McGhee, J. D., & von Hippel, P. H. (1974) *J. Mol. Biol. 86*, 469–489.
McQuistan, R. B. (1968) *Nuovo Cimento 58*, 86–92.
Pirrotta, V., Chadwick, P., & Ptashne, M. (1970) *Nature (London) 227*, 41–44.
Ptashne, M., Backman, K., Humayun, M. Z., Jeffrey, A., Maurer, R., Meyer, B., & Sauer, R. T. (1976) *Science (Washington, D.C.) 194*, 156–161.
Riggs, A. D., Suzuki, H., & Bourgeois, S. (1970) *J. Mol. Biol. 48*, 67–83.
Schellman, J. A. (1975) *Biopolymers 14*, 999–1018.
Schmitz, A., & Galas, D. J. (1979) *Nucleic Acids Res. 6*, 111–137.
Schmitz, A., & Galas, D. J. (1980) *Nucleic Acids Res. 8*, 487–506.
Strauss, H. S., Burgess, R. R., & Record, M. T., Jr. (1980) *Biochemistry 19*, 3496–3504.
Strauss, H. S., Boston, R. S., Record, M. T., Jr., & Burgess, R. R. (1981) *Gene 13*, 75–87.
Thang, M. N., Graffe, M., & Grunberg-Manago, M. (1968) *Biochem. Biophys. Res. Commun. 31*, 1–8.
Wyman, J. (1948) *Adv. Protein Chem. 4*, 407–531.
Wyman, J. (1964) *Adv. Protein Chem. 19*, 223–286.
Yarus, M., & Berg, P. (1967) *J. Mol. Biol. 28*, 479–490.
Yarus, M., & Berg, P. (1970) *Anal. Biochem. 35*, 450–465.

Cooperative, Excluded-Site Binding and Its Dynamics for the Interaction of Gene 5 Protein with Polynucleotides†

Dietmar Pörschke* and Hannelore Rauh

ABSTRACT: The binding of gene 5 protein to various single-stranded polynucleotides is investigated by fluorescence titrations and stopped-flow measurements. The association state of gene 5 protein itself is analyzed by equilibrium sedimentation: the monomer–dimer equilibrium found in the micromolar concentration range is described by a stability constant of 8×10^5 M^{-1}. The fluorescence quenching upon binding to polynucleotides, studied over a broad concentration range and analyzed in terms of a cooperative excluded-site binding model, provides binding constants for "isolated" and for "cooperative" sites. The cooperativity for various ribo- and deoxyribopolymers is between 400 and 800 and is virtually independent of the ionic strength. The binding to isolated sites is strongly dependent upon the ionic strength; analysis in terms of polyelectrolyte theory indicates the compensation of 4 ± 0.5 charges upon complex formation. The number of nucleotide residues covered by one protein molecule is also found to be 4 ± 0.5 units. The affinity of gene 5 protein for polynucleotides increases in the series poly(C) < poly(dA) < poly(A) < poly(U) ≪ poly(dT); the binding constant for poly(dT) is roughly a factor of 1000 higher than that for the other polymers. Model studies with Lys-Tyr-Lys and Lys-Trp-Lys suggest that the preferential interaction with poly(dT) is not simply due to enhanced stacking interactions between the aromatic amino acids and the thymine residues. Stopped-flow reaction curves obtained by mixing of gene 5 protein with poly(dT) in the micromolar concentration range show three relaxation processes with time constants between 1 ms and 1 s. From the concentration dependence, the fast process is assigned to the formation of isolated complexes with a rate constant of 8.1×10^9 M^{-1} (polynucleotide) s^{-1}. The medium and the slow process observed at moderate degrees of binding are partly due to excluded-site effects; the medium and slow process observed at low degrees of binding are assigned to some conformational transition and rearrangement of ligands on the lattice, respectively. This interpretation is supported by Monte Carlo simulations of the binding process using the approach developed by Epstein [Epstein, I. R. (1979) *Biopolymers 18*, 2037–2050]. Stopped-flow data obtained for the "weakly" binding poly(A) are consistent with a nucleation mechanism. The formation of cooperatively bound protein clusters can be explained without postulating a special translocation mechanism of the protein along the polynucleotide chain. A possible misinterpretation of data for the kinetics of nucleation with respect to fast translocation is discussed. However, evidence for some translocation process is obtained from the kinetics of binding close to saturation of the polymer lattice. The redistribution of cooperative clusters is faster than expected from Monte Carlo simulations. According to these results, the clusters are translocated in the time range of approximately a second.

Gene 5 protein is one of the best characterized DNA binding proteins (Kowalczykowski et al., 1981). It has been investigated by numerous techniques including NMR (O'Connor & Coleman, 1982; Alma et al., 1982), fluorescence spectroscopy (Pretorius et al., 1975; Veiko et al., 1981), CD (Day, 1973; Anderson et al., 1975; Tyaglov et al., 1980), ultracentrifugation (Cavalieri et al., 1976; Pretorius et al., 1975), X-ray crystallography (McPherson et al., 1980), and neutron scattering (Torbet et al., 1981; Gray et al., 1982). From these investigations, it is known that gene 5 protein binds cooperatively to single-stranded polynucleotides and due to this binding reduces the melting temperature of DNA by about 40 °C. The association seems to be mainly driven by stacking interactions between tyrosine and phenylalanine residues of the protein with base residues of the polynucleotides as well as electrostatic interactions between lysine and arginine residues of the protein with phosphate residues of the polynucleotides. The structure of the complex is not yet known in detail; various models have been suggested from NMR and chemical modification data (Coleman & Armitage, 1978), from the crystal structure of the free protein together with some data on oligonucleotide–

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protein complexes (McPherson et al., 1980), and recently from neutron scattering (Torbet et al., 1981; Gray et al., 1982).

Since the biological function of gene 5 protein depends not only upon the structure of its complex but also upon its dynamics, we have investigated the kinetics of gene 5 protein binding to various polynucleotides. The quantitative analysis of kinetic data requires knowledge of thermodynamic parameters. Thus, we have also determined these parameters by equilibrium titrations. The results are discussed in terms of the biological function of gene 5 protein.

Materials and Methods

Gene 5 protein was prepared by a modification of the procedure described by Alberts et al. (1972). After lysis of fd-infected *Escherichia coli* K12-1101 cells, the protein fraction was separated from the nucleic acids by a "phase separation" (Okazaki & Kornberg, 1964). The proteins were then dialyzed with buffer A [0.05 M tris(hydroxymethyl)-aminomethane hydrochloride (Tris-HCl), pH 7.5, 0.01 M β-mercaptoethanol, 1 mM ethylenediaminetetraacetic acid (EDTA), 10% glycerol, and 0.05 M NaCl] and subsequently loaded on a DNA–Sepharose column (Arndt-Jovin et al., 1975). After the column was washed with buffer A containing increasing concentrations of NaCl (0.15 and 0.4 M), gene 5 protein was eluted at concentrations of 0.6 and 2.0 M NaCl. The sample was then dialyzed against buffer A without NaCl and washed through a DEAE-cellulose column to remove contaminations of proteins with a higher charge density (and higher molecular weight). Finally, the gene 5 protein was dialyzed into the standard buffer B (0.1 M NaCl, 5 mM sodium phosphate, pH 6.73, and 1 mM EDTA) and stored at −70 °C. The purity of our preparations was controlled by gel electrophoresis and also by determination of free SH residues (Glazer et al., 1975). The concentration of gene 5 protein was calculated with the absorbance coefficient 7.08×10^3 M^{-1} cm^{-1} at 276 nm (Day, 1973).

