PRACA ORYGINALNA

Activation energy of viscous flow for some globular and non-globular proteins obtained from viscosity measurements and modified Arrhenius equation

Energia aktywacji przepływu lepkiego dla kilku globularnych i nie-globularnych białek otrzymana z pomiarów lepkości i zmodyfikowanego równania Arrheniusa

Karol Monkos

ABSTRACT

BACKGROUND:
The aim of the present paper was investigation of the temperature dependence of the activation energy of viscous flow for some proteins in aqueous solutions.

MATERIAL AND METHODS:
The viscosity of hen egg-white lysozyme, bovine β-lactoglobulin, and human, bovine and porcine IgG immunoglobulin aqueous solutions was measured at temperatures ranging from 5°C to 55°C and in a wide range of concentrations. The measurements were performed with an Ubbelohde-type capillary microviscometer.

RESULTS:
The average value of the activation energy of viscous flow ΔE can be experimentally 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}). The functional dependence of ΔE on temperature can be obtained from strict definition $\Delta E = R[d\ln\eta/d(T^{-1})]$, where R is the gas constant and from a three parameters modified Arrhenius formula which gives an analytical function describing the viscosity-temperature dependence for proteins solutions in a wide range of temperatures. As appears, ΔE obtained in such a way decreases with increasing temperature according to a square function. The parameters of this function have been obtained for all studied proteins.

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CONCLUSIONS:
The obtained results show that square function describes the temperature dependence of ΔE both for water, solutions and proteins themselves. One of the main factor which influence the activation energy is a molecular mass of protein. However, the results obtained for the studied immunoglobulins IgG show that this factor is not the only one.

KEY WORDS:
activation energy, viscosity, bovine ß-lactoglobulin, lysozyme, immunoglobulin IgG

STRESZCZENIE

WSTĘP:
Celem niniejszej pracy było zbadanie temperaturowej zależności energii aktywacji przepływu lepkiego dla kilku białek w roztworach wodnych.

MATERIAŁ I METODY:
Lepkość wodnych roztworów lizozymu jaja kurzego, β-laktoglobuliny bydlęcej oraz ludzkiej, bydlęcej i świńskiej immunoglobuliny IgG zmierzono w zakresie temperatur od 5°C do 55°C i w szerokim zakresie stężeń. Pomiary wykonano przy pomocy kapilarnego mikrowiskozymetru typu Ubbelohde'a.

WYNIKI:
Średnią wartość energii aktywacji przepływu lepkiego ΔE można doświadczalnie otrzymać z nachylenia prostej przedstawiającej zależność lepkości cieczy η (w skali logarytmicznej) od odwrotności temperatury bezwzględnej (T^{-1}). Funkcyjną zależność ΔE od temperatury można otrzymać z dokładnej definicji $\Delta E = R[d\ln\eta/d(T^{-1})]$, gdzie R jest stałą gazową oraz z trójparametrycznego zmodyfikowanego równania Arrheniusa, które daje analityczną funkcję opisującą temperaturową zależność lepkości dla roztworów białek w szerokim zakresie temperatur. Jak się okazuje, ΔE otrzymana w ten sposób maleje ze wzrostem temperatury zgodnie z funkcją kwadratową. Parametry tej funkcji zostały wyznaczone dla wszystkich badanych białek.

WNIOSKI:
Otrzymane wyniki pokazują, że funkcja kwadratowa opisuje temperaturową zależność ΔE zarówno dla wody, roztworów jak i samych białek. Jednym z głównych czynników wpływających na energię aktywacji jest masa molowa białka. Jednakże, wyniki otrzymane dla badanych immunoglobulin IgG pokazują, że nie jest to czynnik jedyny.

SŁOWA KLUCZOWE:
energia aktywacji, lepkość, ß-laktoglobulina bydlęca, lizozym, immunoglobulina IgG

INTRODUCTION

Hen egg-white lysozyme (HEWL) is a small globular protein of the molecular mass M_p = 14.32 kDa [1]. It is an enzyme that acts as a glycoside hydrolase. The protein consists of two functional domains located on each side of the active site cleft and consists both helices and regions of **ß** sheet, together with loop regions, turns and disulfide bridges [2]. In solution HEWL can be treated as prolate ellipsoid of revolution with the main axes 4.5 nm and 3 nm [1]. Its hydrodynamic properties have been investigated by different experimental techniques [3-6].

Bovine β-lactoglobulin (BLG) is the major whey protein of the cow's milk. This globular protein consists – in its mature form – 162

amino acid residues, what corresponds to a molecular mass M_p = 18.155 kDa [7]. BLG is predominantly β-sheet protein consisting of a β-barrel of eight continuous antiparallel β-strands, an additional β-strand and one major and four short α-helices [8]. It is able to bind a wide range of ligands, aromatic molecules and alkanone species. At physiological pH BLG forms dimmers, in which the monomers are noncovalently linked. The monomeric native state of BLG is stable at pH values below 3 [9]. It serves as a model protein for different biophysical investigations of folding, stability and self-association and has been the subject of many physicochemical studies for many years [10-15].

The immunoglobulins from the circulatory system of the mammalian body comprise a heterogeneous family of proteins with antibody activity, which can be divided into five classes designated as IgG, IgA, IgM, IgD and IgE. They constitute about 20% of total plasma proteins and the IgG immunoglobulins, in turn, constitute approximately 75% of them [16]. On the basis of minor antigen-binding specificities, the last class can be divided into several subclasses, but the gross conformation of the IgG molecules in all subclasses is the same. The immunological functions of these proteins are related to their partial flexibility, which is strongly correlated with their three-dimensional structure. Molecules of each IgG subclasses consist of four polypeptide chains structure. Two identical light chains and two identical heavy chains are folded in stretched segments running parallel or antiparallel to each other in tightly packed arrangements with hydrophobic amino acid residues dominating in the interior of the protein. Such structure consists of three globular subunits: two F_{ab} fragments and one F_c fragment which are connected by a single joint acting as a semiflexible hinge and allowing internal motions of the fragments [16-18]. The structure looks like to a T or a Y letter, and it means that IgG immunoglobulins are non-globular poteins. Segmental flexibility enables immunoglobulins to bind optimally to multivalent antigens and to carry out certain effector functions but does not influence their hydrodynamic properties [19]. As has been proved by several authors [19-23] hydrodynamic properties - such as sedimentation, diffusion coefficient or viscosity - depend mainly on the overall conformation of immunoglobulins.

