A SIMPLE MODEL OF THE REACTION BETWEEN POLYADENYLIC ACID AND POLYURIDYLIC ACID [1]

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The data of Ross and Sturtevant (1960) on the rate of the reaction between polyadenylic acid and polyuridylic acid, which X-ray studies (Rich and Davies, 1956) have shown to form a two stranded helix, are unusual in that the rate decreases with increasing temperature. Furthermore, the forward rate, as distinguished from the net rate, apparently falls to zero or becomes small at the helix melting temperature. This rate data does not fit an Arrhenius equation; even one with a positive ΔH^*. We wish to present a simple model which accounts for these observations in terms of a series of ordinary kinetic steps. Similar considerations may be important in understanding the mechanisms of thermal denaturation of DNA and of proteins.

Processes involving polymers may often be regarded as involving many identical steps, each involving one unit of the chain. In cases where the process is a cooperative one, each step requires the completion of the previous step. Often the free energy change per step is small even when the total free energy change is large. In such cases each individual step must be readily reversible even though the overall process may be irreversible. In considering the rate of such a process one must consider the probability at each step of going backward in addition to going forward. It is important to note that in such a process no single step can be considered rate determining and no single ΔH^* will characterize the dependence of the rate of the reaction on temper-

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ature. In general, a reaction of the kind described includes a step at the beginning, the initiation, which is different in character from the others. A rate constant k_1 is assumed for this step. For simplicity, all the remaining steps are assumed similar, having forward rate constants k_f and backward rate constants k_b. If n steps are to be performed, there will be n-1 intermediates. In cases where the concentration of each intermediate species remains small relative to the starting material concentration over the course of reaction, one can make the steady state approximation. The overall rate of reaction will then equal the net rate of passage across any of the barriers separating the intermediates. If, as in the particular case, the initiation involves a bimolecular reaction between A and B to product C_1, which then goes to C_2 and eventually goes on to C_n, the product, then:

$$\text{rate} = v = k_1 \left[A\right] \left[B\right] - k_b \left[C_1\right] \quad \text{for the first barrier} \qquad (1)$$

$$= k_f \left[C_i\right] - k_b \left[C_{i+1}\right] \quad \text{for the remaining barriers} \qquad (2)$$

multiplying by $\left(\frac{k_b}{k_f}\right)^i$ one gets:

$$v \left(\frac{k_b}{k_f}\right)^i = k_b \left(\frac{k_b}{k_f}\right)^{i-1} \left[C_i\right] - k_b \left(\frac{k_b}{k_f}\right)^i \left[C_{i+1}\right] \qquad (3)$$

adding all the equations the intermediate concentrations drop out to give:

$$\sum_{i=1}^{i=n-1} v \left(\frac{k_b}{k_f}\right)^i = k_i \left[A\right]\left[B\right] - k_b \left[C_n\right] \left(\frac{k_b}{k_f}\right)^n \qquad (4)$$

Summing the series and rearranging:

$$v = \frac{k_i \left[A\right] \left[B\right]\left(1 - \frac{k_b}{k_f}\right)}{1 - (k_b/k_f)^n} - \frac{k_b \left[C_n\right]\left(\frac{k_b}{k_f}\right)^n \left(1 - \frac{k_b}{k_f}\right)}{1 - (k_b/k_f)^n} \qquad (5)$$

$\left(\frac{k_b}{k_f}\right)$ is the equilibrium constant for each step. Thus below the helix melting point, $\left(\frac{k_b}{k_f}\right)$ is less than one. When n is large, $\left(\frac{k_b}{k_f}\right)^n$ becomes quite small, and the second term becomes negligible. The first term then

reduces to:

$$v = k_i [A][B]\left(1 - \frac{k_b}{k_f}\right) \tag{6}$$

This is simply the expected forward rate over the first barrier times the factor $\left(1 - \frac{k_b}{k_f}\right)$. Assuming normal temperature dependence for the rate and equilibrium constants (6) becomes:

$$v = C \exp \frac{-\Delta H^*}{RT}\left(1 - D \exp \frac{-\Delta H}{RT}\right) \tag{7}$$

Since $\left(D \exp \frac{-\Delta H}{RT}\right)$ increases to approach one at the helix melting temperature, the factor $\left(1 - D \exp \frac{-\Delta H}{RT}\right)$ should decrease and the rate of the reaction would become small as the temperature approaches the helix melting temperature. If $\left(D \exp \frac{-\Delta H}{RT}\right)$ became greater than one, the equilibrium would shift to favor A and B, and the reaction would go in the reverse direction.

