EXPERIMENTAL WORKS

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EFFECT OF TRIFLUOROETHANOL ON ANTIBODIES BINDING PROPERTIES

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The studies on the influence of organic co-solvents on the structure and function of antibodies are of key interest, especially in view of antibodies broad use as recognizing elements in different analytical systems. Here we studied the effect of co-solvent 2,2,2-trifluoroethanol (TFE) on the ability of anti-ovalbumin monoclonal antibodies to interact with its specific antigen. Antibody affinity to antigen and the rate constants of antibody binding to immobilized antigen were analyzed. Changes in antibody reactivity with incubation time which depended on TFE concentration and temperature were revealed. When treatment of antibodies with TFE was carried out at 0°C, we observed nonlinear, non-monotonous changes of antibody reactivity with initial fast decrease and substantial increase as incubation continued that may be related to the loss of antigen binding reactivity by some part of antibodies at the start but its restoration when the incubation proceeds.

K e y w o r d s: 2,2,2-trifluoroethanol, ovalbumin, monoclonal antibodies, antigen-antibody interaction, antibody affinity.

The application of organic co-solvents in protein research extends to different basic science and application areas. In this regard, antibodies are of special interest due to their specific recognition power that can be efficiently used in different chemical sensor devices [1, 2]. They often need to operate in different non-natural media and, particularly, on exposure to different analytes that are low-soluble in water, which requires the use of different solvent mixtures [3, 4]. Notably, most methods for chemical modification of antibodies require organic co-solvents to be added because of hydrophobicity and better reactivity of many chemical modifiers [5]. Therefore, the stability and reactivity of antibodies in these media become the key issues. Usually the buffered aqueous solutions are optimal for protein activity, and the presence of water-miscible organic solvents results in antibody complete or partial loss. However, some examples on increase of antigen-binding efficiency of antibodies can be found in the literature [6]. To our knowledge, the regularities regarding the co-solvent properties, their concentrations and experimental conditions (e.g. temperature) are still lacking.

In this regard, 2,2,2-trifluoroethanol (TFE) can be particularly interesting as a co-solvent due to its strong, known for years, but not always predictable effect on both protein and peptide structures [7, 8]. At low concentrations, the effect of TFE can be attributed to disrupting (or strengthening!) intramolecular H-bonds that can be related to its H-bond donor acidity [9], which is among the strongest of all alcohols [10]. Thus, TFE induces the formation of secondary structure of small amino acid chains by favoring α-helices or even β-sheets [11, 12]. The arrangement of β-hairpin peptide conformations was reported [13]. These effects may be due to selective destabilization of solvent-exposed amide groups in the peptide chain favoring the compact conformations maximizing the intramolecular hydrogen bonding [12, 14]. Such a view is supported by molecular dynamics studies of several peptides that also suggested their salvation by TFE [15]. Important are also the observations that the denaturation

of proteins on application of TFE did not lead to unfolded conformations but rather to the stabilization of their molten globular states [16]. For instance, TFE addition to β-lactoglobulin, a predominantly β-sheeted protein, lead to denaturing the molecule leads to the formation of an α-helical structure [17]. In albumin denatured under alkaline conditions, TFE increases the α-helicity [18]. TFE induces the formation of β-sheeted amyloid-like fibrils, but on a pathway to their formation the α-helical intermediates were detected [19]. It can induce the aggregation of proteins [20] but also produces the opposite effect – disruption of molecular associates [21], so that the switching between aggregating and dissociating effect may be the TFE concentration dependent [22]. The preferential solvation combined with the decrease of local polarity may contribute to the observed effects together with the competition for protein H-bonding with water. All those features suggest that TFE is a noteworthy tool for probing the molecular structure and dynamics of such macromolecules and for the modulation of their function. However, the application of this approach is hampered by our inability to use it for a well understandable and predictable manner.

Such thoughts stimulated our own studies on the effect of different TFE concentrations on antibody features. In addition, our preliminary studies on the effect of TFE on antibody-antigen interactions have revealed unexpected and highly unusual properties of this interaction [32]. For this reason antibody affinity to corresponding antigen and the rate constants of the process of antibody binding to the immobilized antigen were analyzed. We observed that the significant changes of antibody reactivity were nonlinear and even non-monotonous functions of incubation time depending strongly on the TFE concentration and temperature. The obtained results are presented below.

Materials and Methods

Antigens and antibodies. In this study hen egg albumin (ovalbumin) obtained from Sigma, USA was used as antigen. Mouse monoclonal antibodies (mAbs) specific to ovalbumin were also from Sigma, USA. 2,2,2-trifluoroethanol (TFE) was purchased from Fluka.

ELISA. The amount of antibodies attached to ovalbumin that was immobilized on immunological microplates was evaluated using a standard enzyme-linked immunosorbent assay (ELISA). Ovalbumin was coupled to 96-cell NUNC immunological plates by incubation of its solution (5 mg/ml) in 1% of NH_4HCO_3+0.01%NaN_3 at 4°C during 20-24 h. Shortly before using these plates, the solution with molecules of unbound ovalbumin was removed and the plates were washed three times with phosphate buffered (pH 7.2) physiological solution NaCl (PBS)+0.1% Twin 20 (TBS).