The polydeoxyribonucleotides poly(dA) and poly(dT) were purchased from P-L Biochemicals and Miles, respectively; the polyribonucleotides poly(A), poly(U), and poly(C) were obtained from Boehringer Mannheim. The average chain lengths of poly(dT) and poly(A) were determined by sedimentation velocity to be 300 and 1000 nucleotides, respectively. The oligomer $d(pT)_4$ was obtained from Collaborative Research, Inc.

Most measurements have been performed in the following standard buffer: 0.1 M NaCl, 5 mM sodium cacodylate, pH 6.7, and 1 mM EDTA. The ionic strength in the fluorescence titrations was adjusted by an increase or a reduction of the NaCl content. For ultracentrifuge measurements at 230 nm the EDTA concentration was reduced to 0.1 mM. The stopped-flow data for poly(A) were obtained in 0.15 M NaCl, 7.5 mM sodium cacodylate, pH 6.7, and 1.5 mM EDTA. All data were measured at 20 °C.

Fluorescence titration curves were measured with an Aminco SPF 500 spectrofluorometer. The fluorescence was usually excited at 284 nm and the emission collected at 305 nm with a bandwidth of 20 nm by using a WG 305 cutoff filter. The fluorescence intensities were averaged by a Commodore 3032 computer interfaced to the SPF 500. For each sample the absorbance at the excitation wavelength was read by a Zeiss PMQ II spectrophotometer. These absorbance values (A) (given per unit length) were used for inner filter correction by the factor

$$f = \frac{2.303A(d_2 - d_1)}{10^{-Ad_1} - 10^{-Ad_2}} \quad (1)$$

This factor results from a model assuming that fluorescence emission is seen by the multiplier in the space range between d_1 and d_2 along the excitation beam. The d_1 and d_2 values were determined by test experiments to be 0 and 8.3 mm for our conditions.

The gene 5 protein and its fluorescence turned out to be sensitive to shaking. Thus, mixing with added ligand had to be performed gently. The fluorescence of gene 5 protein–polynucleotide mixtures was always taken as the ratio to the fluorescence of another gene 5 sample with the same "history" except addition of polynucleotide.

Kinetic data were mainly obtained by a stopped-flow apparatus constructed in this department. The "dead time" of our apparatus was determined according to Peterman (1979) to be below 1 ms. Fluorescence intensities as a function of time were first stored on a Biomation 1010 transient recorder, which had been modified for simultaneous use of pretrigger and dual time base options. The data were then transferred to the Univac 1108 of the Gesellschaft für wissenschaftliche Datenverarbeitung mbH (Göttingen, West Germany) and evaluated by a relaxation fitting procedure, which can be applied for data that were convoluted by an exponential response curve of the detection system (Pörschke & Ronnenberg, 1981; Diekmann et al., 1982).

The kinetics of $d(pT)_4$ binding were studied by temperature jump measurements using the instrument described by Rigler et al. (1974).

The sedimentation equilibrium was measured with a Spinco Model E analytical ultracentrifuge equipped with photoelectric scanner, multiplexer, and electronic speed control. The ultraviolet optics were modified by the introduction of a collimator developed by Flossdorf & Schillig (1979). All runs were made with 12-mm charcoal-filled epon double-sector cells in a six-hole An-G Ti rotor. The solutions were scanned at 230 nm (or 280 nm). The output signal of the scanner was fed via a Keithley 192 multimeter into a commodore 8032 computer for a first evaluation. For joint least-squares fits of several sedimentation profiles, the data were transferred to the Univac 1108 of the Gesellschaft für wissenschaftliche Datenverarbeitung (Göttingen, West Germany).

Results

Dimerization of Gene 5 Protein. Gene 5 protein is known to form a rather stable dimer (Oey & Knippers, 1972; Pretorius et al., 1975; Cavalieri et al., 1976). The dimerization constant has not been determined yet. Estimates given previously are partly contradictory. Due to the high stability of the dimer, a very sensitive technique is required for a quantitative analysis. We have used the equilibrium sedimentation method, taking advantage of the increased sensitivity offered by the collimator optics of Flossdorf & Schillig (1979), which provides enough light intensity for scans at 230 nm. The equilibrium scans measured at various protein concentrations were fitted simultaneously to a dimerization model, which considers the coupling of sedimentation and association at any point of the cells and also uses the information obtained by integration of the profiles. The procedure is equivalent to that described previously for a different system (Pörschke & Labuda, 1982). As shown by the data in Figure 1, the dimerization model provides a satisfactory fit over a broad concentration range with a stability constant $K_D = 8 \times 10^5$ M^{-1}. Measurements of the fluorescence intensity as a function of the protein concentration in the range from 0.5 to 2.5 μM did not reveal any deviation from linearity, indicating that the fluorescence intensity is virtually independent of the dimerization state.

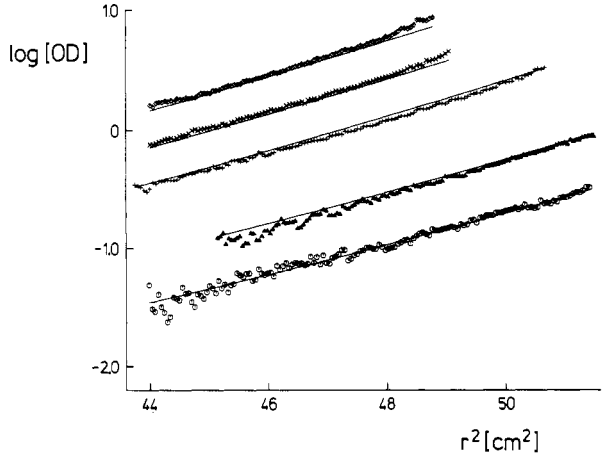

FIGURE 1: Equilibrium sedimentation curves measured at 230 nm for five different gene 5 protein concentrations [(○) 2.44 μM; (△) 5 μM; (+) 10 μM; (×) 15 μM; (◇) 20 μM] before sedimentation of the protein (20 °C, 18 000 rpm). For clarity, subsequent curves are shifted on the log OD scale by 0.2 unit each. For the three highest concentrations, the scans could not be traced down to the bottom of the cells because of too high OD values; in these cases, the material contained in the lower part of the cells has been considered in the conservation of mass by extrapolation of the fitted curves down to the bottom of the cells. The continuous lines represent the joint least-squares fit according to the dimerization model with $K_D = 8 \times 10^5$ M^{-1} (specific volume, 0.74; molecular weight of the monomer 10 000).

