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# NONSPECIFIC BINDING OF *lac* REPRESSOR TO DNA

## II. A SMALL-ANGLE NEUTRON-SCATTERING STUDY

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Complexes between *lac* repressor and DNA fragments from mononucleosomes have been studied by small-angle neutron scattering. Both the radius of gyration and the molecular weight of the complexes were measured, and the experimental results were interpreted according to a model with two types of complex (M. Charlier and J.-C. Maurizot, Biophys. Chem. 18 (1983) 303), and a statistical distribution of repressor on the DNA fragments. Good agreement between the model calculations and the experimental results was obtained. We concluded that there was an absence of strong cooperativity and of network formation between the complexes. The second type of binding, which does not induce any spectroscopic change, is marked by an increase in molecular weight of the complexes. Kinetic measurements were also made, which allowed the determination of the lifetime of the nonspecific DNA-repressor complexes.

## 1. Introduction

The nonspecific binding of *lac* repressor to DNA has been studied extensively by various methods (see section 1 of ref. 2 and refs. 1–24 quoted therein). Most of these studies have concentrated on the spectroscopic and thermodynamic aspects of the binding process, while only electron microscopy work has been applied to investigate the morphology of the complex [1]. Another approach using small-angle neutron scattering seemed to us to be advantageous for investigating any complex formation and to obtain structural data concerning the arrangement of the repressor along the DNA, provided the DNA was carefully chosen. We used DNA fragments of definite length from mononucleosomes, which were large enough to bind several repressor molecules, and small enough to be studied easily by neutron scattering. Analysis of the results needed the development of a model for the interaction between proteins and DNA fragments, which is presented in this paper.

## 2. Materials and methods

### 2.1. Biochemicals

The purification of *lac* repressor and of the DNA fragments, the preparation of complexes by dialysis and their spectrophotometric analysis to determine the concentrations of both components have been described and discussed in the accompanying paper [2]. In the case of neutron-scattering experiments, protein concentrations were in the range from 0.8 to 3.2 mg/ml. All samples were prepared in $^2H_2O$ (> 98% isotopic enrichment) to enhance the signal-to-noise ratio [3]. Preparation of the complexes by dialysing for 48 h ensures that the exchange of all exchangeable protons is

achieved. The buffer used was 1 mM potassium phosphate, 0.1 mM dithioerythrytol, $p^2H = 7.25$.

### 2.2. Neutron-scattering experiments

The measurements were performed at the Institut Laue-Langevin in Grenoble, France, using the D11 small-angle camera. The neutron wavelength used was $\lambda = 10$ Å ($\Delta\lambda/\lambda = 0.08$) and the distance between the sample and the detector was 10 m, to give an observable angular range of 0.003 $Å^{-1} \leqslant Q \leqslant 0.020$ $Å^{-1}$ ($Q = 4\pi \sin\theta/\lambda$, $2\theta$ is the scattering angle).

### 2.3. Theory

#### 2.3.1. Small-angle scattering from solution

For a solution of different particles (the polydisperse case), the small-angle scattering could be analysed in the following way [4,5]

$$I(Q) = I_0\{1 - \tfrac{1}{3}Q^2R_G^2\} \tag{1}$$

The experimental values $I(0)$ and $R_G^2$ are given by:

$$I(0) = \sum_N C_N(\rho_N V_N)^2 \tag{2}$$

$$R_G^2 = \frac{\sum_N C_N(\rho_N V_N)^2 R_{GN}^2}{\sum_N C_N(\rho_N V_N)^2} \tag{3}$$

where $C_N$, $\rho_N$, $V_N$ and $R_{GN}$ are the particle molar concentration, mean contrast, volume, and contrast radius of gyration * of species $N$ in the solution.

In the special case of a solution made up of DNA and *lac* repressor complexes, we shall call $p_N(X)$ the relative population of DNA fragments bearing $N$ repressor molecules, for an input value of $X$ repressors per fragment in the solution. Obviously, $p_N(X)$ depends on the nature of the binding, the number and the types of complexes, etc. Let us call $I_X(Q)$ the scattered intensity of the same solution containing complexes of on the average $X$ repressors bound per fragment, and $V_N$ the volume of a complex bearing $N$ repressors. Then using eqs. 1–3:

$$I_X(Q) = K\sum_N \left[ p_N(X)(\rho_N V_N)^2(1 - \tfrac{1}{3}Q^2R_{GN}^2)\right] \tag{4}$$

(where now $C_N$ is expressed as $Kp_N(X)$ with $\Sigma_N p_N(X) = 1$, and $K$ the total number of particles.

For example, in a complex of two repressors per DNA fragment, the forward scattered intensity is independent of the location of the proteins on the DNA. On the other hand, this is not the case for the radius of gyration. Furthermore, we assume that all possible configurations of repressor location are equally probable, for a given value of $N$. If there are $n$ configurations, $R_{GN}^2 = 1/n\Sigma_N R_{iN}^2$, where $R_{iN}$ is the radius of gyration of the $i$-th configuration of the complex bearing $N$ repressors per fragment.