In order to estimate the amount of mAbs attached to immobilized ovalbumin, the plates were incubated with horseradish peroxidase-conjugated goat anti-mouse IgG antibodies (Sigma, USA) for 60 min at 4°C. After that the unbound conjugates were thoroughly washed away, and peroxidase substrate (the solution of orthophenilendiamine (1 mg/ml) in 0.01 M phosphate buffer, pH 5.0 and 0.003% H_2O_2) was added. After color development the reaction was stopped by adding 50 µl of 2.0 M sulfuric acid per well, and then the color was measured using microphotocolorimeter ELx800 (BIO-TEK) at 490 nm.

It is important to underline that at proper high dilution of the antibody solution, the amount of antibodies attached to immobilized antigen during incubation time usually must be proportional to the concentrations of antibodies in the samples. If so, in these conditions the color developed in microplate wells obtained in ELISA experiment must be directly proportional to the amount of the antibodies attached to immobilized antigen. Because of this reason it is possible to use the color of microplate wells obtained by ELISA for calculation of the kinetic characteristics for studying antigen-antibody reaction instead of the real antibody concentrations.

The investigation of THF effect on mAbs affinity and binding parameters. Anti-ovalbumin mAbs from Sigma, USA, were diluted 1:25 in PBS, stored at 4°C not more than for one week, and used in our experiments. In order to study the TFE effect on mAbs reactivity, different concentrations of TFE were prepared in PBS buffer (from 2% to 30% TFE) and, before use, these aliquots were incubated at 0°C or 24°C during 30-40 min to get their temperature close to desired. Then 5 µl of mAbs was added to 200 µl of TFE solution (at 0°C or 24°C) and incubated for certain time. During this incubation, 10 µl of antibody samples were taken at appropriate intervals and was at first transferred into 1 ml of PBS, and after 10 min of incubation at room temperature the samples were diluted 1 : 5 with TBS. As a control we used similar diluted mAbs without TFE treatment.

After that, the antibodies either treated with TFE or non-treated (control) were transferred to immunological microplates that were covered with ovalbumin. After incubation of these samples of mAbs in microplate at room temperature during 1 h the antibody solutions were removed from microplate wells by shaking out, they were carefully washed with TBS and the amount of the antibodies attached to immobilized antigen were estimated with ELISA.

Evaluation of mAbs affinity. In order to estimate the affinity of the interaction between either intact (control) or TFE-treated anti-ovalbumin mAbs and correspondent antigen, we used the method proposed by Friguet et al. [23]. The samples of studied antibodies were mixed in 1:1 proportion with antigen solutions of different ovalbumin concentrations (from $4 \cdot 10^{-7}$ to $6.25 \cdot 10^{-9}$ M) in TBS and incubated 20-24 h at room temperature to get the state of equilibrium between reagents. Then the samples of these antibody-antigen mixtures were transferred to plate covered with antigen. The plates were incubated for 1 h at room temperature in order to estimate the amount of antibodies that were not occupied by antigen after reaching equilibrium in antigen-antibody reaction.

The estimation of the affinity of bivalent antibodies was performed using two different methods. In one case, we used Eq. (1) that was proposed by Stevens [24]:

$$\sqrt{\frac{A_0}{A_0 - A_i}} = \frac{K_d}{l_i} + 1, \quad (1)$$

where A_0 is the color intensity in the wells containing mAbs samples, to which ovalbumin was not added, and A_i is the color intensity in plate wells, to which antigen concentration l_i was added. K_d is the dissociation constant of the studying antigen-antibody reaction, $K_a = 1/K_d$ (K_a is the affinity constant).

From Eq. (1), it follows that K_d must be equal to the tangent of the linear relation between $\sqrt{\frac{A_0}{A_0 - A_i}}$ and $1/l_i$.

In second case, we used Eq. (2) that was derived earlier by us [25] and that also allows to determine the affinity of bivalent antibodies:

$$\alpha + \sqrt{\alpha^2 + \alpha} = K_a l_i, \quad (2)$$

where $\alpha = \frac{A_0 - A_i}{A_i}$.

From Eq. (2), it follows that the association constant of antigen-antibody reaction, K_a, is equal to the tangent of the slope of the linear dependence $\alpha + \sqrt{\alpha^2 + \alpha}$ on l_i.

It is important to note that Eqs. (1) and (2) are quite similar and they allow to determine identical values of affinity constant ($K_a = 1/K_d$) based on assigned A_i and A_0 data. However, this may not happen in a real experiment, because in Eq. (1) the values in the left side are proportional to reverse values of concentration of competing antigen, and in Eq. (2) the left side is proportional to direct values of antigen concentration. Because of this, if in the studied antibody-antigen mixture there is a small fraction of mAbs with affinity lower than the bulk values, this cannot be detected with Eq. (1) as far as at high concentrations of competing antigen l_i, the experimental points will be located very closely and the deviations from linearity between $\sqrt{\frac{A_0}{A_0 - A_i}}$ and $1/l_i$ could not be detected. In contrast, if one uses Eq. (2) for estimation of K_a in antibody samples containing admixture of low-affinity antibodies, then instead of linear dependence $\alpha + \sqrt{\alpha^2 + \alpha}$ on l_i at high concentrations of competing antigen an obvious convex curve will be obtained. Thus, using Eq. (2) is preferable in many cases.

