A RE-INTERPRETATION OF THE DISSOCIATION KINETICS OF THE DNA–RNA POLYMERASE COMPLEX MEASURED BY THE FILTER RETENTION ASSAY

Paolo U. GIACOMONI

Laboratoire de Pharmacologie Moléculaire No. 147 associé au C.N.R.S. Institut Gustave Roussy, 94800, Villejuif, France

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1. Introduction

The equilibrium constant K_{eq} of a complex is an important parameter governing the association of chemical species. From the values of K_{eq} one can calculate the free energy changes of the system accompanying the binding reaction. As far as the interactions between nucleic acids and proteins are concerned, the equilibrium constant is sometimes so large that only kinetic measurements allow its determination [1–3]. It is in fact well known that $K_{eq} = k_a/k_d$, where k_a is the rate constant of association kinetics and k_d is the rate constant of dissociation kinetics. But with the knowledge of the values of k_a and k_d it is also important to learn about the rate of equilibration of regulatory proteins within a cell, thus giving an idea of the molecular mechanisms in physiological processes.

Recalling that $k_d = 1/\tau$, where τ is the mean life of the complex, knowing k_d allows one to fix the time of exposure of such a complex to DNAases in order to purify and analyze those DNA regions which are protected by the protein against nucleolytic attack [4–6].

As far as the interaction of RNA-polymerase with DNA is concerned, Hinkle and Chamberlin [2,3] thoroughly studied the binding and the release of *E. coli* DNA-dependent RNA-polymerase to and from the DNA of the coliphage T7. These authors used the filter retention assay.

Taking advantage of the fact that one bound enzyme promotes the retention of DNA on nitrocellulose filters [2], they mixed RNA-polymerase with ^{3}H-labeled T7 DNA and, at time zero, added an excess of unlabeled T7 DNA in order to trap free polymerases. They measured the decrease of the radioactivity retained on the filter as a function of time. From their measurements, they deduced that the half life of the complex ranged between 20–60 h, and they estimated the value of the constant for the first order dissociation kinetic as $k_d = 3 \times 10^{-6}$ sec^{-1} in 0.05 M NaCl (corresponding to a mean life of 92 h). As they were able to determine the constant of the kinetic of association ($k_a = 10^8$ M^{-1} sec^{-1}) [3] they could estimate the value of the equilibrium constant:

$$K_{eq} = \frac{k_a}{k_d} \cong 10^{14} \text{ M}^{-1}$$

The value of the mean life of the complex appears thus to be strikingly long, and the value of the equilibrium constant is such that the free energy change upon binding turns out to be

$$\Delta G = -RT \ln K_{eq} \cong -20 \text{ k cal/mol}$$

and is comparable to the energy of some co-valent bonds.

In another paper [7] it is reported that the mean life of the complex formed by RNA-polymerase with the replicative form of the DNA from the phage fd, has a value $\tau = 50$ min in 0.12 M NaCl, and $\tau = 120$ min in 0.01 NaCl. This value is strikingly smaller than the value reported by Hinkle and Chamberlin. I have tried to work out the reasons of such a big discrepancy. This paper deals with the interpretation of their results, according to a rigorous statistical analysis.

2. Results and discussion

The amount of radioactive DNA retained on the nitrocellulose filter is a direct measure of the fraction of DNA complexed to at least one RNA-polymerase. The fraction of radioactivity passing through the filter corresponds to the fraction of free DNA at time t.

Let us assume that there are n sites per genome to which RNA-polymerase strongly binds and that they are all equivalent. In this case first-order dissociation kinetics are expected. The mean number of polymerase molecules bound to DNA (r) should change during the time (t) according to the equation:

$$r(t) = r(0) \exp(-t/\tau) \quad (1)$$

where τ is the mean life of the complex.

From the binomial law of distribution, the probability that one DNA molecule is free of polymerase is given by

$$\pi_0 = \left(1 - \frac{r(t)}{n}\right)^n = \left(1 - \frac{r(0)\ e^{-t/\tau}}{n}\right)^n \quad (2)$$

Three unknown parameters (n,τ and r (0)) appear in this equation. The handling of this equation for the analysis of experimental results can be quite heavy. On the contrary, the conditions to use the law of Poisson are fulfilled if $r(t) \ll n$.

If $n = 3$ and $r(t) = 1$, then the discrepancy between the values computed by the binomial law and the law of Poisson is about 20%. For smaller values of $r(t)$, the discrepancy vanishes: it is less than 5% for $r(t) = 0.5$ and about 1% for $r(t) = 0.3$.

According to the law of Poisson, the fraction of DNA molecules passing through the filters $P_0(t)$ can be expressed as the probability for a DNA to be free of polymerase if the number of enzyme per genome is $r(t)$:

$$P_0(t) = \exp(-r(t)) \quad (3)$$

This quantity is simply $P_0(t) = 1 - q(t)$, where $q(t)$ is the fraction of DNA retained on the filter at time t.

From equations (1) and (3) we obtain:

$$\ln P_0(t) = -r(0) \exp(-t/\tau). \quad (4)$$

By taking the logarithm of the absolute values, equation (4) becomes

$$\ln|\ln P_0(r(t))| = \ln r(0) - t/\tau \quad (5)$$

The slope of the strainght line expressed by equation (5) gives the opposite of the constant of the dissociation kinetic ($k_d = 1/\tau$).

