

Correspondence

Enthalpy–entropy compensation in the transition of a monospecific antibody towards antigen-binding promiscuity

Abstract

In a recent article published in *Molecular Immunology* (Dimitrov, J.D., Lacroix-Desmazes, S., Kaveri, S.V., Vassilev, T.L., 2007. Transition towards antigen-binding promiscuity of a monospecific antibody. *Mol. Immunol.* 44, 1854–1863.), the authors perform kinetic and thermodynamic analyses using a surface plasmon resonance-based technique to explain polyspecificity of antibodies in terms of molecular mechanisms. They found that after the antibody was exposed to urea, a protein destabilizing agent, it had the same affinity as the native state and that the two forms of the antibody use two distinct thermodynamic pathways for binding to the same antigen. In this letter, we report our analysis of these results in terms of equilibrium thermodynamics to show that the specificity transition of the antibody exhibits enthalpy–entropy compensation.

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In a recent article, Dimitrov et al. (2007) report that a monospecific antibody treated with urea in a reversible way gains polyspecificity. To explain the specificity transition of the antibody in terms of molecular interaction energy, the authors perform kinetic and thermodynamic investigations of antigen binding, before and after a urea treatment. They found that the two forms of the antibody have similar affinity despite the considerable difference in their kinetic rate constants. In addition, they show that the similar affinity exhibited by the two forms of the antibody is due to different types of noncovalent energy in antigen binding. While they clearly explain the transition of antibody specificity in terms of kinetics and thermodynamics, further analysis of their results could show that they exemplify a thermodynamic property of weakly coupled systems, enthalpy–entropy compensation (Haynie, 2001). In this letter, we show that their results can be explained using this property.

Antigen–antibody interaction is one of the most specific molecular recognition processes (Finkelstein and Ptitsyn, 2002). The high affinity and specificity observed in this interaction plays an important role in the host defense system. However, several studies demonstrate the existence of monoclonal antibodies that can bind to a variety of antigens. Such antibodies are called polyreactive or polyspecific antibodies. The best explanation for the polyreactivity is a conformational hypothesis that the antigen-binding sites of such antibody molecules are flexible enough to accommodate different antigens (for recent review, Notkins, 2004). One of the mechanisms for gaining the polyspecificity is a transient exposure of antibody to certain protein-destabilizing agents. For example, exposure to oxidizing agents can change binding specificity of autoantibodies in

the blood of patients with autoimmune diseases in such a way that they can be detected (McIntyre et al., 2006).

To explain the molecular mechanism for the induction of polyspecificity, Dimitrov et al. (2007) performed kinetic analysis of the antigen binding reaction of a single monoclonal antibody before and after exposure to mild denaturing conditions by surface plasmon resonance. The major finding in their research is that after a reversible treatment of the antibody with urea, a protein destabilizing agent, the rate constants of the antigen binding reaction were altered in a compensatory manner. The treatment decreased the antibody's association rate constant to 25% of its native state value. The decrease in the association rate constant was accompanied by the reduction of the dissociation rate constant to 17% of its native value. The ratio of association and dissociation rate constants gives an equilibrium constant representing binding affinity (Chang, 2000). Although each rate constant changed significantly due to the urea treatment, there was no substantial change in affinity to the cognate antigen because both rate constants decreased to a similar extent (75 and 83% for the on- and off-rate constants, respectively).

To further elucidate this compensatory phenomenon, we analyze the results in the Dimitrov et al. paper (2007) with respect to equilibrium thermodynamics. Binding free energy (ΔG) is composed of enthalpy (ΔH) and entropy energy ($T\Delta S$) as shown in Eq. (1), where T is the temperature in Kelvin (Chang, 2000).

$$\Delta G = \Delta H - T\Delta S. \quad (1)$$

The difference in ΔG between two different reactions ($\delta\Delta G$) can be expressed as

$$\delta\Delta G = \delta\Delta H - \delta T\Delta S. \quad (2)$$

Table 1

Thermodynamic quantity values in the binding to the antigen of two different forms of antibody at 25 °C

Antibody	ΔG	ΔH	$T\Delta S$
Native	−39.03	−9.35	−29.68
Urea-treated	−39.96	3.97	−43.93

The unit of energy is kJ mol^{-1} .

If the difference of enthalpy between two reactions ($\delta\Delta H$) has the same value as the entropy energy difference ($\delta T\Delta S$), these two reactions have the same binding free energy because there is no resulting difference in ΔG according to Eq. (2), even though each energy component ($\delta\Delta H$ and $\delta T\Delta S$) can vary significantly between the two reactions. This is the underlying principle of enthalpy–entropy compensation.

The thermodynamic parameters for the antigen-binding reactions of the two different forms of the antibody in the paper (Dimitrov et al., 2007) are shown in Table 1. The difference of ΔH in binding to the antigen between the native and urea-treated antibody, $\delta\Delta H$, is $13.32 \text{ kJ mol}^{-1}$, which is 5.4 times the thermal energy at 25 °C, while $\delta T\Delta S$ is $14.25 \text{ kJ mol}^{-1}$, which is 5.8 times the thermal energy. This indicates that the urea-treatment increases both ΔH and $T\Delta S$ to a similar extent. As a consequence, $\delta\Delta G$ has a very small value, $-0.93 \text{ kJ mol}^{-1}$, which is 0.4 times thermal energy at 25 °C. Even though each component of free energy (enthalpy and entropy) changes significantly, they compensate for each other and the resulting $\delta\Delta G$ value is small.

Enthalpy–entropy compensation has been observed in many weakly coupled systems. For example, point mutations in proteins frequently lead to relatively large changes in ΔH and ΔS but a small change in ΔG in protein folding/unfolding reactions (Haynie, 2001). The result of our analysis indicates that the urea treated antibody shows enthalpy–entropy compensation in binding to the antigen. This indicates that the conformational properties of antigen-binding pockets in an antibody

can change in a compensatory manner responding to subtle perturbations such as point mutations or exposure to protein destabilizing agents, although the overall structure of an antibody is rigid (Finkelstein and Ptitsyn, 2002). It will be interesting to see whether this thermodynamic compensation is observed in the affinity maturation process caused by the accumulation of somatic mutations or in the binding to different antigens by a polyspecific antibody. This will provide valuable information on the thermodynamic properties of the antigen-binding sites of antibodies.

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