Determination of the rate constant forbinding antibodies to immobilized antigen and the concentrations of bound antibodies. It is known that the binding of bivalent antibodies with antigen immobilized on a plate is the process practically irreversible [26-28] and because of that the kinetics of binding can be described by Eq. (3):

$$A_i = A_0 e^{-k_1 t_i}, \quad (3)$$

where t_i is the recorded time of antibody binding with immobilized antigen; A_0 is the concentration of antibodies in solution at $t_i = 0$ and this value is equal to the amount of antibodies attached to immobilized antigen if $t \to \infty$; A_i is the concentration of antibody bound with immobilized antigen at time t_i; k_1 is the rate constant of the process of binding.

In Eq. (3) the experimentally determined values are A_i, obtained at known points of time t_i, and we know that A_i is proportional to the amount of bound antibodies. Thus, studying the dependence of A_i on t_i we could find the unknown values of k_1 and A_0 for binding of antibodies to immobilized antigen.

However, Eq. (3) is irrational and because of that its analytical solution in general case is not possible. To solve this problem, several graphical methods were developed [29]. Meantime we were able to get an analytical solution of Eq. (3) for a special case when between the time points t_i exists the relation: $2t_i = t_{2i}$ [30]. It was shown that in this case k_1 and A_0 can be calculated using the following equations:

$$\ln\left(\frac{A_i}{A_{2i}-A_i}\right) = k_1 t_i \,, \qquad (4)$$

where A_i and A_{2i} are the values (in our case it is the optical density) that are proportional to the amount of antibodies attached to immobilized antigen at time points t_i and t_{2i} (if $2t_i = t_{2i}$).

$$A_0 = \frac{A_i^2}{2A_i - A_{2i}} \,. \qquad (5)$$

From Eq. (4), it follows that k_1 is equal to the tangent of the slope in linear dependence between $\ln\left(\frac{A_i}{A_{2i}-A_i}\right)$ and t_i, and from Eq. (5) we can derive that A_0 is equal to the tangent of the slope for linear dependence between A_i^2 and $2A_i - A_{2i}$. Thus, using Eqs. (4) and (5) it becomes possible to determine quantitatively both the rate constant of antibody binding to immobilized antigen, k_1, and the value of A_0, which is proportional to the concentration of antibodies in the studied system.

Results

Antibody reactivity of studied anti-ovalbumin mAbs changes after their incubation in 30% TFE from 0.5 min to 20 min either at 24°C or at 0°C (Fig. 1). We observed that at 24°C the mAbs reactivity decreases gradually with the time of treatment with 30% TFE, which seems to be quite natural simply because the antibodies can be partially inactivated in 30% TFE at this temperature. However, when similar investigations were made at 0°C, quite unexpected results were obtained. As it can be seen in Fig. 1, the kinetics of mAbs reactivity change was quite different from that obtained at 24°C showing the complexity of this process. At 0°C the reactivity of mAbs initially goes down very fast, much faster than at 24° and only then it starts to increase. Thus, we confirm our previously obtained data [32] on very unusual kinetics of change in reactivity of antibodies in 20-30% TFE at 0°C. Essentially new finding of the present study was establishing the fact that after sharp decrease of reactivity of antibodies during 1-2 min of incubation, on extended incubation (up to

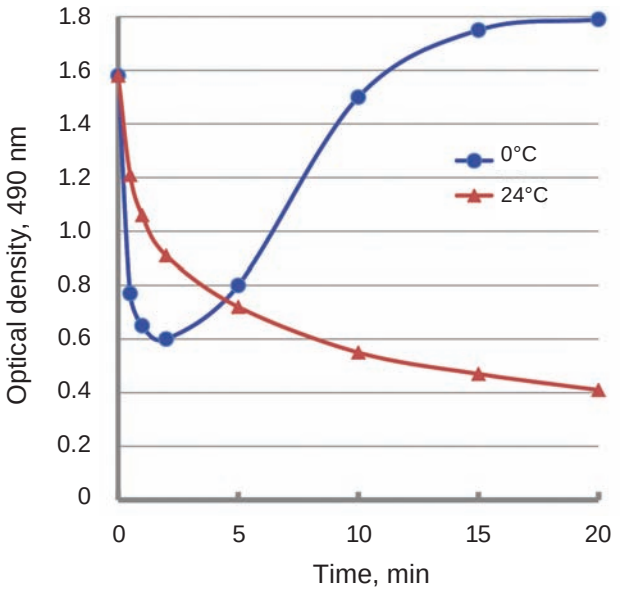

Fig. 1. Dynamics of changing in reactivity of anti-ovalbumin mAbs after their incubation during 20 min in 30% TFE solution either at 24°C or at 0°C

20-40 min) the antibody reactivity not only restored its value to initial level but this value was noticeably exceeded (Fig. 2).

Such unexpected behavior should find its explanation. Apparently, it is impossible to explain such dynamics of antibody reactivity by the process of mAbs inactivation and reactivation only. A natural question arises, how an additional reactivity of antibodies could appear? Is that a result of the change of properties of these antibodies (such as their affinity or the rate of binding with antigen), or their quantity increase with their properties unchanged? We made an attempt to obtain answers to these questions.

