

THERMODYNAMICS OF INTERACTION BETWEEN POLYREACTIVE IMMUNOGLOBULINS AND IMMOBILIZED ANTIGEN

S. A. BOBROVNIK¹✉, O. V. OGLOBLYA²,
M. O. DEMCHENKO¹, S. V. KOMISARENKO¹

¹Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv;

²ESC Institute of Biology and Medicine,
Taras Shevchenko National University of Kyiv, Ukraine;

✉e-mail: s-bobrov@ukr.net

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In order to determine thermodynamic parameters of the interaction between polyreactive immunoglobulins (PRIGs) and immobilized antigen, several of experimental kinetic curves of PRIGs binding to immobilized ovalbumin were obtained at different temperatures. This allowed determining the rate constants for every step of the binding process for each temperature. Then, using appropriate equations, thermodynamic parameters, such as activation energy, enthalpy, entropy, and standard free energy (Gibbs energy), were calculated. Thermodynamic values obtained show that the main energy consuming step in the study process of PRIGs binding is the transformation of “inactive” PRIGs into “active” PRIGs, i.e. formation of hydrophobic patches on the surface of PRIGs molecules. In contrast, the following step of the binding of “active” PRIGs to an immobilized antigen is not an energy dependent process.

Key words: polyreactive immunoglobulins, antigens, kinetics of interaction, rate constants, thermodynamic parameters.

The mechanism of interaction between specific antibodies and antigens, its kinetics and thermodynamics are well-studied processes [1]. However, the discovery of PRIGs, which are capable of non-specific binding to a variety of unrelated antigens, raised the question about the mechanism of this interaction, as well as thermodynamics of the process. Despite the fact that the study of PRIGs (which in the literature are more often referred to as polyreactive antibodies, which is not quite correct) has recently been given more and more attention [2-11], the above questions have not yet been solved, although they are of a great interest.

We have previously established that the interaction of PRIGs with antigens is a process that is much more complex than the interaction of specific antibodies with the antigen, as well as the fact that this process is much more temperature dependent than that of specific antibodies with the corresponding

antigen [12]. The elucidation of the mechanism of interaction between PRIGs and antigens made it possible to describe the dynamics of this process by means of a system of differential equations. The solution of these equations made it possible to obtain algebraic expressions describing the dynamics of PRIG concentration changes at various stages of PRIG-antigen interaction [13].

Later, we proposed a way to numerically solve these complex equations, which were both transcendental and irrational. It is known that such equations do not have an analytical solution, but they can be solved using numerical methods and a computer. The possibility to find a numerical solution of these equations allowed us to find the values of the rate constants of each of the interaction stages, using the experimental curves of the PRIG binding dynamics to the immobilized antigen [13], which depends on the temperature. Finally, the analysis of the dependence

of these rate constants on temperature made it possible to calculate the thermodynamic parameters of the interaction of PRIG with the immobilized antigen. The results of these studies are presented below.

Materials and Methods

PRIGs were obtained by transformation of control (not immune) BALB/c mice serum by incubation of this serum with 3.5 M KSCN during 5 min at 25°C and subsequent dilution of this mixture 1000-5000 times with physiological buffered solution (PBS, pH 7.2). It was shown earlier [12], that such treatment of serum immunoglobulins results in transformation of the majority of them into PRIGs that are able to bind nonspecifically to various antigens. These PRIGs were used to study their binding to antigen (ovalbumin, Sigma, USA) immobilized on polystyrene plates.

Ovalbumin was immobilized on polystyrene plates by the method developed by us before, which increases the hydrophobicity of proteins being adsorbed on polystyrene surface [14]. Briefly, ovalbumin solution (0.1 mg/ml) in 1% (NH₄)₂CO₃ with added 0,01% NaN₃ was placed in the wells of polystyrene plates and then dried out in a thermostat at 37°C. Plates were carefully washed by tap water with subsequent PBS washing to remove poorly associated ovalbumin molecules immediately before experiment, and were used for the study of PRIGs binding. As it was shown before [15], this method of protein immobilization allows obtaining the layer of the partially denatured molecules associated with polystyrene. Such immobilized protein molecules exhibit hydrophobic areas on their surfaces, allowing to study nonspecific PRIGs binding.

