

The Case for Cytidyl-2',3'-phosphate as an Obligatory Intermediate in the Hydrolysis of Cytidyl-3',5'-cytidine catalysed by Ribonuclease

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WILLIAMS¹ reported recently that in the hydrolysis of cytidyl-3',5'-cytidine (I) by ribonuclease, the formation of cytidyl-2',3'-phosphate (II) as an intermediate is not obligatory and that the second-order rate constant (k_3) for the pathway which does not involve the formation of (II) may be calculated from equation (1) in which k_1 is the second-order

$$k_3 = k_1\{[I_0] - [II_m]\} / [II_m] \quad (1)$$

rate constant for the information of (II) from (I), $[I_0]$ is the initial concentration of (I), and $[II_m]$ is

the maximum concentration of (II) formed during the catalysis. This contradicts the work of Witzel and Barnard² who, from a study of the hydrolysis of several dinucleoside-3',5'-phosphates by ribonuclease, suggested that the formation of (II) is obligatory in these catalyses.

Williams reported that the maximum concentration of (II) formed from (I) during the catalysis (pH 7, 25°) as measured by the change in ultraviolet absorption at 286 m μ , consequent upon the conversion of (II) into 3'-cytidylic acid (III) was

“much less” than that expected from $[I_0]$, $k_1 = 7.2 \times 10^4 \text{ mole}^{-1} \text{ sec.}^{-1}$ and $k_2 = 1.57 \times 10^3 \text{ l.mole}^{-1} \text{ sec.}^{-1}$, where k_2 is the rate constant for the hydrolysis of (II) to provide (III). These values of k_1 and k_2 are in reasonable agreement with the corresponding values of k_{cat}/K_m (6.0×10^4 and $1.33 \times 10^3 \text{ l.mole}^{-1} \text{ sec.}^{-1}$ respectively) reported by Witzel and Barnard.² From the reported¹ value of $[II_m]/([I_0] - [II_m]) = 6.75$, calculation gives the observed value of $[II_m]$ as 87% of $[I_0]$. Since equation (1) is valid only if k_2 may be neglected in comparison with k_1 (in which case $[II]/[III] = k_1/k_3$) this observed value of $[II_m]$ was compared presumably with an “expected value” of $[II_m]$ of *ca.* 100% of $[I_0]$. If k_2 is not neglected, however, the catalysis $[(I) \rightarrow (II) \rightarrow (III)]$ may be treated as a system of consecutive, irreversible, pseudo-first-order reactions and the expected value of $[II_m]$ calculated from equation (2) using the reported¹ values of k_1 and k_2 . The value of t is determined experimentally as the time taken to reach the steady state in

which the concentration of (II) is a maximum for a given enzyme concentration $[E]$. Equation (2) gives the expected value for $[II_m]$ as 92% of $[I_0]$.

$$[II_m] = k_1[I_0] \frac{\{\exp -(k_1[E]t) - \exp -(k_2[E]t)\}}{(k_2 - k_1)} \quad (2)$$

We found that when ribonuclease catalyses the hydrolysis of a solution of (I) freshly prepared in tris buffer (pH 7.00, 0.1M, 25.0°) the maximum concentration of (II) formed during the catalysis, as measured^{2,3} by the change in ultraviolet absorption at 286 m μ in a Cary 15 recording spectrophotometer, was 90–95% of $[I_0]$. This value for $[II_m]$ and also that reported by Williams (87%) are in reasonable agreement with the value (92%) predicted by equation (2). Thus, the spectrophotometric data provide no evidence for a pathway for the catalysis which does not involve the formation of (II) as an intermediate.

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¹ A. Williams, *Chem. Comm.*, 1966, 590.

² H. Witzel and E. A. Barnard, *Biochem. Biophys. Res. Comm.*, 1962, 7, 295.

³ E. M. Crook, A. P. Mathias, and B. R. Rabin, *Biochem. J.*, 1960, 74, 234.