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TEMPERATURE DEPENDENCE OF THE MARK-HOUVINK-KUHN-SAKURADA EXPONENT FOR LYSOZYME IN AQUEOUS SOLUTIONS

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The viscosity of lysozyme aqueous solutions was obtained as a function of temperature and of protein concentration. The measurements were conducted at temperatures ranging from 5 to 55ºC and viscosity-temperature dependence was discussed on the basis of the Vogel-Tammann-Fulcher's equation. Viscosity-concentration dependence, in turn, was discussed on the basis of Mooney's formula. A master curve relating the specific viscosity η_{sp} to the reduced concentration $[\eta]c$, over the whole range of temperatures, was obtained. The existence of three ranges of concentrations: diluted, semi-diluted and concentrated, on the log-log plot of the η_{sp} versus $[\eta]c$, was shown. By applying Lefebvre's formula for the relative viscosity in the semi-dilute regime, the Mark-Houvink-Kuhn-Sakurada exponent – over the whole range of temperatures – was evaluated.

INTRODUCTION

Hen egg-white lysozyme is a small globular protein of the molecular weight M = 14 320 Da (Squire & Himmel, 1979). Its structure and hydrodynamic properties have recently been studied by a wide range of experimental techniques (Blanch, Morozowa-Roche, Cochran, Doig, Hecht & Barron, 2000; Blanch, Morozowa-Roche, Cochran, Doig, Hecht & Barron, 2000; Gregory, Gangoda, Gilpin & Su, 1993; Hadden, Chapman & Lee, 1995; Miura, Asaka, Shinyashiki & Mashimo, 1994; Smith, Mark, Dobson & van Gunsteren, 1995; Smith, Sutcliffe, Redfield & Dobson, 1993; Smyth, Syme, Blanch, Hecht, Vasak & Barron, 2001; Turula & de Haseth, 1996) and by some sophisticated theoretical methods (Roth, Neal & Lenhoff, 1996; Zhou, 1995). However, very little attention has been devoted to the viscosity of lysozyme aqueous solutions (Lefebvre, 1982; Monkos, 1997). And viscometric measurements still play an important role in investigations of biopolymers in solution, especially in the study of molecular conformations. This paper presents the results of viscosity measurements for lysozyme aqueous solutions at temperatures ranging from 5 to 55ºC and at a wide range of concentrations. The Vogel-Tammann-Fulcher's equation was used to describe the viscosity-temperature dependence. On the basis of this equation, at each concentration, the experimental data were approximated up to 1ºC. The viscosity-concentration dependence, in turn, was described by using the modified Mooney's formula. Using the product of the intrinsic viscosity $[\eta]$ and the solute concentration c, the existence of three ranges of concentrations: diluted, semi-diluted and concentrated, on the log-log plot of the specific viscosity versus $[\eta]c$ was shown. By applying Lefebvre's formula for the relative viscosity in semi-dilute regime, the Mark-Houvink-Kuhn-Sakurada (MHKS) exponent for lysozyme was determined.

MATERIAL

Crystallized hen egg-white lysozyme was obtained from Sigma Chemical Co. and was used without further purification. Aqueous solutions of the lysozyme were prepared by dissolving the material in distilled water and then filtered by means of filter papers in order to remove possible undissolved fragments. The samples were cooled at refrigerator until just prior to viscometry measurements, when they were warmed from 5ºC to 55ºC by step of 5ºC. The pH values of such prepared samples were about 7.0 and changed only insignificantly during the dilution of the solutions.

VISCOMETRY

Capillary viscosity measurements were conducted using an Ubbelohde microviscometer placed in a water bath controlled thermostatically at 5 to 55ºC.

