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THE BINDING OF ARG- AND LYS-PEPTIDES TO SINGLE STANDED POLYRIBONUCLEOTIDES AND ITS EFFECT ON THE POLYMER CONFORMATION

Dietmar PORSCHKE

Max-Planck-Institut fur biophysikalische Chemie, 34 Gottingen, Fed. Rep. Germany

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The interactions between basic oligopeptides (Lys_2, Lys_3, Arg_2 and Arg_3) and single stranded polynucleotides (poly(A), poly(C), poly(I) and poly(U) were investigated at low ion concentration by UV spectroscopy, circular dichroism and field jump relaxation. Various domains of binding were detected: 1) High concentrations (up to 1 mM) of some peptides induce opalescence followed by coacervation. Arg_3 causes coacervation in all polynucleotides used, yet Lys_3 only in poly(I). In the case of poly(I) the threshold concentration for coacervation is much lower for Arg_3 (150 μM) than for Lys_3 (500 μM). 2) Medium concentrations (>10 μM) of Arg_3 and Lys_3 induce helix formation in poly(U). In the case of poly(I) cooperative helix formation is only induced by Lys_3, but not by Arg_3. 3) The onset of peptide association is observed at very low peptide concentrations ($\geq$1 μM) already by using the field jump method. The association is reflected by a relaxation process, that can be described by a single exponential within experimental accuracy. Measurements of relaxation time constants as a function of the peptide concentration provide information on the association constants K, the number of nucleotide residues per binding place n and the rate constants k_R and k_D. Using a simple model with independent and "separate" binding sites, K for Arg_3 and Lys_3 is found to be in the range of 10^6 to 10^7 M^{-1}. In the case of Arg_2 and Lys_2 K is lower by a factor of about 10. For various polynucleotides K_{Arg_3} is only slightly higher than K_{Lys_3}, except in the case of poly(I), where $K_{Arg_3}/K_{Lys_3} \approx 5$. Similar data are obtained by application of a "sphere model" (see below). These results provide quantitative evidence for specific hydrogen bonding between the guanidino group of Arg and inosine. They also explain the absence of helix formation for poly(I) + Arg_3: Arg blocks the hydrogen bonding sites of inosine. Thus cooperative coupling leads in this case to a considerable amplification of specificity in the peptide-polynucleotide interaction.

Both field jump and stopped flow data demonstrate a high mobility of the peptide ligands along the polymer, resulting in a redistribution being fast compared with the overall binding step. Based on this result the relaxation data are analysed by a "sphere" model, which considers a) excluded binding under the condition of fast ligand distribution along the lattice and b) the connection of sites into a polymer sphere. The rate constants obtained by this model are in the range of 4×10^{11} M^{-1} s^{-1}. These high values reflect the large reaction distance for polymers of chain lengths around 1000. A comparison with rate constants obtained previously for oligomer complexes indicates that the recombination rate is approximately a function of the square root of the nucleotide chain length, which is directly related to the mean radius of coiled polymers.

1. Introduction

The interaction between basic polypeptides and polynucleotides has frequently been studied as model systems for protein nucleic acid interactions in general [1]. Although these model systems have been quite useful, it is obvious that some types of problems cannot be solved conveniently by using polypeptide-polynucleotide models. This is mainly due to the fact that the complex formation between basic polypeptides, like polylysine and polyarginine, and polynucleotides is irreversible under most conditions and moreover leads to precipitation in many cases [2–4]. Under such conditions the possibilities for the derivation of thermodynamic or kinetic parameters are greatly restricted and any specificity of interaction can hardly be assessed quantitatively.

These difficulties can be avoided by selection of model systems with reversible reactions. Such model systems are characterised in the present investigation.