In the present paper, the results of viscosity measurements on aqueous solutions of HEWL, BLG and IgG immunoglobulins of human (HIgG), bovine (BIgG) and porcine (PIgG) at temperatures ranging from 5°C to 55°C and at a wide range of concentrations are discussed. Based on the three parameters modified Arrhenius equation, the viscosity-temperature dependence - for a fixed concentration of each protein - is analyzed. One of the most important quantity characterizing the viscous flow is an activation energy. Temperature dependence of this quantity has been obtained from its strict definition and the modified Arrhenius equation. As appears, the activation energy decreases with increasing temperature according to the square function. The parameters characterizing this function have been obtained for each protein studied here.

MATERIAL

Highly purified HEWL, BLG, BIgG and PIgG were purchased from Sigma Chemical Co. and HIgG from Polish Chemical Reagents factories and were used without further purification for all the measurements. From the crystalline state the material was dissolved in distilled water and this way obtained solutions were treated with filter paper in order to remove possible undissolved dust particles. The samples were stored at 5°C in a refrigerator until just prior to viscometry measurements, when they were wormed from 5 to 55°C. The pH values of such prepared solutions of immunoglobulins were close to their isoelectric points - in the whole range of measured concentrations – and were as follows: 5.6 for HIgG, 5.65 for BIgG and 5.9 for PIgG. The isoelectric point for HIgG is in the range (5.8 – 6.6) [24]. Contrary to this the pH values of HEWL and BLG solutions were outside of their isoelectric points, and were as follows: 7.0 for HEWL and 7.2 for BLG. The isoelectric point for HEWL is in the range (11-11.2) [24] and for BLG is equal to 5.6 [25].

VISCOMETRY

The viscosity measurements of studied here proteins were made by using an Ubbelohde-type capillary microviscometer with a flow time for water of 28.5 s at 25°C. The same mi-

croviscometer - placed in a water-bath controlled thermostatically with a precision of ±0.1°C – was used for all measurements. Measurements started after several minutes delay to ensure the system reached equilibrium. Flow times were recorded to within 0.1 s. For most concentrations the viscosity measurements were conducted from 5°C to 55°C by steps of 5°C. Such a range of temperatures was chosen because slightly above 55°C flow times of the studied proteins solutions increase with increasing temperature and this indicates that thermal denaturation of proteins begins. In this case the proteins show a pronounced tendency to aggregate, but this aggregation reverses upon cooling [26]. The temperature of denaturation slightly changes with concentration and the lower the protein concentration the higher denaturation temperature.

Solution densities were measured by weighing, and proteins concentrations were determined by a dry weight method in which samples were dried at high temperatures for several hours. In the above indicated range of temperatures, the viscosity has been measured from 24.9 kg/m^3 up to 343 kg/m^3 for HEWL, from 16.9 kg/m^3 up to 285 kg/m^3 for HIgG, from 8.8 kg/m^3 up to 208 kg/m^3 for BIgG and from 28.4 kg/m^3 up to 223 kg/m^3 for PIgG. The viscosities of the BLG solutions were measured for concentrations from 20 kg/m^3 up to 444 kg/m^3. However, analysis of the viscosity data has been made only for solutions above the concentration of 119 kg/m^3, because in the above mentioned range of concentrations and temperatures BLG molecules exist in the dimeric form [27].

RESULTS AND DISCUSSION

Viscous flow of solutions or one component liquids can be described – as each thermally activated process – by an equation of Arrhenius type. It has the form:

$$\eta = A \exp\left(\frac{\Delta E}{RT}\right) \qquad (1)$$

and η, ΔE, R and T are viscosity, activation energy of viscous flow, gas constant and absolute temperature, respectively. The pre-exponential factor A is, in the first approximation, independent of temperature. The Arrhenius equation is widely used to the viscosity-temperature dependence description [28-41], but

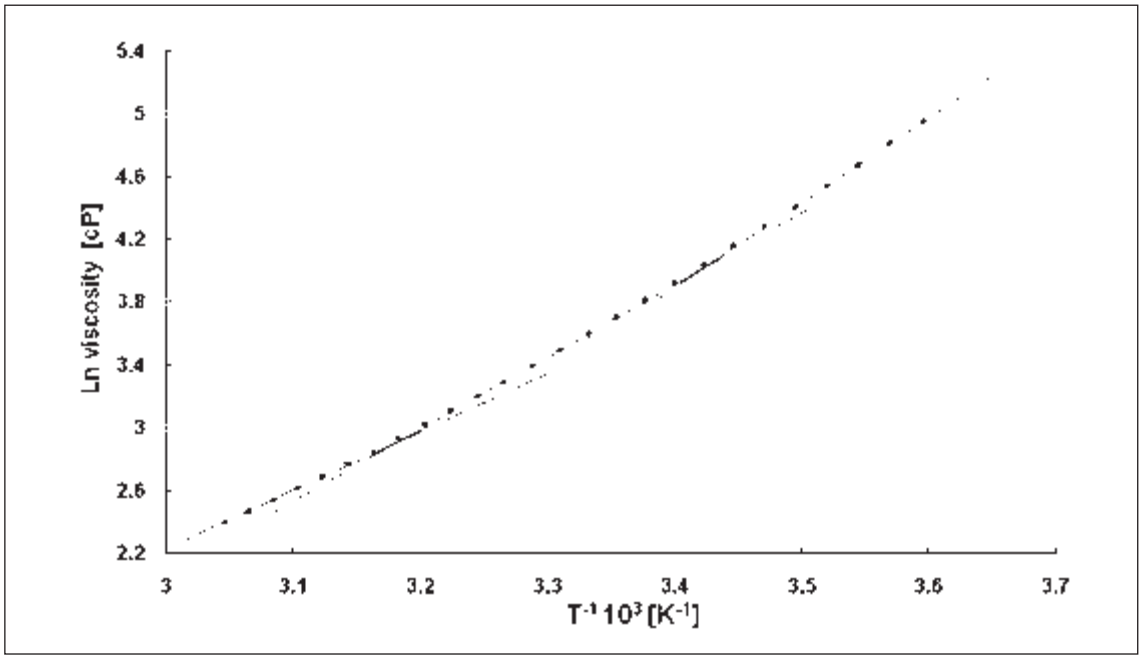

Figure 1. Temperature dependence of the viscosity of human immunoglobulin IgG aqueous solution for the concentration c = 257 kg/m^3 in a log-normal plot. (•) – experimental points; straight lines show different slopes at different temperatures.