PRIGs bound to immobilized ovalbumin was determined by ELISA as it has been described previously [16]. For this purpose, plates with bound PRIGs were incubated with 1:2000 diluted in PBS + 0.1% Tween 20 (TBS) horseradish peroxidase-conjugated goat anti-mouse IgG antibodies (Sigma, USA) during 60 min at 4°C. Then, after careful washing from unbound conjugates, the substrate for peroxidase, namely, the solution of o-phenylenediamine (1 mg/ml) in 0.01 M phosphate buffer (pH 5.0) and 0.003% H₂O₂ solution was added to plates. The reaction was stopped after color developing by adding 0.05 ml 2 M sulfuric acid per wells, and optical density of solutions was measured by microphotocolorimeter ELx800, BIO-TEK, at wave length of 490 nm.

It was shown earlier [17] that in order to use the units of absorption instead of the real PRIGs con-

centrations we had to do preliminary experiments to determine the initial PRIGs dilutions which allow us to obtain 2-fold, 4-fold, 8-fold etc. decrease of absorption with 2-fold, 4-fold, 8-fold etc. increase of dilution, respectively. In this study, according to our pilot experiments, PRIGs concentrations in binding experiments performed at 0°C should exceed those at 37°C by 30 times.

In order to allow reaching the state of equilibrium between an amount of “active” and “inactive” PRIGs in the studied PRIGs solutions, we prepared appropriate PRIGs solutions at 4°C and incubated them during 18-20 hours before the experiment, followed by incubation at the appropriate temperature for at least 2 h. After such incubation, the samples of PRIGs were placed in the wells of plates with adsorbed antigen in different time intervals in order to finalize the different incubation periods simultaneously. After finishing the incubation PRIGs solutions in plates, the solutions with unbound PRIGs were removed from the wells by the plates shaking and subsequent rinsing. Finally, plates were treated, as described earlier, by peroxidase-conjugated anti-Ig serum, and the amount of PRIGs bound to ovalbumin was determined by ELISA.

The acquired curves of PRIGs binding at different temperatures were used to determine the rate constants of this process by the method described below.

Mathematical derivations. In order to determine activation energy, E_a , for each step of PRIGs binding to an antigen one can use the Arrhenius equation:

$$k = A \cdot e^{-\frac{E_a}{RT}}. \quad (1)$$

Where k is the rate constant; R is the gas constant ($R = 8.314\text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$); T is reaction absolute temperature (in Kelvins).

From (1) it follows that

$$\ln k = \ln A - \frac{E_a}{RT}. \quad (2)$$

Thus, the Arrhenius plot can be obtained by plotting the logarithm of the rate constant, k , versus the inverse temperature, $1/T$, and the slope of this linear plot is equal to E_a .

It is known [18] that the Van't Hoff equation relates a temperature-dependent change in equilibrium constant, K_e , to its standard enthalpy, ΔH^0 :

$$\frac{d \ln K_e}{dT} = \frac{\Delta H^0}{RT^2}. \quad (3)$$

Integration of Eq. (3) gives the following relation between the value of standard enthalpy ΔH^o and the reaction temperature T_1 and T_2 :

$$\Delta H^o = \frac{RT_1T_2 \ln \frac{K_{e2}}{K_{e1}}}{T_2 - T_1}, \quad (4)$$

where K_{e1} and K_{e2} – equilibrium constants at temperatures T_1 and T_2 , correspondingly.

The same relation can be obtained by a quite different way. According to the main equation of thermodynamics we have:

$$\Delta G^o = \Delta H^o - T\Delta S^o. \quad (5)$$

Where ΔG^o is the change of standard free energy (Gibbs energy) and ΔS^o is the change of standard entropy at a given temperature T .