The mean contrast of repressor in 100% $^2H_2O$ was obtained from contrast variation experiments, which gave a match point of $41 \pm 1\%$ $^2H_2O$ (results not shown). For DNA, a value was calculated taking a match point of 63% $^2H_2O$ [3]. Because it depends on partial volume and interaction parameters, this value is a function of salt conditions [6]; nevertheless, it is shown below that the experiment is not very sensitive to the DNA contribution.

The mean scattering densities, $\rho$, are as follows for: the solvent, $\rho_{^2H_2O} = 0.064 \times 10^{-12}$ cm $Å^{-3}$; the DNA, $\rho_{DNA} = 0.41 \times 10^{-12}$ cm $Å^{-3}$; the repressor, $\rho_{REP} = 0.030 \times 10^{-12}$ cm $Å^{-3}$. We also deduced the contrast, $\bar{\rho}$, for: the DNA, $\bar{\rho}_{DNA} = -0.023 \times 10^{-12}$ cm $Å^{-3}$; the repressor, $\bar{\rho}_{REP} = -0.034 \times 10^{-12}$ cm $Å^{-3}$. For a DNA fragment of volume $v_{DNA}$ bearing $N$ repressor molecules of volume $V_{REP}$, the mean value of $\bar{\rho}_N V_N$ is given by:

$$\bar{\rho}_N V_N = \bar{\rho}_{DNA} v_{DNA} + \bar{\rho}_{REP} V_{REP} N,$$

and

$$(\bar{\rho}_N V_N)^2 = N^2(\bar{\rho}_{REP} V_{REP})^2\left(1 + \frac{\alpha}{N}\right)^2 \tag{5}$$

with $\alpha = \bar{\rho}_{DNA} v_{DNA}/\bar{\rho}_{REP} V_{REP}$. From the data quoted by Jacrot [3] and the sequence of the repressor [7], we can evaluate $v_{DNA} = 84000$ $Å^3$, $V_{REP} = 197000$ $Å^3$ and $\alpha = 0.3$.

* The contrast radius of gyration is the radius of gyration of excess scattering length (see ref. 3).

Introducing this result in eq. 4, we find:

$$I_X(Q) = K(\bar{\rho}_{REP}V_{REP})^2\left\{\sum_N N^2\left(1+\frac{\alpha}{N}\right)^2 p_N(X)\right\} \times\left\{1-\frac{Q^2}{3}\frac{\sum_N N^2\left(1+\frac{\alpha}{N}\right)^2 p_N(X)R_{GN}^2}{\sum_N N^2\left(1+\frac{\alpha}{N}\right)^2 p_N(X)}\right\} \quad (7)$$

From the experimental data $I_X(Q)$, we can obtain a measured extrapolated value $I_X(0)$:

$$I_X(0) = K(\bar{\rho}_{REP}V_{REP})^2\left\{\sum_N N^2\left(1+\frac{\alpha}{N}\right)^2 p_N(X)\right\}$$

Writing $C$ for the mass concentration of the protein:

$$C = K\frac{M_r}{N_A}\sum_N N p_N(X) \quad (8)$$

where $M_r$ is the molecular weight of the repressor and $N_A$ Avogadro's number. Then

$$\frac{I_X(0)}{C} = (\bar{\rho}_{REP}V_{REP})^2\frac{N_A}{M_r}\frac{\sum_N N^2\left(1+\frac{\alpha}{N}\right)^2 p_N(X)}{\sum N p_N(X)} \quad (9)$$

Expressing eq. 9 in terms of the moments of the distribution $p_N(X)$, $M_r = \Sigma_N N' p_N(X)$, we obtain:

$$\frac{I_X(0)}{C} = (\bar{\rho}_{REP}V_{REP})^2\frac{N_A}{M_r}\left[\frac{M_2}{M_1}+2\alpha+\frac{\alpha^2}{M_1}\right] \quad (10)$$

and from eqs. 3 and 7

$$R_G^2(X) = \frac{\sum_N\left(N^2\left(1+\frac{\alpha}{N}\right)^2 p_N(X)R_{GN}^2\right)}{M_2} \quad (11)$$

We can remark that for $X$ large enough to make $2\alpha + \alpha^2/M_1 \ll M_2/M_1$, $I_X(0)/C$ is a measure of the weight average molecular weight of the complexes.

*2.3.2. Scattering models*

The binding of *lac* repressor to DNA fragments takes place in two steps. At first, 10 repressors bind per fragment, followed by a second step with up to 16 more repressors binding to the complex, as discussed in the accompanying paper [2]. We shall determine distributions $p_N(X)$ for different scattering models.

