TEMPERATURE EFFECTS ON THE KINETICS OF THE PRIMARY ANTIGEN-ANTIBODY COMBINATION

by

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Summary

Kinetic investigations utilizing fluorescence polarization techniques afford a means by which the activation energy for the primary combination between an antigen and its antibody can be determined. Initial rate studies have shown the reaction between fluorescein-labeled ovalbumin and antiovalbumin to be second-order in neutral buffered 0.15 M NaCl over wide concentration ranges between 1.5° and 28°. An activation energy of 12 Kcal/mole for this system suggests that the primary combination between antigen and antibody is not diffusion-controlled and, therefore, that some sort of structural changes occur prior to or during reaction.

Kinetic investigations of various hapten-antihapten systems (Day, et. al, 1962; Froese et. al, 1962; Day et. al, 1963; Froese and Sehon, 1965; Froese, 1968) have indicated that the rate of combination between hapten and antibody molecules is essentially diffusion-controlled and, hence, few if any conformational changes occur prior to or during the process. This conclusion has been reached on the basis of the large values of the second-order rate constants reported (10^7 to 10^8 liters $mole^{-1}$ sec^{-1}), and the small activation energy of about 4 Kcal/mole for the second-order process. These results involving hapten-antibody systems contrast with previous work which has centered largely on antigen-

antibody systems. For example, reported activation energies for various antigen-antibody systems (Talmadge, 1960; Talmadge and Cann, 1961; Poulsen, 1966) are in the range of 10 to 20 Kcal/mole. Unfortunately, however, either because of the method of monitoring the antigen-antibody reaction, or because of failure to test the second-order rate law over wide ranges of concentration, there is considerable uncertainty as to whether these high activation energies relate directly to the primary combination between antigen and antibody.

Recent interpretations of electronmicrographs involving both hapten-antibody (Valentine and Green, 1967) and antigen-antibody systems (Feinstein and Rowe, 1965) have hypothesized that the antibody molecule during reaction with either hapten or antigen, clicks open around a central hinge and acquires a rod-like shape, compared to its more globular shape, prior to reaction. Furthermore, while it has been proposed that the larger antigen may induce greater conformational changes in the antibody than would smaller hapten molecules (Valentine and Green, 1967), it is strange that the "clicking open" of the antihapten does not result in a higher activation energy than 4 Kcal/mole.

In view of these developments, it would be highly desirable to determine directly the activation energy involved in the primary bimolecular combination between a protein and its antibody. Fluorescence polarization kinetic techniques afford a direct means by which the initial binding rates of such an antigen-antibody system can be measured (Dandliker and Levison, 1967). In this manner, the temperature dependence of the second-order rate constant for the reaction between fluorescein-labeled ovalbumin and antiovalbumin has been studied directly and the activation energy determined.

MATERIALS AND METHODS

Antibody: Rabbit antiserum was prepared as previously described (Dandliker and Levison, 1967). Rabbit antiovalbumin was purified

by ammonium sulfate precipitation followed by DEAE cellulose chromatography (Campbell, et. al, 1963).

Antigen: Ovalbumin was prepared from fresh egg-white by a chromatographic method previously described (Levison and Dandliker, 1968).

Fluorescent labeling with fluorescein, and the quantitative precipitin determination used to determine concentration of antibody, were as described previously (Dandliker and Levison, 1967).

Kinetic experiments were performed with the use of a direct readout fluorescence polarometer with temperature control at about $\pm 0.5^{\circ}$ (White, et. al, 1968).

RESULTS AND DISCUSSION

The initial rate of fluorescence polarization change $(dp/dt)_o$ for the fluorescein-labeled ovalbumin-antiovalbumin system in 0.15 M NaCl, neutral 0.01 M Tris can be formulated as follows (Dandliker and Levison, 1967):

$$\left(\frac{dp}{dt}\right)_o = (p_b - p_f)\, k\, (AB)_o^{N_1} (AG)_o^{N_2 - 1} \qquad (1)$$

where p_b and p_f designate polarizations of fluorescence of the bound and free forms of the fluorescent-labeled material, k is the usual second-order rate constant, and $(AB)_o$ and $(AG)_o$ are initial molar concentrations of antibody and antigen, respectively. (The value of $p_b - p_f$ (0.05) was found to be independent of temperature between 1° and 28°.