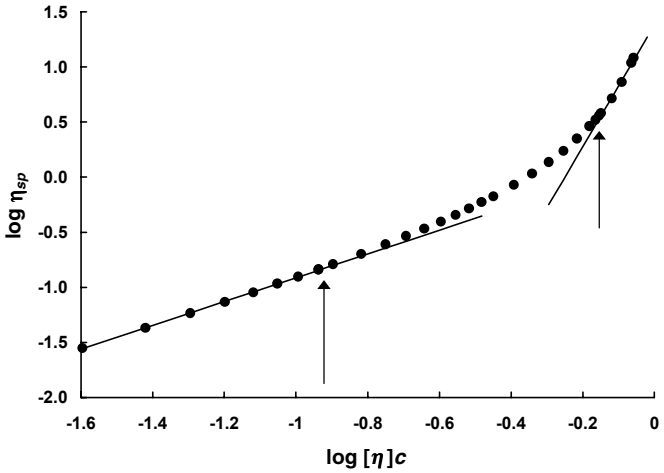

Fig. 1. Specific viscosity as a function of $[\eta]c$ in a log-log plot for lysozyme at 35^{O}C. The arrows show the boundary concentrations c^{*} (left arrow) and c^{**} (right arrow).

For most concentrations the measurements were made at 5ºC intervals. At the temperatures higher than 55ºC the thermal denaturation occurs and the lower protein concentration the higher denaturation temperature. Solution densities and lysozyme concentrations were measured by weighing. The details of the method are described elsewhere (Monkos & Turczynski, 1991; Monkos, 1994). The viscosities of the lysozyme solutions were measured for concentrations from 24.9 kg/m^3 up to 342.6 kg/m^3.

RESULTS AND DISCUSSION

Viscosity data can be presented in a different way. One of the methods of the experimental results presentation, for different polymer systems, consists of using reduced variables. In the case of the viscosity-concentration dependence, this parameter is a dimensionless quantity $[\eta]c$, where $[\eta]$ is the intrinsic viscosity in m^3/kg and c is the solute concentration in kg/m^3. The intrinsic viscosity:

$$[\eta] = \lim_{c \to 0} \frac{\eta_{sp}}{c}$$

where the specific viscosity $\eta_{sp} = \eta_r - 1$. The relative viscosity $\eta_r = \eta/\eta_o$, where η and η_o denote the viscosity of the solution and solvent, respectively. The principal method of determination of the magnitude of intrinsic viscosity consists of plotting the η_{sp}/c against concentration and extrapolating it to the intercept which is equal to $[\eta]$. However, this linear extrapolation gives a serious error in $[\eta]$. In the present paper, we have used our own method described elsewhere (Monkos, 1996; Monkos, 1997).

The dependence of the specific viscosity on $[\eta]$c in a log-log plot exhibits – for lysozyme - classical behaviour, with transitions from dilute to semi-dilute solution at concentration c^{*}, and from semi-dilute to concentrated solution at concentration c^{**}. In Fig. 1, the master curve for lysozyme at 35ºC is shown. The relative viscosity on the basis of Mooney's formula was calculated, and such ob-

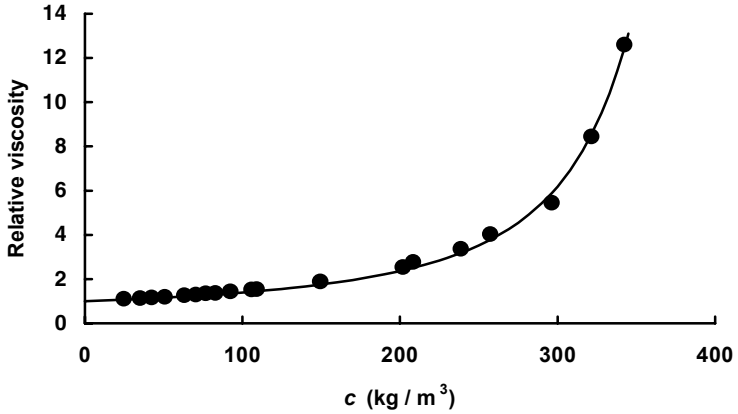

Fig. 2. Plot of the relative viscosity η_r versus concentration c for lysozyme at t = 35^{O}C; the curve shows the fit obtained by using equation (1) with parameters $A = 2.718 \cdot 10^{-3}$ m^3/kg and $B = 1.842 \cdot 10^{-3}$ m^3/kg.