There is no doubt, that the effect of TFE solution on molecular structure of mAbs is more complex process than that previously observed on treatment of mAbs either with chaotropic ions, or drastic changes of pH [31]. We can assume that the observed changes of antibody reactivity can be due to the change in binding properties of the whole population of antibodies that can be at first partially inactivated and then reactivated with time. Alternatively, there may appear dramatic changes only in some part of mAbs population inducing strong antibody heterogeneity that may be described as the variation of efficient concentration of the active antibodies in the TFE-treated samples in comparison to untreated antibodies. To determine which of these possibilities is more likely (or both are correct), we first evaluated

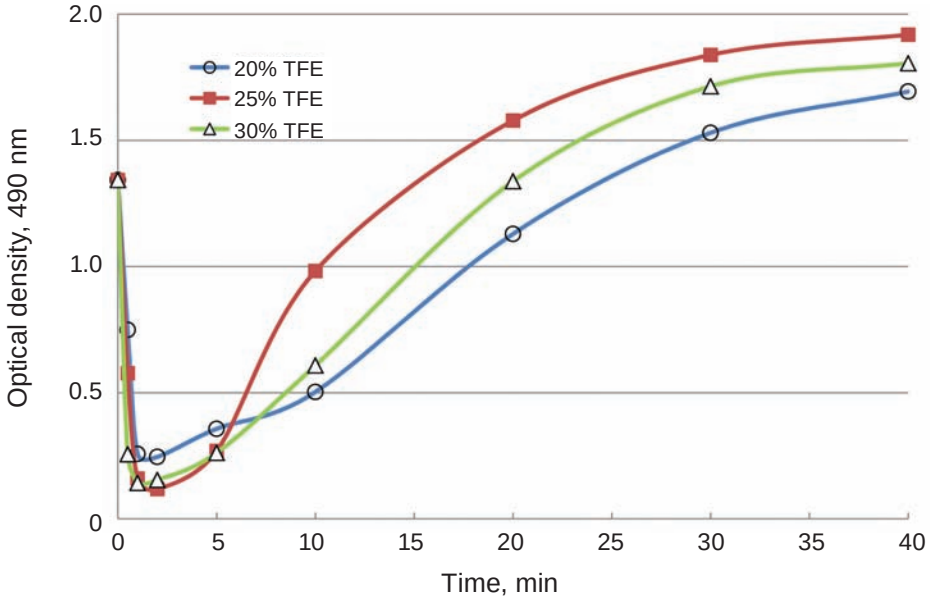

Fig. 2. Effect of incubation of mAbs at 0°C in 20%, 25%, and 30% TFE solutions on the reactivity of anti-ovalbumin mAbs

the affinity of interaction between ovalbumin and antibodies either without treatment or treated during 40 min by incubation in 25% TFE solution at 0°C. For doing that, we used two different approaches for evaluating the antibodies affinity, as it was described above (see Materials and Methods).

Fig. 3,*A* presents the linear relation obtained on titration of both samples of antibodies (either control or treated by TFE at 0°C during 40 min). Fig. 3,*B* shows the binding curves of these antibody samples in the presence of different ovalbumin concentrations after reaching equilibrium in the antigen-antibody reaction. It can be seen (Fig. 3, *A*) that the titration of the antibody samples in both cases resulted in linear relationships. This fact demonstrates that there is a direct correlation between the antibody concentration and the intensity of the staining of the microplate wells (optical density, A_i) that we obtained using ELISA. This fact confirms the validity of using values A_i for calculation the binding parameters for antigen-antibody interaction instead of real antibody concentrations that are unknown.

From Fig. 3, *A*, it is also clear that the reactivity of mAbs sample treated for 40 min by 25% TFE at 0°C is really higher than the antibody reactivity in control samples. However, in spite of the found differences in antibody reactivity, the affinity of these antibody samples was quite similar. The use of the data from Fig. 3,*B* for plotting the relations either $\sqrt{\frac{A_0}{A_0 - A_i}}$ on reverse ovalbumin concentration, $1/l_i$, (Fig. 4, *A*) or $\alpha + \sqrt{\alpha^2 + \alpha}$ (where $\alpha = \frac{A_0 - A_i}{A_i}$) on ovalbumin concentration, l_i, (Fig. 4, *B*), allows obtaining linear dependences, the angle of the decline of which in the first case (Fig. 4, *A*) is equals to the dissociation constant $K_d = 1/K_a$, and in the second case (Fig. 4, *B*) it is equal to the affinity constant K_a (see Materials and Methods).

Since linear functions are observed for both intact and 25% TFE-treated mAbs samples (Fig. 4, *A*), this fact indicates the homogeneity of the antibodies in the samples studied, i.e. for the absence of any antibody admixtures with higher or lower affinities. In addition, these liner functions are practically superimposed, which demonstrates almost the same antibody affinity either before or after 25% TFE treatment at 0°C for 40 min. If we used Eq. (1) for affinity evaluation, then the values of dissociation constants were found to be equal to $K_d = 1.64 \cdot 10^{-9}$ M for control mAbs, and $K_d = 1.55 \cdot 10^{-9}$ M for 40 min TFE-treated sample. Thus, the obtained values of dissociation constants, K_d, were, in fact, practically equal for these samples of studied antibodies.