By plotting $\log(dp/dt)_o$ vs. $\log(AB)_o$ at constant $(AG)_o$, the order with respect to (AB), i.e., N_1, can be determined. Alternatively, plotting $\log (dp/dt)_o$ vs. $\log(AG)_o$ at constant $(AB)_o$ yields N_2, the order with respect to AG. Examples of such plots in the temperature range from 1.5° to 28° are shown in Figures 1 and 2, which indicate that N_1 and N_2 are each equal to 1. These plots indicate that the reaction obeys

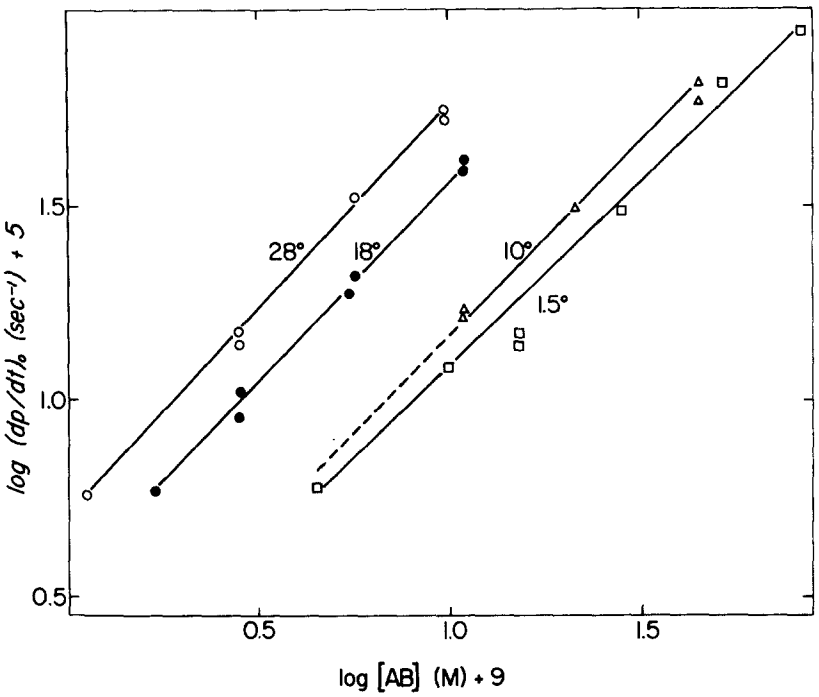

Figure 1. Determination of the order of reaction, N_1 (equation 1) with respect to antibody (antiovalbumin) by initial rate measurements at various temperatures. Reaction solutions at a particular temperature contained constant antigen concentration (AG), but varying antibody concentration (AB), in 0.15 M NaCl, 0.01 M Tris neutral buffer. $(dp/dt)_o$ denotes rate of change of polarization as $t \rightarrow 0$. O, $28^o \pm 0.5^o$, (AG) - 2.6×10^{-8} M; ●, $18^o \pm 0.5^o$, (AG) - 1.3×10^{-8} M; △, $10^o \pm 0.5^o$ (AG) - 2.7×10^{-8} M; □, $1.5^o \pm 0.5^o$ (AG) - 3.2×10^{-8} M.

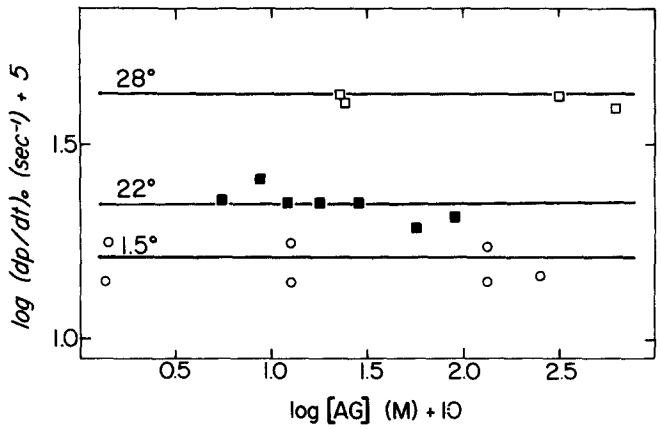

Figure 2. Determination of order N_2 (equation 1) with respect to antigen (AG), by initial rate method at various temperatures. Reaction solutions at a particular temperature contained constant antibody concentration (AB), as well as 0.15 M NaCl, 0.01 M Tris neutral buffer. □, $28^o \pm 0.5^o$ (AB) - 2.8×10^{-9} M; ■, $22^o \pm 0.5^o$, (AB) - 4.5×10^{-9} M; O, $1.5^o \pm 0.5^o$ (AB) - 4.9×10^{-8} M.

