# INTERPRETATION OF THE KINETICS OF HELIX FORMATION

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An explanation of the kinetic behavior observed in the reaction between single strands of poly A and poly U which results in formation of the double-stranded poly (A + U) helical complex has recently been proposed by Ross and Sturtevant (1962), based on a simplified kinetic model of Saunders and Ross (1960). This model treats the reaction as a multi-stage "zipping" process, in which all steps except the first have identical rate constants in the forward direction and in which all steps have the same rate constant in the backward direction. We propose here a modification of this model to include a different backward rate constant for the first step, and we show that this appreciably affects the resulting rate equations and the interpretation of the experimental data in terms of them.

We take the kinetic scheme for the reaction as follows:

$$A + B \underset{k_u}{\overset{k_o}{\rightleftarrows}} C_1 \qquad \text{initial step} \qquad (A)$$

$$C_1 \underset{k_b}{\overset{k_f}{\rightleftarrows}} C_2 \underset{k_b}{\overset{k_f}{\rightleftarrows}} \cdots C_{N-1} \underset{k_b}{\overset{k_f}{\rightleftarrows}} C_N \qquad (B)$$

by analogy with the treatment of Saunders & Ross. In this notation A and B denote the concentrations of the single-stranded species, while $C_i$ represents the concentration of state of the combined species with i bound pairs. Where

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Saunders and Ross assumed that the rate constant for the backward step in (A) is $k_b$, the same as for the steps in (B), we write it as $k_u$. It seems intuitively clear that $k_u$ should be greater than $k_b$, since the last step in the reverse direction represents the physical separation of the strands and would therefore be accompanied by a large entropy change. Furthermore, the effect of the Brownian motion of the long unbonded chains would be again to favor a large value for $k_u$.

The rate of the initiation reaction then follows the equation

$$v_o = k_o[A][B] - k_u C_1, \tag{1}$$

while the succeeding steps are given by

$$v_i = k_f C_i - k_b C_{i+1} \qquad (1 \leq i \leq N - 1). \tag{2}$$

For temperatures below the melting temperature, where $k_b/k_f \leq 1$, we find

$$v_o = k_o AB (1 - \frac{k_b}{k_f}) + (k_b - k_u) C_1 (1 - \frac{k_b}{k_f}) \tag{3}$$

instead of equation (3) in Ross and Sturtevant. Qualitatively then, the rate is again in accord with the requirement that the rate approaches zero at the melting temperature.

If we examine the concentration of species 1 we find under steady state conditions with $dC_1/dt = 0$,

$$C_1 = \frac{k_o}{k_u + k_f} AB + \frac{k_b}{k_u + k_f} C_2. \tag{4}$$

For $j \geq 2$ we have the recursion formula

$$C_j = pC_{j+1} + qC_{j-1}, \tag{5}$$

where

$$p = \frac{k_b}{k_b + k_f}, \qquad q = \frac{k_f}{k_b + k_f}.$$

This is identical to the difference equation for the well-known problem of the gambler's ruin, as recognized by Flory (1961).

This equation as a difference equation has the general solution

$$C_j = \alpha + \beta \, (q/p)^j,$$

where $\alpha$ and $\beta$ are constants. Since we require $C_{N+1} = 0$, and writing the remaining constant in terms of $C_N$ we formally eliminate $\alpha$ and $\beta$:

$$C_j = C_N \left(\frac{p}{q}\right)^{N-j} \frac{p}{p-q}\left[1 - \left(\frac{q}{p}\right)^{N-j+1}\right] .$$

In particular, with $K = p/q$,

$$C_2 = C_N \left(\frac{1 - K^{N-1}}{1 - K}\right) . \tag{6}$$

Neglecting $K^{N-1} << 1$, we find from equations (4) and (6)

$$C_1 = \frac{k_o\, AB}{k_u + k_f} + \frac{k_b\, C_N}{k_u + k_f} \left(\frac{1}{1-K}\right) . \tag{7}$$

The final expression for the rate is, from equation (3),

$$v = k_o AB(1-K)\left(1 + \frac{k_b - k_u}{k_u + k_f}\right) + \frac{k_b(k_b - k_u)}{k_u + k_f} C_N , \tag{8}$$

which reduces to the rate equation of Saunders & Ross when $k_u = k_b$.

We now consider the temperature dependence predicted by such a model. We assume, as do Ross and Sturtevant, that the temperature dependence of each rate constant can be represented by

$$k_i = \frac{RT}{Nh} \exp\left(\frac{\Delta S_i^{+}}{R}\right) \exp\left(-\frac{\Delta H_i^{+}}{RT}\right) , \tag{8a}$$

where i = o, u, b or f. Since we are interested in comparing our prediction with experiment, we look at the case in which the empirical law holds:

$$v = k_{app}\, AB . \tag{9}$$

This corresponds to the initial stages of the reaction, so we neglect the last term in equation 8, and obtain

$$v \cong k_o\, AB\, (1-K^2)\ \frac{1}{1 + k_u/k_f} . \tag{10}$$

Equating equations 9 and 10, we find, with equation 8a, and letting $\Delta S^o = \Delta S_b^{+} - \Delta S_f^{+}$, $\Delta H^o = \Delta H_b^{+} - \Delta H_f^{+}$:

$$\frac{Nh}{RT} k_{app} \exp\left(\frac{\Delta H_o^{+}}{RT}\right) = \frac{\exp\left(\frac{\Delta S_o^{+}}{R}\right)\left[1 - \exp\left(\frac{2\Delta S^o}{R} - \frac{2\Delta H^o}{RT}\right)\right]}{1 + \left[\exp\left(\frac{\Delta S_u^{+}}{R} - \frac{\Delta H_u^{+}}{RT}\right)/\exp\left(\frac{\Delta S_f^{+}}{R} - \frac{\Delta H_f^{+}}{RT}\right)\right]} \tag{11}$$

The left-hand side of equation 11 is the function plotted by Ross and Sturtevant as a function of $\exp(-\Delta H^o/RT)$ for various values of $\Delta H^{+}$ and $\Delta H^o$.

However, the right-hand side of equation 11 differs from their equation 6. Note, that if $\Delta H_f^{+} \cong \Delta H_u^{+}$, our equation 11 would predict a linear dependence on exp $(-2\Delta H^o/RT)$, instead of exp $(-\Delta H^o/RT)$.

The above considerations therefore significantly affect any attempt at a simple interpretation of the observed rate data. The enthalpies of activation which appear as parameters in the treatment of the data of Ross and Sturtevant cannot be unambiguously assigned to a specific stage of the reaction, as is evident from equation (11). The rate can vanish when $k_b = k_f$ or when $k_u$ becomes larger than $k_b$ and $k_f$, which corresponds to short chains. Furthermore, if one is concerned with heats of activation, the difference between $k_u$ and $k_b$ in the first step cannot be incorporated into $k_1$. It must also be emphasized that the number of bonded base pairs which effectively constitutes a "step" and to which the heat of activation corresponds is not necessarily unity.*

## References

P. J. Flory, J. Polymer Science XLIX, 105 (1961).

P. D. Ross & J. M. Sturtevant, J. Am. Chem. Soc., 84, 4503 (1962).

M. Saunders & P. D. Ross, Biochem. and Biophys. Res. Comm., 3, 314 (1960).

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