Rycina 1. Temperaturowa zależność lepkości wodnego roztworu ludzkiej immunoglobuliny IgG o stężeniu c = 257 kg/m^3 w skali pół-logarytmicznej. (•) – punkty doświadczalne; linie proste przedstawiają różne nachylenia przy różnych temperaturach.

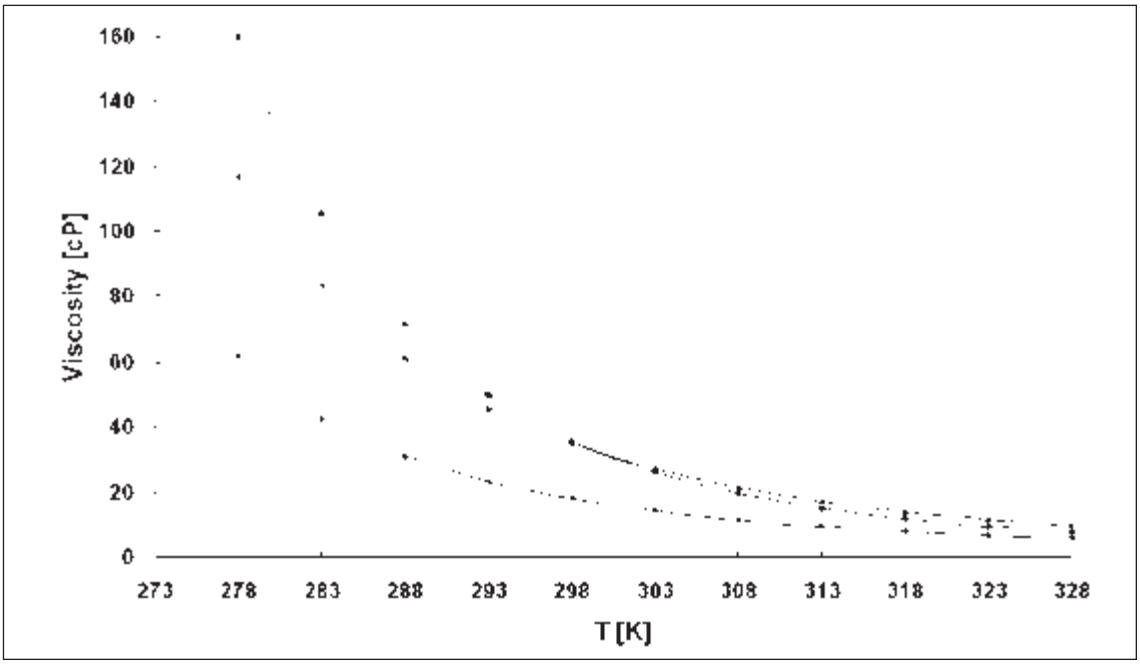

Figure 2. Temperature dependence of the viscosity of bovine (●), human (▲) and porcine (◆) immunoglobulin IgG aqueous solutions for concentrations: c = 208, 246 and 217 kg/m^3, respectively. The curves show the fit obtained by using equation (3) with the parameters: $B_s(c)$ = 69.76, $D_s(c) = 7.908\times10^{-2}$ K^{-1} and $\Delta E_s(c)$ = 106.2 kJ/mol for bovine IgG; $B_s(c)$ = 51.53, $D_s(c) = 5.46\times10^{-2}$ K^{-1} and $\Delta E_s(c)$ = 79.08 kJ/mol for human IgG; $B_s(c)$ = 75.7, $D_s(c) = 9.51\times10^{-2}$ K^{-1} and $\Delta E_s(c)$ = 107.4 kJ/mol for porcine IgG.

Rycina 2. Temperaturowa zależność lepkości wodnych roztworów immunoglobuliny IgG: bydlęcej (●), ludzkiej (▲) i świńskiej (◆) o stężeniach, odpowiednio, c = 208, 246 and 217 kg/m^3. Krzywe przedstawiają dopasowanie otrzymane przy wykorzystaniu równania (3) z parametrami: $B_s(c)$ = 69.76, $D_s(c) = 7.908\times10^{-2}$ K^{-1} i $\Delta E_s(c)$ = 106.2 kJ/mol dla bydlęcej IgG; $B_s(c)$ = 51.53, $D_s(c) = 5.46\times10^{-2}$ K^{-1} i $\Delta E_s(c)$ = 79.08 kJ/mol dla ludzkiej IgG; $B_s(c)$ = 75.7, $D_s(c) = 9.51\times10^{-2}$ K^{-1} i $\Delta E_s(c)$ = 107.4 kJ/mol dla świńskiej IgG.

only in the relatively narrow range of temperatures. The activation energy of viscous flow ΔE is usually defined on the basis of applications of the absolute rate theory to the process of flow and means the minimum energy required for a molecule to escape the influence of its neighbouring molecules or the energy required for the jump of a molecule from the cage formed by the nearest neighbours to one of the adjoining cages [42]. This quantity can be experimentally obtained from the Arrhenius plot, i.e. the plot of the viscosity versus T^{-1} in a ln-normal scale. The activation energy can be then directly calculated from the slope of the straight line in this plot. If the Arrhenius plot is linear in the whole range of temperatures, it means that the activation energy is temperature independent.