a second-order rate law over wide concentration ranges, and at different temperatures. Hence, it is unlikely that there is any abrupt change in mechanism over the ranges of concentration or temperature studied. The simplest mechanism consistent with the observed rate data involves a simple bimolecular reaction between antibody and protein. Furthermore, as indicated in Figure 3, the plot of log k vs. 1/T yields an activation energy of about 12 Kcal/mole for this second-order reaction. This activation energy is decidedly higher than the 4 Kcal/mole reported for hapten systems (Day, Sturtevant and Singer, 1963). If, as in the smaller hapten system, the initial ovalbumin-antiovalbumin combination was truly diffusion-controlled, one would expect an apparent activation energy of only 4 Kcal/mole resulting mainly from the temperature dependence of solvent viscosity (Longsworth, 1954). The relatively large activation energy observed in this work, together with the fact that the rate constant for the antiovalbumin system is at least a factor of 50 less than

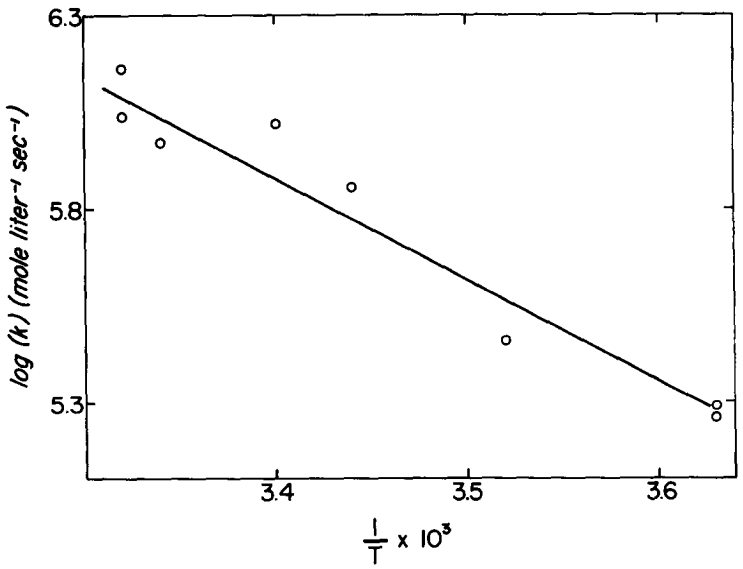

Figure 3. Effect of temperature on the second-order rate constant of the fluorescein-labeled ovalbumin-antiovalbumin combination. The second-order rate constants at each temperature were averaged before the plot was made. The activation energy was determined to be 12 Kcal/mole.

that predicted for reacting non-polar spheres (Dandliker and Levison, 1967),implies that the reaction is not diffusion-controlled, and that unlike hapten-antihapten systems, some sort of structural changes occur prior to or during combination with the antigen. Moreover, the initial rate of reaction is essentially independent of pH in the range of 6.4 to 7.8, which implies that electrostatic contributions to the free energy of activation for the binding process is small.

Finally, it is instructive to estimate the entropy of activation utilizing the Eyring equation (Glasstone et. al, 1941) for the hapten-antihapten and the ovalbumin-antiovalbumin systems. The entropy of activation for the ovalbumin-antiovalbumin combination is unusual in that it is much more positive (6.6 cal $mole^{-1}$ deg^{-1})than that for the hapten-antihapten reaction (-11 cal/$mole^{-1}$ $degree^{-1}$), and for many other reactions in solution in which two molecules combine to form one (Laidler, 1965). The positive entropy of activation implies that formation of the transition state may involve unfolding, dehydration, or both (Edsall, et. al, 1954).

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