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TEMPERATURE DEPENDENCE OF THE ACTIVATION ENERGY OF VISCOUS FLOW FOR OVALBUMIN IN AQUEOUS SOLUTIONS.

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The activation energy of viscous flow ΔE is usually defined as a minimum energy required for a molecule to escape the influence of its neighbouring molecules. In many cases, it is obtained from the slope of the line that represents the dependence of the liquid viscosity η (in logarithmic scale) versus a reciprocal of the absolute temperature (T^{-1}). More strict definition, which allows calculation of ΔE at the individual temperature is: $\Delta E = R[d\ln\eta/d(T^{-1})]$, where R is the gas constant. A modified Arrhenius formula gives an analytical function which describes the viscosity-temperature dependence for globular proteins solutions – in a wide range of temperatures. Such function applied to the above definition shows that square function describes the dependence of ΔE for proteins solutions on temperature. To apply it for ovalbumin, the viscosity measurements for ovalbumin aqueous solutions were performed over a wide range of concentrations and at temperatures ranging from 5^0C to 55^0C in 5^0C intervals. Analysis of the data showed that the activation energy of viscous flow for ovalbumin $\Delta E_p(T)$ fulfils the relation: $\Delta E_p(T) = \Delta E_p - RD_pT^2$, where the parameters ΔE_p and D_p were obtained from a modified Arrhenius formula, and for ovalbumin: $\Delta E_p = (8.49 \pm 0.46)\times10^7$ J/mol and $D_p = (86.4 \pm 5.4)$ K^{-1}.

INTRODUCTION

Ovalbumin – the major protein of chicken egg white – consists of a single polypeptide chain of 385 amino acid residues that folds into a globular conformation with three β-sheets, nine α-helices and three short helical segments of three to four residues (Stein *et* al, 1991; McCarthy & Worrall, 1997; Onda *et* al, 1997). It has been the subject of many physicochemical studies by using different experimental techniques for many years (Monkos, 2000 and references therein) and it can serve as a model protein for different biophysical studies. Particularly, analysis of the crystal structure of the protein by means of X-ray crystallography shows that the ovalbumin molecules can be approximated by tri-axial ellipsoids with overall dimensions 7×4.5×5 nm and with molecular mass M_p = 45 kDa (Stein *et al,* 1991). This paper presents the results of viscosity measurements for ovalbumin aqueous solutions (from diluted up to concentrated ones) at temperatures ranging from 5 to 55^0C. Based on these results the viscosity-temperature relationship is discussed and the temperature dependence of the activation energy of viscous flow of ovalbumin is obtained.

MATERIALS

Highly purified hen ovalbumin (grade V) was purchased from Sigma Chemical Co. and was used without further purification for all the measurements. From the crystalline form the material was dissolved in distilled water and the solution was treated to remove dust particles with filter papers. The samples were stored in a refrigerator until just prior to viscometry measurements, when they were wormed from 5^0C to 55^0C, mainly by steps of 5^0C. The pH values of such prepared samples changed only slightly in the whole range of concentrations with the average value 6.4, i.e. were outside of isoelectric point for ovalbumin which is in the range (4.59 – 4.71).

VISCOMETRY

The viscosity of ovalbumin solutions was previously studied by Lefebvre (Lefebvre, 1982). The author showed that the flow behaviour of those solutions is Newtonian. This is important information because it justifies the use of viscometry for viscosity measurements of ovalbumin solutions. Viscosity measurements were made by using an Ubbelohde-type capillary micro-viscometer with a flow time for water of 28.5 s at 25^0C. The same microviscometer – placed in a waterbath

controlled thermostatically - was used for all measurements. It was mounted so that it always occupied the same position in the bath. For most concentrations the viscosity measurements were made from 5^0C to 55^0C. The upper limit of temperature has been established by the temperature of denaturation of ovalbumin. The viscosities of the ovalbumin solutions were measured for concentrations from 6.2 kg/m^3 up to 430 kg/m^3. Solutions densities were measured by weighing and protein concentrations were determined by a dry weight method in which samples were dried at high temperature for several hours.