Figure 1 shows the Arrhenius plot for HIgG for the concentration c = 257 kg/m^3. As seen the plot is non-linear, and this is also the case both for other concentrations of HIgG and for the other proteins investigated here. It means that for the studied proteins ΔE depends on temperature. The activation energy obtained on the basis of Arrhenius plot represents only the ΔE averaged over the range of temperatures in which the slope has been calculated. So, the activation energy at a single-chosen temperature is equal to the slope of the tangent to the curve in a concrete point on the Arrhenius plot. Taking it into consideration, the activation energy at the individual temperature for a solution, where ΔE depends both on concentration and temperature, can define in the following way:

$$\Delta E(c,T) = R\frac{d\ln\eta(c,T)}{dT^{-1}} \qquad (2)$$

As mentioned above the Arrhenius equation (1) describes the viscosity-temperature dependence only in a narrow range of temperatures. However, it can be modified in such a way that it describes this relation in a wide range of temperatures, and for protein's solutions from the neighbourhood of solution freezing point up to the vicinity of the temperature of ther-

mal denaturation of the protein. This modified Arrhenius equation has the form [43]:

$$\eta(c,T) = \exp\left[-B_s(c) + D_s(c)T + \frac{\Delta E_s(c)}{RT}\right] \quad (3)$$

where $B_s(c)$, $D_s(c)$ and $\Delta E_s(c)$ are parameters depending on the concentration of the solution. The modified Arrhenius equation has been successfully applied to description of the viscosity-temperature dependence for several proteins [22,43-47]. To fit the above equation to the experimental values of η and T, the numerical values of $B_s(c)$, $D_s(c)$ and $\Delta E_s(c)$ are needed. They were calculated – for each fixed concentration – by using the least squares method, as described previously [43]. Figure 2 shows the temperature dependence of the viscosity for the studied immunoglobulins at different concentrations. As seen, curves obtained by using the function from the relation (3) give a very good fit to the experimental points over the whole range of temperatures. This is also the case for HEWL and BLG. It justifies the using of function (3) in relation (2) for obtaining the temperature dependence of the activation energy of viscous flow of the studied proteins.

After insertion of the relation (3) into equation (2), differentiation and simple transformations one obtains the temperature dependence of the activation energy of a solution as follows:

$$\Delta E(c,T) = \Delta E_s(c) - RD_s(c)T^2 \quad (4)$$

The above equation shows that – at each fixed concentration – the activation energy of viscous flow for a solution decreases with increasing temperature according to the square function. It is worth to note that $\Delta E_s(c)$ means the activation energy at T = 0 and $D_s(c)$ describes the rate of decreasing of the activation energy with increasing temperature. The numerical values of ΔE(c,T) obtained on the basis of equation (4) – for PIgG for three temperature – are presented in Figure 3, for HIgG and BIgG at 5°C in Figure 4 and for HEWL and BLG at 5°C in Figure 5.

The parameters of the modified Arrhenius equation $B_s(c)$, $D_s(c)$ and $\Delta E_s(c)$ depend on concentration in the same way: they monot-

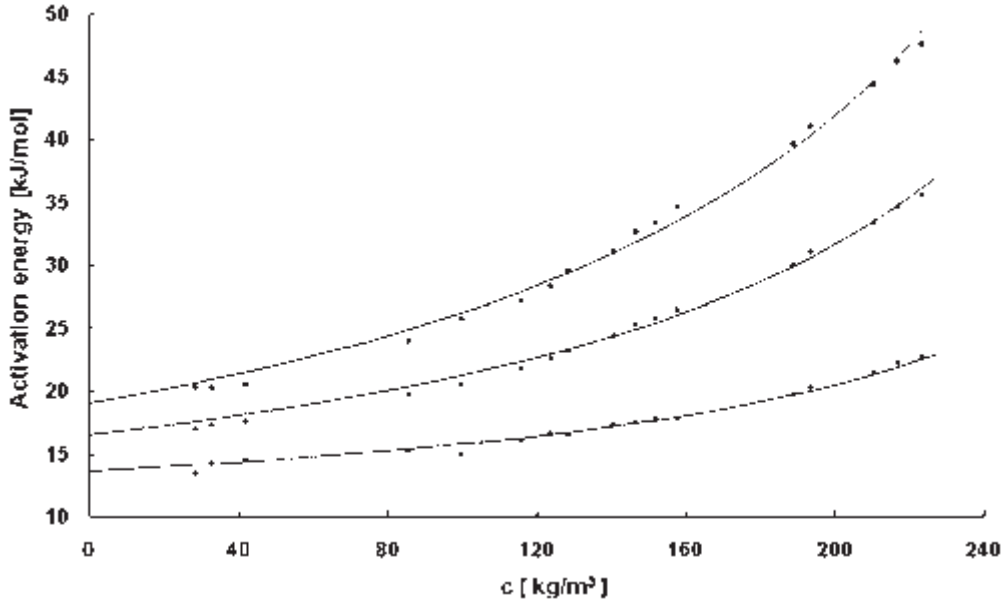

Figure 3. Plot of the solution activation energy ΔE(c,T) versus concentration c at t = 5°C (●), t = 30°C (▲) and t = 55°C (◆) for porcine immunoglobulin IgG. Experimental points were obtained on the basis of Eq. (4); the curves show the fit according to Eq. (7) with the parameters: $\alpha = 1.358\times10^{7}$ kg/m³, $\xi = 2.71\times10^{-3}$ m³/kg and $\Delta E_p(T) = 7.089\times10^{5}$ kJ/mol, $\Delta E_w(T) = 19{,}09$ kJ/mol at t = 5°C; $\Delta E_p(T) = 4.71\times10^{5}$ kJ/mol, $\Delta E_w(T) = 16.5$ kJ/mol at t = 30°C; $\Delta E_p(T) = 2.127\times10^{5}$ kJ/mol, $\Delta E_w(T) = 13.69$ kJ/mol at t = 55°C.

Rycina 3. Wykres energii aktywacji roztworu ΔE(c,T) od stężenia c dla t = 5°C (●), t = 30°C (▲) i t = 55°C (◆) dla świńskiej immunoglobuliny IgG. Punkty doświadczalne otrzymano na podstawie równania (4); krzywe przedstawiają dopasowanie zgodnie z równaniem (7) z parametrami: $\alpha = 1.358\times10^{7}$ kg/m³, $\xi = 2.71\times10^{-3}$ m³/kg i $\Delta E_p(T) = 7.089\times10^{5}$ kJ/mol, $\Delta E_w(T) = 19{,}09$ kJ/mol dla t = 5°C; $\Delta E_p(T) = 4.71\times10^{5}$ kJ/mol, $\Delta E_w(T) = 16.5$ kJ/mol dla t = 30°C; $\Delta E_p(T) = 2.127\times10^{5}$ kJ/mol, $\Delta E_w(T) = 13.69$ kJ/mol dla t = 55°C.