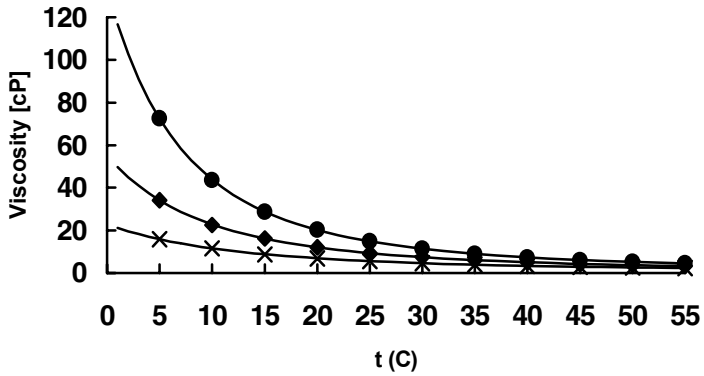

Fig. 3. Temperature dependence of the viscosity of lysozyme aqueous solutions for concentrations c_1 = 342.57 kg/m^3 (●), c_2 = 321.81 kg/m^3 (◆) and c_3 = 296.37 kg/m^3 (×). The curves show the fit obtained by using formula (2) with the parameters: W = 3.745 cP, Z = 285.18 K and T_0 = 227.25 K for c_1; W = 0.2326 cP, Z = 283.95 K and T_0 = 221.24 K for c_2 ; W = 0.1759 cP, Z = 300.82 K and T_0 = 211.34 K for c_3.

tained values of η_r were used in plotting of the Fig. 1. This method allows to obtain precisely the boundary concentrations c^* and c^{**}. As has been shown in our earlier papers (Monkos, 1994, 1996, 1997), in the case of aqueous solutions of globular proteins, Mooney's formula is the most useful functional form describing the dependence of relative viscosity on concentration (Mooney, 1951). To our purposes we have used it in a somewhat modified form:

$$\eta = \exp\left(\frac{Ac}{1-Bc}\right), \tag{1}$$

where A and B are two adjustable parameters. Numerical values of the parameters were calculated, for all temperatures, by applying a least square method described in the appendix. In Fig. 2, the experimental results of the relative viscosity at t = 35ºC and the curve obtained on the basis of equation (1) is shown.

For each concentration, the measurements were conducted at temperatures ranging from 5ºC to 55ºC. However, the most significant changes in viscosity occur at low temperatures. So, we have extrapolated the viscosity data up to 1ºC using the Vogel-Tammann-Fulcher's formula (Vinogradov & Malkin, 1980). This semi-empirical equation describes the viscosity-temperature dependence over a wide range of temperatures and has the form:

Table 1. The numerical values of the intrinsic viscosity, critical concentrations, reduced critical concentrations and MHKS exponent for lysozyme. Except for $[\eta]$, the parameters were obtained from the fit of the curves in Figs. 1 and 4 and from formula (3).

t [°C]	$[\eta]\cdot 10^3$[m^3/kg]	c^* [kg/m^3]	c^{**} [kg/m^3]	c^* $[\eta]$	c^{**} $[\eta]$	a
1	3.158	39.92	197.6	0.1261	0.6241	0.3147
2	3.131	40.06	199.0	0.1254	0.6232	0.3148
3	3.105	40.17	200.3	0.1247	0.6220	0.3149
4	3.079	40.51	203.1	0.1248	0.6255	0.3141
5	3.055	40.73	206.5	0.1244	0.6308	0.3132
10	2.937	41.87	213.7	0.1230	0.6277	0.3115
15	2.834	42.43	224.0	0.1202	0.6347	0.3079
20	2.742	43.27	239.0	0.1187	0.6554	0.3044
25	2.663	44.02	256.2	0.1172	0.6822	0.3006
30	2.595	44.89	265.5	0.1165	0.6889	0.2973
35	2.537	45.51	276.0	0.1155	0.7003	0.2955
40	2.490	45.87	291.7	0.1142	0.7264	0.2940
45	2.453	46.42	306.0	0.1139	0.7506	0.2925
50	2.425	46.91	312.5	0.1138	0.7578	0.2922
55	2.406	47.11	325.1	0.1133	0.7821	0.2907

$$\eta = W \exp\left(\frac{Z}{T - T_o}\right), \qquad (2)$$

where T is the absolute temperature and W, Z and T_o are parameters. In Fig. 3 the experimental results for three concentrations, and the curves obtained on the basis of relation (2) are shown. As seen, a very good fit over the whole range of temperatures was obtained. So, from the above relation — at each measured concentration — the viscosity at 1, 2, 3 and 4ºC was calculated.