*2.3.2.1. Cooperative case for the first type of binding.*

The only case which we can deal with easily is the infinitely cooperative case. In this case, for $X \leqslant 10$, only two types of fragments can exist: fragments which are fully covered by 10 repressors, and fragments with none. Then:

$$\left.\begin{array}{ll} p_N(X) = \frac{X}{10} & \text{for } N = 10 \\ p_N(X) = 1-\frac{X}{10} & \text{for } N = 0 \\ p_N(X) = 0 & \text{for } 1 < N < 9 \end{array}\right\} M_1 = X \quad M_2 = 10X$$

Eq. 10 will be transformed as follows, considering that $2\alpha$ and $\alpha^2/M_1 \ll M_2/M_1$, since $X \geqslant 1$,

$$\frac{I_X(0)}{C} = (\bar{\rho}_{REP}V_{REP})^2\frac{N_A}{M_r}\times 10 \quad (12)$$

which is a constant. Putting in $(I(0)/C)_{REP}$ for a solution of repressor alone, we have

$$\frac{I_X(0)}{C} = 10\times\left(\frac{I(0)}{C}\right)_{REP} \quad (13)$$

Let us call $r_G$ the radius of gyration of the DNA fragment; eq. 11 will be transformed as follows (considering that $\alpha/10 \ll 1$): $R_G^2(X) = R_{G.10}^2 + \alpha^2/10r_G^2(1/X - 1/10)$. As $\alpha = 0.3$ and $(1/X - 1/10) < 1$, we can write: $R_G^2(X) = R_{G.10}^2 + \beta r_G^2$, with $\beta < 0.009$.

As the DNA fragment can be considered as a rigid rod of $146 \times 3.4$ Å length, $r_G$ could be equal to 143 Å. And we can write that:

$$R_G^2(X) = R_{G.10}^2 \quad (14)$$

with a maximum error of 1.3 Å, since $X \geqslant 1$.

*2.3.2.2. Anticooperative case for the first type of binding*

For such a process, the result does not differ strongly from the case of noninteracting ligands (see below) except that the length of the excluded site will vary from $k$ (noninteracting ligands) to $k+1$ (infinite nearest-neighbor anticooperativity) as discussed by McGhee and Von Hippel [8]. But this consideration does not modify the distribution of the repressor along the DNA.

*2.3.2.3. Noncooperative case*

As long as $X$ is smaller than 10, the population

of the complexes follows a binomial distribution:

$$\left.\begin{aligned} p_N(X) &= C_{10}^N \left(\frac{X}{10}\right)^N \left(1-\frac{X}{10}\right)^{10-X} \\ M_1 &= X,\ M_2 = X^2 + X - \frac{X^2}{10} \end{aligned}\right\} \quad (15)$$

For $X$ greater than 10, we can assumed a Poisson distribution for $p_N(X)$:

$$\left.\begin{aligned} p_N(X) &= \frac{e^{-(X-10)}(X-10)^{(N-10)}}{(N-10)!} \\ M_1 &= X,\ M_2 = X^2 + X - 10 \end{aligned}\right\} \quad (16)$$

Introducing these values in eq. 10, then

$$\left.\begin{aligned} \frac{I_N(0)}{C} &= \left(\frac{I(0)}{C}\right)_{REP}\left(X+1-\frac{X}{10}+2\alpha+\frac{\alpha^2}{X}\right): \quad X \leqslant 10 \\ \frac{I_N(0)}{C} &= \left(\frac{I(0)}{C}\right)_{REP}\left(X+1-\frac{10}{X}+2\alpha+\frac{\alpha^2}{X}\right): \quad X > 10 \end{aligned}\right\} \quad (17)$$

$(I(0)/C)_{REP}$ has been measured under the same conditions and is equal to $0.6 \pm 0.06$.

*Radius of gyration*

The radius of gyration can be calculated from eq. 11, however, with difficulty, since $R_{G,N}$ must be evaluated. For a value of $N$, there are different arrangements of the $N$ repressors along the DNA matrix. We thus calculated $R_{G,N}$ by two different procedures which led to the same result. We hypothesised that: the DNA is a rigid rod of length $L = 146 \times 3.4$ Å whose scattering power is negligible compared to those of the proteins (i.e., $\alpha$ is neglected, since $N$ is greater than 1): the complexes behave as linear arrangements of proteins along the DNA matrix.