The application of Eq. (2) also allowed detecting negligibly small differences between association constants, K_a, for the antibody samples either intact or treated with 25% TFE mAbs at 0°C

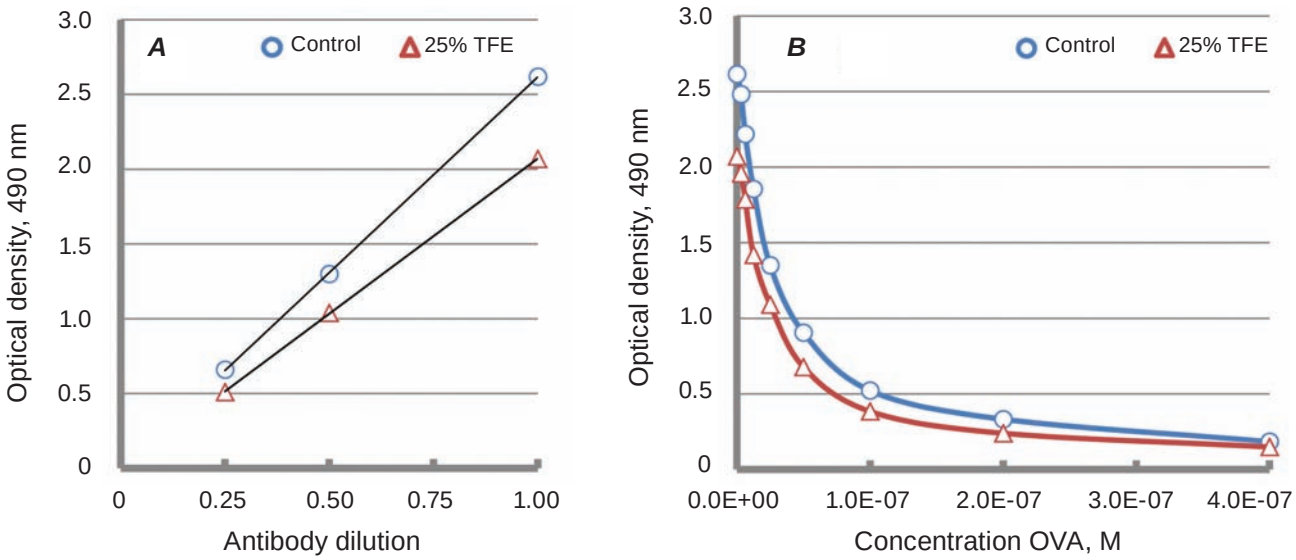

*Fig. 3. Titration of mAbs before (control) and after their incubation for 40 min in 25% TFE solution at 0°C (**A**) and relation between concentration of competing antigen l_i and the values of A_i, obtained by ELISA (**B**). Plot (**A**) shows that A_i values are proportional to the concentration of antibodies in studied samples*

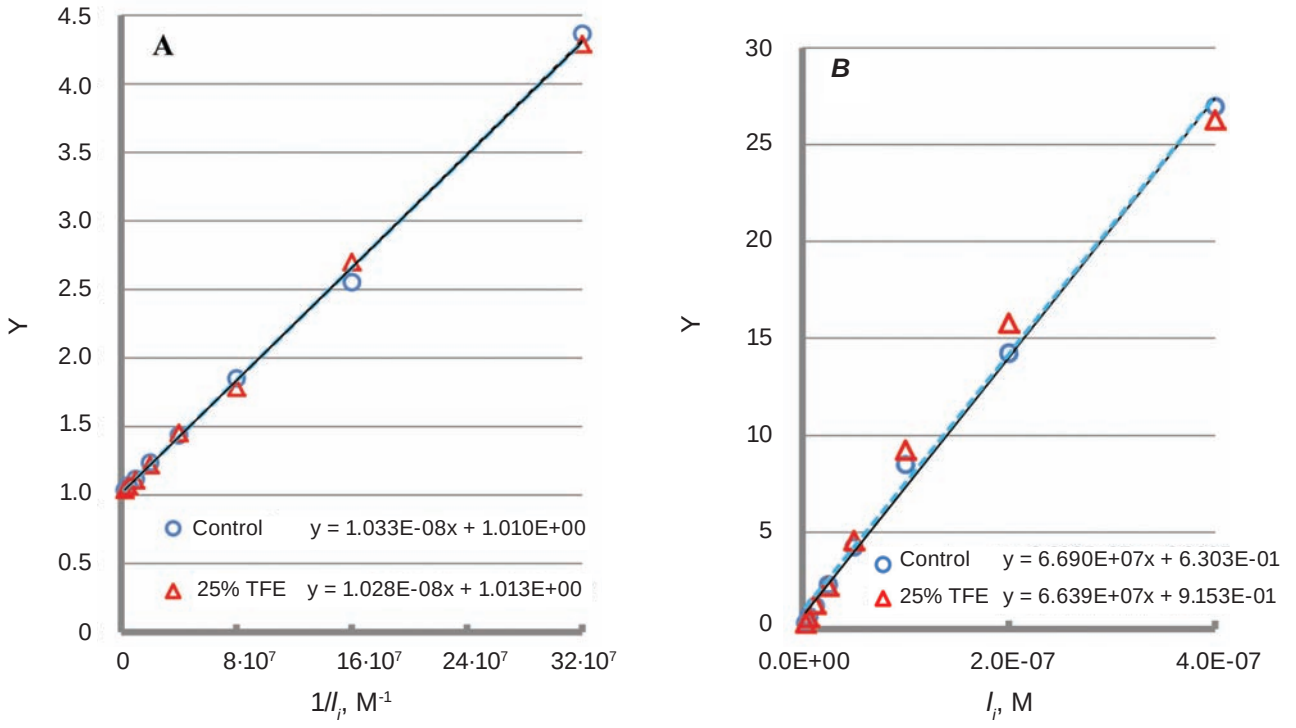

*Fig. 4. Evaluation of the anti-ovalbumin mAbs affinity before (control) and after their incubation in 25% solution of TFE at 0°C for 40 min using either Eq. 1 (**A**) or Eq. 2 (**B**).*