The reaction between simple basic oligopeptides and single stranded polyribonucleotides is described and analysed quantitatively. Most of the information, both on thermodynamics and kinetics of the reactions, is obtained by the field jump technique [5,6]. This technique is very useful for the characterisation of reactions, which are, at least partially, driven by electrostatic attraction. The approach is quite similar to that described previously for the case of Mg^{2+} condensation to single stranded polyribonucleotides [7]. A short electric field pulse is used to dissociate positively charged ligands from the polynucleotide chain, which has a high negative charge density due to its many phosphate residues. The reaction between the ligands and the polynucleotides can then be followed by recording the polynucleotide absorbance, since ligand binding usually facilitates stacking of the nucleotide bases. If the ligand binding is sufficiently strong, conditions can be selected such that the stacking reaction provides a fast indication of the ligand binding reaction [7]. Measurements of the concentration dependence of the relaxation time constants can then be used for the derivation of both kinetic and thermodynamic parameters.

2. Materials and methods

The polyribonucleotides poly(A), poly(C), poly(I) and poly(U) were obtained from Boehringer, Mannheim. These polymers were dialysed extensively against EDTA solutions in order to remove traces of bivalent ions. Sedimentation measurements yielded the following $S^0_{20,w}$ coefficients: poly(A): 8.9, poly(C): 7.5, poly(I): 10.9 and poly(U): 8.1. From these sedimentation coefficients the degrees of polymerisation were estimated [8] to be around 1000 for poly(A), 750 for poly(C), 1700 for poly(I) and 1000 for poly(U).

The peptides Arg_2, Arg_3, Lys_2 and Lys_3 were purchased from Bachem Feinchemikalien AG, Switzerland. Excess salt, Br^--ions etc. were removed by ion exchange chromatography. Mg^{2+} and Ca^{2+} contaminations were removed by paper chromatography on carefully washed papers. Finally, the pH of the peptide solutions was adjusted to 8.0 by addition of HCl and the peptide concentrations determined, after acidic hydrolysis, by ninhydrin according to the method described by Moore and Stein [9]. The concentrations of the peptides are given in mol oligomer units per liter, whereas the polynucleotide concentrations are given in mol monomer residues per liter. Most measurements were performed in 1 mM Tris-HCl pH 8.0, 50 μM EDTA. The field jump apparatus used in the present investigation was constructed by Grünhagen [6]. The optical detection system of the apparatus was modified in order to achieve an undisturbed, reliable operation with polarised light [7]. All the chemical relaxation effects were recorded using polarised light with the polarisation plane inclined at 54.74° with respect to the vector of the electric field. In most cases field effects were recorded at 248 nm, taking advantage of the strong light intensity of the mercury line. Field jump cells made from plexiglas were used with optical pathlengths of 10 and 20 mm and with electrode distances around 6 mm.

The time course of the transmission changes induced by electric field pulses were stored by a datalab DL 920 transient recorder. The relaxation curves were then displayed and directly compared with the signal of a relaxation function generator (C.R. Rabl, in preparation) using a two channel oscilloscope. Exact matching of the measured and the simulated curve led to the evaluation of relaxation time constants.

Stopped flow measurements were performed with a machine produced in the MPI. The construction allows exact thermostating and avoids any contact of the solutions with metal parts (except of a diaphragm made out of gold).

Unless specified otherwise the measurements reported in the present investigation were performed at 20°C.

3. Theoretical part

3.1. "Simple" model

The most "simple" model for ligand binding to polymers is as follows (cf. fig. 1):

a) The polymer is separated into binding sites. The total site concentration c_s^0 is given by

$$c_s^0 = c_p^0/n, \tag{1}$$

where c_p^0 is the total concentration of polymer residues and n the number of residues occupied by one ligand.

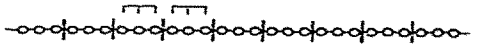

Fig. 1. Scheme of reaction sites according to the simple model. In this scheme it is not represented, that the simple model assumes uniform distribution of the (separated) reaction sites in the solution.

b) The sites are also assumed to be independent and the equilibrium constant is given by

$$K = c_c/c_s c_\ell, \tag{2}$$

where c_c, c_s and c_ℓ are the equilibrium concentrations of complexes, sites and ligands respectively.

