ANALYSIS OF PROTEIN–NUCLEIC ACID FILTER BINDING USING THE POISSON DISTRIBUTION: A METHOD TO ESTIMATE CO-OPERATIVITY

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1. Introduction

Several proteins that bind to nucleic acid exhibit cooperative binding; examples include the λ repressor [1], the T4 gene 32 protein [2], and the fd gene 5 protein** [3,4]. The basis of the very useful filter binding assay [5,6] is that radiolabeled nucleic acid becomes filter-bound in the presence, but not in the absence, of the protein under study. In this communication we calculate the expected fraction of filter-bound nucleic acid as a function of (protein)/(nucleic acid) molar ratio assuming a Poisson distribution of the protein among the nucleic acid binding sites. The observed binding curve for 5P with DNA deviates from the calculated curves in a manner indicative of cooperative binding.

2. Materials and methods

2.1. *Preparation of 5P and fd DND*

Log phase *E. coli* DM48 (derived from strain S26 provided by A-Garen Hohn et al. [7] at 10^8 cells/ml were infected with wild type fd [8] at a multiplicity of 3–10. Six hr after infection, cells were harvested. Cell disruption, degradation of cellular DNA by DNAase I in the presence of Ca^{++} and Mg^{++}, removal of Ca^{++} and Mg^{++} to inactivate the DNAase I, and isolation of 5P by DNA-cellulose chromatography were basically as described by Alberts et al. [2]. Concentrations of 5P were determined spectrophotometrically using 1 A_{280} = 0.72 mg/ml [4,9].

[^{14}C]Thymidine labeled fd DNA, which was a gift from Dr Ben Tseng, was prepared from purified [^{14}C]thymidine labeled fd by the phenol extraction procedure outlined by Mavin and Schaller [10]. DNA concentrations were determined spectrophotometrically using 1 A_{260} = 42 μg/ml [10].

2.2. *Filter binding assay*

5P at an initial concentration of about 1 mg/ml was diluted serially in 0.05 M NaCl, 0.01 M Tris, 0.001 M EDTA, and 0.1% (v/v) β-mercaptoethanol, pH 7.8. In each experiment 3–4 dilutions were performed and to each diluent a prescribed amount of ^{14}C-labeled fd DNA was added without delay. After 15 min., the samples (about 5 ml) were slowly filtered through S & S B-6 nitrocellulose filters. The filters were washed four times with 5 ml of buffer followed by an alcohol wash, dried, and then counted in toluene containing 0.4% PPO and 0.01% POPOP. In various experiments (5P)/(DNA) molar ratios from about 1 to about 4×10^{-5} were achieved. The lowest activity filters, containing about 20 cpm above background, were counted for 100 min. In every set a control with no 5P was run to monitor residual DNA and essentially no counts (<5 cpm) could be detected above background.

3. Results

3.1. *Analysis of the filter binding assay*

If the 5P·DNA interaction were infinitely co-operative (i.e., once a particular DNA molecule has a

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** *Abbreviation:* 5P, fd gene 5 protein.

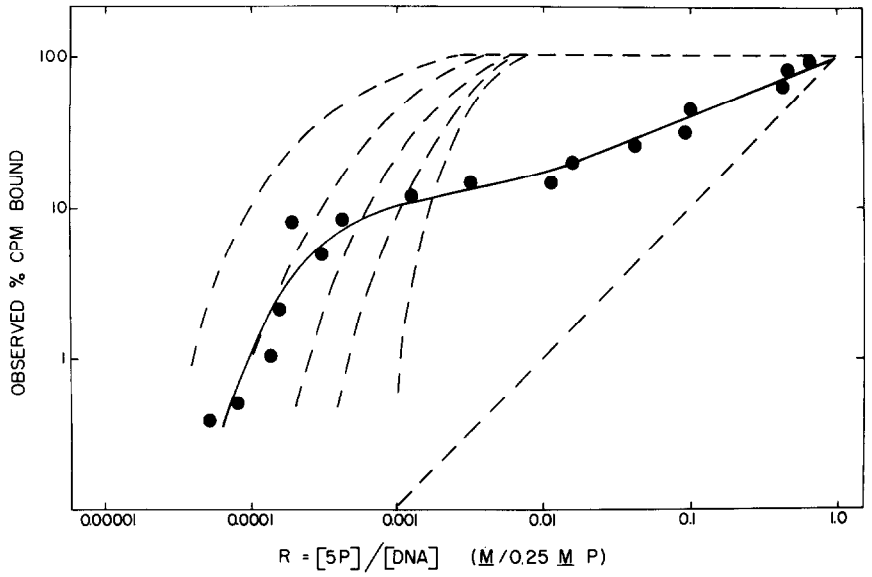

Fig.1. Calculated and observed f_b curves. The dashed curves represent the expected binding based on a Poisson distribution (nonco-operativity); the dashed straight line represents the limit of infinite cooperativity (see text). The data points show the results of several experiments employing fd DNA bound with 5P.