A) $Y = \sqrt{\frac{A_0}{A_0 - A_i}}$; ***B)*** $Y = \alpha + \sqrt{\alpha^2 + \alpha}$, where $\alpha = \frac{A_0 - A_i}{A_i}$

(Fig. 4, *B*). Namely, $K_a = 3.22 \cdot 10^8$ M^{-1} was found for control antibodies, and $K_a = 3.20 \cdot 10^8$ M^{-1} for mAbs treated during 40 min. Thus, we can derive that dramatic change in antibody reactivity nevertheless very slightly (if at all) changes the affinity of the interaction between antibodies and ovalbumin. Based on this analysis, a conclusion can be drawn that enhancement after 40 min treatment by 25% TFE at 0°C cannot be due to the change of antibody affinity but to some other still unknown antibody property that influences the process of binding mAbs to immobilized antigen. Thus, the change in antibody affinity is not the reason of the observed reactivity changing induced by 20-30% TFE at 0°C.

Two alternative explanations of the observed modulation of mAbs reactivity after their treatment with TFE at 0°C could be suggested. First, it can be the change in concentration of "active" antibodies, and second, it could be the changing in the rate constant, k_1. It is obvious that on simultaneous several-fold decrease or increase of the rate constant of antigen binding k_1 and the rate constant of dissociation k_2 the affinity constant $K_a = k_1/k_2$ will remain unchanged. But in contrast to affinity constant, K_a, the rate of antibody binding to immobilized antigen should change proportionally to the value of k_1, since such mAbs binding is known to be bivalent and therefore this reaction is practically irreversible [26-28]. This means that the rate constant of antibody dissociation, k_2, is practically equal to zero and therefore the amount of bound antibodies to immobilized antigen will strongly dependent only on k_1.

In order to verify this suggestion, we studied the kinetics of mAbs binding to immobilized antigen before mAbs treatment (control antibodies) and antibodies after their treatment with 25% TFE at 0°C for 2, 5 and 40 min. The results of one of such experiments are presented in Fig. 5,*A* and 5,*B*. It can be seen that during 2 min of incubation the antibody reactivity went down, then after 5 min it was partially restored and after 40 min of incubation it went up to become almost equal to the reactivity of control (untreated) antibodies (Fig. 5, *A*).

Then the extracted antibodies samples at these time periods were used for studying the kinetics of antibody binding to immobilized ovalbumin. The obtained kinetics curves (Fig. 5, *B*) were transformed either in coordinates $\ln\left(\frac{A_i}{A_{2i} - A_i}\right)$ versus t_i (in order to determine k_1) or A_i^2 versus $2A_i - A_{2i}$ (in