The reaction of ligands ℓ with sites s to complex c

$$\ell + s \underset{k_D}{\overset{k_R}{\rightleftharpoons}} c \tag{3}$$

is described by the reciprocal relaxation time constant [5]

$$1/\tau = k_R(c_\ell + c_s) + k_D. \tag{4}$$

Under certain conditions this simple model can also be used, when the association step is followed by a conformation change:

$$\ell + s \underset{k_{12}}{\overset{k_{01}}{\rightleftharpoons}} c \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} c^*. \tag{5}$$

If the second step is fast compared to the first step, the reciprocal relaxation time constant obtained for the slow process is a linear function of $(c_\ell + c_s)$ [cf. ref. 5]. If such data are evaluated according to eq. (4), the apparent rate constants k_R and k_D are equivalent to

$$k_R = k_{01}, \tag{6}$$

$$k_D = k_{10}/(1 + k_{12}/k_{21}). \tag{7}$$

The equilibrium constant is given by

$$K = k_R/k_D = (k_{01}/k_{10})(1 + k_{12}/k_{21}) = K_1(1 + K_2) \tag{8}$$

Thus the K value obtained by this procedure is equivalent to that obtained by equilibrium titrations. The same arguments apply for the sphere model described in sect. 3.2.3.

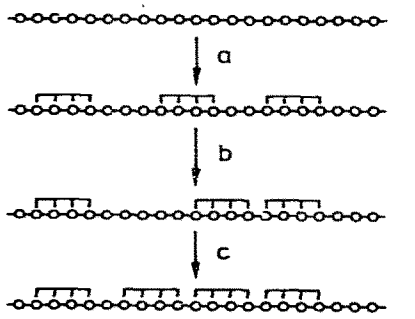

Fig. 2. Binding reactions of large ligands to a polymer lattice: a) Statistical binding of 3 ligands to a polymer segment resulting in a configuration excluding further direct ligand binding. b) Diffusion of a ligand along the polymer lattice. c) After rearrangement b a further ligand can be bound.

3.2. Kinetics of large ligand-polymer association

It has been shown by various authors [cf. ref. 10] that a simple model like that described in the previous section may lead to erroneous results for the binding of large ligands to polymers due to "excluded" binding phenomena (In the following "excluded" binding is used as a short term to designate the specific binding effects expected for large ligand binding to polymers as described e.g. in ref. [10]). Most of the investigations were concerned with the equilibrium theory of excluded binding, whereas the problems connected with the kinetics of excluded binding so far received very little attention. Since the data reported in the present investigation were mainly obtained by kinetic procedures, the influence of excluded binding phenomena upon the kinetics had to be accounted for. It will not be attempted to derive a complete theory of excluded binding kinetics. In the following a simple experimental test is described to explore the type of ligand binding. Then a relaxation equation derived for excluded binding kinetics by Jovin and Striker [11] is tested by numerical calculations. Finally a simple model is proposed which accounts also for "polymer sphere effects".

3.2.1. Kinetics at low ligand mobility

The kinetics of large ligand binding to a polymer will depend upon the mobility of the ligand along the polymer. Two limit cases may be considered: If the mobility of the ligand is low, i.e. if the rate of rear-

rangement of the ligand distribution on the polymer lattice is low compared with the rate of ligand binding to the polymer, two separate binding phases should be expected. In the first binding phase the ligands occupy the binding places at random, until binding places with the required number of lattice sites are not directly available any more. However, due to the particular nature of excluded binding more binding places can be produced by rearrangement of the ligands along the polymer (cf. fig. 2). Thus during the phase of rearrangement more ligands can be bound to the polymer. Model calculations by Epstein [12] demonstrate that the amplitude of this second binding phase may be up to 20% of the total amplitude. The absence or presence of a second binding phase may be used as a test for the mobility of the ligands along the polymer. The data described in the experimental section indicate that this ligand mobility is relatively high in the present model systems.