5P start that DNA becomes complexed to completion or until the 5P is depleted; but, unstarted DNA molecules remain naked), then the fraction of filter-bound DNA would be in direct proportion to the amount of 5P present. This possibility is illustrated by the dashed 45° line of fig.1. If, on the other hand, 5P binding displayed no cooperativity, then the distribution of 5P among the various DNA molecules should obey a Poisson distribution:

$$P_n(x) = \frac{e^{-kx}(kx)^n}{n!}$$

where $P_n(x)$ is the probability of having n molecules of 5P per x length of DNA, and k is the average number of 5P molecules/unit length of DNA. There are about 6600 nucleotides per fd molecule [6] and one 5P molecule spans about 4 nucleotides [4,8]. As an example, if $x = 1$ fd length of DNA and $k = 1$ 5P/100 nucleotides, then $kx = 66$, or there would be an average of 66 5P molecules per fd DNA. At saturation, $kx = 6600/4 \sim 1600$ molecules 5P per unit length. Furthermore, suppose that the attachment of at least m molecules of 5P to a DNA molecule is required for the DNA to become filter bound. Thus, the fraction bound, f_b, would be the fraction having m or more 5P molecules per DNA, or

$$f_b = \sum_{n=m}^{1600} P_n(x) \Big/ \sum_{n=0}^{1600} P_n(x) \qquad (1)$$

Since $\sum_{n=1600}^{\infty} P_n(x) \ll 1$ (for $kx \ll 1600$) and since $\sum_{n=0}^{\infty} P_n(x) = 1$, we can replace $\sum_{n=0}^{1600} P_n(x)$ by $\sum_{n=0}^{\infty} P_n(x) = 1$.

Furthermore, by the same reasoning, if m is sufficiently small and if $kx \ll 1600$, then

$$\sum_{n=m}^{1600} P_n(x) \sim \sum_{n=m}^{\infty} P_n(x).$$

Thus, the fraction bound is given approximately by:

$$f_b \approx \sum_{n=m}^{\infty} P_n(x) = \sum_{n=m}^{\infty} \frac{e^{-kx}(kx)^n}{n!} \quad (2)$$

which is much more convenient than equation 1.

For R = [5P] / [DNA], where [5P] is in mol/liter and [DNA] is in 0.25 mol P/liter, (so that R = 1 corresponds to saturation of the DNA), kx is given simply by $(R) \times (1600)$. Thus, for homogeneously sized DNA, the fraction bound, f_b, is a function of R and m only. Using standard statistical tables, it is a straightforward to generate the family of curves showing this functional dependence. The dashed curves in fig.1 are, from left to right, the f_b curves for m = 1, 2, 3, 4 and 5.

If 5P binding is cooperative, but not infinitely so, then one expects a curve intermediate between the extremes presented in fig.1.

Suppose the binding constant to naked DNA is given by k_n and the binding to a site adjacent to an already bound 5P is given by k_a. Then the probability of binding to an adjacent site is proportional to $[DNA_a] \times k_a$ and to a nonadjacent site is similarly $[DNA_n] \times k_n$. When $[DNA_n] \times k_n \geq [DNA_a] \times k_a$, there is no preference for an adjacent site; the cooperativity becomes swamped out by excess free DNA. Thus, when $[DNA_n] \times k_n = [DNA_a] \times k_a$, it would be expected that the observed f_b curve should inflect towards one of the calculated f_b curves described above (depending on m, the number of protein molecules required for filter binding).

At low numbers of 5P per fd DNA, $[DNA_n] \sim$ total DNA and $[DNA_a] \sim 2$ [5P] (the factor of 2 becomes unity if addional 5P can add only to 1 side of already bound 5P). Thus,

$$\frac{k_a}{k_n} \approx \tfrac{1}{2} \left(\frac{[DNA]}{[5P]_I}\right) = \frac{1}{2R_I}$$

where R_I denotes the value for R at the inflection on the observed f_b versus R graph.

3.2. *5P Binding as a function of R*

The closed circles of fig.1 represent data from a series of filter binding assays performed as described in Materials and methods. It is evident that

$$\frac{1}{2R_I} \sim 1\text{–}2 \times 10^3$$

4. Discussion

The high ratio of cooperative to nonco-operative binding found in this study favors a relatively low number of starts for 5P binding to DNA. One start per DNA would assure a smooth, continuous, unbranched 5P · DNA complex, which fits nicely with the rod shaped 5P · DNA complex isolated from fd infected cells [11]. Thus, the current results have relevance to the in vivo formation of the 5P · DNA complex.

By entirely different procedures, 5P was found to bind to R_{17} RNA about 300 times less effectively than to fd DNA (manuscript in preparation). The value of 300 is not too different from the value of 1000 estimated as the lower limit for $\frac{1}{2R_1}$. Thus, 5P binding to RNA is about the same as 5P binding non-cooperatively to DNA. Two complementary hypotheses are that thymidine or deoxyribose or both are necessary for the full expression of the cooperative effects, or that uracil or ribose or both inhibit cooperativity.

Finally, the data of fig. 1 suggest that at least 2 5P molecules are required for DNA to become filter bound. One possible interpretation is that the active form of 5P is a dimer rather than a monomer, which is consistent with previous observations suggesting that aggregates of 5P are more competent than monomers for 5P binding to DNA [4,12].

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