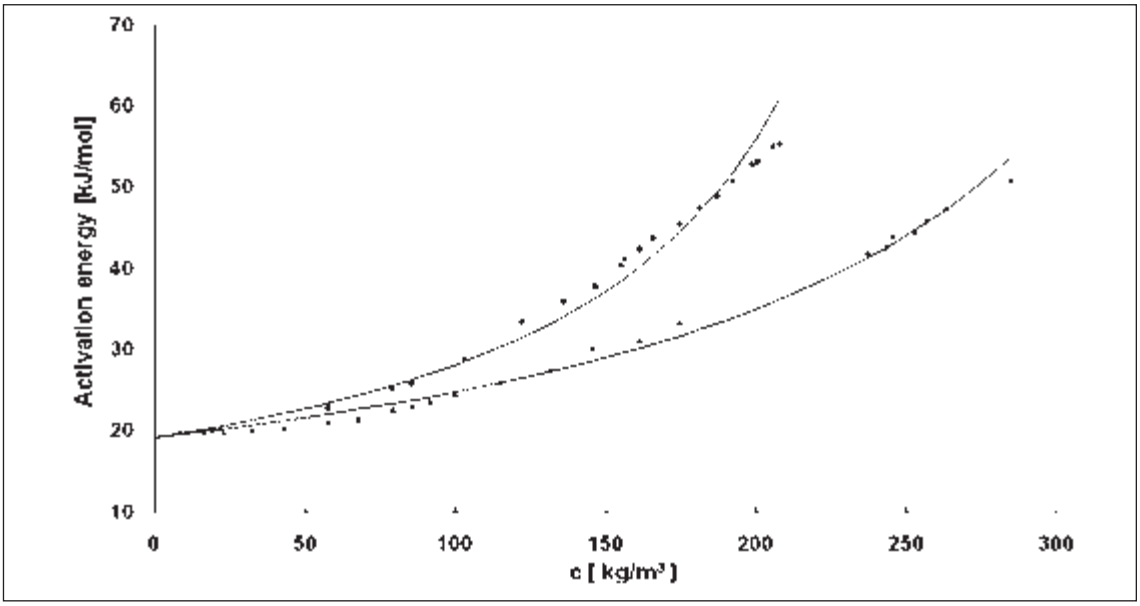

Figure 4. Plot of the solution activation energy $\Delta E(c,T)$ versus concentration c for human (▲) and bovine (●) immunoglobulin IgG at t = 5°C. Experimental points were obtained on the basis of Eq. (4); the curves show the fit according to Eq. (7) with the parameters: $\alpha = 1.358\times10^{7}$ kg/m³ and $\Delta E_w(T) = 19.09$ kJ/mol and: $\xi = 2.25\times10^{-3}$ m³/kg, $\Delta E_p(T) = 5.923\times10^{5}$ kJ/mol for human immunoglobulin IgG; $\xi = 3.4\times10^{-3}$ m³/kg, $\Delta E_p(T) = 8.039\times10^{5}$ kJ/mol for bovine immunoglobulin IgG.

Rycina 4. Wykres energii aktywacji roztworu $\Delta E(c,T)$ od stężenia c dla ludzkiej (▲) i bydlęcej (●) immunoglobuliny IgG dla t = 5°C. Punkty doświadczalne otrzymano na podstawie równania (4); krzywe przedstawiają dopasowanie zgodnie z równaniem (7) z parametrami: $\alpha = 1.358\times10^{7}$ kg/m³ i $\Delta E_w(T) = 19.09$ kJ/mol i: $\xi = 2.25\times10^{-3}$ m³/kg, $\Delta E_p(T) = 5.923\times10^{5}$ kJ/mol dla ludzkiej immunoglobuliny IgG; $\xi = 3.4\times10^{-3}$ m³/kg, $\Delta E_p(T) = 8.039\times10^{5}$ kJ/mol dla bydlęcej immunoglobuliny IgG.

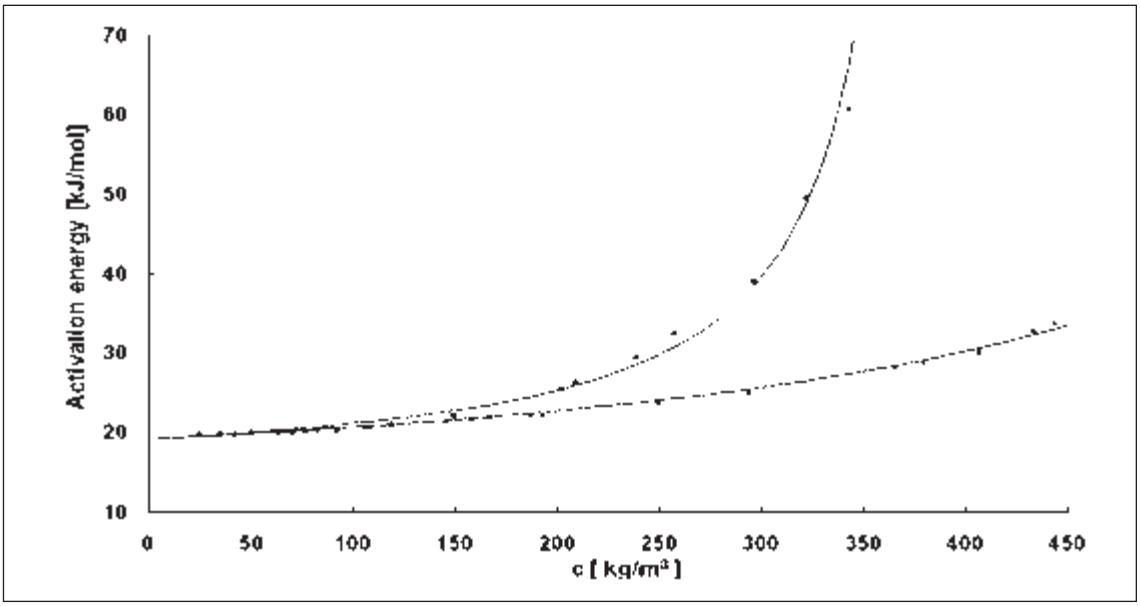

Figure 5. Plot of the solution activation energy $\Delta E(c,T)$ versus concentration c for bovine β-lactoglobulin (▲) and hen egg-white lysozyme (●) at t = 5°C. Experimental points were obtained on the basis of Eq. (4); the curves show the fit according to Eq. (7) with the parameters: $\Delta E_w(T) = 19.09$ kJ/mol and $\alpha = 2.743\times10^{6}$ kg/m³, $\xi = 1.29\times10^{-3}$ m³/kg, $\Delta E_p(T) = 3.7\times10^{4}$ kJ/mol for bovine β-lactoglobulin; $\alpha = 1.098\times10^{6}$ kg/m³ $\xi = 2.6\times10^{-3}$ m³/kg, $\Delta E_p(T) = 1.636\times10^{4}$ kJ/mol for hen egg-white lysozyme.