*2.3.2.3.1. The 'string of beads' model.* We shall assume that the $N$ repressor molecules are equidistant, with a distance between them $d = L/N$ (see fig. 1). Let us call $d_i$ the distance between the center of mass of the complex and the $i$-th repressor molecule. Using the parallel axes theorem,

$$R_{G,N}^2 = R_O^2 + \frac{1}{N}\sum_{i=1}^{N} d_i^2$$

with $R_O$ the radius of gyration of the repressor. For further calculation, we have to distinguish

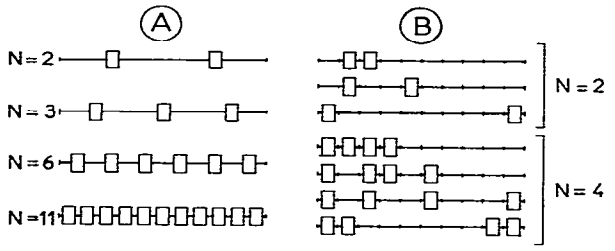


Fig. 1. Schematic drawing of the two models. (A) The string of beads model, with the repressors distant from each other by $d = L/N$. (B) The ten binding sites model, with ten discrete sites per DNA fragment. Only a small number of configurations among the $C_{10}^N$ existing for $N$ are presented as examples.

between odd and even values of $N$, but both lead to the same expression.

$$R_{G,N}^2 = R_O^2 + \frac{L^2}{12}\frac{N^2-1}{N^2} \quad (18)$$

Introducing this value in eq. 11 (and neglecting $\alpha/N$ compared to 1), we obtain:

$$R_G^2(X) = R_O^2 + \frac{L^2}{12}\left[1-\frac{1}{M_2}\right]$$

which gives:

$$\left.\begin{aligned} R_G^2(X) &= R_O^2 + \frac{L^2}{12}\left[1-\frac{1}{X^2+X-\frac{X^2}{10}}\right] \quad \text{for } X \leqslant 10 \\ R_G^2(X) &= R_O^2 + \frac{L^2}{12}\left[1-\frac{1}{X^2+X-10}\right] \quad \text{for } X > 10 \end{aligned}\right\} \quad (19)$$

As can be seen, since $X$ is greater than 3, the value of $R_G(X)$ differs from $R_O^2 + L^2/12$ by less than 10%. In this calculation, we have assumed that the repressor's center of mass is always on the DNA. Clearly, this cannot be the case, especially for large values of $X$ ($> 10$). However, this approximation breaks down for repressors which are close to the center of mass, whose contribution to the total radius of gyration is small.

*2.3.2.3.2. The 'ten binding sites' model.* We assumed that the DNA bears 10 discrete binding sites equidistant by $L/10$, numbered from 0 to 9: each binding site can received only one repressor; each free site is equally probable for the binding of one repressor.

Table 1

Radii of gyration calculated from the two models

(A) The string of beads model. $R_{GN}$ is calculated from eq. 18 and $R_G(X)$ from eq. 19. (B) The ten binding sites model. $R_{GN}$ are obtained as described in the text. In both calculations, $R_0$ was taken as equal to 40 Å (ref. 11) and $L$ equal to 493 Å (for a 146 base-pair rigid fragment of DNA in the B form).

| $N$ or $X$ | A | | B |
|---|---|---|---|
| | $R_{GN}$ (Å) | $R_G(X)$ (Å) | $R_{GN}$ (Å) |
| 1 | 40 | 107 | 40 |
| 2 | 130 | 135 | 113 |
| 3 | 140 | 142 | 128 |
| 4 | 143 | 144 | 135 |
| 5 | 145 | 145 | 139 |
| 6 | 146 | 146 | 142 |
| 7 | 146 | 146 | 144 |
| 8 | 147 | 147 | 145 |
| 9 | 147 | 147 | 146 |
| 10 | 147 | 147 | 147 |
| 11 | 147 | 148 | |
| 12 | 147 | 148 | |
| 13 | 147 | 148 | |
| 15 | 148 | 148 | |
| 18 | 148 | 148 | |
| 20 | 148 | 148 | |

For a given number $N$ ($N \leqslant 10$) we have $C_{10}^{N}$ different configurations with the repressors at positions $a_1, a_2, \ldots, a_n$ ($a_i \in N$, $0 \leqslant a_i \leqslant 9$). For each configuration, we calculated the radius of gyration $R_C(N)$ using:

$$R_C^2(N) = R_0^2 + \frac{L^2}{100}\frac{1}{N}\left\{\sum_{i=1}^{N} a_i^2 - \frac{1}{N}\left(\sum_{i=1}^{N} a_i\right)^2\right\}$$

and

$$R_{GN}^2 = \frac{1}{C_{10}^{N}}\sum R_C^2(N)$$

over all the configurations. The computed values (using $R_0 = 40$ Å and $L = 493$ Å) are given in table 1. This calculation is valid only for $X \leqslant 10$, and in this range, gives for $R_{GN}$ values very close to those obtained by the string of beads model.

*2.3.3. Kinetic studies*

We have assumed that the binding of the repressor on the DNA obeys $R + F \underset{k_{OFF}}{\overset{k_{ON}}{\rightleftharpoons}} C$ for the first type of binding, with R, the repressor; F, a free site of $k$ contiguous base plates (see ref. 2); C, the complex: $K_A = k_{ON}/k_{OFF}$, the association constant.

