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Communications

The Negative Rate Constants of Breslow and Huang

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Summary: The arguments of Breslow and Huang (Breslow, P.; Huang, D.-L. *J. Am. Chem. Soc.* **1990**, *112*, 9621), in which a mechanistic scheme is supported by negative rate constants, are shown to be untenable.

Breslow and Huang[1] recently published a communication (hereafter called B/H) on the cleavage and isomerization of 3′,5″-adenyladenine (ApA). A related article on the cleavage and isomerization of 3′,5″-uridyluridine was coauthored by Anslyn and Breslow (A/B).[2]

A reading of the above papers led to a measure of concern on several fronts (listed in a roughly increasing order of seriousness): (a) extremely slow isomerization kinetics (e.g., an isomerization rate of 0.19 × 10^{-3} h^{-1} equivalent to a $t_{1/2}$ of 151 days at 80 °C);[1] (b) a paucity of kinetic data (e.g., only two kinetic points per line in Figures 1 and 2 of B/H); (c) a high experimental error (e.g., $k_{isom} = 0.19 \pm 0.72$);[1] (d) pseudo-first-order rate constants given in the wrong units (e.g. $k\psi$ in μM/min in Figures 1–9 of A/B; their Table I contains both first- and second-order rate constants in the same unit);[2] (e) no control of ionic strength; (f) unbuffered kinetic runs (e.g., when $[Im]/[ImH^+] = 100/0$ in Table I of B/H); (g) rate constants tacked onto the steady-state rate equation as ad hoc corrections for an expression that otherwise does not fit the data (e.g. $k'[Im]$ and $k''[ImH^+]$ in eq 1 of A/B and B/H; k' and k'' are never further incorporated into any mechanistic scheme).

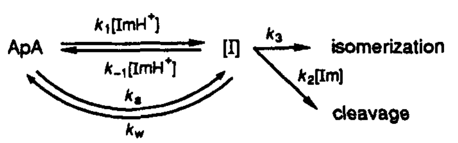
Two points in particular bear special mention. A rate equation of A/B,[2] duplicated below in eq 1, corresponds to the mechanism shown in Figure 1. It is seen that the principle of microscopic reversibility has been ignored.

$$k_{isom} = \frac{k_1k_3[ImH^+]}{k_{-1}[ImH^+] + k_2[Im] + k_3 + k_w} \qquad (1)$$

But perhaps the most striking feature of the Breslow kinetics, and the one to which I will confine the remainder of the discussion, pertains to the title of the B/H paper: "A Negative Catalytic Term Requires a Common Intermediate in the Imidazole Buffer Catalyzed Cleavage and Rearrangement of Ribodinucleotides". Thus, pseudo-first-order rate constants reported for the isomerization process included many negative values (Table I). These negative rate constants served as the basis of the B/H communication. It is, therefore, important to understand exactly how the constants were derived. Raw rates of imidazole-catalyzed isomerization were "corrected" by subtracting the background "water" reaction. Since reactions with imidazole were often found to be *slower* than those in water without imidazole, negative rate constants ensued. Note that the negative rate constants were not simply presented as a euphemism for an ordinary rate inhibition where one positive rate diminishes to a smaller positive rate. If this were the case, then why would they claim, as they did, that negative rate constants "do not seem to be widely used or known"?[1] In actual fact, the experimental rate constants, in and of themselves, were found to be subzero and extolled as such.

Since an experimental rate constant can never be less than zero, the negative numbers must represent an experimental or procedural artifact. One possibility is that lack of pH control somehow inflated the "water" rate. More likely, however, the negative rates arose from a faulty data treatment discussed below.

It is the correction for the "imidazole-independent rate" that appears inappropriate. This would follow if the mechanism of the reactions in pure water overlaps with that of the imidazole-catalyzed processes as shown in eq 2.[3] Note that by including a k_a step in eq 2 the mechanism



(2)

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[1] Breslow, R.; Huang, D.-L. *J. Am. Chem. Soc.* **1990**, *112*, 9621.
[2] Anslyn, E.; Breslow, R. *J. Am. Chem. Soc.* **1989**, *111*, 4473.

