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Interaction between bacteriophage T_4 coded gene 32 protein and poly(rA)

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The cooperative binding of T_4 gene 32 protein with polynucleotides, of which the quantitative aspects in the literature have not satisfied the requirements of thermodynamics, is studied by adopting a modified formula of the lattice theory. A moderate value is found for the cooperativity parameter ($q \sim 200$ at 0.2 M NaCl), which is weakly dependent on salt concentration. The cation effect on the binding suggests that the shielding of negative charges of the protein or a loose cation bridge between the bound protein molecules plays a role in the cooperative binding process.

Gene protein; DNA-binding protein, single-stranded; Cooperativity; DNA-protein interaction; Thermodynamics

1. INTRODUCTION

The cellular concentration of gene 32 protein encoded by bacteriophage T_4 (g32p) is regulated by its cooperative binding to a variety of polynucleotides [1–7]. The interactions have quantitatively been analysed in terms of a lattice theory [4,5] and the analysis is regarded as representative of the quantitative study of DNA-protein interactions. It was found, however, that the result in [4] is not compatible with the requirements of equilibrium thermodynamics: when the binding isotherm (the degree of saturation, θ, versus the concentration of free g32p, c_A) was drawn from a titration curve in fig.8 of [4] with the stoichiometric number $n = 7$, one value of c_A corresponded to three different values of θ around $\theta \approx 0.5$ (a value of $n \geq 9.0$ was required to overcome this difficulty). In other words, the criterion of the best fit of the theory to the experimental curves is very uncertain. Indeed, the same authors reported considerably different values for the strength of cooperativity, ≈ 2500 [4] and $\geq 10^4$ [8]. In addition, the value of n is also to be established; a wide range of values have been reported, $n = 4.7–11$ [1–7].

In view of the fact that parameters such as n are fundamental to the understanding of the mechanism of DNA-protein interactions in general, it is necessary to establish a reliable method of data analysis. Adopting a linear formula of the lattice theory [9] which is different from [4], the cooperative binding of g32p to poly(rA) was re-examined according to the procedure described [9,10]. The result is now compatible with the circumstantial evidence.

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2. EXPERIMENTAL

The purified g32p was kindly donated by Dr T. Bickle. The concentration of g32p was determined using the extinction coefficient of 3.7×10^4 $M^{-1} \cdot cm^{-1}$ [2]. Poly(rA) was purchased from P-L Biochemicals and the concentration was determined using the extinction coefficient per mole of phosphate at 260 nm, 10300 $M^{-1} \cdot cm^{-1}$ [4]. Sample solutions were adjusted to pH 7.5 with 4 mM Tris-HCl buffer.

Fluorescence was measured with a Farrand MK1 fluorospectrophotometer. The tryptophan fluorescence of g32p was monitored at 345 nm (292 nm for the excitation). Temperature was maintained at 20°C.

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3. RESULTS

The quenching efficiency Z of tryptophan fluorescence of g32p upon the complex formation was estimated by the addition of a large excess of poly(rA) over the protein. The value $Z = 0.47 \pm 0.07$ was obtained. This value was confirmed by the method of [10] (not shown).

The fluorescence intensity, F, for a solution containing a fixed concentration of poly(rA), c_p, was measured by increasing the g32p concentration, c_A^o as shown in fig.1. The final straight line indicating saturation of the binding sites ($\theta = 1$) is parallel to the control line obtained in the absence of nucleic acid. The first experimental point came close to the control line. This implies no binding ($\theta = 0$), confirming the existence of cooperativity in the binding.

The value of n was obtained by extrapolating the final line to $F = 0$ where the relation $n = Zc_p/c_A^o$ holds [9]. Using the value of $Z = 0.47$, $n = 9.4 \pm 1.4$ (bases/monomer) was obtained. There was no effect of NaCl concentration (0.2 and 0.4 M) on the values of Z and n. The result $n = 9.4$ comes close to the higher value of $n \approx 10$ in the literature [1,6,7], a value clearly higher than those reported elsewhere ($n = 4.7$–7.5 [2–4]).

The titration data were analysed in terms of the large ligand model [10,11] according to [9,10]. The magnitude of θ was graphically determined as shown in the diagram; $\theta = d/d_\infty$. The free g32p concentration c_A was evaluated by eqn 1

$$c_A = c_A^o - \theta c_p/n \quad (1)$$

For the evaluations of the cooperative binding constant K and the cooperativity parameter q the following equation was used:

$$(2\theta - 1)/\sqrt{[\theta(\theta - 1)]} = \sqrt{[q/n]}(Kc_A - 1); \quad \text{for } q/n \geq 4 \quad (2)$$

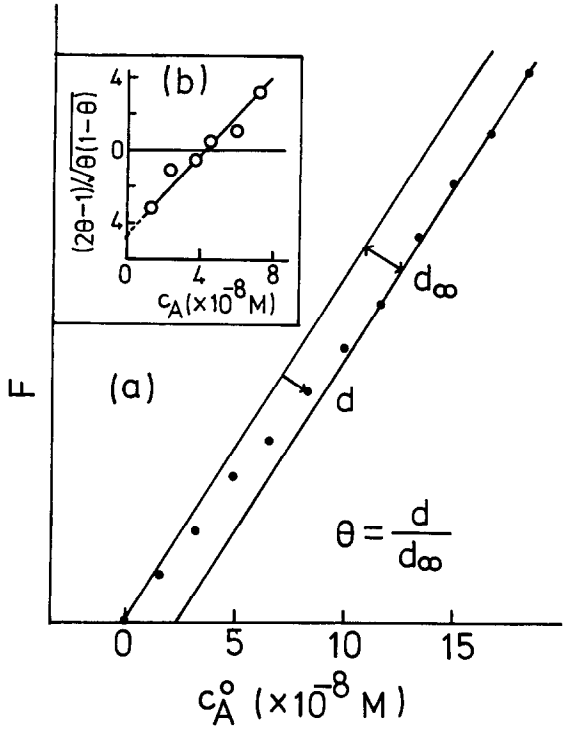

Fig.1. (a) Fluorescence intensity F at constant poly(rA) concentration ($c_p = 2.9 \times 10^{-7}$ M, 0.2 M NaCl) was plotted against total g32p, c_A^o. (b) $(2\theta - 1)/\sqrt{[\theta(1-\theta)]}$ of the data in a was plotted against c_A according to eqn 2; $K = 2.3 \times 10^7$ M^{-1} and $q = 210$ with $n = 10$.