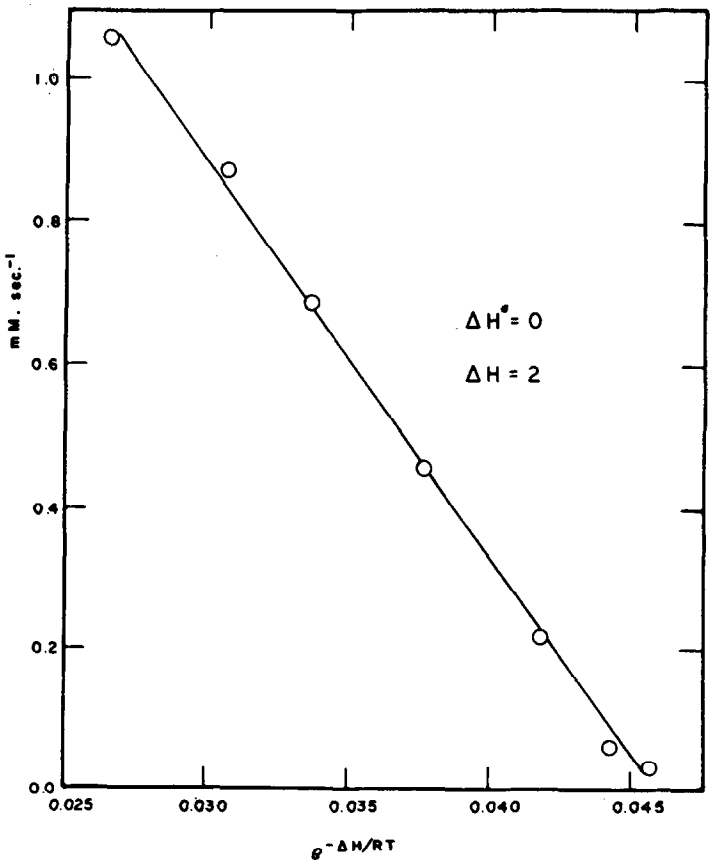

Fig. 1

The data of Ross and Sturtevant which shows a rate change of a factor of 30 over a range of 40° in 0.1 M NaCl can be fitted to (7) for several combinations of the parameters ΔH^* and ΔH. Unfortunately, it is possible to fit the data for increasingly negative values of ΔH^* by choosing compensatingly large positive magnitudes for ΔH. This result suggests that small values for these parameters are likely since ΔH is probably not excessively large. Thus, for example, by adjusting the constants, good fits can be obtained assuming $\Delta H^* = 0$ for $0 \text{ kcal/mole} < \Delta H < 5 \text{ kcal/mole}$. Figure 1 shows a typical fit.

One would expect that application of similar considerations would be useful in any case where a process requires a definite number of steps of a similar type for its completion. The formation and breaking of hydrogen bonds in some thermal denaturation processes of proteins and nucleic acids are such processes and might, with profit, be analyzed in a similar way. Since the tendency toward denaturation as well as the rate of the initial steps become greater with increase of temperature, both factors operate in the same direction and one should get a very large temperature coefficient leading to an apparently large ΔH^* if an Arrhenius plot is made. It should be noted that deviation from an Arrhenius plot should occur eventually but that data is seldom available over a wide enough temperature range as it is in the case of helix formation, since the reactions become too fast to measure at the higher temperature.

We should like to thank Professor Onsager for pointing out that a mathematically similar model has been used (Volmer, 1926, 1927) in interpreting data on the rate of nucleus formation in supercooled vapors, where the individual steps are adding and losing molecules to droplets in the critical region of size. We also wish to thank Dr. Bruno Zimm and Professor J. M. Sturtevant for helpful discussions.

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