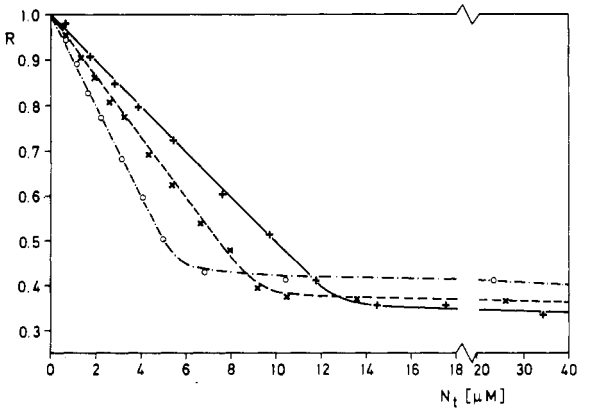

FIGURE 2: Fluorescence (R) of the protein with added polynucleotide relative to that of the free protein as a function of the poly(dA) concentration ([N_t]) for three different concentrations of gene 5 protein [4 μM (+); 3 μM (×); 2 μM (○); standard buffer]. The lines represent the fit according to the cooperative excluded-site binding model with $K_i = 2.4 \times 10^3$ M^{-1} and $\omega = 1000$.

Table I: Results from Fluorescence Titrations according to the Cooperative Excluded-Site Binding Model[a]

	log $K_i\omega$ at 1 M NaCl	$K_i\omega$ (M^{-1}) at 0.1 M NaCl	ω	z
poly(A)	2.3	3.8×10^6	500	4.3
poly(U)	3.6	8.4×10^6	400	3.3
poly(C)		4.8×10^5	400	
poly(dA)		2×10^6	800	
poly(dT)	6.1	3.2×10^9	500	3.4

[a] Estimated accuracies: $K_i\omega$, ±15%; ω, ±25%; z, ±15%. The data for poly(dT) were measured at ionic strengths between 0.5 and 1 M, whereas the data for the other polymers were obtained at ionic strengths between 0.1 and 0.25 M; $K_i\omega$ and z are evaluated by linear regression (cf. Figure 3); ω is the average obtained from the measurements at different ionic strengths.

Analysis of the Cooperative Binding to Polynucleotides. As was observed previously (Pretorius et al., 1975), the fluorescence of gene 5 protein is quenched upon binding to polynucleotides. We have used this effect for the quantitative characterization of equilibrium parameters according to the following procedure: the fluorescence quenching is measured as a function of the polynucleotide concentration at different gene 5 protein concentrations. These data are compared in a computer with binding curves predicted by an excluded-site binding model allowing for cooperative association (McGhee & von Hippel, 1974). The parameters required for this comparison are the following: (1) the equilibrium constant (K_i) for association of a protein ligand to an isolated site on the polynucleotide; (2) the cooperativity (ω) corresponding to the equilibrium constant for the combination of two isolated bound protein ligands; (3) the number of nucleotide residues (n) covered by one protein molecule; (4) the fluorescence intensity of gene 5 protein in the complex (α) relative to that of free protein. (It is assumed that the fluorescence quenching is identical for isolated and contiguously bound protein. This assumption will not cause problems in the present system, since its high cooperativity leads to a very low equilibrium population of isolated complexes.)

By a systematic variation of the parameters and continuous comparison of the model curves with experimental data, finally a set of parameters is evaluated which provides an optimal fit to the experimental data with a minimum in the error sum. The significance of these parameters is tested by independent variations demonstrating the depth of the minima for individual parameters. This procedure leads to an estimate for the accuracy of the various parameters. An example for a set of experimental data and its representation by the model is given in Figure 2. Corresponding data were obtained for various polynucleotides at different salt concentrations. The results may be summarized as follows:

The number of nucleotide residues n covered by one protein is close to 4 in all cases with a variation of about ±0.5. The fluorescence of the protein in the bound state is between α = 0.25 and 0.4 relative to that of the free protein; its exact value is somewhat dependent upon the nature of the polynucleotide and also slightly dependent upon the ion conditions. The cooperativity ω cannot be determined with a high degree of accuracy. We found values between 300 and 1000. Our data do not show any systematic dependence of the cooperativity upon the ionic strength, whereas a clear ionic strength dependence is found for the stability constant K_i. An increase in the ionic strength leads to a strong decrease of K_i. Plots of log K_i against the logarithm of the ion concentration according to polyelectrolyte theory (Manning, 1978; Record et al., 1978) show linear correlations. From the slopes we obtain the number of charges (z) compensated upon complex formation [definition of z according to Manning (1978)]. As shown in Table I, the values obtained for z are close to the value expected from the number n of nucleotide residues covered by one protein molecule. The equilibrium constants K_i exhibit a clear dependence upon the composition of the polynucleotide. A particularly high affinity is found for poly(dT).

Comparative Analysis of Peptide Binding. The exceptionally high equilibrium constant for the binding of gene 5 protein to poly(dT) raises the question of the nature of the interactions leading to the high affinity. As mentioned in the introduction, both electrostatic and stacking interactions have been discussed as major sources of stabilization. Since the electrostatic interactions are expected to be similar for the various polynucleotides, it remains possible that the stacking

interactions of aromatic amino acids are particularly strong in the case of poly(dT). This explanation appears to be plausible also in terms of results obtained for stacking of modified nucleic acid bases, showing that methylation may strongly promote stacking (Pörschke & Eggers, 1972).

For a test of this explanation, we have analyzed the binding of Lys-Tyr-Lys and Lys-Trp-Lys to poly(dT) by fluorescence titrations. In a buffer containing 1 mM NaCl, 1 mM sodium cacodylate, and 0.2 mM EDTA, we found binding constants of 3.9×10^4 M^{-1} for Lys-Tyr-Lys ($n = 2$, $\alpha = 0.50$) and 3.3×10^4 M^{-1} for Lys-Trp-Lys ($n = 2$, $\alpha = 0.17$). These binding constants are very close to those obtained previously for, e.g., poly(A) (3.7×10^4 M^{-1}; Brun et al., 1975; Pörschke, 1980). Thus, we do not find any indication for a strongly enhanced stacking interaction of aromatic amino acids to poly(dT) in the case of simple model peptides. This result indicates that the sequence specificity of gene 5 protein is due to a more complex mechanism of recognition.

Association Kinetics at High Ligand Affinity: Poly(dT). The sequence of reaction steps involved in the binding of gene 5 protein to single-stranded polynucleotides was studied by stopped-flow measurements. We used the fluorescence quenching of tyrosine residues to follow the reaction (Figure 3). Most of the measurements were conducted with a large excess of polynucleotide for two reasons: (1) With an excess of one reagent, we reduce the reaction from second to first order and may analyze our reaction curves in terms of exponentials. (2) When we have an excess of polynucleotide, the binding density on the polymer lattice remains low, and complications expected for high binding densities due to excluded-site binding are avoided to some degree (cf. below).