RESULTS AND DISCUSSION

The activation energy of viscous flow ΔE is usually defined as a minimum energy required for a molecule to escape the influence of its neighbouring molecules (Vinogradov & Malkin, 1980). From the experimental point of view, ΔE can be obtained from the slope of the line that represents the dependence of $\ln\eta$ versus T^{-1},

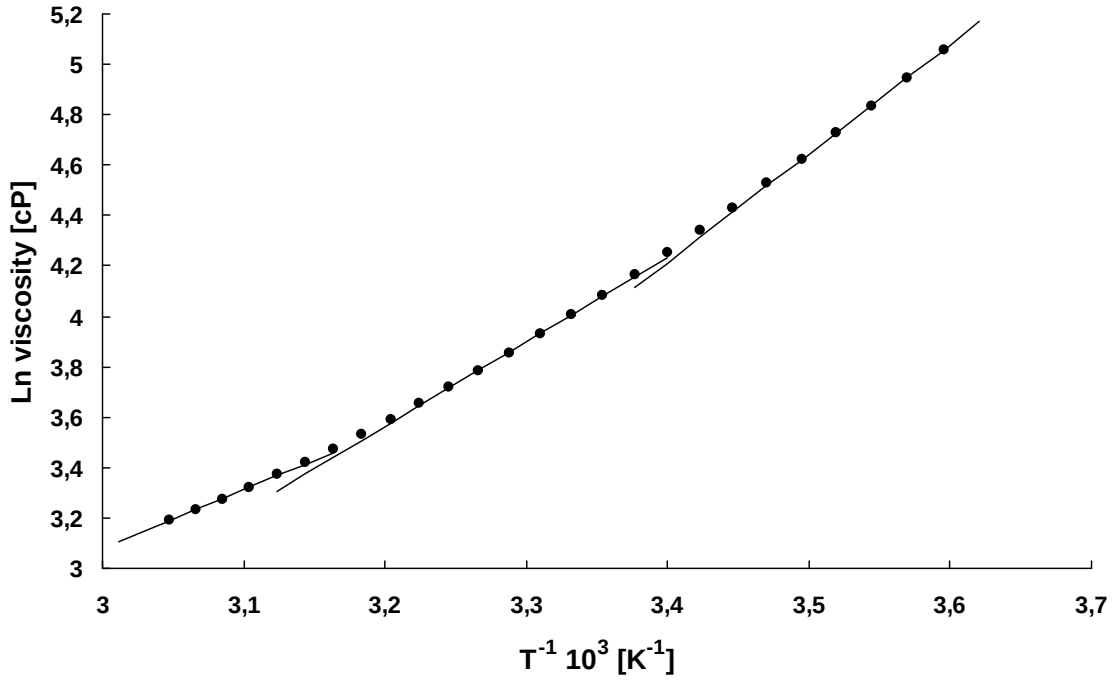

Fig. 1. Temperature dependence of the viscosity of ovalbumin aqueous solution for the concentration c = 422 kg/m^3 in a log-normal plot. (•) – experimental points; straight lines show different slopes at different temperatures.

where η and T denote a viscosity of liquid and temperature in which the viscosity has been measured, respectively. The activation energy is constant when such dependence is linear. Figure 1 shows the results of the viscosity measurements for ovalbumin aqueous solutions for the concentration c = 422 kg/m^3. As seen the plot of $\ln\eta$ versus T^{-1} is nonlinear, and it means that ΔE depends on temperature. This way obtained activation energy is only the mean value of ΔE in the range of temperatures in which the slope is calculated. *T*o obtain the activation energy at the individual temperature, the more strict definition has to be applied. For a solution, where ΔE depends both on concentration and on temperature, it has the form:

$$\Delta E(c,T) = R\frac{d\ln\eta(c,T)}{dT^{-1}}. \qquad (1)$$

For protein's solutions the viscosity-temperature dependence - from the neighbourhood of solution freezing point up to the vicinity of the temperature of thermal denaturation of the protein – can be described by a somewhat modified Arrhenius equation. It has the form (Monkos, 1996):

$$\eta(c,T) = \exp\left[-B_S(c) + D_S(c)T + \frac{\Delta E_S(c)}{RT}\right], \qquad (2)$$

where $B_s(c)$, $D_s(c)$ and $\Delta E_s(c)$ are parameters depending on the concentration of the solution and R is a gas constant. The above equation has been successfully applied to the description of the temperature dependence of viscosity of aqueous solutions of ovalbumin (Monkos, 2000), lysozyme (Monkos, 1997), some mammalian IgG immunoglobulins (Monkos & Turczynski; 1999) and mammalian albumins (Monkos, 1996, 2004, 2005).

