A Kinetic Model for Nucleohistone-Dependent DNA Synthesis

The nucleohistone component of pea embryo chromatin prepared according to the procedure of HUANG and BONNER[1], inert as a template for RNA synthesis, functions as a primer in an *Escherichia coli* DNA polymerase (deoxynucleoside triphosphate: DNA nucleotidyl transferase, EC. 2.7.7.7) reaction system[2]. The resulting limited synthesis ceases over a time interval in which DNA-primed DNA synthesis progressively increases.

The product of the nucleohistone primed DNA synthesis comprises mostly nucleohistone bound DNA and some free unbound DNA[3]. The fraction of DNA product bound to the nucleohistone, which also appears to complex the polymerase enzyme to form an inactive complex, is sensitive to endonuclease but insensitive to exonuclease action[4]. However, there is very little transfer of primer histone to newly synthesized DNA product[5]. The present investigation is concerned with the rate of DNA polymerase action with DNA and nucleohistone as primers as affected by concentration of primer and deoxynucleoside triphosphates. The experimental observations are in accord with a quasi equilibrium model in which a complex polymerase template and product is reversibly transformed to a state in which the polymerase is no longer available for catalyzing further DNA synthesis.

Results. Figure 1 shows the effect of varying DNA concentrations on the rates of DNA synthesis by *E. coli* DNA polymerase with DNA and with nucleohistone of pea embryo as primers. As previously shown[2], DNA synthesis with either primer proceeds in an essentially linear fashion over the measured time interval, 30 min. The ratio of DNA- to nucleohistone-primed reaction rates decreased with increasing primer concentration. The rate vs. concentration profiles obeyed, with experimental error, simple Michaelis kinetics at low DNA concentrations. Both K_m and V were greater for the DNA-primed reaction than for the nucleohistone-primed reaction. The slopes of the double reciprocal 1/c V. 1/DNA plots (K_m/V) were the same.

An analogous situation pertains to the effect of deoxynucleoside triphosphate (dNTP) concentration (Figure 2). At low concentrations of dNTP the rates are comparable. At high concentrations the rate decreased with DNA, but not with nucleohistone as primer. K_m and V_{max}, as in the case of varying DNA concentration, were greater for the DNA- than for the nucleohistone-primed reaction; and the slopes of the double reciprocal 1/v vs. 1/dNTP plots were the same. In experiments in which both nucleohistone and DNA were present in the polymerase reaction mixture, the rate of DNA synthesis was always closer to that of the nucleohistone-primed reaction than to that of the DNA-primed reaction alone.

When various components of the DNA polymerase reaction mixture were added after 30 min of reaction, very little, if any, further increase in DNA synthesis was observed with nucleohistone as primer (Table). With DNA as primer further DNA synthesis ensued upon addition of enzyme and enzyme plus dNTP.

We propose the following mechanism to account for the finding that the straight lines resulting from the reciprocal plots with nucleohistone and DNA a primer appear to be parallel upon varying either DNA or dNTP concentration.

