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Kinetics and Template-Dependency of Ribonucleic Acid Synthesis by Bacterial Ribonucleic Acid Polymerase

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The rate of RNA synthesis catalysed by DNA-dependent RNA polymerase shows a Michaelis–Menten-type saturation curve with increasing template concentration. However, the apparent K_m is proportional to enzyme concentration, indicating that the reaction does not obey a simple kinetic scheme. The action of inhibitors also indicates a more complex interaction between the enzyme and the DNA template; many inhibitors of RNA synthesis either decrease V_{max} , without affecting K_m , or increase K_m without affecting V_{max} . All of these observations can be accounted for quantitatively by a reaction pathway in which the non-specific binding sites of the viral DNA template inhibit competitively the binding of the enzyme to the initiation sites. In terms of this pathway the two classes of inhibitors of RNA synthesis must then act predominantly either on the rate of elongation or on the availability of the binding sites respectively.

Many compounds of important pharmacological and biological activity, such as intercalating drugs (Waring, 1968), chromosomal proteins (Huang & Bonner, 1962; Paul & Gilmour, 1968) and steroid receptors (Buller *et al.*, 1976), have been shown to interact with DNA and alter its template properties for RNA synthesis. The effects of ethidium bromide and its structural analogues (Waring, 1964; Panayotatos, 1977), proflavine and actinomycin D (Hurwitz *et al.*, 1962), 4,5',8-trimethylpsoralen (N. Panayotatos, unpublished work), as well as histones (Marushige & Bonner, 1966; Keshgegian & Furth, 1972), on the DNA-dependency of RNA synthesis have been studied extensively; some of these compounds decrease V_{max} , without affecting K_m , whereas others increase K_m without affecting V_{max} . However, owing to the complexity of the process of RNA synthesis, only empirical kinetic equations have been proposed for relating the rate of the reaction to the DNA-template concentration. It has been shown (Shih & Bonner, 1970) that the dependence of the rate of RNA synthesis on the concentration of the DNA template follows an equation formally analogous to the Michaelis–Menten equation:

$$v = V_{max} \cdot [A] / (K_m + [A]) \quad (1)$$

where v is the rate of total RNA synthesis, $[A]$ is the concentration of DNA, V_{max} is the rate of RNA synthesis at saturating DNA concentrations, and K_m is the concentration of DNA required for half-maximal rate. The last parameter is not a true kinetic

constant, however, since K_m was found to be proportional to the enzyme concentration (Shih & Bonner, 1970; Wood & Berg, 1964), indicating that the reaction does not obey a simple kinetic scheme. At present, no mechanisms have been proposed to account for the observed kinetics, and the effects of inhibitors on K_m and V_{max} have only been interpreted empirically. In the present paper we propose a reaction pathway in which the non-specific binding sites of the viral template (B sites) inhibit competitively the binding of the enzyme to the initiation sites (A sites). In terms of this pathway the enzyme-concentration-dependence of K_m can be accounted for quantitatively, and the two classes of inhibitors of RNA synthesis must then act predominantly either on the rate of elongation or on the availability of the binding sites.

Experimental evidence indicates that the interaction of RNA polymerase with the DNA template and the substrates involves the formation of non-specific enzyme–template complexes (C), two forms of specific complexes at the initiation site(s) (I and RS), as well as the ternary enzyme–DNA–nucleotide complex; it is the last complex that leads to RNA chain growth (Mangel & Chamberlin, 1974*a,b*; Hinkle & Chamberlin, 1972). Under the most widely used experimental conditions, where the molar concentrations of enzyme $[E_0]$ and template initiation sites (A sites) are of the same order of magnitude but the ribonucleotide concentration is in great excess, all of the enzyme is associated with the template, and forms C,

Scheme 1. *Proposed mechanism of RNA synthesis*

Unbound enzyme (E) can interact with an unoccupied non-specific binding site (N) and form a non-specific complex (C) that, in turn, can interact with an unoccupied specific binding site (J) and form the first specific complex (I). Alternatively, I can be formed directly from E and J. I is in equilibrium with form RS. The latter can rapidly form the irreversible initiation complex. k represents the rate-limiting constant of the elongation reaction. $K_C = [E][N]/[C]$; $K_I = [C][J]/[I][N]$; $K_J = [E][J]/[I]$; $K = [I]/[RS]$.

I and RS are in equilibrium, i.e. rapidly interconvertible. Under these conditions, the maximum rate of RNA synthesis occurs only when the enzyme concentration is at least 10 times higher than the estimated concentration of initiation sites (A sites) (Bautz & Bautz, 1970). Therefore the non-productive binding of the enzyme to the non-specific sites of the template (B sites) must be kinetically significant, resulting in suboptimal utilization of the enzyme.

On the basis of these observations, a kinetic scheme can be proposed (Scheme 1).

From Scheme 1, a general rate equation can be derived, relating the rate of RNA synthesis to the template and enzyme concentrations. This rate equation shows that the rate of RNA synthesis (v) as a function of template concentration $[A_0]$ obeys a hyperbolic relationship if, and only if, $n > (1 + K)/KK_I > 1$, where n is the molar ratio of B to A sites. This mathematical condition reflects the preferential distribution of the enzyme into the non-productive sites, even under conditions of maximal rate of RNA synthesis; i.e. $[E_0] > [I] + [RS]$. If indeed the values of K and K_I satisfy the above condition, the rate equation can be simplified into the following expression:

$$v = k[RS] = \frac{[A_0]k[E_0]/KK_I n}{[E_0](1 + K - KK_I a/KK_I n + [A_0])} \quad (2)$$

where a is the molar ratio of template deoxynucleotide concentration $[A_0]$ to the total concentration of specific binding sites $[J_0]$, and $[E_0]$ is the total enzyme concentration.