Our purpose was to deduce $k_{OFF}$ from measurements of $I(0)/C$ for a solution of complexes, to which DNA was added. We assume a site length of $k = 14$ base-plates, and no cooperativity or anti-cooperativity. Originally, the solution contains $X_0$ repressors per DNA fragment (we took $X_0 = 10$ to cover all fragments with repressor). According to McGhee and Von Hippel [8], the number of sites of $k$ base-plates length available per DNA fragment is equal to:

$$F = (B - Xk)\left(\frac{B - Xk}{B - (k-1)X}\right)^{k-1} \qquad (20)$$

where $B$ is the length of the fragment expressed as base-plates, $k$ is the length of the excluded site and $X$ is the average number of repressors per fragment.

If we add to the complex solution a small volume of concentrated DNA fragments solution, to double the total amount of DNA present in the solution, the repressor will redistribute over the fragments and a new equilibrium will be reached.

At zero time, the solution contains: $M$ DNA fragments of type 1, bearing $X_0$ repressors per fragment on average ($X_1(0) = X_0$) and $M$ DNA fragments of type 2, free of repressors ($X_2(0) = 0$); a total number of free sites $F_0$ distributed on the fragments of type 1, [$F(X_0)$] and of type 2, [$F(0)$]; a concentration $R_0$ of free repressor.

At time $t$, the solution contains: $M$ DNA fragments of type 1 bearing on average $X_1$ repressors and $F(X_1)$ free sites; $M$ DNA fragments of type 2 bearing on average $X_2$ repressors and $F(X_2)$ free sites; a concentration $R$ of free repressor.

In this case, $R$ is always small compared to the total concentration of protein, as discussed in the accompanying paper. Consequently, $dR/dt$ remains small, and we can assumed the steady-state hypothesis $dR/dt = 0$. Then:

$$\frac{dX_1}{dt} = -k_{OFF}X_1 + k_{OFF}X_0\frac{F(X_1)}{F(X_1) + F(X_0 - X_1)} \qquad (21)$$

and the same differential equation is obtained by exchanging subscripts 1 and 2 for $dX_2/dt$.

During the first part of the process ($10 < X_1 < 8$) $F(X_1)/F(X_1)+F(X_0-X_1)$ remains smaller than 0.02 and $F(X_0-X_1)/F(X_1)+F(X_0-X_1)$ is very close to 1.We obtain:

$$\frac{dX_1}{dt} = -k_{OFF}X_1$$

$$\frac{dX_2}{dt} = -k_{OFF}(X_2 - X_0)$$

Whose solutions are:

$$X_1 = X_0 e^{-k_{OFF}t}$$

$$X_2 = X_0(1 - e^{-k_{OFF}t})$$

At time $t$, the solution contains a bimodal distribution of fragments bearing $N$ repressors. The probability of finding a fragment bearing $N$ repressors is given by $P_N(t) = (p_N(X_1) + p_N(X_2))/2$ with $p_N(X)$ defined by eq. 15.

The two first moments of the distribution $p_N(t)$ are:

$$M_1(t) = \tfrac{1}{2}(X_1 + X_2) = X_0/2$$

$$M_2(t) = \tfrac{1}{2}\left(X_1^2 + X_2^2 + X_1 + X_2 - \frac{X_1^2 + X_2^2}{10}\right)$$

and

$$\frac{M_2}{M_1} = 1 + 0.9X_0(1 - 2e^{-k_{OFF}t} + 2e^{-2k_{OFF}t})$$

Introducing this value in eq. 10, we obtain:

$$\frac{I(0,t)}{C} = (\bar{\rho}_{REP}V_{REP})^2\frac{N_A}{M_r}\left[1 + 2\alpha + 2\frac{\alpha^2}{X_0} + 0.9X_0(1 - 2e^{-k_{OFF}t} + 2e^{-2k_{OFF}t})\right]$$

At time 0,

$$\frac{I(0,0)}{C} = (\bar{\rho}_{REP}V_{REP})^2\frac{N_A}{M_r}\left[1 + 2\alpha + \frac{2\alpha^2}{X_0} + 0.9X_0\right]$$

and

$$\frac{\Delta I}{I_0} = \frac{\frac{I(0,0)}{C} - \frac{I(0,t)}{C}}{\frac{I(0,0)}{C}} = \frac{1.8X_0(e^{-k_{OFF}t} - e^{-2k_{OFF}t})}{1 + 2\alpha + 0.9X_0 + \frac{2\alpha^2}{X_0}}$$

$\Delta I/I_0$ is the relative variation of the extrapolated intensity to zero angle measured at various times.

For $X_0 = 10$,

$$\frac{\Delta I}{I_0} = 1.7(e^{-k_{OFF}t} - e^{-2k_{OFF}t}) \qquad (22)$$

## 3. Results

All the experiments reported in this paper were performed with a $^2H_2O$ buffer. CD measurements did not display any differences in the binding behavior of repressor in $^2H_2O$ and $H_2O$, allowing us to correlate the results of this paper with those reported in the previous one.