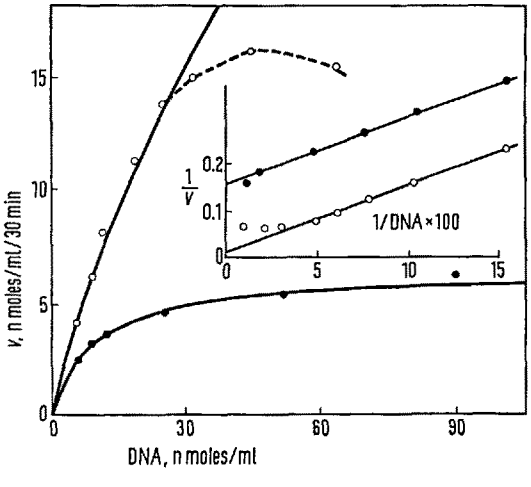

Fig. 1. Effect of DNA concentration on the rate of DNA synthesis with pea embryo DNA (O) and with nucleohistone (●) as primers. The reaction mixtures contained in μmoles/ml; potassium phosphate buffer pH 7.2, 40 μmoles; $MgCl_2$, 4.0 μmoles; 2-mercaptoethanol 0.6 μmole; dCTP, dGTP, dTTP and [H^3] dATP (10 μC/μmole), 12.5 nmoles each = 50 nmoles of dNTP. In addition, the mixture contained 1.3 units of DNA polymerase and varying amount of primer. Preparation and characterization of primers and enzyme are given in previous publications[1,4]. Rate (v) expressed as nmoles of DNA/ml reaction mixture synthesized · ml/30 min at 37 °C. The smooth curves were calculated from the Michaelis equation $v = VS/(K_m + S)$ where S is the DNA concentration. The values for K_m are 80 and 8.8 nmoles/ml reaction mixture for the DNA- and nucleohistone-primed reactions, respectively. The corresponding values for V are 55.6 and 6.1 nmoles of DNA synthesized/ml reaction mixture · 30 min. The inset shows the corresponding double reciprocal plots.

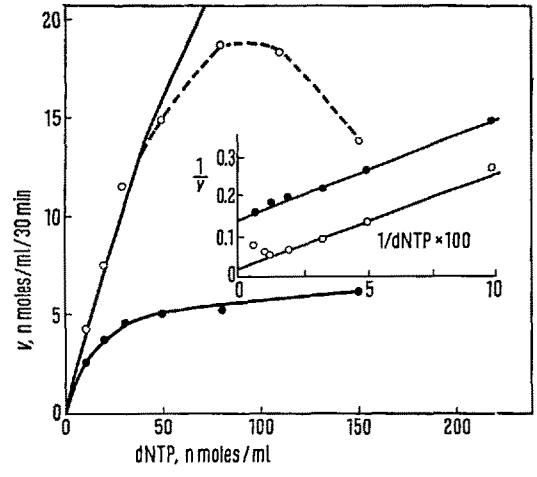

Fig. 2. Effect of deoxynucleoside triphosphate (dNTP) concentration on the rate of DNA synthesis in the presence of 32 nmoles/ml of pea embryo DNA. Other conditions are the same as those for Figure 1. The values for K_m are 150 and 16 nmoles/ml for the DNA and nucleohistone-primed reactions. The corresponding values for V are 63 and 6.7 nmoles DNA synthesized/ml · 30 min. The inset shows the corresponding reciprocal plots.

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(a) $$T + X^\circ \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [T \sim X^\circ]$$

(b) $$[T \sim X^\circ] + E \xrightarrow{k_3} [T \sim X^\circ]\, E$$

(c) $$[T \sim X^\circ] + X^\circ \xrightarrow{k_4} [T \sim X^\circ X]\, E$$

(d) $$[T \sim X^\circ X]\, E + X^\circ \xrightarrow{k_4} [T \sim X^\circ X_2]\, E$$

(e) $$[T \sim X^\circ X_{(n-2)}]\, E + X^\circ \xrightarrow{k_4} [T \sim P]\, E \xrightarrow{k_5} [T \sim P] + E$$

where T is template DNA; X°, nucleoside triphosphate; X, nucleotide residue; E, enzyme; P, the product DNA $[= X^\circ X_{(n-1)}]$; k_1 to k_5 are rate constants; and $(\sim)$ denotes bonding to primer. The first 2 reactions are the reversible bonding of the base of a nucleoside triphosphate to the base at the 3′-OH end of the template (a) followed by complexing with enzyme (b). It is further assumed that the template unwinds and that the unwinding, hydrogen bonding, and coupling of nucleoside triphosphate to form a phosphodiester linkage (release of pyrophosphate not shown) proceed virtually simultaneously (c)[6]. The product DNA chain thus grows from the phosphorylated 5′-OH end (d)[7] to yield template-product complex[8] and free enzyme (e).