Eqn. (2) is of the same form as eqn. (1) if:

$$K_m = [E_0](1 + K - KK_I a/KK_I n) \quad (3)$$

and

$$V_{\max} = k[E_0]/KK_I n \quad (4)$$

Thus K_m is indeed proportional to the total enzyme concentration (eqn. 3), in full agreement with the experimental observations.

The conformity of eqn. (2) with the experimental findings warrants the assumptions used in its derivation. According to the proposed reaction scheme, the enzyme-concentration-dependence of K_m is a direct consequence of the predominantly non-productive binding of the enzyme to DNA. Since $([RS] + [I])$ is much smaller than $[E_0]$, most of the enzyme molecules are bound non-productively, and therefore, under the usual experimental conditions *in vitro*, the rate of RNA synthesis is suboptimal. Most importantly, the experimentally observed K_m is a complex constant that bears little relationship to the affinity constant of the enzyme-template interaction.

The conditions required for a hyperbolic relationship between the rate of RNA synthesis and the concentration of the template impose restrictions on the relative values of n , K and K_C . For bacteriophage-T₇ DNA and bacterial RNA polymerase, the value of the formation constant of the I complex from the enzyme and DNA, $1/K_I$, is experimentally determined to be of the order of 10^{12} M^{-1} (M. Chamberlin, unpublished work, cited by Chamberlin, 1974), whereas the value of K_C varies between $1 \mu\text{M}$ and 1 nM , depending on the estimated value of n (10^3 – 10^5) (Chamberlin, 1974; P. de Haseth, T. M. Rohman, T. M. Record, & R. R. Burgess, unpublished work). Since $K_I = K/K_C$, the value of K_I may then be calculated to be 10^{-2} – 10^{-3} . In view of the large uncertainty in the values of these parameters, the restrictions imposed by the mathematical conditions are easily met and appear to be reasonable. Therefore the proposed kinetic scheme is consistent with all pertinent experimental observations, and it suggests that, before chain elongation, equilibrium is indeed

established between the C, I and RS complexes. The derivation of eqn. (2) implies that, during chain elongation, no re-initiation occurs, at least until the synthesized RNA is released from the template. In other words, initiation of the elongation process 'freezes' the equilibrium distribution of the enzyme between A and B sites, which results in linear elongation rates. Experimental observations of RNA synthesis *in vitro* seem to support fully this assumption (Bremer, 1970; Richardson, 1970).

The above kinetic scheme also provides a means for analysing the kinetic behaviour of inhibitors of RNA synthesis. Since the kinetic scheme involves multiple binding sites for the enzyme on the template, addition of an inhibitor complexing with the DNA could affect the kinetics of RNA synthesis in a variety of ways. Experimentally, at least two different types of inhibition are observed: (a) the competitive type, exemplified best by structural analogues of ethidium bromide (Panayotatos, 1977), which increase K_m without affecting $V_{max.}$, and (b) the purely non-competitive type, exhibited by histones (Shih & Bonner, 1970), actinomycin D (Panayotatos, 1977) or 4,5',8-trimethylpsoralen bound covalently on the DNA (N. Panayotatos, unpublished work), which decrease $V_{max.}$ without affecting K_m . Actinomycin D is used effectively at very low concentrations where less than one inhibitor molecule is bound per 500 DNA base-pairs (Hyman & Davidson, 1970). Under these circumstances, interference with the binding of the enzyme to the template must be negligible and, as a result, inhibition of chain elongation is the only observable effect. Ethidium bromide is an effective inhibitor only when at least one ethidium molecule is bound per 50 DNA base-pairs. These conditions result in competitive inhibition of enzyme binding, since the presence of ethidium on the template effectively decreases the number of total nucleotides $[A_0]$ available for binding by a factor x . As a result the value of K_m appears to be increased by the same factor, as shown in eqn. (5):

$$v_i = V_{max.}[A_0]/xK_m + [A_0] \quad (5)$$

The dissociation rate of ethidium ($10^2 s^{-1}$; Bresloff & Crothers, 1975) is faster than the rate of RNA elongation (≤ 50 nucleotides/s; Bremer, 1970). Even if the translocation step during chain elongation were rate-limiting, the binding of this drug would only have a minor effect on $V_{max.}$. In view of these considerations, the proposed kinetic scheme is not inconsistent with the observed behaviour of these inhibitors on RNA synthesis.

The analysis of the kinetic behaviour of bacterial RNA polymerase indicates that interference of template segments other than the initiation sites is an important facet of gene expression and gene control.

RNA polymerase requires strong binding sites on the template to be able to transcribe it efficiently. The presence of a large number of non-specific binding sites, however, is a source of extensive unfavourable interactions. It appears that even the presence of σ factor is compensating only partially for this drawback. The situation will most probably be encountered with most nucleic acid-recognizing enzymes, and in the kinetic analysis of these systems more than a cursory glance should be given to intramolecular competition. Finally, the transcription rate of a given template should depend on its ratio of non-specific to specific binding sites, n . With eukaryotic DNA containing long non-transcribed regions, the value of n might be large enough to become the predominant factor regulating activity. On the other hand, with homogeneous synthetic templates such as poly(dA)·poly(dT) and poly(dG)·poly(dC), every other binding site is a potential initiation site, and little non-productive binding should be observed.

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