The neutron-scattering results have been analysed taking into account the interparticular effect. The mean distance between two complex molecules at 5 mg/ml protein and $X = 10$ is 800 Å. The shape of the scattering curve obtained for charged polymers (as DNA fragments) exhibits a strong dependence on the concentration, especially at low ionic strength (and it is the case for our measurements). The partial regular arrangement of the scattering particles induces an interference term, which leads to apparently lower values of the radii of gyration [4]. As the Guinier plots are linear whatever the concentration we used (fig. 2) we are justified in extrapolating to infinite dilution both the square of the radii of gyration and the intensity to the origin [23], to obtain their corrected values.

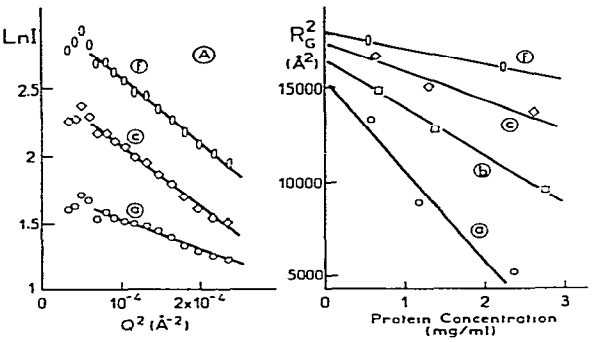


Fig. 2. (A) Guinier plots for three different complexes at the maximum concentration. (B) Extrapolation of $R_G^2$ at infinite dilution for several types of complexes see table 2 for the significance of a–c and f.

### 3.1. Radii of gyration

Fig. 2 shows the Guinier plots for three types of complexes, and the extrapolation curves for several of the complexes studied. In table 2, the extrapolated values for the radii of gyration of the complexes are given. We can observe that these radii of gyration are nearly independent of $X$, in agreement with our calculations (see section 2.3.2.3). Nevertheless, the experimental values of $R_G(X)$ are smaller by 10% than those expected for particles of DNa of 146 base-plates in length. Because of a weak signal-to-noise ratio, we have not been able to measure with sufficient accuracy the radius of gyration of the uncomplexed DNA fragment.

### 3.2. Scattered intensities at the origin

Fig. 3 shows the variations of $I(0)/C$ extrapolated at infinite dilution for different values of $X$. The experimental values are normalized by $(I(0)/C)_{REP}$ obtained for the repressor alone and equal to $0.60 \pm 0.06$. The observed values are slightly higher than the theoretical ones especially for small values of $X$. For $X$ greater than 10, the theory and the experiment are in good agreement. For $X$ smaller than 10, strong cooperativity can be ruled out. Nevertheless, we cannot exclude any low cooperativity.

### 3.3. Dissociation of the complex

Fig. 4 shows the scattering curve for a complex bearing an average number of repressors equal to 10.5, and the scattering curve for the same solution

Table 2

Radii of gyration extrapolated at zero concentration for complexes at various values of $X$

| Complex | $X$ | $R_G$ extrapolated (Å) |
|---|---|---|
| a | 2.4 | 127 |
| b | 4.9 | 129 |
| c | 8.2 | 131 |
| d | 11.3 | 130 |
| e | 13.5 | 131 |
| f | 15.2 | 134 |

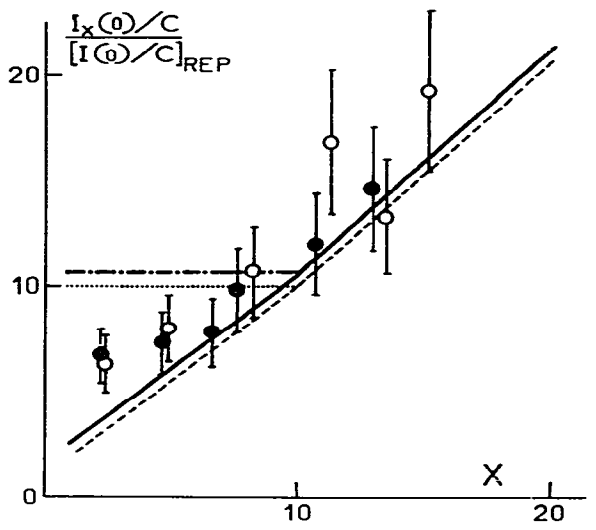


Fig. 3. Normalized weight average molecular weight of the complexes. Open and full circles correspond to two different experiments. Theoretical calculations of the same parameter are plotted in the figure. Noncooperative case: (———) according to eq. 17; (- - - - - -) the same, neglecting the DNA contribution ($\alpha = 0$). Infinitely cooperative cases: (· · · · · ·) according to eq. 13; (·—·—·) the same, taking into account the DNA contribution.