Taking into account that

$$\Delta G^o = -RT \ln K_e \quad (6)$$

then the following two equations can be obtained for two different temperatures, T_1 and T_2 :

$$\begin{cases} -RT_1 \ln K_{e1} = \Delta H^o - T_1\Delta S^o \\ -RT_2 \ln K_{e2} = \Delta H^o - T_2\Delta S^o \end{cases} \quad (7)$$

Algebraic solution of Eqs. (7) allows one to get not only the same formula as Eq. (4) for ΔH^o evaluation, but also to obtain the following expression for ΔS^o calculation:

$$\Delta S^o = \frac{RT_2 \ln K_{e2} - RT_1 \ln K_{e1}}{T_2 - T_1}. \quad (8)$$

If values of K_{e1} and K_{e2} are known at different temperatures T_1 and T_2 , using Eqs. (4), (6), and (8) one can also determine the values of thermodynamics characteristics ΔH^o , ΔG^o , and ΔS^o . The accuracy of ΔH^o and ΔS^o determination could be increased if one can do several measurements of K_e at correspondent temperatures T_i . In this case we can represent Eqs. (4) and (8) as following:

$$RT_1T_2 \ln \frac{K_{e2}}{K_{e1}} = \Delta H^o(T_2 - T_1) \quad (9)$$

$$RT_2 \ln K_{e2} - RT_1 \ln K_{e1} = \Delta S^o(T_2 - T_1). \quad (10)$$

From Eq. (9) it follows that the plot $RT_1T_2 \ln \frac{K_{e2}}{K_{e1}}$ versus values $(T_2 - T_1)$ should be a straight line with the slope ΔH^o . Thus, building this plot for several points T_i and measuring its slope allows to determine ΔH^o with high accuracy.

The same is also true for the calculation the values of ΔS^o . According to Eq. (10), ΔS^o is equal to the slope of linear plot for the relation between $RT_2 \ln K_{e2} - RT_1 \ln K_{e1}$ and $(T_2 - T_1)$.

The mechanism of nonspecific interaction between PRIGs and any immobilized antigen [13] was determined earlier and the following scheme of this interaction was proposed:



where N – concentration of “inactive” PRIGs in the solution; A – concentration of “active” PRIGs in the solution; C – concentration of PRIGs bound to immobilized antigen; k_1 – rate constant of the transformation of “inactive” PRIGs into “active” PRIGs; k_2 – rate constant of the transformation of “active” PRIGs into “inactive” PRIGs; k_3 – rate constant of the binding of “active” PRIGs to immobilized antigen.

The following system of differential equations describes such interaction:

$$\begin{cases} \frac{dN}{dt} = k_2A - k_1N \\ \frac{dA}{dt} = k_1N - (k_2 + k_3)A \\ \frac{dC}{dt} = k_3A \end{cases} \quad (12)$$

The solution of the system of differential equations (12) results in equation (13) that describes the dynamics of PRIGs bounding to immobilized antigen, i.e. the value of C :

$$\begin{aligned} C = S & \frac{S(r_2+k_3)(r_1+k_2+k_3)}{(k_1+k_2)(r_2-r_1)} e^{r_1t} - \\ & - \frac{S[k_2(r_2-r_1)-(r_2+k_3)(r_1+k_2+k_3)]}{(k_1+k_2)(r_2-r_1)} e^{r_2t} - \\ & - \frac{k_1S(r_2+k_3)}{(k_1+k_2)(r_2-r_1)} e^{r_1t} - \\ & - \frac{k_1S[k_2(r_2-r_1)-(r_2+k_3)(r_1+k_2+k_3)]}{(k_1+k_2)(r_2-r_1)(r_2+k_2+k_3)} e^{r_2t}, \end{aligned} \quad (13)$$

where

$$\begin{aligned} r_1 &= \frac{-(k_1+k_2+k_3) + \sqrt{(k_1+k_2+k_3)^2 - 4k_1k_3}}{2}; \\ r_2 &= \frac{-(k_1+k_2+k_3) - \sqrt{(k_1+k_2+k_3)^2 - 4k_1k_3}}{2}; \end{aligned}$$

S is the total amount of PRIGs in the study solution, i.e. $S = N + A + C$.