The master curves, such as seen in Fig. 1, for cellulose derivatives (Castelain, Doublier & Lefebvre, 1987), citrus pectins (Axelos, Thibault & Lefebvre, 1989), randomly coiled globular proteins (Lefebvre, 1982), native globular proteins (Monkos, 1994, 2000, 2001) and some other biopolymers (Launay, Cuvelier & Martinez-Reyes, 1997; Durrani & Donald, 2000) were yet previously obtained. The parameters describing the curves for lysozyme at temperatures ranging from 1 to 55ºC are gathered in Table 1.

In the dilute region ($c[\eta] < c^*[\eta]$), the molecules move freely without interactions as in the infinitely diluted solution. The plot of $\log \eta_{sp} - \log [\eta]c$ is linear (with the correlation coefficient $r = 0.99985$). As is seen in Table 1, the values of c^* increase with increasing temperature but the reduced concentration $c^*[\eta]$ decreases. So, the higher temperature the narrower the dilute region is.

In the semi-dilute region ($c^*[\eta] < c[\eta] < c^{**}[\eta]$), the molecules begin progressively interact each other and the master curve begins to be non-linear. As is seen in Table 1, the second boundary concentration c^{**} increases with increasing temperature too, but the product c^{**} $[\eta]$ up to 10ºC is constant (within the experimental errors) and at $t > 10$ºC increases with increasing temperature. It means that the higher temperature the broader the semi-dilute domain is. As was shown by Lefebvre (1982), in the semi-dilute region, the following equation for the relative viscosity is fulfilled:

$$\ln \eta = 2a[\eta]c^*\left(\frac{c}{c^*}\right)^{\frac{1}{2a}} - (2a-1)[\eta]c^*, \qquad (3)$$

where a is the MHKS exponent. This parameter is often used as an indicator of polymer conformation in solution. For relatively flexible molecules it depends on temperature. The effect of the solution temperature on the MHKS exponent is insignificant only for stiff macromolecules. For example, this is the case for ovalbumin (Monkos, 2000) and bovine IgG immunoglobulin (Monkos, 2001). The numerical values of MHKS exponent are: $a = 0$ for hard spherical particles, $a < 0.5$ for compact molecules like globular proteins, $0.5 < a < 1$ for random coils and $1.8 < a < 2$ for hard long rods.

Fig. 4 shows a plot of $\ln \eta_r$ versus c for lysozyme at 35ºC, in the semi-dilute region. As seen, the points obtained on the basis of Mooney`s relation are in good agreement with the curve resulting from the equation (3). The MHKS exponent a and the boundary cocentration c^* had to be taken as two adjustable parameters in it. On the other hand, the boundary concentration c^* can be immediately estimated from the master curve (Fig. 1). To obtain the values of c^* from the master curve and from the relation (3) in good accordance we had to precisely choose the second boundary concentration c^{**}. The values of the parameters, obtained by the above-described method, are gathered in Table 1. As seen the MHKS exponent up to 4ºC is (within the experimental errors) constant and then slowly decreases with increasing temperature. It means that up to 4ºC the lysozyme conformation does not