Even under these simplifying experimental conditions, we find relatively complex reaction curves. An accurate representation of these curves requires three exponentials with time constants ranging from 1 ms to 1 s. The fastest process with time constants around 1 ms could only be resolved at very low concentrations in the micromolar range. The nature of the different reaction steps may be elucidated as usual by an analysis of concentration dependences. This could be done most directly for the fast step in the case of gene 5 protein binding to poly(dT): the reciprocal relaxation time ($1/\tau_1$) is a linear function of the poly(dT) concentration (Figure 4) and thus indicates a bimolecular step

$$P + N \underset{k_d^i}{\overset{k_a^i}{\rightleftharpoons}} PN_i \qquad (2)$$

with

$$1/\tau = k_a^i[P + N] + k_d^i \qquad (3)$$

From the slope we obtain an association rate constant $k_a^i = 2.7 \times 10^7$ M^{-1} s^{-1}. Since the intercept at the $1/\tau$ axis is rather small, the k_d^i value cannot be determined with high accuracy. From the value $k_d^i \sim 2.7$ s^{-1} obtained by linear regression, we may calculate an equilibrium constant $k_a^i/k_d^i \sim 10^7$ M^{-1}, which should correspond to the binding constant for the "isolated" gene 5 protein–poly(dT) complex. A comparison with the value determined by fluorescence titrations, $K_i = 6.4 \times 10^6$ M^{-1} (cf. Table I), reveals a satisfactory agreement.

The k_a^i value is obtained from measurements at a protein concentration of 0.5 μM, where most of the gene 5 protein is in its monomer state. In order to test for the influence of the protein dimerization upon the association rate, some experiments were conducted at various protein concentrations up to 2 μM, where most of the protein is in its dimeric state. Stopped-flow mixing with an excess of poly(dT) revealed relaxation time constants that were independent of the protein

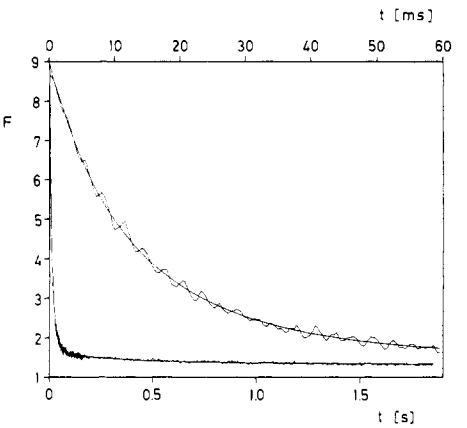

FIGURE 3: Change of the fluorescence intensity (F) (arbitrary units) as a function of time (t) observed in a stopped-flow apparatus after mixing of gene 5 protein and poly(dT) to concentrations of 0.5 and 5 μM, respectively. The signal is convoluted with a detector rise time of 0.55 ms. The upper and lower time scales are valid for the upper and lower curves, respectively. The line without noise represents a least-squares fit with $\tau_1 = 6$ ms, $\tau_2 = 17$ ms, and $\tau_3 = 300$ ms (0.1 M NaCl, 5 mM sodium cacodylate, pH 6.7, and 2 mM EDTA).

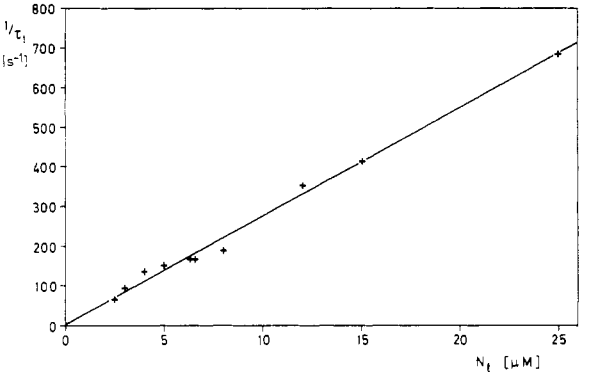

FIGURE 4: Reciprocal relaxation time constant ($1/\tau_1$) observed for the fast process in the reaction of 0.5 μM gene 5 protein with poly(dT) as a function of the poly(dT) concentration ([N_t]) (monomer units). By linear regression, we obtain the rate constants $k_a^i = 2.7 \times 10^7$ M^{-1} (nucleotide) s^{-1} and $k_d^i = 2.7$ s^{-1} (0.1 M NaCl, 5 mM sodium cacodylate, pH 6.7, and 2 mM EDTA).

concentrations (within the limits of accuracy ±20%) as expected according to eq 3. These data indicate that the binding reactions of the protein monomer and dimer are very similar. Obviously the rate constants are not affected by the choice of protein concentration units under the present reaction conditions.

As shown by the data given in Figure 4, the $1/\tau$ values obtained for the fast process at high degrees of binding (θ) do not show deviations from the linear dependence, even though the relaxation conditions are not fulfilled at high θ, and furthermore, deviations due to excluded-site binding may be expected [θ = (number of residues covered by ligands)/(total number of residues)]. It is likely that this simple relationship only holds during the first part of the reaction, when the degree of binding (θ) still is very low.

The interpretation of the medium and slow process is more difficult for several reasons. First of all, the relaxation time constants cannot be determined with high accuracy due to mutual coupling of the fitted parameters upon each other. This is mainly a problem for the medium process, whereas the evaluation of the slow process is rendered more difficult by its relatively small amplitude. Since it should be expected that medium and slow process are strongly influenced by excluded-site binding (cf. the following section), these processes should be analyzed at particularly low θ values. Due to

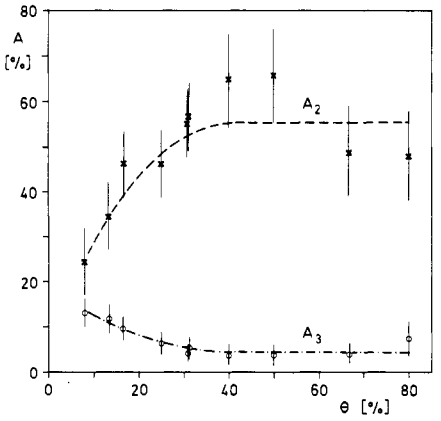

FIGURE 5: Relative amplitude of the medium ($A_2/\sum A$) and slow ($A_3/\sum A$) processes for the reaction of gene 5 protein with poly(dT) as a function of the degree of binding (θ) (standard buffer, 20 °C).

technical difficulties (either too low fluorescence intensity at low protein concentrations or too high rates at high polymer concentrations) only a limited range of concentrations is accessible for measurements of the complete reaction curve. For this reason, the medium and slow process are evaluated only in a semiquantitative manner.

The influence of excluded-site binding becomes apparent, when the relative contributions of the medium ($A_2/\sum A$) and slow processes ($A_3/\sum A$) are given as a function of the degree of binding (θ) (Figure 5). The decrease of $A_2/\sum A$ at low θ indicates that at $\theta > 0.1$ a relatively large part of the amplitude A_2 is due to reactions associated with excluded-site binding. However, part of the amplitude A_2 remains clearly visible at low θ and high poly(dT) concentrations, where Monte Carlo simulations of the processes associated with excluded-site binding do not predict any "medium" or "slow" process anymore (cf. the following section). Thus, our stopped-flow experiments indicate the existence of a reaction step in the medium time range independent of excluded-site binding phenomena; its time constant is about 10 ms in the concentration range between 10 and 25 μM poly(dT). [At high θ and low poly(dT) concentrations ([N]), where the medium process is mainly due to excluded-site effects, its time constant shows an increase with decreasing [N], which is consistent with Monte Carlo simulations.]