Then, using Eq. (13) and experimental kinetic curves of nonspecific PRIGs binding to an immobilized antigen that were obtained at different temperatures allows us to determine the values of the rate constants k_1 , k_2 and k_3 [13].

Let's denote $Y(t) = C(t)/S$, then, after some simple transformations, this function can be written in the form (14).

It's easy to see

$$Y(t) = 1 - e^{-\frac{1}{2}(k_1+k_2+k_3)t} \left(\frac{\cosh\left(\frac{1}{2}\sqrt{(k_1+k_2+k_3)^2-4k_1k_3}t\right) + \left((k_1+k_2)^2+(k_2-k_1)k_3\right)\sinh\left(\frac{1}{2}\sqrt{(k_1+k_2+k_3)^2-4k_1k_3}t\right)}{(k_1+k_2)\sqrt{(k_1+k_2+k_3)^2-4k_1k_3}} \right) \quad (14)$$

$$-\frac{Y''(0)}{Y'(0)} = k_3 \quad (15)$$

and

$$k_1 \left(\frac{k_3}{Y'(0)} - 1 \right) = k_2. \quad (16)$$

For non-linear fitting of curve (Eq. 14) to experimental data seed values $(\bar{k}_1, \bar{k}_2, \bar{k}_3)$ close to the accurate values (k_1, k_2, k_3) should be provided. For the task of finding seed values we interpolated the experimental data and find the first and second derivatives of the interpolation function $Y_{interp}(t)$ at zero time. From (15) we obtain the following formula for the seed value

$$\bar{k}_3 = -\frac{Y_{interp}''(0)}{Y_{interp}'(0)}. \quad (17)$$

Because $r_1 > r_2$ then for large values of time $t > t_{plato}$, when the function has almost reached a plateau $Y(t) \approx 1$ (and experimental data for this area are usually already lacking, because it can happen after a significant interval of time, for example after a week), the most essential for the logarithmic derivative $\frac{d \ln(1-Y(t))}{dt}$ is the term with algebraically greater value of the decrements in exponents (Eq. 13). This is the term with the lesser modulus (the numbers r_1, r_2 are negative), i.e. r_1 . Since $Y(t) = 1 - A_1 e^{r_1 t} - A_2 e^{r_2 t}$ and $\frac{d \ln(1-Y(t))}{dt} = \frac{A_1(r_1 - r_2)}{A_1 + A_2 e^{(r_2 - r_1)t}} + r_2$ at large values of time the exponent in the denominator tends to zero under the condition $r_1 > r_2$, we obtain the value r_1 . This allows us to estimate the seed value of the parameter \bar{r}_1 :

$$\bar{r}_1 = \left. \frac{d \ln(1-Y(t))}{dt} \right|_{t=t_{plato}} \quad (18)$$

$$\text{But } r_1 = \frac{-(k_1+k_2+k_3) + \sqrt{(k_1+k_2+k_3)^2 - 4k_1k_3}}{2},$$

let's substitute here the value for the seed value of

the parameter $\bar{k}_2 = \bar{k}_1 \left(\frac{\bar{k}_3}{Y'_{interp}(0)} - 1 \right) \bar{k}_1$ and we get \bar{k}_1 :

$$\bar{k}_1 = -\frac{Y'_{interp}(0)\bar{r}_1(\bar{k}_3 + \bar{r}_1)}{\bar{k}_3(Y'_{interp}(0) + \bar{r}_1)} \quad (19)$$

Now we have founded values \bar{k}_1, \bar{k}_3 and with help of the formula (16) we could get \bar{k}_2 :

$$\bar{k}_2 = \bar{k}_1 \left(\frac{\bar{k}_3}{Y'_{interp}(0)} - 1 \right) \quad (20)$$