Figure 2 shows the results of ovalbumin solution viscosity measurements for three concentrations. The curves were obtained on the basis of equation (2) with the parameters $B_s(c)$, $D_s(c)$ and $\Delta E_s(c)$ which – at each fixed concentration – were obtained by using the least square method (Monkos, 1996). As seen, this way obtained curves give a very good fit to the experimental points over the whole range of temperature.

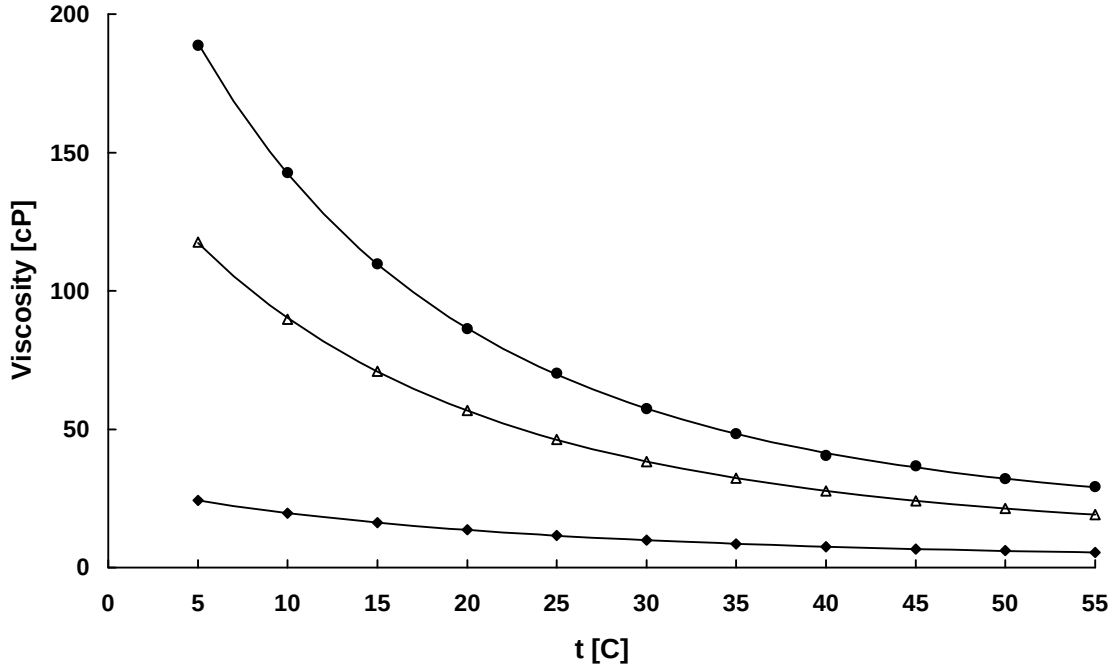

Fig. 2. Temperature dependence of the viscosity of ovalbumin aqueous solutions for concentrations c = 430 kg/m^3 (•), 409 kg/m^3 (Δ) and 332 kg/m^3 (♦). The curves show the fit obtained by using equation (2) with the parameters: $B_s(c)$ = 66.4, $D_s(c)$ = 8.65×10^{-2} K^{-1} and $\Delta E_s(c)$ = 94.09 kJ/mol for c = 430 kg/m^3; $B_s(c)$ = 53.78, $D_s(c)$ = 6.55×10^{-2} K^{-1} and $\Delta E_s(c)$ = 77.29 kJ/mol for c = 409 kg/m^3; $B_s(c)$ = 40.48, $D_s(c)$ = 4.45×10^{-2} K^{-1} and $\Delta E_s(c)$ = 56.37 kJ/mol for c = 332 kg/m^3.