Rycina 5. Wykres energii aktywacji roztworu $\Delta E(c,T)$ od stężenia c dla bydlęcej β-laktoglobuliny (▲) i lizozymu (●) dla t = 5°C. Punkty doświadczalne otrzymano na podstawie równania (4); krzywe przedstawiają dopasowanie zgodnie z równaniem (7) z parametrami: $\Delta E_w(T) = 19.09$ kJ/mol i $\alpha = 2.743\times10^{6}$ kg/m³, $\xi = 1.29\times10^{-3}$ m³/kg, $\Delta E_p(T) = 3.7\times10^{4}$ kJ/mol dla bydlęcej β-laktoglobuliny; $\alpha = 1.098\times10^{6}$ kg/m³ $\xi = 2.6\times10^{-3}$ m³/kg, $\Delta E_p(T) = 1.636\times10^{4}$ kJ/mol dla lizozymu.

onically increase with increasing concentration of protein [22,43-47]. By assuming that the concrete parameter for a solution is a weighted sum of this parameter for water and dissolved proteins, the following relation for $D_s(c)$ and $\Delta E_s(c)$ - which determine the activation energy of a solution $\Delta E(c,T)$ - can be obtained [43]:

$$\Delta E_s(c) = \frac{c}{\alpha - \beta c}(\Delta E_p - \Delta E_w) + \Delta E_w \qquad (5)$$

$$D_s(c) = \frac{c}{\alpha - \beta c}(D_p - D_w) + D_w \qquad (6)$$

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. The effective specific volume is the constant of proportionality between the specific molar volume and the molar mass of a macrosolute [48]. The parameters ΔE_p, D_p characterize the dissolved protein, and ΔE_w, D_w the water molecules. In particular, ΔE_p and ΔE_w mean the activation energy of protein and water, respectively. The study of the concentration dependence of the activation energy of viscous flow of the solutions conducted by several authors [29,31-34] showed that the activation energy always increases with increasing concentration. Unfortunately, the functional description of such dependence has been proposed only by Durand [34], and the obtained fit to the experimental values of the activation energy is – in this case – not the best one. Contrary to this, the relation (5) describes very well the concentration dependence of the activation energy of globular proteins aqueous solutions in the whole of mono-disperse range [22,43-47].

At c = 0, the modified Arrhenius equation (3) describes the viscosity-temperature dependence for water. Taking the values of water viscosity from the standard physicochemical tables, one can obtain – for the temperature range from 5°C to 55°C – the following values of the parameters of this equation: ΔE_w = 32.88 kJ/mol and $D_w = 2.143\times10^{-2}$ K^{-1}. If the parameters ΔE_w and D_w are known, the pairs of parameters (ΔE_p, ξ) and (D_p, ξ) in equations (5) and (6) can be treated as adjustable parameters, and they can be calculated when the molecular mass of hydrated proteins is known. Proteins in solution are surrounded by water molecules, whose structure differs from that of bulk water. Internal water molecules, which fill cavities inside of proteins play an important role in the process of folding of a protein polypeptide chain and in maintaining its stability. Ordered water molecules on a protein surface, in turn, are attached through hydrogen bonds to the atoms of oxygen and nitrogen, or to the polar groups and contribute to the hydrodynamic friction in the same way as protein atoms. Water molecules from these two groups are an integral part of a protein and therefore contribute to its hydrodynamic mass [49,50]. So, the molecular mass of hydrated protein can be considered as a sum of the molecular mass of unhydrated protein M_p and the mass of hydration shell of water surrounding the protein molecules: $M_h = M_p(1 + \delta)$. The quantity δ means the level of protein hydration and is equal to the amount of grams of water associated with the protein per gram of protein.

In the case of IgG immunoglobulin the level of hydration for F_{ab} fragments and F_c fragment is equal to 0.5 and 0.7, respectively [51]. So, one can assume that for the entire IgG molecule: $\delta = 0.567$. Because the molecular mass of unhydrated IgG immunoglobulin: M_p = 156 kDa it gives the hydrodynamic mass of IgG: M_h = 244.4 kDa and in this case $\alpha = 1.358\times10^7$ kg/m^3. For HEWL $\delta = 0.38$ [52], M_h = 19.762 kDa, $\alpha = 1.098\times10^6$ kg/m^3 and for BLG $\delta = 0.36$ [14], M_h = 49.382 kDa, $\alpha = 2.743\times10^6$ kg/m^3. The pairs of parameters (ΔE_p, ξ) and (D_p, ξ) in equations (5) and (6) can be calculated by using the least squares method. The results of such calculations for unhydrated IgG and HEWL have been presented earlier [22,44]. In Table I the parameters ΔE_p, D_p and ξ obtained for hydrated proteins studied here are gathered.

It is quite natural to assume that the activation energy of a solution – at any individual temperature – can be treated as a superposition of the activation energy of dissolved protein molecules $\Delta E_p(T)$ and water molecules $\Delta E_w(T)$ at the same temperature. This assumption leads to the relation analogous to that presented in equation (5):

$$\Delta E(c,T) = \frac{c}{\alpha - \beta c}\left[\Delta E_p(T) - \Delta E_w(T)\right] + \Delta E_w(T) \qquad (7)$$

On the other hand, definition (2) and the modified Arrhenius equation for water allow obtaining the relation connecting the activation energy of water molecules with temperature:

$$\Delta E_w(T) = \Delta E_w - RD_w T^2 \qquad (8)$$

The activation energy of water molecules, calculated from the above equation changes from $\Delta E_w(T)$ = 19.09 kJ/mol (t = 5°C) up to $\Delta E_w(T)$ = 13.69 kJ/mol (t = 55°C). When $\Delta E_w(T)$ in relation (7) is known, $\Delta E_p(T)$ is the only unknown parameter. It can be calculated by using once more the least squares method. The results of such calculations for the studied proteins are presented in Figures 6 and 7. Figures 3, 4 and 5, in turn, show that for the studied proteins the curves obtained on the basis of relation (7) give good fit to the values obtained from equation (4).