From here, we wrote a program in Mathematica (Wolfram Research) to find the kinetic parameters of the process. The program uses the exact solution to the differential equations discussed above as fitting function (Eq. 14). As (Eq. 14) asymptotically approaches 1 as time increases $\lim_{t \rightarrow \infty} Y(t) = 1$,

we added additional data points at a sufficiently long time with values equal 1. Then we build interpolating function for experimental data with added some extra points at a sufficiently long time. We use this interpolating function for finding its first and second derivatives at time zero and logarithmic derivative of one minus interpolating function at a sufficiently long time. Obtained quantities allows us to calculate approximate values of needed parameters (Eq. 17, 19, 20), which were used on the next step as seed values for the kinetic parameters of the model. Finally, using Computational Intelligence Packages (CIP) for Mathematica, which implements the Levenberg-Marquardt algorithm, we found more precise parameters by non-linear fitting starting from these seed values and made to match our experimental data. This provided precise parameters for (Eq. 13, 14).

Results and Discussion

In our previous paper [13] we presented the data demonstrating that the earlier suggested by us mechanism of PRIGs nonspecific binding to an im-

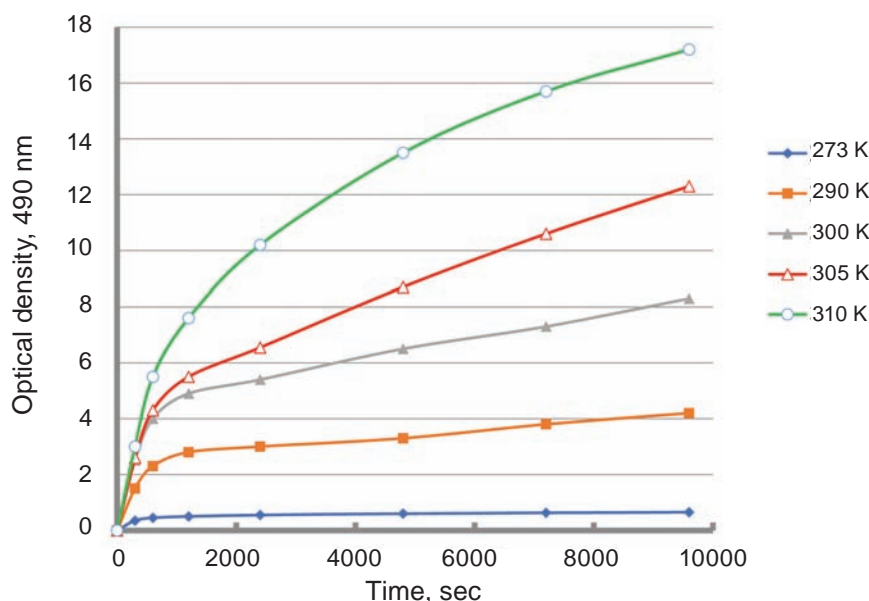


Fig. 1. Kinetic curves of PRIGs binding to immobilized antigen at different temperatures: 273, 290, 300, 305, and 310 K

mobilized antigen allows determining the rate constants for each step of the process.

By doing measurements at different temperatures (Fig. 1), it was possible to determine the relations between rate constants and temperature (Table 1) and by utilizing presented above equations (1)-(13) to evaluate thermodynamic parameters of PRIG-antigen interaction.

From the above table it is obvious that the observed value of k_3 does not depend on the temperature. It does not increase with the increasing of the temperature and even has some tendency to diminish at temperatures higher than 290 K. And conversely, the constants k_1 and k_2 gradually increase with temperature.