The slow process with an average relaxation time of 230 ms is associated with a relatively small amplitude only. The increase of A_3 at low degrees of binding (cf. Figure 5) suggests that the slow process is associated with a rearrangement of ligands along the lattice. When the protein binds to a large excess of polymer, it is likely that during the first part of the reaction the bound protein molecules are isolated (i.e., without direct protein neighbors) or are associated in a large number of small clusters. Due to the cooperativity the larger clusters will then grow at the expense of the isolated molecules and the small clusters. This rearrangement will only be reflected in our experiments, when the formation of cooperative contacts is associated with some change of fluorescence. Since the number of the initially separate molecules will increase with the excess of polymer, the amplitude will increase with decreasing θ, as observed for our slow process. The time constant of the rearrangement in the absence of facilitating mechanisms like sliding will be determined by the rate of dissociation. According to the data obtained from the fast relaxation process, the lifetime of "isolated" complexes is 240 ms (K_i/k_a^i), very close to the relaxation time constant observed for the slow process. Thus, the rearrangement of ligands resulting in the formation of cooperative contacts seems to be determined by the lifetime of isolated complexes. It is likely that there are also slower reactions resulting from the rearrangement of clusters [cf. Schwarz (1972)], but the amplitude of these reactions at low and medium θ is probably too small to be detected in our flow experiments. Binding experiments leading to saturation of the polymer lattice demonstrate the existence of slower reactions (cf. below).

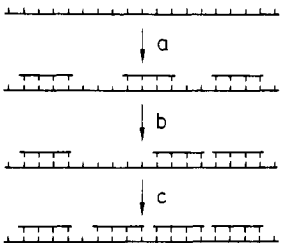

FIGURE 6: Binding scheme for large ligands to a polymer lattice. (a) Statistical attachment of ligands to a polymer segment resulting in a distribution excluding further binding; (b) rearrangement of the ligand distribution either by dissociation and recombination or by diffusion along the chain; (c) subsequent attachment of a further ligand.

Monte Carlo Simulation of the Kinetics for Cooperative Excluded-Site Binding. The binding of large ligands to a linear lattice is a relatively complex reaction due to special effects resulting from the exclusion of binding sites. The phenomenon is illustrated in Figure 6. During the first part (a) of the binding reaction ligands are distributed on the lattice in a statistical manner, until the distribution of ligands does not allow further binding, because the gaps are not large enough to accomodate further ligands. When small gaps are enlarged by redistribution of the ligands along the lattice (b), however, binding of further ligands becomes possible (c). The ligands may be redistributed along the lattice either by dissociation and recombination or by sliding and also by hopping. The sequence of reactions a, b, and c results in relatively complex kinetic curves which cannot be evaluated in terms of analytical equations except for certain limit conditions. A general procedure for the simulation of these kinetic curves has been elaborated by Epstein (1979) using Monte Carlo methods. Details of these simulations have been described by Epstein (1979). We are using the simulation program developed by Epstein and simply introduce the parameters that were determined directly by experiments. The model is appropriate to describe the binding kinetics of gene 5 protein to polynucleotides since, according to all evidence available, the binding is to clearly defined sites (involving insertion of aromatic amino acids between nucleotide bases). The Monte Carlo model includes the possibility of cooperative contacts between adjacent protein ligands. However, it does not include the possibility of additional "dimer" contacts between protein units.

To simulate the binding kinetics of gene 5 protein to poly(dT), we are using the equilibrium parameters determined by fluorescence titrations and the association rate constant k_a^i evaluated from the fast relaxation process observed in stopped-flow experiments. Since the k_a^i value determined for the formation of isolated complexes is close to the limit of diffusion control, the formation of complexes with a cooperative contact cannot be much faster; thus, we assume that the cooperativity only affects the dissociation rate. The equilibrium parameters required for the assignment of dissociation rates at 0.1 M NaCl are obtained by extrapolation of the data measured in the range of 0.5–1 M ion concentration. The kinetic data obtained for the binding to isolated sites (cf. previous section) indicate that this extrapolation does not give

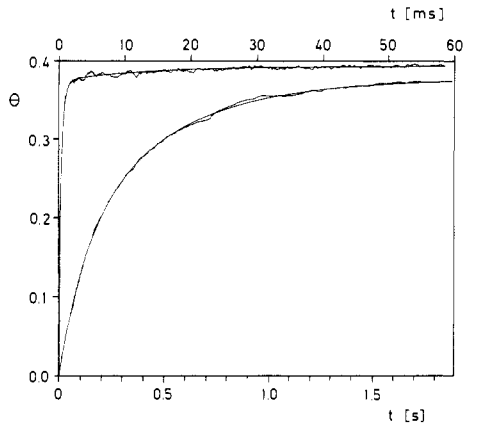

FIGURE 7: Binding kinetics of a ligand (0.5 μM, covers four sites) to a polymer lattice (5 μM monomers, chain length 100 nucleotides) simulated by a Monte Carlo procedure according to Epstein (1979). Rate constants for isolated sites, $k_a^i = 2.7 \times 10^7$ M^{-1} s^{-1}, $k_d^i = 4.2$ s^{-1}; for cooperative sites, $k_a^c = 2.7 \times 10^7$ M^{-1} s^{-1}, $k_d^c = 8.4 \times 10^{-3}$ s^{-1}.

rise to any serious error. A simulation for protein and polymer concentrations of 0.5 and 5 μM, respectively, provides a reaction curve that is very close to the experimental one (Figure 7). The concentrations are such that both medium and slow binding processes are controlled by reactions associated with cooperative excluded-site binding. The close agreement of measured and simulated reaction curves demonstrates that the model and its parameters provide a rather faithful representation of the real reaction, even though the dimerization reaction of the protein was not included in the Monte Carlo model. Probably the dimerization is not directly reflected in the reaction curve, since under the present conditions most of the protein binding to the polymer is relatively slow compared to the dimerization reaction (cf. below). The close agreement also indicates that the fluorescence quenching is a rather reliable measure for the degree of binding, even though the formation of cooperative contacts probably results in an additional small contribution to the quenching.

Further simulations for various concentrations demonstrate that the slow binding effects coupled to the redistribution of ligands disappear when the polymer concentration is higher than that of the protein by a factor of about 15. In this case a simple binding process is observed, which can be represented by a single exponential. However, the simulation program in its present form only delivers the overall degree of binding and does not show directly the formation of cooperative contacts after rearrangement of bound ligands.