The numerical values of the activation energy of the studied proteins $\Delta E_p(T)$ presented in Figures 6 and 7 show that this quantity decreases with increasing temperature. Because the activation energy both for solution (Eq. 4) and for water (Eq. 8) decreases with increasing temperature according to square function, one can suspect that the same function describes the temperature dependence of the activation energy for proteins, namely: $\Delta E_p(T) = a - RbT^2$. Using the numerical values of $\Delta E_p(T)$ calculated for the studied proteins on the basis of formula (7), one can obtain – by applying once more the least squares method – the adjustable parameters "a" and "b". This way calculated parameters are presented in Table I. A comparison – for each studied protein – the numerical values of ΔE_p with "a" and D_p with "b" shows that this pairs of quantities are identical (in the range of estimated errors). So, one can assume that the following relation is fulfilled:

$$\Delta E_p(T) = \Delta E_p - RD_p T^2 \qquad (9)$$

The curves of dependence of $\Delta E_p(T)$ versus temperature obtained on the basis of the above relation for the studied proteins are presented in Figure 6 and 7. As seen, the curves give very good fit to the values obtained from equation (7). It strongly suggests that the activation energy of viscous flow of a protein at any individual temperature can be calculated from relation (9) if the parameters ΔE_p and D_p are known. They should be calculated from formula (5) and (6). Quite recently the relation analogous

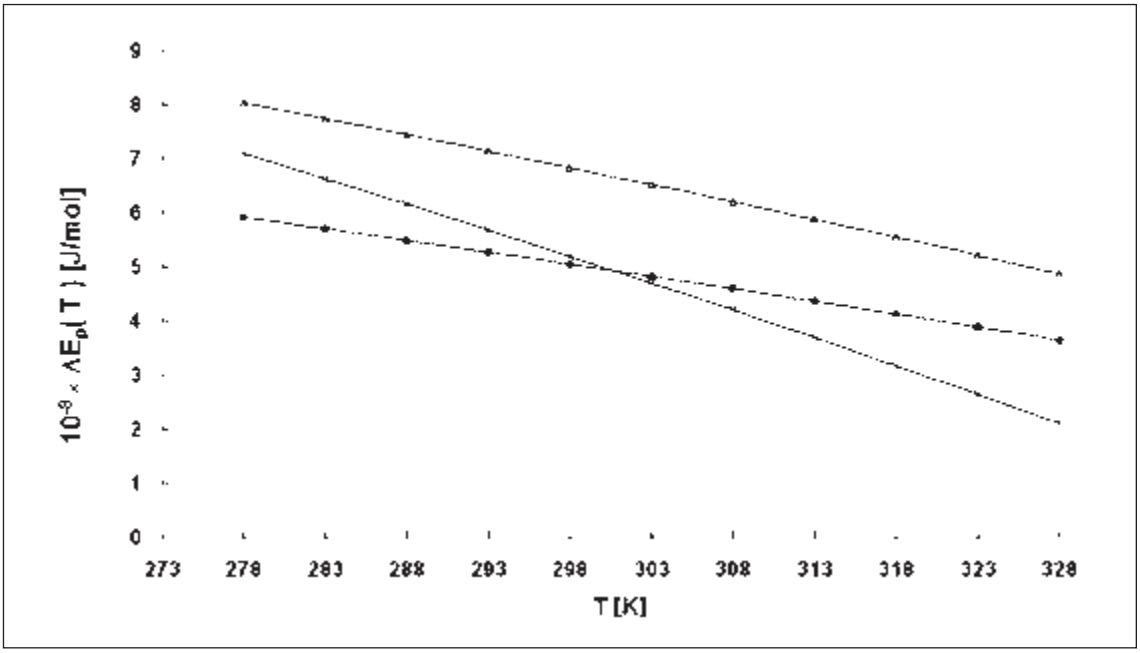

Figure 6. Plot of the activation energy of viscous flow $\Delta E_p(T)$ versus temperature for human (•), porcine (×) and bovine (Δ) immunoglobulin IgG. Points have been obtained from relation (7) in which $\Delta E_p(T)$ is the adjustable parameter. The curves show the fit according to formula (9) with: $\Delta E_p = 1.17\times10^9$ J/mol, D_p = 902 K^{-1} for human IgG, $\Delta E_p = 1.98\times10^9$ J/mol, D_p = 1968 K^{-1} for porcine IgG, $\Delta E_p = 1.61\times10^9$ J/mol, D_p = 1254 K^{-1} for bovine IgG.

Rycina 6. Wykres energii aktywacji przepływu lepkiego $\Delta E_p(T)$ od temperatury dla immunoglobuliny IgG: ludzkiej (•), świńskiej (×) i bydlęcej (Δ). Punkty otrzymano z relacji (7) w której $\Delta E_p(T)$ jest parametrem dopasowania. Krzywe przedstawiają dopasowanie zgodnie z formułą (9) z: $\Delta E_p = 1.17\times10^9$ J/mol, D_p = 902 K^{-1} dla ludzkiej IgG, $\Delta E_p = 1.98\times10^9$ J/mol, D_p = 1968 K^{-1} dla świńskiej IgG, $\Delta E_p = 1.61\times10^9$ J/mol, D_p = 1254 K^{-1} dla bydlęcej IgG.