According to the Arrhenius' theory, the absence of the effect of temperature on the reaction rate most likely means that the activation energy of this

reaction, E_a , is close to zero and it does not need to overcome a potential barrier equal to the activation energy value for the interaction of the reacting molecules. In the above example, k_3 is the rate constant of the binding reaction between the activated PRIGs molecules and the antigen immobilized on the plate due to their hydrophobic interaction. Thus, we can assume, at least in this case, that the hydrophobic interaction between PRIGs and antigen is very little dependent on temperature, or that this relationship is more complex than the directly proportion dependence of the logarithm of the rate constant on the inverse value of temperature, expressed in Kelvin units. Note that similar patterns of hydrophobic interaction dependence on temperature were observed by other authors when studying completely different reagents and under different conditions [19, 20].

A completely different situation was found when we studied the dependence of k_1 and k_2 values on temperature. This allowed us to construct linear dependences corresponding to equations (1)-(11) and to determine the values of several thermodynamic parameters of the investigated process. Fig. 1 shows the dependence of the logarithms of the rate constants k_1 and k_2 on the inverse temperature according to Eq. (2):

As can be seen from Fig. 2 (and as it should be according to Eq. 2), the values of the logarithms of the rate constants k_1 and k_2 are proportional to the inverse of the reaction temperature, expressed in

Table 1. Rate constants of PRIGs binding to immobilized ovalbumin at different temperatures

T (K)	k_1 (s ⁻¹)	k_2 (s ⁻¹)	k_3 (M×s ⁻¹)
273	8.9031E-07	3.4000 E-05	3.7300E-03
290	1.81963E-05	1.55600E-04	4.87690E-03
300	2.99750E-05	9.49800E-05	2.64300E-03
305	8.00240E-05	2.43694E-04	2.71960E-03
310	2.04400E-04	3.16340E-04	1.75080E-03

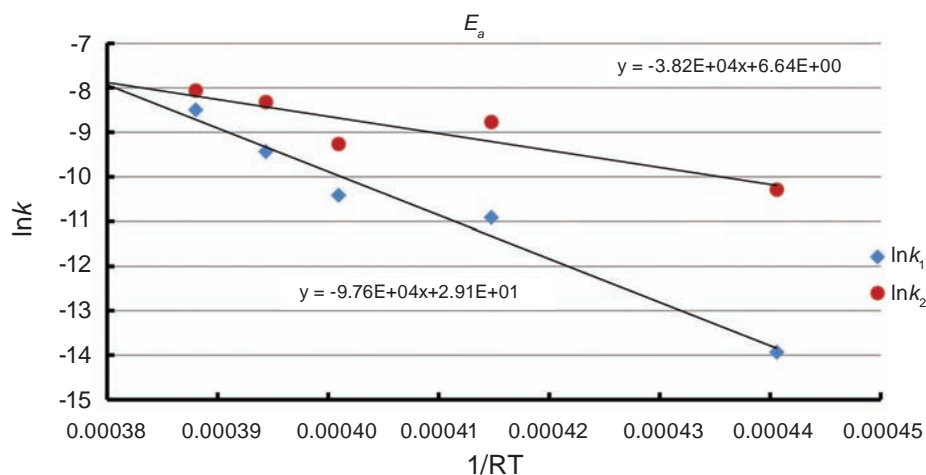


Fig. 2. Linear dependences between the logarithms of the rate constants k_1 and k_2 and the inverse values of the reaction temperature, expressed in degrees Kelvin

degrees Kelvin and multiplied by the value of the gas constant, R . Due to this fact, we obtained linear dependences, the negative values of the tangents of the slope which are equal to the activation energy of these processes, E_a , could be easy to calculate. Using Microsoft Excel, we found that for the PRIGs “activation” process, the equation for the linear relationship between $\ln(k_1)$ and $1/(RT)$ is $y = -97600x + 29.1$, and for the reverse process, i.e. for the “inactivation” of the PRIG, the linear dependence of $\ln(k_2)$ on $1/(RT)$ will be represented by the equation $y = -38200x + 6.64$. Consequently, E_a for “activation” of PRIGs is $97600 \text{ J}\cdot\text{mol}^{-1}$, and for the process of “inactivation” of PRIGs $E_a = 38200 \text{ J}\cdot\text{mol}^{-1}$.