Association Kinetics at Low Ligand Affinity: Poly(A). The kinetics of cooperative binding is strongly influenced by the affinity of the ligands for the polymer. In the case of poly(A) the affinity of gene 5 protein for isolated sites is relatively low, such that the extent of binding to isolated sites remains very small. Stabilization by cooperative contacts is required for a clearly detectable extent of binding. This fact is reflected in the binding kinetics by a nucleation phenomenon. The protein molecules bound to isolated sites, although present only at a low concentration, serve as nucleation sites for the association of further protein molecules. This mechanism is clearly demonstrated by a decrease of the relaxation time observed for the first relaxation process with increasing protein concentration, even when the polymer is present in a large excess. This behavior is in contrast to that observed in the case of poly(dT), where the relaxation is independent of the protein concentration, when the polymer is present in large excess. The two-step mechanism for poly(A) may be represented by

$$P + N \underset{k_d^i}{\overset{k_a^i}{\rightleftharpoons}} PN_i \quad (4)$$

$$PN_i + P \xrightarrow{k_a^c} PN_c \quad (5)$$

where PN_i and PN_c are isolated and clustered protein–nucleic acid complexes, respectively. Due to the relatively low concentration of PN_i the first step of the reaction is not directly detected in the stopped-flow experiments. However, the existence of the first step is reflected by its influence on the rate of the second step, which is accessible to direct investigation. A simplified procedure for the evaluation of kinetic data has been proposed by Lohman & Kowalczykowski (1981), using the following arguments: (a) the first step is fast compared to the second one and thus can be treated as a fast preequilibrium; (b) the back-reaction of the second step may be neglected; (c) excluded-site effects are avoided by a large excess of the polynucleotide with respect to the protein. Under these conditions, the relaxation time constant τ_f associated with the growth process may be described (Lohman & Kowalczykowski, 1981) by

$$\tau_f = \frac{\tau_f^\infty}{K_i} \frac{1}{[N]} + \tau_f^\infty \quad (6)$$

where $K_i = k_a^i/k_d^i$ and τ_f^∞ is the growth relaxation time constant at infinite concentration of the polynucleotide ([N]). From the data measured for gene 5 protein + poly(A), we obtain by linear regression according to eq 6 a value $K_i = 800$ M^{-1}. The accuracy of this value is limited due to the difficulty in the extrapolation of τ_f^∞. Under these circumstances, the agreement with the value $K_i = 1.4 \times 10^3$ M^{-1} obtained from fluorescence titrations is satisfactory. The simple nucleation model has also been used to evaluate the growth rate constant k_a^c (Lohman & Kowalczykowski, 1981) according to the equation

$$\tau_f^{-1} = 2k_a^c[PN_i] \quad (7)$$

The data obtained for gene 5 protein + poly(A) can be represented by this equation with reasonable accuracy and provide a value $k_a^c = 2.3 \times 10^8$ M^{-1} s^{-1}. (For this evaluation, $[PN_i]$ was calculated by using the excluded-site model and the binding constant for isolated sites according to the parameters given in Table I.)

In addition to the "fast" process analyzed above, the relaxation curves observed for gene 5 protein + poly(A) also contain slower components with time constants around 100 ms, which may be attributed to the rearrangement of bound protein molecules.

Simple Nucleation Model Overestimates Growth Rate. The growth rate constant obtained for gene 5 protein + poly(A) at 0.15 M NaCl ($k_a^c = 2.3 \times 10^8$ M^{-1} s^{-1}) is much higher than that found for the formation of isolated complexes in the case of poly(dT) at 0.1 M NaCl ($k_a^i = 2.7 \times 10^7$ M^{-1} s^{-1}). Although these rate constants were obtained for slightly different conditions of ionic strength and polymer chain length, the relatively high growth rate may be interpreted to be due to facilitating mechanisms like sliding or hopping of protein molecules along the polymer chain (Lohman & Kowalczykowski, 1981). The facilitating mechanisms are of considerable biological interest. Thus, we have checked our results by Monte Carlo simulations of the nucleation-dependent association mechanism. Interpretation of the simulated data according to eq 7 always led to a much higher value of k_a^c than that used in the simulation, even though the simulation does not imply any facilitation mechanism. An examination of the

reaction scheme leading to eq 7 reveals a simplification, which will strongly affect the evaluated rate constant. The concentration of nuclei is calculated from the equilibrium constant for isolated complexes. This value is correct before growth reactions occur. The formation of any cooperative contact results in a considerable decrease of the dissociation rate for the complex used as a nucleus, which is thus "excluded from the preequilibrium". Since the preequilibrium results from the balance of association and dissociation rates, the inhibition of dissociation by cooperative contacts will lead to the formation of an increasing population of protein complexes that may be used for the growth reaction. This process will continue until the concentration of free protein is decreased to a low level (note that the polynucleotide is present in excess). Obviously, the number of nucleation sites is underestimated by the simple reaction scheme, and thus the rate constant for the growth reaction is overestimated. Using the rate constant for the formation of isolated complexes found in the case of poly(dT) as a basis for the Monte Carlo calculations in the case of poly(A), we find a simulated acceleration effect, which is equivalent to that found in the experiments (within the limits of accuracy). Thus, our kinetic data for the nucleation-dependent growth reaction do not indicate any significant contribution from facilitating mechanisms like sliding or hopping in the case of gene 5 protein.

Approach to Saturation of the Polymer Lattice. In the case of poly(dT) with its high affinity for the gene 5 protein, it is relatively simple to drive the reaction to very high degrees of binding close to saturation of the lattice. Under these conditions the Monte Carlo calculations predict the existence of a separate slow process corresponding to the rearrangement of cooperative clusters. In the absence of facilitating mechanisms, the rate-limiting step for the rearrangement is the dissociation of a cooperatively bound protein ligand, which is associated with a time constant of ~120 s. Thus, the rearrangement of cooperative clusters is a very slow reaction as long as the rearrangement is not facilitated by sliding or hopping. According to the Monte Carlo simulations the slow reaction should also be associated with a clearly detectable amplitude (around 10% of the total amplitude). Mixing experiments of gene 5 protein with poly(dT) leading to a degree of binding (θ) close to 1 do not show any slow process with a time constant of around 120 s. The slowest process detected in these experiments is associated with a time constant of about 7 s. Thus, the rearrangement of cooperative clusters appears to be accelerated by some facilitating mechanism resulting in an apparent distribution time constant of approximately a second.

Some Properties of an Oligonucleotide Complex. For comparison we have also analyzed a complex formed between gene 5 protein and $d(pT)_4$. By measurements of the UV absorbance we determined an association constant for a 1:1 complex of 1.7×10^4 M^{-1} (estimated accuracy ±20%). Equilibrium sedimentation experiments show that the binding of the oligonucleotide induces some association of the protein beyond the dimer state. However, from simulations of the sedimentation profiles, we estimate that only a relatively small part of the protein (10–20%) is involved in higher aggregates. Temperature-jump experiments reveal the existence of two relaxation processes with time constants of around 10 and 100 μs. The time constant observed for the fast process shows some decrease with increasing free reactant concentration, whereas the slow process is almost independent of the concentration. Due to the high rate of these processes together with rather small amplitudes, it has not been possible to characterize the relaxation to a high accuracy. Nevertheless, the relaxation time constants measured at various concentrations may be interpreted in terms of a two-step mechanism:

$$\text{oligomer} + \text{protein} \underset{k_d^{I}}{\overset{k_a^{I}}{\rightleftharpoons}} \text{complex I}$$

$$\text{complex I} \underset{k_d^{II}}{\overset{k_a^{II}}{\rightleftharpoons}} \text{complex II}$$

By least-squares fitting of the experimental data using the equilibrium constant determined from UV absorbance titrations, we obtained the following parameters:

$$k_a^{I} = 5 \times 10^8\ M^{-1}\ s^{-1} \qquad k_d^{I} = 5 \times 10^4\ s^{-1}$$

$$k_a^{II} = 5 \times 10^3\ s^{-1} \qquad k_d^{II} = 8 \times 10^3\ s^{-1}$$

Due to the problems discussed above, the accuracy is limited to about ±50%. The nature of the second step remains to be elucidated. Due to the limited accuracy of the experimental data it is not possible at present to exclude other mechanisms.