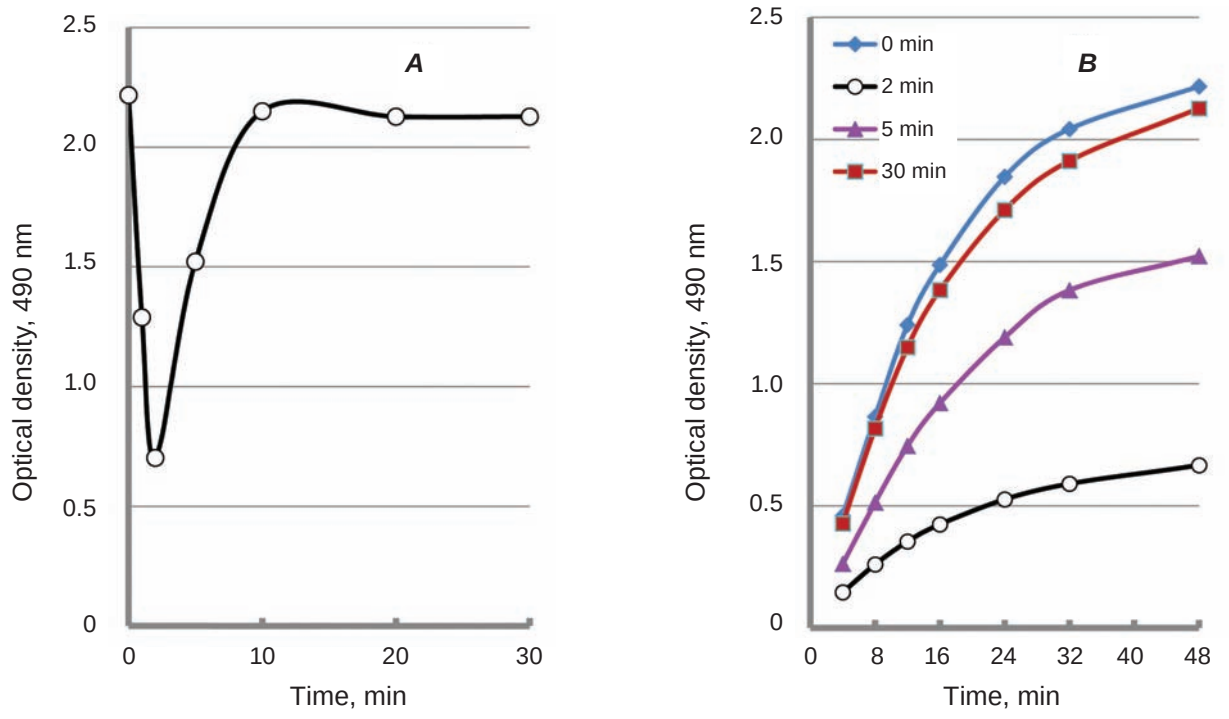

Fig. 5. ***A*** *– Effect of incubation time of mAbs in 25% TFE at 0°C on mAbs reactivity against ovalbumin;* ***B*** *– Dynamic of the binding of mAbs to antigen immobilized on plate for control (0 min) mAbs or mAbs pretreated during 2 min, 5 min or 30 min with 25% TFE at 0°C*

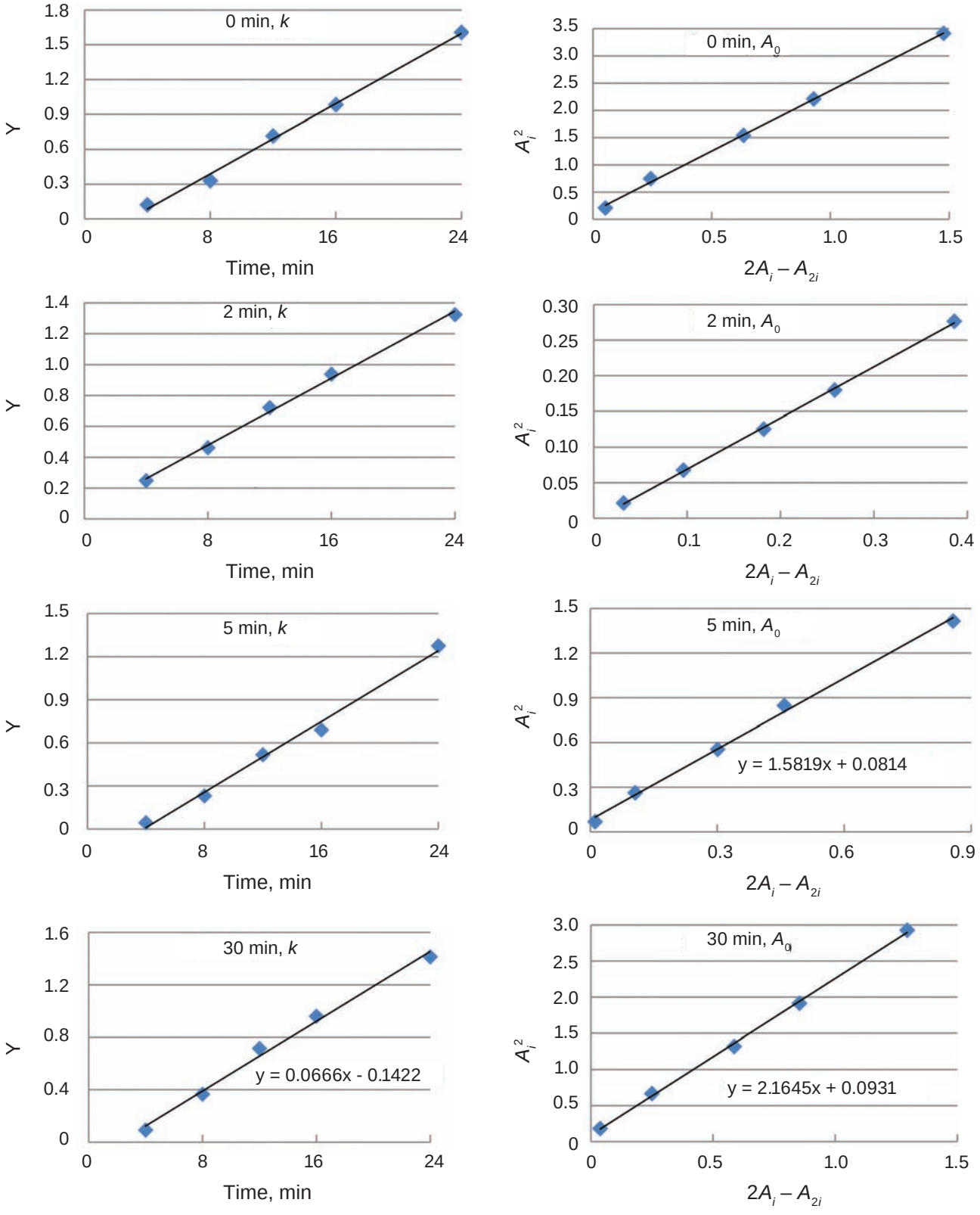

Fig. 6. Calculations of k_1 and A_0 from the data on antibody binding kinetics to immobilized antigen presented in Fig. 5, B using linear functions $Y = \ln\left(\frac{A_i}{A_{2i} - A_i}\right)$ *vs. t_i and A_i^2 vs. $2_{Ai} - A_{2i}$. The linear functions obtained with Microsoft Excel are presented. The tangents of their decays are equal to k_1 (on graphs to left) or A_0 (on graphs to right) for antibody samples (0 min as control) or treated 2, 5 or 30 min in 25% TFE at 0°C*

order to determine A_0) and presented in Fig. 6. As it can be expected, the obtained plots (Fig. 6) gave the straight lines, the tangents of decline angles of which allowed calculating the values of k_1 and A_0. It is reasonable to remind that values of A_0 are proportional to the amount of antibodies in the studying samples.