The parameters $B_s(c)$, $D_s(c)$ and $\Delta E_s(c)$ depend on concentration exactly in the same way; they monotonically increase with increasing concentration. By assuming that – for instance – $\Delta E_s(c)$ is a superposition of this parameter for water ΔS_w and dissolved protein ΔS_p, one can obtain the following relation (Monkos, 1996):

$$\Delta E_s(c) = \frac{c}{\alpha - \beta c}\left(\Delta E_p - \Delta E_w\right) + \Delta E_w, \qquad (3)$$

where $\alpha = \rho_w M_h / M_w$ and $\beta = \alpha\xi$ - 1. The quantities ρ_w, ξ, M_h and M_w denote the water density in kg/m^3, the effective specific volume of a protein and the molecular masses of the dissolved protein and water, respectively. At c = 0, equation (3) gives $\Delta E_s(c) = \Delta E_w$. At temperature ranging from 5 to 55^{0}C one can obtain for water: ΔE_w = 32.88 kJ/mol. To calculate the parameters ΔE_p and ξ in equation (3), the molecular mass of hydrated ovalbumin is needed. This quantity is composed of the molecular mass of unhydrated ovalbumin and the mass of hydration shell of water surrounding the protein molecules in solution: $M_h = M_p(1 + \delta)$, where δ denotes the amount of grams of water associated with the protein per gram of protein. For ovalbumin δ = 0.36 (Young, 1963) and it gives the molecular mass of hydrated ovalbumin M_h = 61.2 kDa. By using the least square method in equation (3) the following values one can can obtain: ξ = 1.95×10^{-3} m^3/kg and ΔE_p = (8.49 ± 0.46)×10^4 kJ/mol. The parameter $D_s(c)$ fulfils the analogous equation to (3) and it gives D_p = (86.4 ± 5.4) K^{-1} (Monkos, 2000).

Now, let us insert expression (2) into equation (1). After simple calculations one can obtain the temperature

dependence of the activation energy of viscous flow of a solution:

$$\Delta E(c,T) = \Delta E_S(c) - R\,D_S(c)\,T^2 . \quad (4)$$

It is now clear the physical meaning of the parameters of the modified Arrhenius equation: $\Delta E_s(c)$ denotes the activation energy at $T = 0$ and $D_s(c)$ describes the rate of decreasing of the activation energy with increasing temperature. The values of $\Delta E(c,T)$ calculated from equation (4) – for three temperatures - are shown in Figure 3.