Discussion

Equilibrium Parameters. It has been known from previous investigations of gene 5 protein in solution (Pretorius et al., 1975; Cavalieri et al., 1976) and also in the crystal (McPherson et al., 1980) that the formation of dimers is an important property of this DNA binding protein. The dimerization affinity is unusually high, and its determination requires very sensitive techniques. The binding constant determined in the present investigation shows that—in spite of the high affinity—the dissociation of dimers and the reaction of monomers have to be considered in the binding process. The dimerization of the gene 5 protein is, however, not directly reflected in the data obtained from the equilibrium titration. The parameters evaluated from these titrations are based upon units of the monomer concentration. According to the double-helical model of the gene 5 protein–polynucleotide complex proposed by McPherson et al. (1980), the protein forms dimer contacts between monomers associated with two different strands in addition to cooperative contacts between adjacent monomers on the same strand. If this model represents the structure in solution, the nature of the cooperativity is more complex than that described by the excluded-site binding model according to McGhee & von Hippel (1974). However, Torbet et al. (1981) and Gray et al. (1982) concluded from neutron scattering measurements that the model proposed by McPherson et al. (1980) is not valid for the complex in solution.

Our present analysis confirms previous results on the number of four-nucleotide residues covered by a protein monomer (Pretorius et al., 1975). In addition, we identified the number of electrostatic contacts. The observed compensation of four charges per protein unit indicates that all phosphate residues covered by the protein are in close contact with lysine and/or arginine residues. A large part of the affinity for nucleic acids found for gene 5 protein at physiological salt concentration is due to electrostatic interactions. However, extrapolation to an ionic strength of 1 M shows that there is also a considerable contribution from other interactions, which is strongly dependent upon the nature of the nucleotide residue. Most of this additional contribution is probably due to stacking between the nucleic acid bases and tyrosine or phenylalanine residues of the protein. It is known that stacking may be strongly enhanced by methylation (Pörschke & Eggers, 1972). Thus, the presence of methyl groups could provide an explanation for the particularly high affinity of the protein to

poly(dT). Yet, our experiments with model peptides indicate that the preferential interaction of gene 5 protein with poly(dT) is not due to a simple stacking effect. Our data also do not support the interpretation that the differences in the binding affinity are simply due to differences in base stacking of the single-stranded polynucleotides. The binding constant for poly(dT) is much higher than that for poly(U), although both polymers show very little stacking. Apparently, the difference between poly(dT) and poly(U) is not merely due to the presence of the 2′-OH in poly(U), since poly(A) and poly(dA) show a similar affinity to gene 5 protein and have a similar degree of base stacking. It is remarkable that the two other melting proteins, which have been analyzed quantitatively, also show a strong binding preference to poly(dT) [gene 32 protein (Newport et al., 1981) and *E. coli* SSB protein (Krauss et al., 1981)]. Probably the nature of the molecular interactions is very similar for the complexes of these different melting proteins. In the case of fd phages, the DNA has a relatively high content of thymine residues. However, the T clusters appear to be distributed in a statistical manner and also do not provide a regular binding frame for the gene 5 protein with multiples of four nucleotide residues between subsequent T clusters.

Dynamics. The kinetics observed for the binding of gene 5 protein to polynucleotides is rather complex due to the cooperativity and the excluded-site binding effects. The assignment of relaxation effects is particularly difficult due to the existence of (at least) three different processes in the time range from 1 ms to 1 s. Nevertheless, it has been possible to elucidate the main features of the binding dynamics. We encounter two different limits of the same general reaction scheme: (1) in the case of poly(dT) having a strong affinity, it is possible to characterize the binding to isolated sites, whereas (2) the weakly binding poly(A) exhibits a nucleation-dependent association mechanism. From the data obtained in the first case, we evaluate a rate constant $k_a^i = 2.7 \times 10^7$ M^{-1} (nucleotide) s^{-1} for the binding of gene 5 protein to isolated sites. Using the average chain length of 300 nucleotides for our poly(dT) sample, we may convert this rate constant into 8.1×10^9 M^{-1} (polynucleotide) s^{-1} (based upon units of the polymer concentration). Obviously, the rate constant of association should not exceed the limit of diffusion control. In a previous investigation of gene 32 protein, Lohman & Kowalczykowski (1981) found much lower rate constants of $(0.9–1.5) \times 10^9$ M^{-1} (polynucleotide) s^{-1} for the corresponding binding reaction to isolated sites. Using the model of Berg et al. (1981) for the rate of ligand binding to a polymer chain, Lohman and Kowalczykowski concluded that their value is in good agreement with the theoretical estimates for a diffusion-controlled reaction. The difference in the rate constants for gene 5 and gene 32 protein is beyond the limits of experimental uncertainty and thus requires some explanation. For simplicity, we use the expression for the rate constant k_a of a diffusion-controlled reaction derived by von Smoluchowsky (1916):

$$k_a = 4\pi N_a r_D (D_A + D_B)$$

where N_a is Avogadro's number, r_D is the reaction distance (closest approach at which a spontaneous reaction occurs), and D_A and D_B are the diffusion coefficients of the reactants. Using a diffusion coefficient of 12.4×10^{-7} cm^2 s^{-1} for the gene 5 protein monomer [interpolated from data given by Tanford (1961)] and assuming that the diffusion coefficient of the polynucleotide may be neglected, we may calculate from our experimental rate constant an apparent reaction radius of 86 Å (assuming a steric factor of 1). The length of our poly(dT) sample in a stacked form would be approximately 1000 Å. It can be easily imagined that a more compact, coiled form provides a target with an apparent radius of more than 86 Å, allowing for some effect due to steric hindrance. It may be argued that a coiled target has a relatively large "transparency term" due to the open space between polymer segments. However, this effect will be compensated to a large degree by segment motion and rotation of the polymer sphere. Both segment motion and rotation of polymers are known to be very fast processes for the range of chain lengths used in the present investigation [cf. Diekmann et al. (1982)]. The rate determined for the association of $d(pT)_4$ with gene 5 protein ($\sim 5 \times 10^8$ M^{-1} s^{-1}) demonstrates the strong influence of the chain length. Similar data were found for the binding of oligopeptides to single-stranded nucleotide chains (Pörschke, 1979). According to the evidence available, the rate found for the binding of gene 5 protein is in the range expected for a diffusion-controlled reaction. The lower rate constants observed for gene 32 protein may be explained to some degree by its higher molecular weight. However, most of the difference appears to be due some steric hindrance effect.