Our calculations gave the following results. In control mAbs sample (without 25% TFE treatment), the reactivity of which in this experiment was maximal (Fig. 5, *B*), the rate constant of mAbs binding to immobilized ovalbumin, k_1, is also maximal and equal to 0.075 min^{-1}. The value of A_0 for this sample of antibodies was equal to 2.216 relative units (Fig. 6).

After 2 min of mAbs incubation in 25% TFE at 0°C, the k_1 value decreased not very significantly, namely to 0.054 min^{-1}, but A_0 value dropped as much as to 0.717, i.e. it became three times lower than in control sample. Thus, these data witnessed that the concentration of "active" antibodies also decreased by three times after 2 min of mAbs incubation with 25% TFE at 0°C.

After 5 min of mAbs incubation, a very small increase in k_1 value was observed, compared to 2 min incubation, reaching 0.061 min^{-1}, whereas A_0 increased twice and reached 1.581 relative units. Finally, after 40 min of mAbs incubation in 25% TFE at 0°C, the value of k_1 increased to 0.066 min^{-1} and A_0 increased to 2.164, reaching almost the same values as for the untreated antibody samples.

Thus, in contrast to the values of affinity constant, K_a, which were almost the same for control mAbs and those treated at 0°C with 25% TFE, the value of rate constants k_1 and the value of A_0 (which is proportional to the antibody concentrations in the studied samples) are quite different and depend on the time of treatment. The closest are the values of k_1 and A_0 that were determined for control (untreated) samples of mAbs and the samples that were treated for the longest time period, 40 min. When mAbs were treated in these conditions for either 2 min or 5 min, then the values of k_1 and A_0 were noticeably smaller. These data demonstrate that at temperatures close to 0°C the application of TFE in 20-30% concentration is able to induce some mild transformations in mAbs structure, probably involving the Fv-region of studied mAbs, which is responsible for antigen binding. The result of this transformation could be the loss of antigen binding reactivity by some parts of antibodies at the start of incubation but its restoration when the incubation proceeds.

Conclusion. In this work we found that TFE was able to induce significant changes in the ability of specific to albumin monoclonal antibodies to interact with correspondent antigen. The change in interaction affinity, K_a, with antigen is probably very small, if any at all, and our experiment did not allow detecting them. We found, however, that interaction with TFE induced the change in the number of reactive antibodies (this value is proportional to A_0) and the rate of antigen-antibody binding, k_1. Both these values decreased significantly (during first two minues of incubation with TFE), and then gradually restored. Since the equilibrium constant does not change, (K_a = constant), the rate constant of the antigen-antibody complex dissociation, k_2, (as far as $k_2 = k_1/K_a$), should change in the same manner as k_1.

The fact that after initial rapid drop of specific antibody reactivity the restoration of this reactivity develops in time on a rather long scale (Fig. 4) deserves special attention. It may happen that in some parts of mAbs population the antigen binding sites are destroyed by TFE molecules, probably due to interference with their intramolecular hydrogen bonds. The observed in some experiments increases of reactivity exceeding the control values may be due to the presence in the pool of these antibodies of species with "incorrect" folding of polypeptide chains and because of that they are unable to bind the antigen. Their incubation with TFE at 0°C could probably leads to the restoration of their reactivity. If so, then it can be suggested that in a pool of anti-ovalbumin mAbs there may exist two (or even more) relatively stable forms of mAbs with their paratopes, which are complementary to structurally different epitopes. Further experiments will show if that can be the case.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ВПЛИВ ТРИФТОРЕТАНОЛУ НА ЗДАТНІСТЬ АНТИТІЛ ЗВ'ЯЗУВАТИСЬ З АНТИГЕНОМ

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Вивчення впливу органічних розчинників на структуру і функцію антитіл має ключовий інтерес, особливо з огляду на широке використання антитіл як елементів розпізнавання у різних аналітичних системах. У цій роботі вивчали вплив розчинника 2,2,2-трифторетанолу (TFE) на здатність моноклональних антитіл до овальбуміну зв'язуватись з його специфічним антигеном. Аналізували афінність антитіла до антигену та константи швидкості зв'язування антитіла з іммобілізованим антигеном. Виявлено зміни реактивності антитіл із часом інкубації залежно від концентрації TFE та температури. За інкубації антитіл із TFE при 0°C спостерігали нелінійні, немонотонні зміни реактивності антитіл із початковим швидким зниженням і потім значним підвищенням, що може бути пов'язано з втратою антигензв'язувальної реактивності деякою частиною антитіл на початку, але з її поступовим відновленням під час інкубації.

Ключові слова: 2,2,2-трифторетанол, моноклональні антитіла, взаємодія антиген-антитіло, кінетика взаємодії антитіл із антигеном, афінність антитіл.

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