If the results of Hinkle and Chamberlin, analyzed by this procedure, yield straight lines, it will mean that the hypothesis formulated above can apply. In particular, it will not be necessary to invoke the existence of several classes of tight binding sites to explain the non linearity of the crude dissociation curves reported by these authors.

In fig.1 I report the values of $\ln|\ln P_0(t)|$ versus time at different ionic strenghts. The values of $P_0(t)$ are taken from fig.5 of the quoted paper of Hinkle and Chamberlin [2] and tabulated in table 1.

Figure 2 shows the change of τ versus the concentration of monovalent cation, as determined by the procedure described herein.

From fig.1 it can be seen that the data of Hinkle and Chamberlin yield straight lines, in agreement with the formulated hypothesis that the tight binding sites are all equivalent.

The deviation from linearity occurring for $t < 5$ min has not to be attributed to the existence of several classes of tight binding sites and should not disprove the mathematical treatment just described. When

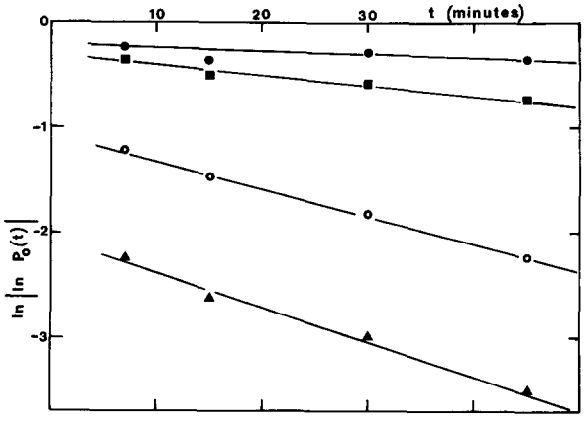

Fig.1. Plot of $\ln|\ln(P_0(t)|$ versus time in (•) 0.05 M NaCl, (▪) 0.1 M NaCl, (○) 0.15 M NaCl, (▲) 0.2 M NaCl.

Table 1
The effect of ionic strength on the rate of dissociation of the RNA-polymerase holoenzyme–T7 DNA complex

Time (minutes)	Fraction of DNA bearing no bound RNA-polymerase, $P_0(t)$, in 0.05M NaCl	0.1M NaCl	0.15M NaCl	0.2M NaCl
7	0.45	0.5	0.72	0.9
15	0.5	0.55	0.8	0.93
30	0.47	0.58	0.85	0.95
45	0.5	0.62	0.9	0.97

The data are taken from fig.5 in ref. [2].

every DNA molecule bears at least one polymerase, then $P_0(t) = 0$ and $\ln|\ln P_0(t)| = +\infty$, and a sufficiently long time has to pass in order to have $\ln|\ln P_0(t)|$ varying linearly with t (i.e. to fulfill the hypothesis for using the Poisson distribution law).

From fig.2 it can be seen that the mean life of the complex formed by T7 DNA with RNA-polymerase in 0.05 M NaCl has a value of 280 min (instead of 92 h). The value of τ diminishes with increasing ionic strength and, in 0.12 M NaCl, it has a value $\tau = 70$ min, which is strikingly close to our result reported in another paper [7], instead of more than two hours as stated in ref. [2]. A possible explanation

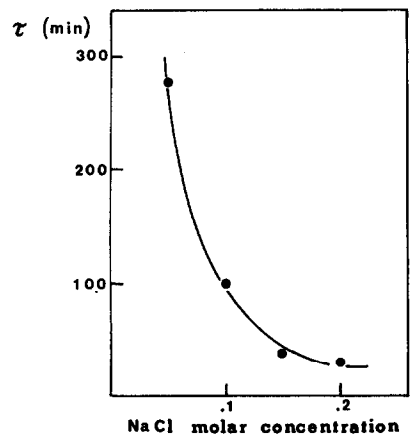

Fig.2. The change of the mean life τ of the RNA-polymerase–T7 DNA complex versus ionic strength. The values of τ are obtained by computing the reciprocal of the slopes in fig.1.

of the large overestimation of τ at low ionic strength in ref. [2] is that the holoenzyme binds at multiple, non-specific sites to DNA at low cationic concentration [7] and a longer time is than necessary for a DNA molecule to be freed of RNA-polymerase.

Thus, it appears that it is possible to re-analyze all the data of Hinkle and Chamberlin, in order to deduce the values of the parameters of the interaction between DNA and RNA-polymerase.

For instance, the rate constant of dissociation of core enzyme from T7 DNA appears to have different values when measured with method 1 or method 2 in the paper of Hinkle and Chamberlin. This discrepancy is strikingly reduced if the results of the measurements performed according to method 1 are analyzed following the procedure described herein.

Moreover, the equilibrium constant of the complex formed by the holoenzyme with T7 DNA can be estimated, taking $k_a = 10^8\ M^{-1}\ s^{-1}$ [3]:

$$\frac{k_a}{k_d} = K_{eq} \cong 4 \times 10^{11}\ M^{-1}$$

in 0.12 M NaCl and $K_{eq} \cong 1.7 \times 10^{12}\ M^{-1}$ in 0.05 M NaCl, which are much smaller values than the ones reported in ref. [2].

These results suggest that the filter retention assay can be used for determining the physico-chemical parameters of interaction concerning the complexes formed by proteins and nucleic acids, when there is more than one binding site per DNA molecule if the analysis of the results is carried out as described.

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