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Short Communication

On the antigen-antibody interaction: a thermodynamic consideration

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Abstract. Despite its relevance to many biomedical fields, relatively little effort has been put into a comprehensible quantitative description of the effect of reaction temperature on the interaction between antigens and their antibodies. In this article, a novel, straightforward mathematical model is proposed, which aims to describe the effect of temperature on antigen-antibody kinetics. The model proposed in this article could hopefully provide clinicians, immunologists, and biochemists with an improved insight into the kinetic effect of fluctuations in reaction temperature on antigen-antibody-dependent processes and therefore into the kinetics of the humoral adaptive immune response.

Keywords: Antigen, antibody, kinetics, temperature, mathematical model

1. Introduction

The binding of antigens by antibodies or immunoglobulins, which are produced by the plasma cells in the human body, is highly specific due to the strong affinity of the variable antigen-binding domains, and is of vital importance to the humoral adaptive immune response [1]. Therefore, understanding the kinetics involved in the antigen-antibody interaction is essential to anyone who studies the fields of medicine, immunology or biochemistry. The reversible binding of an antigen to its specific polypeptide antibody – which is the result of several non-covalent interactions between these molecules – can be viewed as a chemical equilibrium reaction in which the ratio of the unbound antigen (ligand) concentration to the antigen-antibody (ligand-receptor) complex concentration equals an association/dissociation equilibrium constant [2]. It is a well-known chemical dogma that every equilibrium reaction is influenced by the temperature at which it takes place. As a result, reaction temperature swings influence the antigen-antibody kinetics and can therefore have profound biological effects (for instance, as evidenced by the influence of fever or hypothermia on the humoral adaptive immune response) or consequences with regard to the sensitivity and incubation duration of antibody-based analytical assays, such as the enzyme-linked immunosorbent assay or ELISA, immunohistochemistry, and many assays employed in antibody-based proteomics and biomarker research [1,3,4].

Unfortunately, despite its obvious relevance to many biomedical fields, relatively little effort has been put into a comprehensible description of the effects of reaction temperature on the interaction between antigens and their antibodies. In the following section, a straightforward mathematical model is proposed, which aims to describe the effect of temperature on the antigen-antibody kinetics. It should be noted that the presented model provides a theoretical framework; an experimental validation falls beyond the scope of this paper.

2. Mathematical derivation

The molecular interaction between an antigen Ag – such as a bacterial cell wall component – and its anti-

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body Ab can be described by the following straightforward equilibrium reaction [2]:

$$Ag + Ab \rightleftarrows AgAb \tag{1}$$

According to the law of mass action, the following association equilibrium constant K_A can be defined:

$$K_A = \frac{[AgAb]}{[Ag]\,[Ab]} \tag{2}$$

The dissociation equilibrium constant can therefore be defined as follows:

$$K_D = \frac{1}{K_A} = \frac{[Ag]\,[Ab]}{[AgAb]} \tag{3}$$

The fraction θ represents the ratio of the number of occupied antigen-binding sites on an antibody molecule to the total number of antigen-binding antibody sites and can be derived from the Eqs (1) and (3) as follows:

$$\begin{aligned}\theta &= \frac{\textit{Occupied binding sites}}{\textit{Number ofbinding sites}} \\ &= \frac{[AgAb]}{[AgAb]+[Ab]} \\ &= \frac{\frac{[Ag][Ab]}{K_D}}{\frac{[Ag][Ab]}{K_D}+[P]} \\ &= \frac{[Ag]}{K_D+[Ag]}\end{aligned} \tag{4}$$

Equation (4) can be rewritten in order to yield a linear (Hill-like) plot:

$$\ln\left(\frac{\theta}{1-\theta}\right) = \ln([Ag]) - \ln(K_D) \tag{5}$$

A shift in the association/dissociation equilibrium of the antigen-antibody interaction in a closed system (i.e., assuming that the concentration of the available antigen is unaffected by the reaction temperature) will alter the fraction θ as follows:

$$\begin{aligned}&\text{logit}(\theta_2) - \text{logit}(\theta_1) = \ln([Ag]) - \ln(K_{D,2}) \\ &-(\ln([Ag]) - \ln(K_{D,1})) = \ln\left(\frac{K_{D,1}}{K_{D,2}}\right)\end{aligned} \tag{6}$$