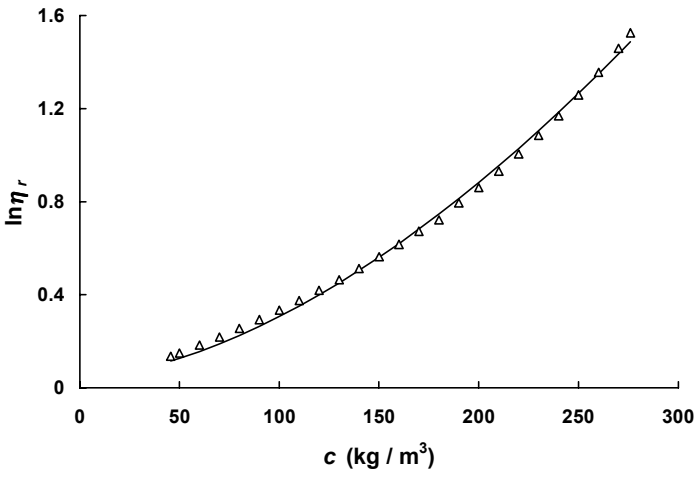

Fig. 4. Plot of the relative viscosity vs. concentration in a log-normal plot in a semi-dilute region for lysozyme at 35ºC. The points were obtained on the basis of equation (1); the curve shows the fit obtained by using relation (3).

change. For the higher temperatures the conformation slowly changes, and the results suggest that the lysozyme molecules become more spherical as the temperature increases.

In the concentrated region ($c[\eta] > c^{**}[\eta]$), the macromolecules are very close to each other and the molecular interactions become important. The movement of the molecules becomes partially correlative; the effects of intermolecular entanglements appear.

CONCLUSIONS

The viscosity-concentration and the viscosity-temperature dependence of the lysozyme aqueous solutions may be quantitatively described by Mooney's and Vogel-Tammann-Fulchers's approximations, respectively. The plot of log η_{sp} – log$[\eta]c$ shows that the three regions of concentrations exist: diluted, semi-diluted and concentrated. The higher temperature the narrower the dilute region. On the other hand, the semi-dilute domain becomes broader with increasing temperature. The MHKS exponent, calculated on the basis of Lefebvre's equation in the semi-dilute region, does not change with temperature up to 4ºC and then decreases with increasing temperature. Lysozyme molecules in aqueous solution behave as hard quasi-spherical particles. However, in the range from 5ºC to 55ºC, their conformation slightly changes with temperature.

APPENDIX

To find the parameters A and B in relation (1), for a given temperature, we have minimized the square form:

$$\Psi = \sum_{i=1}^{n} \left[(1 - Bc_i) y_i - Ac_i \right]^2 , \qquad \text{(A1)}$$

where $y = \ln \eta_r$, with respect to A and B. A differentiation yields to the following set of equations:

$$\left.\begin{aligned} \sum_{i=1}^{n} \left[(1 - Bc_i) y_i - Ac_i \right] c_i = 0 \\ \sum_{i=1}^{n} \left[(1 - Bc_i) y_i - Ac_i \right] c_i y_i = 0 \end{aligned}\right\} . \qquad \text{(A2)}$$

After some calculations, one can obtain the following expressions:

$$A = \frac{\sum_{i=1}^{n} c_i y_i \sum_{i=1}^{n} c_i^2 y_i^2 - \sum_{i=1}^{n} c_i y_i^2 \sum_{i=1}^{n} c_i^2 y_i}{\sum_{i=1}^{n} c_i^2 \sum_{i=1}^{n} c_i^2 y_i^2 - \left(\sum_{i=1}^{n} c_i^2 y_i \right)^2} \qquad \text{(A3)}$$

$$B = \frac{\sum_{i=1}^{n} c_i y_i - A \sum_{i=1}^{n} c_i^2}{\sum_{i=1}^{n} c_i^2 y_i} . \qquad \text{(A4)}$$

Putting the experimental values of η_r and c, for a given temperature, into relations (A3) and (A4) one can obtain the numerical values of A and B.

Acknowledgements

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REFERENCES

Axelos M. A. V., Thibault J. F. & Lefebvre J. (1989). Structure of citrus pectins and viscometric study of their solution properties. *Int. J. Biol. Macromol.*, **11**, 186-191.

Blanch E. W , Morozowa-Roche L. A , Cochran D. A. E., Doig A. J., Hecht L. & Barron L. D. (2000). Is polyproline II helix the killer conformation? Raman optical activity study of the amyloidogenic prefibrillar intermediate of human lysozyme. *J. Mol. Biol.*, **301**, 553-563.

Blanch E. W., Morozowa-Roche L. A., Hecht L., Noppe W. & Barron L. D. (2000). Raman optical activity characterization of native and molten globule states of equine lysozyme: comparison with hen lysozyme and bovine α-lactalbumin. *Biopolymers* **57**, 235-248.