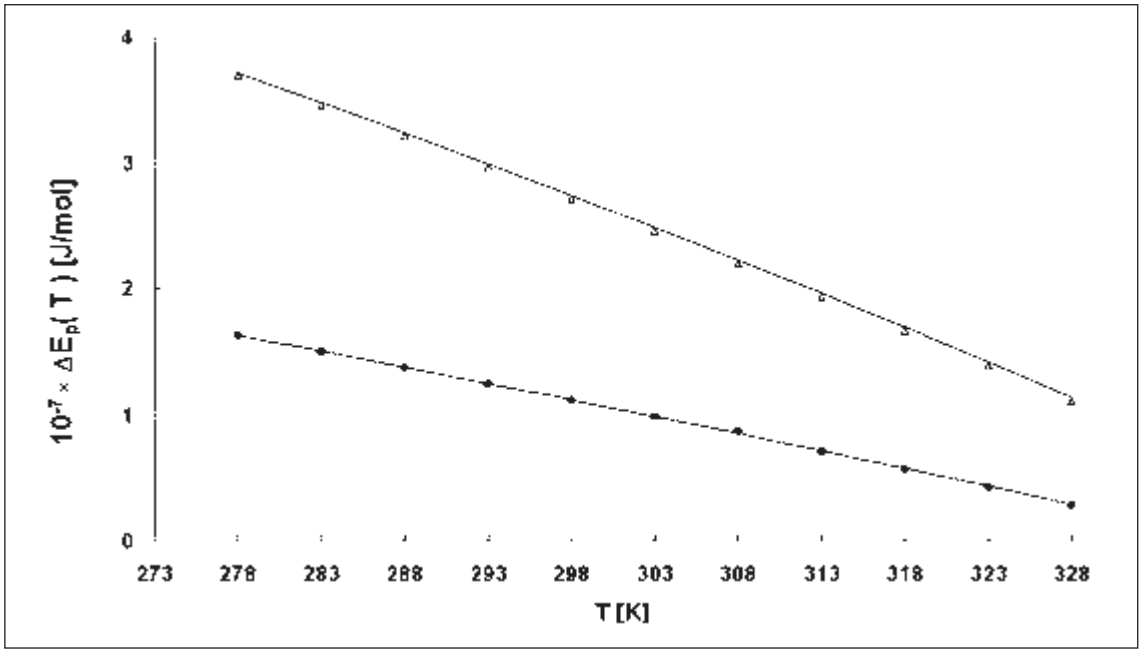

Figure 7. Plot of the activation energy of viscous flow $\Delta E_p(T)$ versus temperature for lysozyme (•) and bovine β-lactoglobulin (Δ). Points have been obtained from relation (7) in which $\Delta E_p(T)$ is the adjustable parameter. The curves show the fit according to formula (9) with: $\Delta E_p = 5.07\times10^7$ J/mol, $D_p = 53.3$ K^{-1} for lysozyme and $\Delta E_p = 1.03\times10^8$ J/mol, $D_p = 102$ K^{-1} for bovine β-lactoglobulin.

Rycina 7. Wykres energii aktywacji przepływu lepkiego $\Delta E_p(T)$ od temperatury dla lizozymu (•) i bydlęcej β-laktoglobuliny (Δ). Punkty otrzymano z relacji (7) w której $\Delta E_p(T)$ jest parametrem dopasowania. Krzywe przedstawiają dopasowanie zgodnie z formułą (9) z: $\Delta E_p = 5.07\times10^7$ J/mol, $D_p = 53.3$ K^{-1} dla lizozymu i $\Delta E_p = 1.03\times10^8$ J/mol, $D_p = 102$ K^{-1} dla bydlęcej β-laktoglobuliny.

to (9) has been obtained for ovalbumin [53] and it suggests that it is universal one.

The results of the activation energy for the studied proteins presented in Figures 6 and 7 show that one of the factor which influences this quantity is molecular mass of protein – the higher molecular mass the higher activation energy. However this is not the only factor. This is evident for the studied immunoglobulins, which have the same molecular mass but quite different values of activation energy.

The electrostatic interactions between two proteins in solution can be divided into two categories: (i) repulsive interaction between net charges of the proteins and (ii) attractive dipole-dipole interaction between their dipole moments. In the conditions in which the viscosity measurements has been conducted for the studied immunoglobulins, i.e. for the pH of the solutions in the vicinity of isoelectric point, the net charges on the proteins are small, the Coulomb repulsion is negligible and the attractive dipole-dipole interaction

Table I. The numerical values of the parameters ΔE_p, D_p and ξ for the studied proteins calculated from equations (5) and (6). The parameters "a" and "b" have been obtained as adjustable parameters in equation $\Delta E_p(T) = a - RbT^2$.

Tabela I. Wartości liczbowe parametrów ΔE_p, D_p and ξ dla badanych białek obliczone z równań (5) i (6). Parametry "a" i "b" zostały otrzymane jako parametry dopasowania w równaniu $\Delta E_p(T) = a - RbT^2$.

Protein	ξ [m^3/kg]	ΔE_p [kJ/mol]	a [kJ/mol]	D_p [K^{-1}]	b [K^{-1}]
HEWL	2.60×10^{-3}	$(5.34\pm0.45)\times10^4$	5.07×10^4	56.4±5.7	53.3
BLG	1.29×10^{-3}	$(1.03\pm0.02)\times10^5$	1.03×10^5	107±5.9	102
HIgG	2.25×10^{-3}	$(1.19\pm0.06)\times10^6$	1.17×10^6	912±58.6	901.7
BIgG	3.40×10^{-3}	$(1.72\pm0.13)\times10^6$	1.61×10^6	1184±77.7	1254
PIgG	2.71×10^{-3}	$(1.97\pm0.09)\times10^6$	1.98×10^6	1986±113	1968

dominates. Despite the similarity in experimental conditions and the similarity in three dimensional conformation of the studied immunoglobulins substantial differences occur in the temperature dependence of their activation energy (see Figure 6). For instance, $\Delta E_p(T)$ at 5°C for BIgG is about 10% greater than for PIgG, but at 55°C is more than 2 times greater. The activation energy at 27.5°C for HIgG and PIgG is the same, but at 5°C $\Delta E_p(T)$ for PIgG is greater than for HIgG and at 55°C the situation is reversed. It shows that in details the activation energy of each mammalian IgG immunoglobulin should be studied separately. To explain which factors influence the rate of decreasing of the activation energy with increasing temperature further experimental and theoretical work is needed. This is interesting problem not only in the case of viscous flow of liquids but also for other activated processes like, for instance, for the processes of biochemical reactions.

CONCLUSIONS

A modified Arrhenius equation – which describes the viscosity-temperature dependence at a wide range of temperatures - allows obtaining the functional dependence of the activation energy of viscous flow for HEWL, BLG and mammalians IgG immunoglobulins in aqueous solutions. As appears, both for solutions, water molecules and proteins the activation energy decreases with increasing temperature according to the two parameters square function. The first of these parameters means the activation energy at T = 0 K, and the second one denotes the rate of decreasing of the activation energy with increasing temperature. Analysis of the results obtained for hen egg-white lysozyme, bovine β-lactoglobulin and human, bovine and porcine IgG immunoglobulin shows that activation energy of protein depends on its molecular mass. However, that this is not the only factor which influence the activation energy of protein. The results obtained for the studied immunoglobulins show that despite the similarity in experimental conditions, the similarity of molecular mass and the similarity in their three dimensional conformation the above mentioned parameters – for each IgG immunoglobulin – are quite different. It indicates that each protein should be studied in details separately.

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