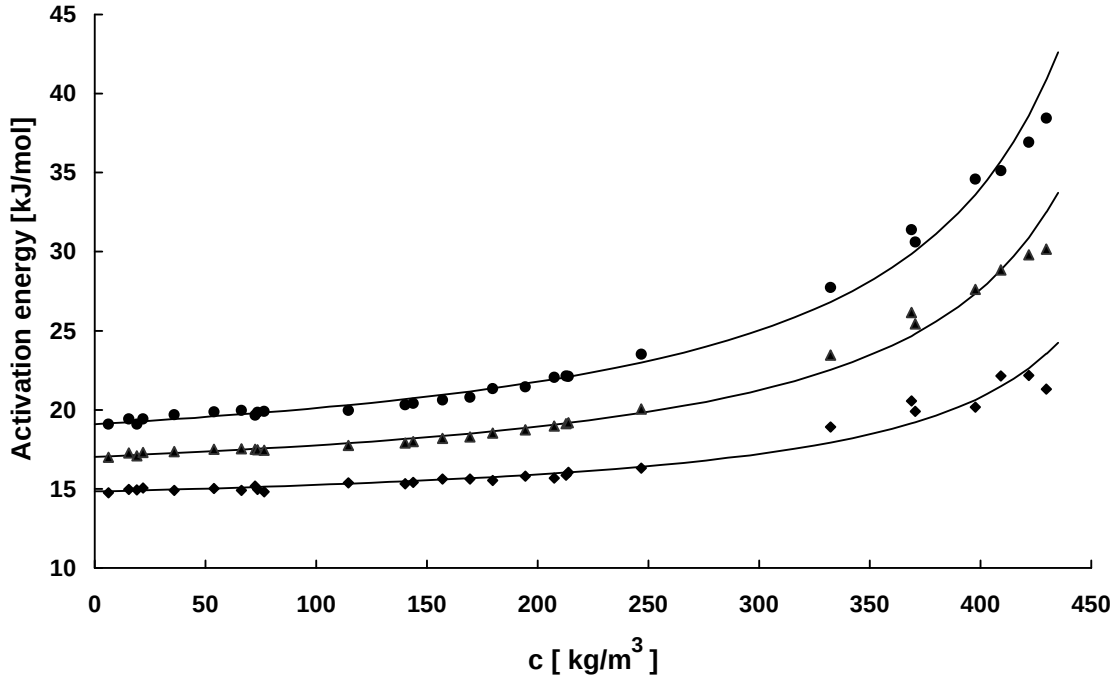

Fig. 3. Plot of the solution activation energy $\Delta E(c,T)$ versus concentration c at $t = 5^0$C (•), $t = 25^0$C (▲) and $t = 45^0$C (♦). Experimental points were obtained on the basis of equation (4); the curves show the fit according to equation (5) with the parameters: $\alpha = 3.4\times10^6$ kg/m^3, $\xi = 1.95\times10^{-3}$ m^3/kg and $\Delta E_p(T) = 2.788\times10^4$ kJ/mol, $\Delta E_w(T) = 19{,}09$ kJ/mol at $t = 5^0$C; $\Delta E_p(T) = 1.979\times10^4$ kJ/mol, $\Delta E_w(T) = 17{,}04$ kJ/mol at t = 25^0C; $\Delta E_p(T) = 1.113\times10^4$ kJ/mol, $\Delta E_w(T) = 14{,}84$ kJ/mol at $t = 45^0$C.

On the other hand, the activation energy of a solution - at any given temperature T – can also be treated as a superposition of the activation energy of water molecules at this temperature $\Delta E_w(T)$ and dissolved protein molecules at this temperature $\Delta E_p(T)$. This leads to the relation analogous to that presented in equation (3):

$$\Delta E(c,T) = \frac{c}{\alpha - \beta c}\left[\Delta E_p(T) - \Delta E_w(T)\right] + \Delta E_w(T) . \quad (5)$$

The activation energy of water – calculated on the basis of equation analogous to (4) - changes from $\Delta E_w(T) = 19.09$ kJ/mol (t = 5^0C) up to $\Delta E_w(T) = 13.69$ kJ/mol ($t = 55^0$C). In the above equation $\Delta E_p(T)$ is a parameter, which can be calculated by using the least square method. The results of such calculations are shown in Figure 4. At the same time, Figure 3 shows that the curves obtained on the basis of equation (5) give good fit to the values obtained on the basis of equation (4).

Figure (4) shows that the activation energy of viscous flow of ovalbumin decreases with increasing temperature. Let us assume that the functional dependence is the same as in the equation (4): $\Delta E_p(T) = a - RbT^2$. Taking into account the values of $\Delta E_p(T)$ obtained for ovalbumin on the basis of equation (5), one can obtain - by applying once more the least square method - the following numerical values: $a = 8.22\times10^4$ kJ/mol and b = 84.4 K^{-1}. These values agree (in the range of estimated errors) with the values of ΔE_p and D_p given earlier for ovalbumin. So, one can postulate that the following equation is fulfilled:

