increase of $[\eta]$ with increasing temperature across the θ temperature has been observed in dilute solutions of several synthetic polymers^[55, 56], which is related to the globule-to-coil transition.

CONCLUSIONS

Gliadin solutions with various concentrations between 20 g L⁻¹ to 200 g L⁻¹ behave as Newtonian fluids with an flow activation energy of 23.5–27.3 kJ mol⁻¹. Intrinsic viscosity $[\eta]$ increases from 6.9 mL g⁻¹ at 15 °C to 14.1 mL g⁻¹ at 30 °C and Huggins constant k_H decreases from 7.5 at 15 °C to 1.7 at 30 °C. The results reveal that gliadins are not spherical shaped and the molecular size tends to increase with temperature due to improved solvation.

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