Castelain C., Doublier J. L. & Lefebvre J. (1987). A study of the viscosity of cellulose derivatives in aqueous solutions. *Carbohydr. Polym.*, **7**, 1-16.

Durrani C. M. & Donald A. M. (2000). Shape, molecular weight distribution and viscosity of amylopectin in dilute solution. *Carbohydr. Polym.*, **41**, 207-217.

Gregory R. B., Gangoda M., Gilpin R. K. & Su W. (1993). The influence of hydration on the con- formation of lysozyme studied by solid-state ^{13}C-NMR spectroscopy. *Biopolymers* **33**, 513-519.

Hadden J. M., Chapman D. & Lee D. C. (1995). A comparison of infrared spectra of proteins in solution and crystalline forms. *Biochim. Biophys. Acta* **1248**, 115-122.

Launay B., Cuvelier G. & Martinez-Reyes S. (1997). Viscosity of locust bean, guar and xanthan gum solutions in the Newtonian domain: a critical examination of the log $(\eta_{sp})_o$ - log $c[\eta]_o$ master curves. *Carbohydr. Polym.*, **34**, 385-395.

Lefebvre J. (1982). Viscosity of concentrated protein solutions. *Rheol. Acta* **21**, 620-625.

Miura N., Asaka N., Shinyashiki N. & Mashimo S. (1994). Microwave dielectric study on bound water of globule proteins in aqueous solution. *Biopolymers* **34**, 357-364.

Monkos K. & Turczynki B. (1991). Determination of the axial ratio of globular proteins in aqueous solution using viscometric measurements. *Int. J. Biol. Macromol*., **13**, 342-344.

Monkos K. (1994). Viscometric study of human, bovine, equine and ovine haemoglobin in aqueous solution. *Int. J. Biol. Macromol*., **16**, 31-35.

Monkos K. (1996). Viscosity of bovine serum albumin aqueous solutions as a function of temperature and concentration. *Int. J. Biol. Macromol*., **18**, 61-68.

Monkos K. (1997). Concentration and temperature dependence of lysozyme aqueous solutions. *Biochim. Biophys. Acta* **1339**, 304-310.

Monkos K. (2000). Viscosity analysis of the temperature dependence of the solution conformation of ovalbumin. *Biophys. Chem*. **85**, 7-16.

Monkos K. (2001). A viscosity analysis of the solution conformation and stiffness of some IGG immunoglobulins. *Polish J. Med. Phys. Eng*. **7**, 49-54.

Mooney M. (1951). The viscosity of a concentrated suspension of spherical particles. *J. Colloid. Sci*. **6**, 162-170.

Roth C. M., Neal B. L. & Lenhoff A. M. (1996). Van der Waals interactions involving proteins. *Biophys. J*. **70**, 977-987.

Smith J. S., Mark A. E., Dobson C. M. & van Gunsteren W. F. (1995). Comparison of MD simulations and NMR experiments for hen lysozyme. Analysis of local fluctuations, cooperative motions, and global changes. *Biochemistry* **34**, 10918-10931.

Smith L. J., Sutcliffe M. J., Redfield C. & Dobson C. M. (1993). Structure of hen lysozyme in solution. *J. Mol. Biol.* **229**, 930-944.

Smyth E., Syme C. D., Blanch E. W., Hecht L., Vasak M. & Barron L. D. (2001). Solution structure of native proteins with irregular folds from Raman optical activity. *Biopolymers* **58**, 138-151.

Squire P. G. & Himmel M. E. (1979). Hydrodynamics and protein hydration. *Arch. Biochem.Biophys.* **196**, 165-177.

Turula V. E. & de Haseth J. A. (1996). Particle beam LC/FT-IR spectrometry studies of biopolymer Conformations in reversed - phase HPLC separations: native globular proteins. *Anal. Chem.* **68**, 629-638.

Vinogradov G. V. & Malkin A. Ya. (1980). *Rheology of polymers*. Moscow: Mir Publishers, 108.

Zhou H. X. (1995). Calculation of translational friction and intrinsic viscosity. Application to globular proteins. *Biophys. J.* **69**, 2298-2303.