The quasi-equilibrium rate equation for free DNA is:

$$v = \frac{k_5\, e\, (T)\, (X)}{K_A + K_B\, (T) + (T)\, (X)} \qquad (1)$$

where $[v = k_5\, e$ is the velocity of formation of acid insoluble DNA], $K_A = k_2\, k_5/k_3\, k_1$, $K_B = (n-1)\, k_5/k_4$, and (T), (X), and e are the concentrations of template, dNTP, and enzyme, respectively.

The mechanism with nucleohistone as template is assumed to be identical with that for DNA except that in the final step the enzyme is not released but is reversibly tied up in a complex such that it is no longer available for further catalysis, i.e.:

$$[T \sim P]\, E \underset{k_6}{\overset{k_5}{\rightleftharpoons}} [T \sim P]\, E^*$$

This introduces an additional form of enzyme, $[T \sim P]\, E^*$, whose concentration is a function of the active enzyme-substrate complex $[T \sim P]\, E$. The resulting quasi-equilibrium rate equation is:

$$v = \frac{k_5\, e\, (T)\, (X)}{K_A + K_B\, (T) + (1 + K_c)\, (T)\, (X)} \qquad (2)$$

where $K_c = k_5/k_6$.

Inspection of equations (1) and (2) when v is expressed as a function of either template (T) or dNTP (X) concentration shows that each obeys simple Michaelis kinetics, i.e. $[v = (VS)/(K + S)]$, and that the slope of the reciprocal plot (K_m/V) is not changed by the introduction of an additional form of the enzyme, as was found experimentally. Failure to respond to increasing enzyme concentration implies that the limited sites on the nucleohistone for combination with enzyme to form an active complex are no longer available. The previous observation that addition of pancreatic deoxyribonuclease, an endonuclease, to a nucleohistone supported polymerase reaction mixture after maximum DNA synthesis has occurred[4], suggests that selective internal phosphodiester bonds of pro duct and/or template are involved in the formation of the putative complex of product, template and inactive enzyme. It is of interest to note that polyamines have also been found to stimulate the rate of nucleohistone-dependent DNA synthesis[9].

An alternate mechanism postulating that the presence of 2 species of template (free DNA and nucleohistone), both of which combine with enzyme, but that only 1 complex (that with free DNA) is enzymatically active, leads to a rate equation with identical K_m's for free DNA and chromatin but different V's, and is therefore excluded. This latter type of behavior has been observed in a comparison of DNA- and liver chromatin-supported RNA synthesis by *E. coli* RNA polymerase[10,11].

Résumé. A basse concentration de «template» et de déoxynucléoside triphosphate, la vitesse de synthèse de DNA par la DNA polymérase de *E. coli* suit la loi simple de Michaelis. Les valeurs de K_m et de V pour les réactions dépendant de la nucléohistone sont plus basses que les valeurs pour les réactions dépendant du DNA, mais les rapports V/K_m sont semblables. Les additions supplémentaires de constituants du mélange réactionnel de la polymérase n'ont eu qu'un très faible effet sur le système dépendant de la nucléohistone.

Effect of adding components after incubation

Added after 30 min[a]	Relative amount of DNA synthesis	
	DNA primer	Nucleohistone
H_2O	100	100
dNTP, 80 nmoles	114	115
Enzyme, 0.7 units	148	94
Enzyme + dNTP	181	121
Nucleohistone	–	94
DNA	–	105

[a] Standard reaction mixture contained per ml: 80 nmoles of dNTP; 65 nmoles of pea embryo DNA or 310 nmoles of nucleohistone (as DNA); and 0.2 unit of DNA polymerase. After 30 min at 37 °C, the components (in 0.1 ml) above were added and the reaction allowed to proceed for an additional 30 min. The amounts of DNA synthesized after addition of H_2O were 3.7 and 1.2 nmoles for the DNA- and nucleohistone-primed reactions, respectively.

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