It is important to notice that constant k_1 increases much faster with increasing temperature than k_2 (Table 1). For this reason, the equilibrium constant of the PRIG “activation” process, K_e , equal to the ratio of k_1 to k_2 , is almost ten times higher at 310 K than at 273 K (Table 2). Consequently, as the temperature increases, the proportion of PRIGs molecules that are “activated”, i.e., exhibit hydrophobic sites on their surface and, due to this, are capable of non-specific binding to antigens, increases significantly. Using Eq. (6) we can also determine the ΔG° values for the transformation of PRIGs into “active” molecules at different temperatures (Table 2). As can be seen from Table 2, ΔG° values stay positive at all temperatures. This suggests that the PRIG “activation-inactivation” process is sharply shifted toward the formation of “inactive” molecules, i.e., those that do not expose hydrophobic sites on their surface.

It important to remember that the ΔG° value is several times lower at 310 K than at 273 K. This

suggests that as the temperature rises to 310 K, the appearance of “active” PRIGs becomes much more likely than at 273 K. As a result, the portion of “active” PRIGs increases drastically with temperature increase leading to dramatic increase in the rate of PRIGs binding to the immobilized antigen.

Furthermore, we can determine the values of ΔH° and ΔS° for the PRIG “activation” process from equations (9) and (10). Fig. 3 shows the dependency of $T_1 T_2 \ln \frac{K_{e2}}{K_{e1}}$ on the value of $(T_2 - T_1)$. According to equation (9), the tangent of the angle of the obtained linear dependency is ΔH° . Using the Microsoft Excel program, it is easy to determine that in this case $\Delta H^\circ = 59895 \text{ J}\cdot\text{M}^{-1}$. Thus, the values of enthalpy of the reaction of “activation” of PRIGs molecules, i.e. induction of appearance of hydrophobic sites on the surface of a protein molecule in hydrophilic medium, which are normally hidden inside the molecule, shows us that it is a very energy-consuming process.

Using similar approach, we can determine the values of ΔS° for the “activation” process of

Table 2. The values of K_e and ΔG° for PRIGs transformation from “inactive” into “active” state

t (K)	$k_1/k_2 = K_e$	ΔG° (J)
273	3.4000 E-05	8267.6
290	1.55600E-04	5174.3
300	9.49800E-05	2876.6
305	2.43694E-04	2823.8
310	3.16340E-04	1125.6

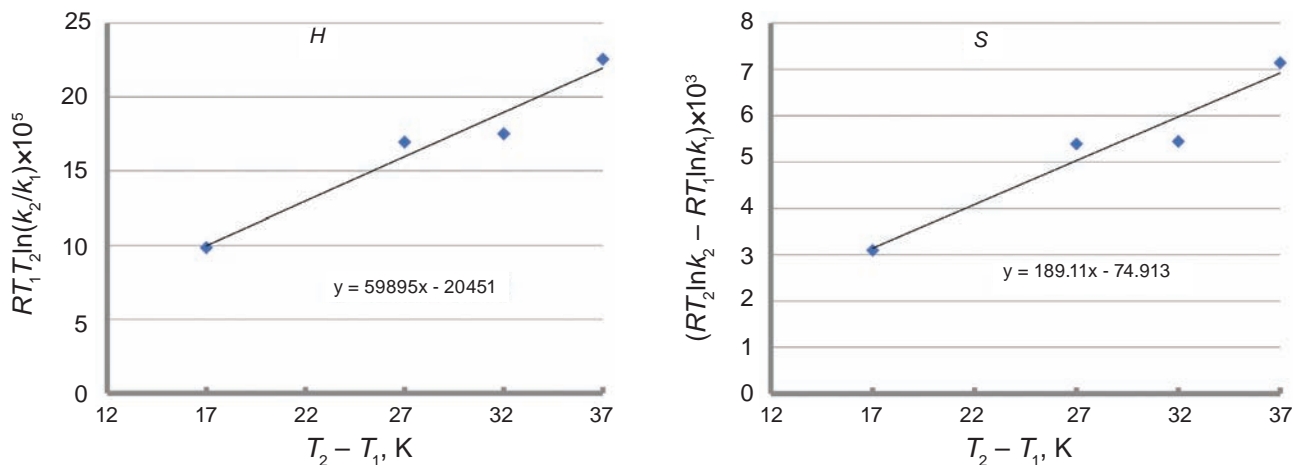


Fig. 3. Evaluation of the values of ΔH° and ΔS° for the PRIG "activation" process according to Eqs. (9) and (10)