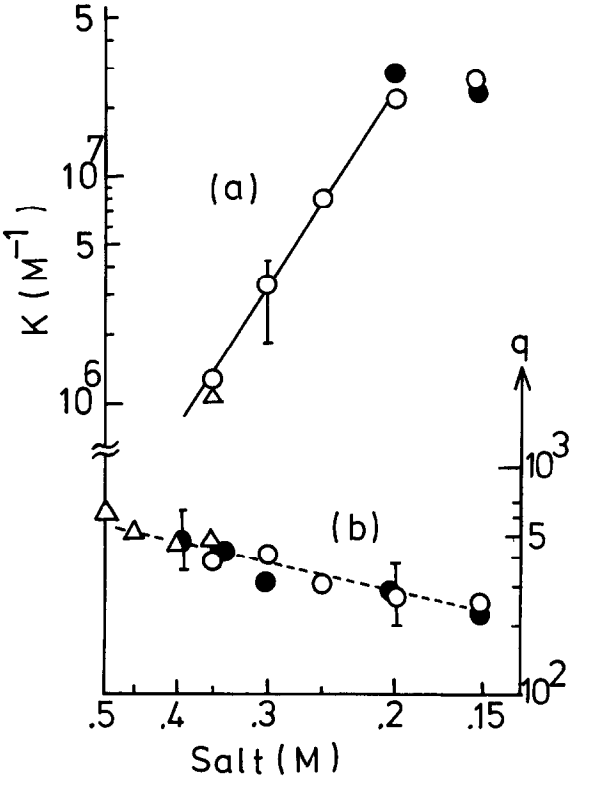

Fig.2. Dependences of the binding parameters on salt concentration. (a) The cooperative-binding constant K and (b) the cooperativity parameter q. (○) NaCl and (●) NaF. The result of re-analysis of the titration curves of [4] according to eqn 2 using the value of $n = 9.0$, is shown with (△).

A plot of $(2\theta - 1)/\sqrt{[\theta(1-\theta)]}$ versus c_A is shown in fig.1. The plot intercepts the c_A axis at $1/K$ and the ordinate at $-\sqrt{[q/n]}$, respectively. The value of q was obtained using the value of n determined above.

When the salt concentration was increased from 0.15 to 0.5 M, the value of K decreased, revealing a contribution of electrostatic interactions to the complex formation, while the value of q increased gradually, as shown in fig.2. The values of $K \approx 1 \times 10^6$ M^{-1} and $q \approx 400$ at 0.35 M were obtained. The salt concentration dependence of K is summarised in fig.2a and that of q in fig.2b. The effect of anion species on the binding was examined with two different sodium salts, NaCl and NaF. There was practically no anion effect on q.

Finally, the titration curves for poly(rA) in figs 3 and 4 of [4] were re-analysed according to eqns (1) and (2) using the value of $n = 9.0$. The result is represented with open triangles in fig.2. The values of K and q and their salt concentration dependences proved to be essentially the same as those in the present study.

4. DISCUSSION

In the present work a linear formula of the lattice model [9] was used for analysis of the interaction, since the analysis of data in [4] using 'curve-fitting' of the Scatchard type formula [4,11] to experimental data is largely uncertain as described in section 1. The result now appears to be self-consistent.

A moderate cooperativity, $q \approx 350$ at 0.3 M NaCl, was observed here, in contrast to the higher values in the literature, $q \approx 2500$ [5] and $q \geq 10^4$ [8]. The binding constant for g32p-rA(pA)$_7$ at 0.3 M NaCl is $\approx 3 \times 10^4$ M^{-1} [4] so that the value of $q = 2500$ predicts the cooperative binding constant K of $\approx 10^8$ M^{-1} at 0.3 M NaCl. However, the results in fig.2a and [4] indicate a K value of $\approx 10^6$ M^{-1} at 0.3 M NaCl. This circumstantial evidence supports the lower value of q. Indeed, the discrepancy in the value of q between the present study and the earlier literature [4,5] disappears, when the original titration curves in [4] are re-analysed with $n = 9$ according to eqn 2, as represented in fig.2b.

The re-analysed data of [4] and present results show a weak salt concentration dependence of q as shown in fig.2b. Similar salt concentration dependence of q was observed in other cooperative binding systems: nucleoproteins [10,12] and gene 5 protein [13]. g32p has a cluster of negative charges in the A region, while its nucleic acid binding domain contains positive charges [14]. Thus, the neutralization of the positive charges of g32p upon binding to nucleic acid will enhance the repulsive force between the two negative charge clusters unless effective shielding is present. This implies the significance of cation species in the cooperative interaction. Indeed, addition of a few mM $MgCl_2$ brings about stronger cooperativity, $q \approx 500$ at 5 mM (not shown). There was no anion effect on the value of q between Cl^- and F^-. These results suggest that neutralization of the negatively charged cluster of the bound g32p by cations or loose cation bridges between bound g32p molecules plays a role in the cooperative binding process.

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