Antibody–antigen interactions play a major role in the adaptive immune system, as antibodies can recognize a wide range of molecules including macromolecules and small chemicals with a high degree of specificity and affinity [1]. This feature of antibodies makes them one of the most potent tools in therapy and diagnostics [2]. The functionality of the specific recognition of antibodies is also utilized for the molecular detection in biotechnology such as in western blotting [3], enzyme-linked immunosorbent assay [4], and immunochemistry [5]. The interaction of antibodies with protein or peptide antigens has been studied as a model system for protein–protein or protein–peptide interactions [6-8]. For this applicability and usefulness of antibodies, detailed investigation on the biochemical and biophysical aspects of antibody–antigen interactions is desirable in order to understand the underlying mechanism of the specific molecular recognition process.

Recently we reported the biophysical mechanism of the interaction between a human pathogenic antibody (BO2C11) and its target antigen, coagulation factor VIII (FVIII) using a surface plasmon resonance-based technique and other binding assays [9]. In the article, we show kinetic and thermodynamic investigation of the binding between BO2C11 and FVIII in different salt conditions of the binding buffer in order to understand the underlying energetic of the binding process. One of the central observations in the paper is the thermodynamic variables (ΔG°‡, ΔH°‡, and ΔS°‡) in the association of BO2C11 and FVIII. In this editorial, we report our analysis of these results in terms of equilibrium thermodynamics to show that the association phase in the interaction of the antibody and its antigen exhibits enthalpy–entropy compensation. We discuss the implication of this finding in the use of antibodies for the molecular recognition in biotechnology.

In the kinetic experiment of the association of BO2C11 and FVIII, we measured the association rate constant at various temperatures. Using the Arrhenius equation, we derived ΔH°‡ of the association. The value of ΔH°‡ is highly dependent on the ionic strength of the binding buffer as it was 159 kJmol⁻¹ and -5.2 kJmol⁻¹ when the NaCl concentration in the binding buffer was 0.15 M or 0.035 M, respectively. The difference of ΔH°‡ (ΔΔH°‡) is -164.2 kJmol⁻¹, which is 66.2 times the thermal energy at 25°C. In the calculation of ΔΔH°‡, we assigned the binding in the buffer containing 0.15 M NaCl as an initial state. According to the value of ΔΔH°‡, the association between BO2C11 and FVIII is more favorable in the lower salt concentration of the binding buffer in terms of enthalpy. The value of ΔG°‡ can be derived using the following equation with the values obtained from the above calculations for ΔΔH°‡ and TΔΔS°‡:

\[ ΔG°‡ = ΔH°‡ - TΔS°‡ \]  

(1)

The value of TΔS°‡ at 25°C was 120 kJmol⁻¹ and -56.7 kJmol⁻¹ when the NaCl concentration in the binding buffer was 0.15 M or 0.035 M, respectively.

The corresponding value of TΔS°‡ is -176.7 kJmol⁻¹, which is 71.3 times the thermal energy at 25°C. Therefore, the association is more favorable in the higher salt concentration of the binding buffer in terms of entropy. The difference in ΔG°‡ between two different reactions of different salt concentrations in the binding buffer (ΔΔG°‡) can be obtained using the following equation with the values obtained from the above calculations for ΔΔH°‡ and TΔΔS°‡:

\[ ΔΔG°‡ = ΔΔH°‡ - TΔΔS°‡ \]  

(2)

The corresponding value of ΔG°‡ is 12.5 kJmol⁻¹, which is just 5 times the thermal energy at 25°C (Figure 1). This calculation clearly indicates that the association between BO2C11 and FVIII is an example of enthalpy–entropy compensation in that the difference of enthalpy between two reactions (ΔΔH°‡) is comparable to the value of the entropic energy difference (TΔΔS°‡) so that two reactions have similar free energy changes (ΔG°‡) since there is no resulting significant difference in ΔG°‡ according to Eq. (2), even though each energy component (ΔΔH°‡ and TΔΔS°‡) can change significantly between the two reactions.

The same analysis as shown above can be applied to the comparison of the association of BO2C11 and FVIII in the binding buffer with different salt types (NaCl and NaSCN) in the paper [9]. In this case, the values of ΔΔH°‡ and TΔΔS°‡ are -155.8 kJmol⁻¹ and -161.4 kJmol⁻¹, which is 62.9 and 65.1 times the thermal energy at 25°C, respectively.
In the calculation of $\Delta \Delta H^\circ$ and $T \Delta S^\circ$, the binding in the buffer containing 0.15 M NaCl was assigned as an initial state. Using Eq. (2), the corresponding value of $\Delta \Delta G^\circ$ is obtained as 5.6 kJmol$^{-1}$, which is just 2.3 times the thermal energy at 25°C (Figure 1). As one can see from our analysis, the underlying thermodynamic energy components, $\Delta H^\circ$ and $T \Delta S^\circ$, can vary at least more than 60 times the thermal energy due to the different salt conditions (concentration or salt type) in the binding buffer, but the resulting changes of the free energy component is quite limited as their values of $\Delta \Delta G^\circ$ are less than 5 times the thermal energy.

Enthalpy–entropy compensation is often observed in many weakly coupled systems such as protein folding [11], antibody–antigen interactions [12-14], and minor histocompatibility antigen–peptide interactions [15]. According to our analysis, this feature is responsible for the relative insensitivity of the $\Delta G^\circ$ value in the association between BO2C11 and FVIII in different salt conditions [9] by mutual compensations between $\Delta \Delta H^\circ$ and $T \Delta S^\circ$ values, although the enthalpy and entropy changes of a reaction occurring in different conditions can differ significantly.

Antibody-based detection has been widely used in biotechnology [3–5]. For example, an antibody can detect its target antigens to assess their quantity in western blot analysis. The binding condition for the molecular detection of an antigen by an antibody is different in most cases from the in vivo condition, where an antibody is selected for high affinity to specific antigens, but the antibody still successfully performs its function of molecular recognition (for example, [16]). This could be a consequence of enthalpy–entropy compensation in the binding of antibodies to antigens as shown in this editorial. However, one should note that the compensation is not perfect, so the affinity of the antibody to its antigen could differ depending on the binding conditions such as salt concentration or salt type. Therefore, if the desired binding affinity of an antibody to its antigens is not attained, one may optimize the binding buffer condition, for example, by changing the salt concentration of the binding medium. In addition, even when the antibody shows a desirable affinity to its target antigens in the in vitro application, the underlying thermodynamics can be quite different from that of the in vivo binding as shown in this letter.

Significance

We analyze an antibody–antigen interaction to deduce a thermodynamic feature of the interaction, enthalpy–entropy compensation, and discuss the implication of this finding in biotechnology.

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