$$\Delta E_p(T) = \Delta E_p - RD_pT^2 . \quad (6)$$

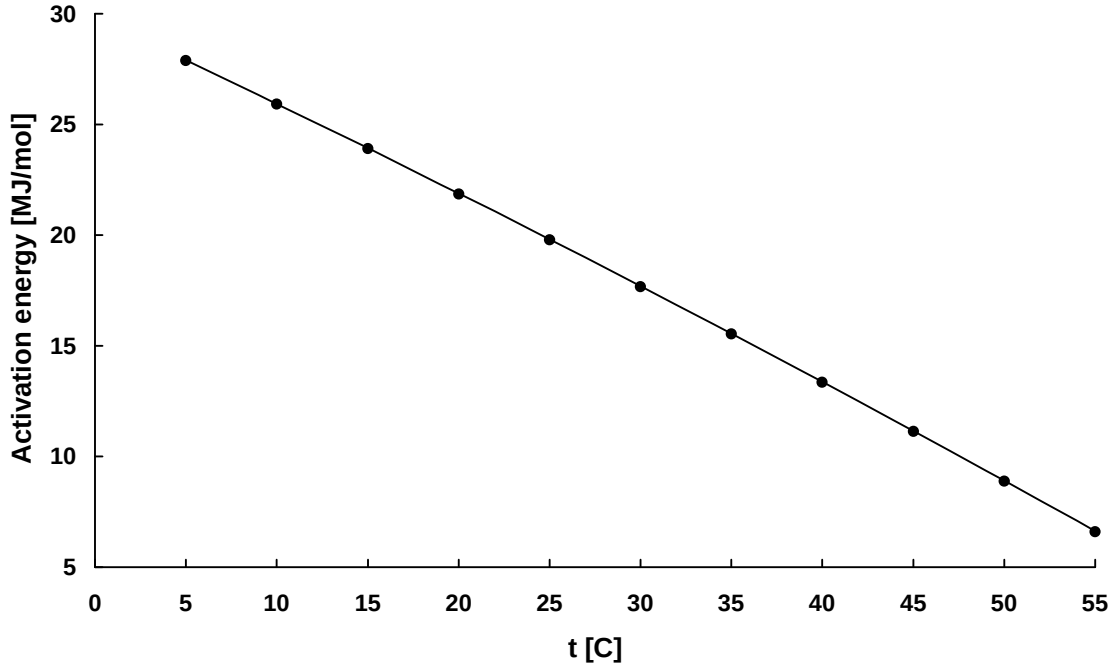

Fig. 4. Plot of the ovalbumin's activation energy $\Delta E_p(T)$ versus temperature. Points (•) have been obtained from equation (5) in which $\Delta E_p(T)$ is the adjustable parameter; the curve shows the fit according to relation (6) with $\Delta E_p = 8.22\times10^4$ kJ/mol and $D_p = 84.4$ K^{-1}.

Figure 4 shows that curve of dependence of $\Delta E_p(T)$ versus temperature obtained on the basis of this equation gives very good fit to the values obtained from equation (5). It means that the activation energy of viscous flow of a protein at any given temperature can be calculated from equation (6) if the parameters ΔE_p and D_p are known. These parameters should be obtained from relation (3) and analogous equation for $D_s(c)$. The equation (6) should give the temperature dependence of $\Delta E_p(T)$ not only for ovalbumin, but also for others proteins. This will be the subject of further investigations.

REFERENCES

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

McCarthy B. J., Worrall D. M. (1997). Analysis of serpin inhibitory function by mutagenesis of ovalbumin and generation of chimeric ovalbumin/PAI-1 fusion proteins. *J. Mol. Biol.,* **267,** 561-569.

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 viscosity in lysozyme aqueous solutions. *Biochim. Biophys. Acta,* **1339**, 304-310.

Monkos K., Turczynski B. (1999). A comparative study on viscosity of human, bovine and pig IgG immunoglobulins in aqueous solutions. *Int. J. Biol. Macromol.,* **26**, 155-159.

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

Monkos K. (2004). On the hydrodynamics and temperature Dependence of the solution conformation of human serum albumin from viscometry approach. *Biochim. Biophys. Acta,* **1700**, 27-34.

Monkos K. (2005). A comparison of solution conformation and hydrodynamic properties of equine, porcine and rabbit serum albumin using viscometric measurements. *Biochim. Biophys. Acta,* **1748,** 100-109.

Onda M., Tatsumi E., Takahashi N., Hirose M. (1997). Refolding process of ovalbumin from urea-denatured state. *J. Biol. Chem.,* **272**, 3973-3979.

Stein P. E., Leslie A. G. W., Finch J. T., Carrell R. W. (1991). Crystal structure of uncleaved ovalbumin at 1.95 Å resolution. *J. Mol. Biol.,* **221,** 941-959.

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

Young E. G. in: Florkin M., Stolz E. H. (Eds.) (1963). Comprehensive biochemistry. Elsevier, Amsterdam, pp. 49-50.