The assignment of the medium and slow process observed for the binding of gene 5 protein to poly(dT) is more difficult than that of the fast process. At low concentrations of poly(dT) and a relatively high degree of binding, these processes can be directly assigned to excluded-site effects as shown by the Monte Carlo simulation. At high concentrations of poly(dT) and low degrees of binding, however, the remaining processes with time constants of ~10 and ~230 ms indicate the existence of other, independent reaction steps. According to both amplitudes and time constants, the "230-ms" process may be assigned to a redistribution of protein ligand and the formation of cooperative contacts. The "10-ms" process may be due to some conformational transition. A reasonable candidate for the assignment of this process would be the relaxation of the protein–dimer equilibrium in the bound state. It is likely that the protein–dimer contacts found in the free protein are also involved in the protein–nucleic acid complex at equilibrium. During the first reaction step protein monomers (and some dimers) will be associated with the polymer in statistical distribution, which may result in some dimer connections between different segments of the chain, but will also inhibit the formation of other connections due to steric constraints. The rate-limiting step in a redistribution of the dimer contacts is expected to be the dissociation of existing dimers. Provided that the formation of protein dimers is a simple diffusion-controlled reaction, the lifetime of a protein dimer (without consideration of cooperative effects) will be approximately 10 ms; thus, formation and rearrangement of these dimer contacts will certainly interfere with the overall binding reaction. The complex formed between gene 32 protein and polynucleotides apparently does not involve protein–dimer contacts (not to be confounded with the cooperative contacts). The relatively simple binding kinetics observed for gene 32 protein (Lohman & Kowalczykowski, 1981) is probably a consequence of this.

The data obtained in the present investigation do not provide evidence for the existence of a *fast* sliding or hopping mechanism. In any case, it would be difficult to imagine a *fast* sliding for a complex with intercalated residues, which are assumed to be essential in the interaction of gene 5 protein with polynucleotides. Fast sliding would be a reasonable mechanism for a "precomplex" without inserted aromatic groups. However, the rearrangement of cooperative clusters is faster than expected according to Monte Carlo simulations.

Our experimental data indicate that gene 5 protein is translocated with an effective time constant of approximately a second. In the absence of any translocation mechanism the time required for saturation of the polymer lattice could be very long [more than 5 min for a poly(dT) lattice; even with an excess of gene 5 protein]. Thus, the translocation mechanism is probably very useful to protect the polynucleotide chain and to support fast processing of the genetic message.

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Registry No. Lys-Tyr-Lys, 35193-18-1; Lys-Trp-Lys, 38579-27-0; poly(C), 30811-80-4; poly(dA), 25191-20-2; poly(A), 24937-83-5; poly(U), 27416-86-0; poly(dT), 25086-81-1.

References

Alberts, B., Frey, L., & Delius, H. (1972) *J. Mol. Biol. 68*, 139–152.

Alma, N. C. M., Harmsen, B. J. M., Van Boom, J. H., Van der Marel, G., & Hilbers, C. W. (1982) *Eur. J. Biochem. 122*, 319–326.

Anderson, R. A., Nakashima, Y., & Coleman, J. E. (1975) *Biochemistry 14*, 907–917.

Arndt-Jovin, D. J., Jovin, T. M., Bähr, W., Frischauf, A. M., & Marquardt, M. (1975) *Eur. J. Biochem. 54*, 411–418.

Berg, O. G., Winter, R. B., & von Hippel, P. H. (1981) *Biochemistry 20*, 6929–6948.

Brun, F., Toulme, J. J., & Helene, C. (1975) *Biochemistry 14*, 558–563.

Cavalieri, S. J., Neet, K. E., & Goldthwait, D. A. (1976) *J. Mol. Biol. 102*, 697–711.

Coleman, J. E., & Armitage, I. M. (1978) *Biochemistry 17*, 5038–5045.

Day, L. A. (1973) *Biochemistry 12*, 5329–5339.

Diekmann, S., Hillen, W., Morgeneyer, B., Wells, R. D., & Pörschke, D. (1982) *Biophys. Chem. 15*, 263–270.

Epstein, I. R. (1979) *Biopolymers 18*, 2037–2050.

Flossdorf, J., & Schillig, H. (1979) *F&M, Feinwerktech. Messtech. 87*, 93–96.

Glazer, A. N., Delange, R. J., & Sigman, D. S. (1975) in *Chemical Modifications of Proteins* (Work, T. S., & Work, E., Eds.) Elsevier/North-Holland, New York, Amsterdam, and Oxford.

Gray, D. M., Gray, C. W., & Carlson, R. D. (1982) *Biochemistry 21*, 2702–2713.

Kowalczykowski, S. C., Bear, D. G., & von Hippel, P. H. (1981) *Enzymes, 3rd Ed. 14*, 373–444.

Krauss, G., Sindermann, H., Schomburg, U., & Maass, G. (1981) *Biochemistry 20*, 5346–5352.

Lohman, T. M., & Kowalczykowski, S. C. (1981) *J. Mol. Biol. 152*, 67–109.

Manning, G. S. (1978) *Q. Rev. Biophys. 11*, 179–246.

McGhee, J. D., & von Hippel, P. H. (1974) *J. Mol. Biol. 86*, 469–489.

McPherson, A., Jurnak, F., Wang, A., Kolpak, F., Rich, A., Molineux, I., & Fitzgerald, P. (1980) *Biophys. J. 32*, 155–170.

Newport, J. W., Lonberg, N., Kowalczykowski, S. C., & von Hippel, P. H. (1981) *J. Mol. Biol. 145*, 105–121.

O'Connor, T. P., & Coleman, J. E. (1982) *Biochemistry 21*, 848–854.

Oey, J. L., & Knippers, R. (1972) *J. Mol. Biol. 68*, 125–138.

Okazaki, T., & Kornberg, A. (1964) *J. Biol. Chem. 238*, 259–268.

Peterman, B. F. (1979) *Anal. Biochem. 93*, 442–444.

Pörschke, D. (1979) *Biophys. Chem. 10*, 1–16.

Pörschke, D. (1980) *Nucleic Acids Res. 8*, 1591–1612.

Pörschke, D., & Eggers, F. (1972) *Eur. J. Biochem. 26*, 490–498.

Pörschke, D., & Ronnenberg, J. (1981) *Biophys. Chem. 13*, 283–290.

Pörschke, D., & Labuda, D. (1982) *Biochemistry 21*, 53–56.

Pretorius, H. T., Klein, M., & Day, L. A. (1975) *J. Biol. Chem. 250*, 9262–9269.

Record, M. T., Anderson, C. F., & Lohman, T. M. (1978) *Q. Rev. Biophys. 11*, 103–178.

Rigler, R., Rabl, C. R., & Jovin, T. M. (1974) *Rev. Sci. Instrum. 45*, 580–588.

Schwarz, G. (1972) *Ber. Bunsenges. Phys. Chem. 76*, 373–468.

Tanford, C. (1961) *Physical Chemistry of Macromolecules*, Wiley, New York.

Torbet, J., Gray, D. M., Gray, C. W., Marvin, D. A., & Siegrist, H. (1981) *J. Mol. Biol. 146*, 305–320.

Tyaglov, B. V., Naidenova, N. M., Minaev, V. E., & Permogorov, V. I. (1980) *Mol. Biol. (Engl. Transl.) 14*, 814–819.

Veiko, N. N., Gromova, E. S., Sigalov, A. B., & Shabarova, Z. A. (1981) *Mol. Biol. (Engl. Transl.) 14*, 814–819.

von Smoluchowsky, M. (1916) *Phys. Z. 17*, 585–599.