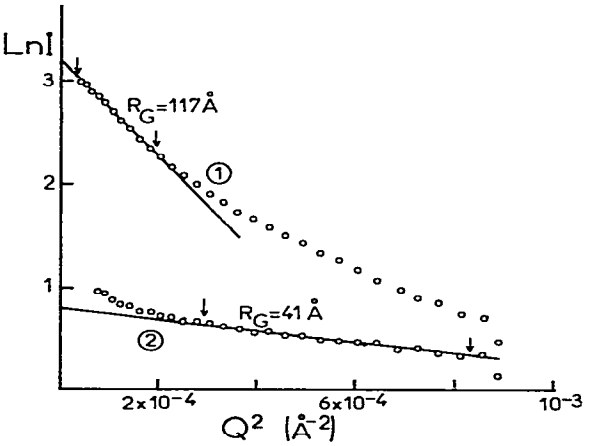


Fig. 4. Scattering curve for the complex at $X = 10.5$ at low ionic strength [1] and at high ionic strength [2]. Radii of gyration are calculated from the straight parts of the curves between the arrows.

after addition of KCl to a final concentration of 0.5 M. The radius of gyration decreases from 117 to 41 Å, as expected for free repressor. The extrapolated intensity to zero angle decreases by a factor of 11, in very good agreement with the molecular weight of the complex expressed in repressor units. In this case, the contribution of the free DNA to the scattering is negligible.

### 3.4. Kinetic measurements

Our purpose was to estimate the lifetime of the nonspecific complex, $\tau = 1/k_{OFF}$, in measuring the molecular weight of the complexes at different times after the addition of free DNA to the solution. The amount of free DNA added was equal to that originally present as a complex. The value of $X_0$ for the initial complex was chosen equal to 10 for two reasons: to have the maximum molecular weight compatible with the existence of the first type of complex, and to justify the introduction of the simple hypothesis $F_1/F_1 + F_2 \simeq 0$ and $F_2/F_1 + F_2 = 1$, as calculated from eq. 20.

As $R_G$ does not change significantly for $X$ going from 5 to 10, this parameter is not useful in following the changes in repressor distribution. We measured the scattered intensity $I(0)/C$, which is related to the distribution of the molecular weight of the particles. Fig. 5 shows the experimental data and several theoretical curves calculated according to eq. 22 using $X = 10$. The best value for $k_{OFF}$ is between 0.020 and 0.033 $h^{-1}$, which gives a lifetime $\tau = 40 \pm 10$ h. This value is in good agreement with the previous observations of Durand and Maurizot [9].

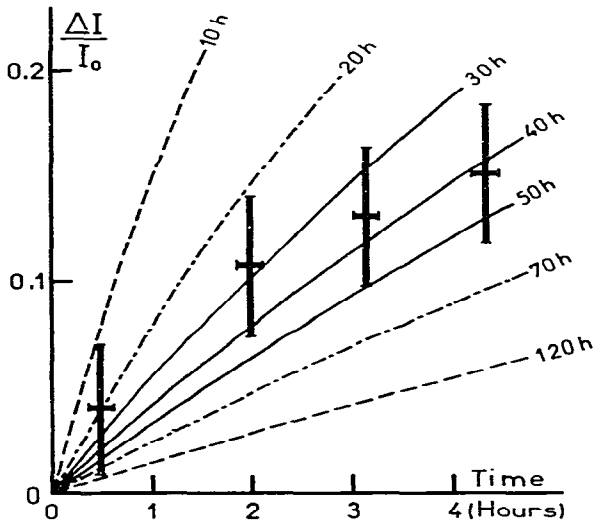

Fig. 5. Kinetic data plotted according to eq. 22.

All our experimental data are obtained in a time range of $t = 4$ h 20 min, i.e., $\Delta t k_{OFF} = 0.11$. Under these conditions, $X_1$ remains greater than 8.96 and $X_2$ smaller than 1.04. The simplifying hypothesis: $F(X_1)/F(X_1) + F(X_2) \simeq 0$ ($1.56 \times 10^{-3}$ in our case) and $F(X_2)/F(X_1) + F(X_2) \simeq 1$ (0.998 in our case) is therefore justified.

## 4. Discussion

As expected from the model calculations, the radii of gyration of the complexes are almost constant for values of $X$ up to approx. 2. Thus, these complexes behave as cylinders of length equal to the length of the DNA fragment and of small cross-sectional area as compared to the square of their length. DNA fragments as small as 145 base-pairs long have the same hydrodynamic properties as rigid rods [10]. Assuming that the DNA is in the B form (3.4 Å per base-plate), and a radius of gyration of 40 Å per repressor [11], the asymptotic value for the radius of gyration of such a complex would be 149 Å. Our experimental values are consistently 10% lower. Although this could be due to end effects, since the arrangement of the repressors may lead to a 'cylinder' slightly smaller than the DNA in B form, a slight shortening of the DNA (10%) upon repressor binding cannot be excluded. Nevertheless, the micrographs of Zingsheim and co-workers [1] did not show any shortening for nonspecific binding on an *Eco*RI fragment of SV 40 DNA.