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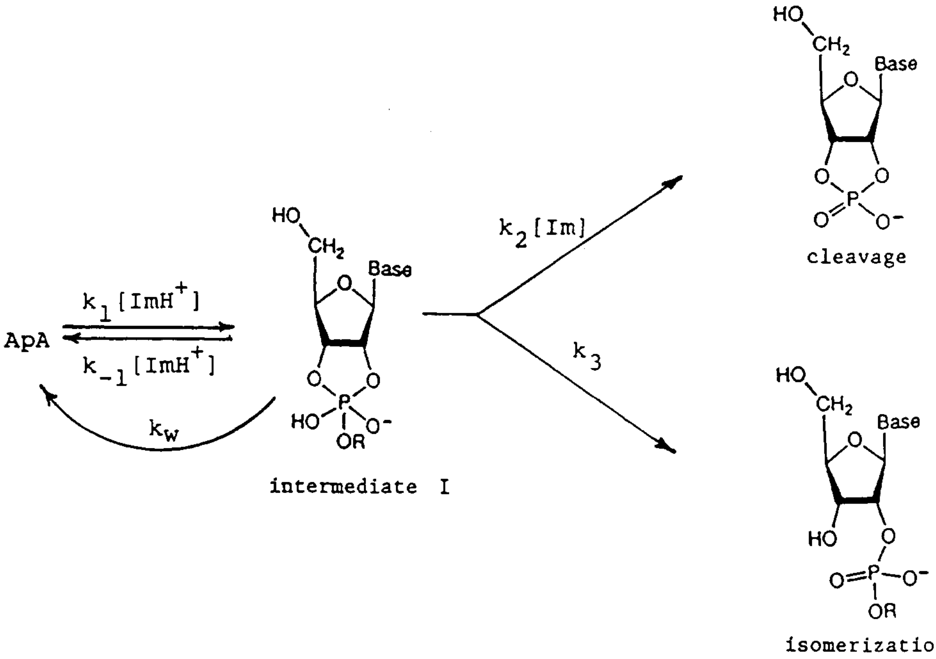

Figure 1. The Anslyn–Breslow mechanism utilizing the five rate constants in eq 1 of their article[2] (and given in eq 1 of the present article). A steady-state treatment of intermediate I generates these equations.

does not now violate microscopic reversibility. The corresponding k_{isom} would then be that shown in eq 3.[4] The background portion of this rate constant (i.e., when [Im] = [ImH$^+$] = 0) is given by eq 4.[5] It is obvious from eqs

$$k_{\text{isom}} = \frac{k_1k_3[\text{ImH}^+] + k_ak_3}{k_{-1}[\text{ImH}^+] + k_2[\text{Im}] + k_3 + k_w} \tag{3}$$

$$k_{\text{bckgrd}} = \frac{k_ak_3}{k_3 + k_w} \tag{4}$$

3 and 4 that one cannot simply subtract the background from the experimentally measured rate constant because the two are not in an additive relationship. In particular, the observed rate constant minus the background will become negative when eq 5 maintains. This will occur

$$\frac{k_a}{k_3 + k_w} > \frac{k_1[\text{ImH}^+] + k_a}{k_{-1}[\text{ImH}^+] + k_2[\text{Im}] + k_3 + k_w} \tag{5}$$

at high [Im], exactly as was observed in Table I. Of course, the negative values would be devoid of physical meaning or mechanistic significance with respect to the isomerization and cleavage steps as claimed by Breslow and Huang.

Table I. Pseudo-First-Order Rate Constants ($\times 10^3$ h^{-1}) for the Isomerization of 3′,5″-Adenyladenine in Imidazole Buffers at 80 °C as Reported by Breslow and Huang[1]

Im/Im·HCl	concn, M	k(isom)[a,b]
100/0	0.8	–0.31
40/60	0.8	–0.51
0/100	0.8	0.19
100/0	1.3	-1.26
40/60	1.3	–0.93
0/100	1.3	1.13
100/0	2.0	-1.20
40/60	2.0	-1.33
0/100	2.0	0.91

[a] As explained in the text, these are observed pseudo-first-order rate constants which have been corrected for the background ("water") reaction in absence of an imidazole buffer. [b] Note that the values are *both* negative and positive. It is difficult to imagine a rate expression in which changing concentrations converts an observed rate constant from positive to negative.

The above errors (in addition to others not specifically cited here[6]) prevent a scholarly analysis of the ApA mechanisms at this time.

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[3] Equation 2 is by no means the only possible mechanism for the background reaction. It is merely the simplest alteration of the unproven Breslow–Huang scheme that explains their negative rate constants.

[4] A rate expression for k_{isom} is presented in B/H which differs from the corresponding rate expression in A/B (see eq 1 above). The former has a k_w tacked onto the numerator of the steady-state equation. The B/H version would be formally correct only if k_w happened to equal k_ak_3 in eq 3; this would be a bizarre coincidence.

[5] Derivations of rate expressions in this paper are available upon request.

[6] Dr. Albert Haim of Stony Brook simultaneously and independently uncovered a variety of other problems with the Breslow manuscripts (e.g., the reported rate constants and equations do not fit the theoretical plots). His analysis will be published elsewhere. Neither of us was permitted to publish our work in the journal where the errors originated.