In which $\text{logit}(\theta) = \ln(\theta/(1-\theta))$ is defined in order to improve mathematical transparency and allow for linear plotting. The Van 't Hoff equation relates a temperature-dependent change in dissociation equilibrium constant to its standard enthalpy $\Delta H^{\ominus}$ and the reaction temperature (in Kelvin) as follows [6]:

$$\frac{\delta \ln(K_D)}{\delta T} = -\frac{\Delta H^{\ominus}}{RT^2} \tag{7}$$

Equation (6) can be solves as follows by integrating on both sides:

$$\ln\left(\frac{K_{D,2}}{K_{D,1}}\right) = -\frac{\Delta H^{\ominus}}{R}\left(\frac{1}{T_2}-\frac{1}{T_1}\right) \tag{8.a}$$

$$\ln\left(\frac{K_{D,1}}{K_{D,2}}\right) = \frac{\Delta H^{\ominus}}{R}\left(\frac{1}{T_2}-\frac{1}{T_1}\right) \tag{8.b}$$

Equations (6) and (8.b) can be combined in order to yield the expression below:

$$\begin{aligned}&\text{logit}(\theta_2) - \text{logit}(\theta_1) \\ &= \frac{\Delta H^{\ominus}}{R}\left(\frac{1}{T_2}-\frac{1}{T_1}\right) = \frac{\Delta H^{\ominus}}{RT_2} - \frac{\Delta H^{\ominus}}{RT_1}\end{aligned} \tag{9}$$

Therefore, the effect of the reaction temperature on antigen-antibody interaction – and therefore on the effect on an important part of the humoral adaptive immune response – can be described by the following straightforward relationship under the condition that the antigen concentration is temperature-independent:

$$\text{logit}(\theta) \propto \frac{\Delta H^{\ominus}}{RT} \tag{10.a}$$

$$\theta \propto \frac{1}{1+\exp\left(-\frac{\Delta H^{\ominus}}{RT}\right)} \tag{10.b}$$

3. Discussion

In the previous section, a comprehensible and straightforward equation has been derived that aims to describe the effect of the reaction temperature on the antigen-antibody interaction, which consists primarily of the association and dissociation of an antigen-antibody complex (also known as an immune complex). The binding of an antigen to the variable domain or F_{AB}-region (fragment, antigen-binding) of its specific antibody, which is composed of the amino-terminal end of both a heavy chain and a light chain, is mediated by several non-covalent interactions between these molecules, such as Van der Waals forces, hydrophobic or hydrophilic interactions and hydrogen bonds [1,4]. The overall temperature-dependence of this antigen-antibody interaction increases the more the standard enthalpy $\Delta H^{\ominus}$ of this association/dissociation reaction differs from zero [5]. This explains why the interaction between peptide antigens and their antibodies is particularly temperature-dependent. The model proposed in this short report rests on two well-described pillars. The first pillar is defining the equilibrium constant of the association/dissociation reaction of an antigen and its an-

tibody. Not every type of non-covalent bond is influenced by temperature to the same extent. For instance, hydrophilic interactions are very temperature-sensitive, whereas the hydrophobic interactions are not [6]. Therefore, this lock-and-key model of antigen-antibody is – by definition – a simplification of reality, but accurate and useful in the analysis of the antigen-antibody interaction [1]. The second pillar is the Van't Hoff equation, which relates the temperature-dependent change in this association/dissociation equilibrium to the reaction temperature (in Kelvin) [4,7]. The proposed equation provides a quantitative insight into the complex interrelationship between reaction temperature and antigen-antibody kinetics, both *in vivo* and *in vitro*, which has been experimentally investigated by several authors. An early example is the paper from 1980 by Mason et al., who found that increasing reaction temperature from 4 degrees Celsius to 18 degrees Celsius increases the dissociation of antibodies from rat thymocytes ten-fold [3]. For the *in vitro* antigen-antibody-interaction of most peptide antigens, raising the incubation temperature from 5 degrees Celsius to 37 degrees Celsius decreases the value for the association/dissociation constant by approximately a factor four [5].

In order to derive the equations above, it is assumed that the (plasma) concentration of an antigen is independent of temperature. Furthermore, it is assumed that no temperature-dependent change in three-dimensional structure of peptide antigens or polypeptide antibodies takes place. This is a valid supposition, unless an extreme rise in temperature occurs, during which denaturation of secondary and tertiary protein structures can take place [7]. Additionally, it is assumed that no significant (temperature-dependent) changes in pH occur, as these are known to affect antigen-antibody affinity as well due to conformational changes in both antigens and antibodies [8].

In conclusion, the straightforward mathematical model proposed in this article could hopefully provide clinicians, immunologists, and biochemists with an improved insight into the kinetic effect of fluctuations in reaction temperature on antigen-antibody-dependent processes. Although an experimental validation would theoretically allow for exact calculations, the presented model should primarily be considered a means to get a quantitative understanding of the temperature-dependence of the interaction between an antigen and its antibody and therefore a better insight into the kinetics of the humoral adaptive immune response.

Conflict of interest

The author declares no conflict of interest.

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