In the range of $X$ from 10 to 16, the measured molecular weights as deduced from the extrapolated forward intensity are in agreement with the hypothetical molecular weight of $X$ repressors bound per fragment. These facts confirm the hypothesis we made in the preceding paper, i.e., all

molecules of repressor are bound to the DNA fragments even after the CD signal ceases to increase.

All our results are in good agreement with the assumption that two types of binding take place. The first type is drastically dependent on the ionic strength and the length of its excluded site is $14 \pm 2$ base-plates. It induces a conformational change in the DNA, observed both by CD and ultraviolet absorption spectroscopy. The second type can be called 'second layer binding'. It does not induce any spectroscopically detectable conformational change, although the molecular weight of the complex increases. DNA cellulose chromatography experiments [12] showed that the first type of binding involves the formation of $12 \pm 2$ ion pairs per repressor. There remain free $172 \pm 20$ phosphate groups per fragment for the second type of binding. But their spatial location cannot offer the repressors an adequate set of free charges for the first type of binding. The most likely possibility is that repressor binding of the second layer does not involve binding to the DNA, but to the repressors of the first layer, in a mechanism similar to that of protein aggregation, which occurs at the ionic strength we used.

Strong cooperativity of the first type of binding can be eliminated from the molecular weight measurements (eq. 13 and on fig. 3). Nevertheless, we cannot exclude a low cooperativity in the binding process. With a 203 base-pair fragment containing the *lac* operator and one pseudo operator site, Fried and Crothers [13] observed discrete DNA-protein complexes by polyacrylamide gel electrophoresis. Taking into account that two repressors are bound on the specific and the pseudo specific sites, their observation of up to eight discrete bands for $X = 6.67$ rules out any strong cooperativity, in agreement with our conclusions.

It has been demonstrated that the repressor has two binding sites for the operator DNA [14,15]. As the binding constant for nonoperator DNA is very large at the ionic strength we used ($K_A > 10^6$ $M^{-1}$), it might be expected that complexes between one repressor and two DNA fragments are formed. These complexes for low values of $X$ should lead to network formation of the same type as antibody-antigen complexes. Nevertheless, we have not observed such a phenomenon which would have been quite obvious in the neutron scattering from a strong curvature of the scattering curve near the origin. It is likely that the association constant for DNA at the second site is greatly decreased by the binding to DNA fragment at the first site. The nonspecific binding of *lac* repressor is essentially ionic, and the electrostatic potential induced by the first DNA fragment may lead to a reduced affinity for the second one. This is not the case for binding to the operator, where the nonelectrostatic contribution to the free energy of interaction make it possible for binding of either two 29 base-pair or 203 base-pair operator bearing DNA fragments (ref. 15 and F. Culard, personal communication).

The determination of $k_{OFF}$ from the measurements of $I(0)/C$ gives only an order of magnitude estimation of $k_{OFF}$ or $\tau$. The difficulty arises from the fact that we cannot measure the scattering intensity over a time range greater than a few hours. The time range used, about a tenth of the determined lifetime, is obviously too small to give a very precise result. But our purpose was not to obtain $\tau$ with a precision of minutes. We can, nevertheless, discard lifetimes longer than 70 h, and shorter than 20 h. This result is not without implications concerning the association constant $K_A = k_{ON}/k_{OFF}$. De Haseth and co-workers [16] studied the variation of $K_A$ in a range of ionic strength around 0.15 M as $Na^+$. Extrapolating their values and considering that our buffer (potassium phosphate) gives the same type of variation, would lead to a value of $10^{21}$ or $10^{22}$ $M^{-1}$ for the association constant. Assuming a maximum value of $10^{11}$ $M^{-1}$ $s^{-1}$ for $k_{ON}$, the calculated lifetime would be of the order of three centuries. This is obviously not the case, and we conclude that the result of De Haseth and co-workers cannot be extrapolated to very small ionic strengths. This nonlinearity of $\ln[K^+]$ vs. $\ln[Na^+]$ has been observed by Barkley [17] and calculated by Berg and Blomberg [18] for the specific interaction between operator and repressor.

The first type of binding involves ten repressor molecules per DNA fragment, each molecule being separated from the adjacent one by $14 \pm 2$ base-plates [2]. For such a distance between the interac-

tion sites, the possibility of positioning the repressor molecules with their longest axis parallel to the DNA helical axis as proposed by Steitz et al. [19] or Dunaway et al. [20] is excluded, since this dimension is of the order of 120–150 Å [11,21]. Such an arrangement would cover a length of 35 base-plates on a DNA helix in the B form, and is ruled out by our experimental results. The only remaining possibility is that the long axis of the repressor is perpendicular to the DNA axis, as shown on the recent models proposed for the specific binding [21,22]. The relative position of the two partners in both types of binding (specific or nonspecific) would be nearly the same. The only difference, which leads to a spectacular increase in the association constant, would arise probably from a few additional interactions, perhaps from the headpieces, or from the core, as suggested by Dunaway et al. [20].

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