PRIGs molecules, based on the linear dependence of $RT_2 \ln K_{e2} - RT_1 \ln K_{e1}$ on $(T_2 - T_1)$ according to equation (10). In this case, we find that the change in entropy under standard conditions $\Delta S^\circ = 189 \text{ J} \times \text{M}^{-1} \times \text{grad}^{-1}$ (i.e., entropy units), which is also a very significant value. In other words, structural changes in the polypeptide PRIG chain stacking occur as a result of which hydrophobic sites briefly appear on the surface of the molecule, and this process, according to our calculations, is accompanied by a significant increase in entropy.

Thus, using the previously proposed model of the nonspecific reaction of interaction of PRIGs with antigens and the series of kinetic curves of PRIGs binding to immobilized antigen at various temperatures obtained in the experiment, we managed to find the rate constants of individual stages of the considered process, and also determine the thermodynamic characteristics of each of the stages. We hope that the data obtained will allow for a better understanding of the process of non-specific PRIG binding to a variety of antigens. Assuming that this interaction also occurs *in vivo*, the data obtained may also be of certain biological significance.

In addition, as we have already mentioned in a previous article [13], the process of PRIGs binding to antigen is similar to nonspecific aggregation of various proteins in solutions. Like the process of interaction of PRIGs with the antigen, the processes of protein aggregation are highly dependent on the temperature of the solution. In this connection, it is possible to assume that the regularities of PRIGs "activation" and subsequent PRIGs binding to antigen can be similar to the processes of protein aggregation. Methods used in this article can be ap-

plied to solve practical problems for example to find ways to store proteins in solutions and to minimize irreversible aggregation of these proteins during storage.

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ТЕРМОДИНАМІКА ВЗАЄМОДІЇ МІЖ ПОЛІРЕАКТИВНИМИ ІМУНОГЛОБУЛІНАМИ ТА ІММОБІЛІЗОВАНИМИ АНТИГЕНАМИ

С. А. Бобровник¹✉, О. В. Оглобля²,
М. О. Демченко¹, С. В. Комісаренко¹

¹Інститут біохімії ім. О. В. Палладіна
НАН України, Київ;

²ІНЦІ Інститут біології та медицини,
Київський національний університет
імені Тараса Шевченка, Україна;

✉e-mail: s-bobrov@ukr.net

Для визначення термодинамічних параметрів взаємодії між поліреактивними імуноглобулінами (ПРІГ) і іммобілізованим антигеном, було одержано кілька експериментальних кінетичних кривих зв'язування ПРІГ із іммобілізованим овальбуміном за різних температур. Це дозволило визначити константи швидкості

для кожного етапу процесу взаємодії для кожної температури. Потім за допомогою відповідних рівнянь було розраховано такі термодинамічні параметри, як енергія активації, ентальпія, ентропія і стандартна вільна енергія (енергія Гіббса). Знайдені термодинамічні величини показують, що найенергетичнішим затратним кроком процесу зв'язування ПРІГ з іммобілізованим антигеном є трансформація «неактивних» молекул ПРІГ в «активні», тобто утворення гідрофобних областей на поверхні ПРІГ. І навпаки, наступний крок взаємодії, а саме зв'язування «активних» ПРІГ із іммобілізованим антигеном є енергетично незалежним.

Ключові слова: поліреактивні імуноглобуліни, антигени, кінетика взаємодії, константи швидкості, термодинамічні параметри.

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