3.2.2. Kinetics at high ligand mobility

When the rearrangement of the ligand distribution at the polymer is a fast reaction compared with the overall binding step, the distribution of the ligands along the polymer chain will always be in equilibrium. For this case it is possible to derive a solution for the relaxation kinetics of excluded binding. According to Jovin and Striker [11] the reciprocal relaxation time $1/\tau$ is given by

$$1/\tau = k_R [\bar{c}_p - f\bar{c}_\ell] + k_D, \qquad (9)$$

with $\bar{c}_p$ being the equilibrium concentration of free binding places according to the excluded binding model, $\bar{c}_\ell$ the free ligand concentration and

$$f = \frac{[nr(n-1) - 2n + 1](1-nr)^{n-1}}{[1-(n-1)r]^n}, \qquad (10)$$

where n is the number of residues covered by one ligand and r is the "binding density" in moles of bound ligand per mole of total lattice residue [10,11].

Since the derivation given by Jovin and Striker involves some approximation, one should like to test their result by an independent approach. This test was made by numerical calculations using a Runge Kutta procedure for the integration of the rate equation

$$dc_c/dt = k_R c_\ell c_p - k_D c_c, \qquad (11)$$

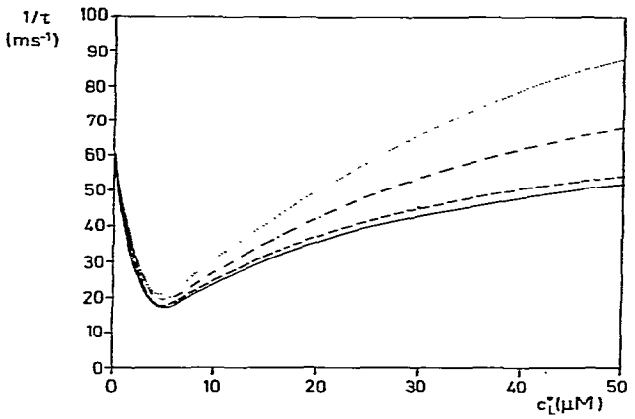

Fig. 3. Reciprocal relaxation time constants as a function of the total ligand concentration according to the excluded binding model given by Jovin and Striker in the limit of zero perturbation (—) and calculated by a Runge Kutta procedure at 1% (– –), 5% (– . –) and 10% (. . . .) perturbation ($K = 10^6\ M^{-1}$, $k_R = 2 \times 10^9\ M^{-1}\ s^{-1}$, $n = 5$, $c_p^0 = 30\ \mu M$).

where c_c is the concentration of the peptide-nucleotide complex. The concentration of free binding places c_p at any time and at any binding density r is calculated according to the equilibrium theory [10]. Thus it is assumed that the equilibrium distribution of ligands along the polymer chain is always maintained. This condition requires a high mobility of ligands along the polymer. The complete equation used in the Runge Kutta integration is

$$\frac{dc_c}{dt} = k_R(c_\ell^0 - c_c)c_p^0(1-nr)\left[\frac{1-nr}{1-nr+r}\right]^{n-1} - k_D c_c, \qquad (12)$$

where $c_\ell^0 (c_p^0)$ is the total concentration of peptide ligands (nucleotide residues). Runge Kutta integrations of eq. (12) were performed for various conditions. In each case the time dependence of c_c was fitted by single exponentials. In most cases the Runge Kutta curves could be fitted by single exponentials without any serious deviation. The time constants obtained by this procedure closely agreed with those obtained according to eq. (9) as long as the perturbation of the bound ligand concentration remained below 1%. Higher perturbations resulted in time constants, which were clearly different from those expected according

to eq. (9). A graphical representation of reciprocal relaxation times calculated for various degrees of perturbation is given in fig. 3. These data demonstrate that an application of eq. (9) does lead to erroneous results when the perturbation is around 10%. Perturbations of this magnitude are usually considered to be sufficiently small in relaxation experiments to justify linearisation of rate equations. The relaxation experiments described in sect. 4.4. of the main paper involved perturbations of about 20%, in order to attain a sufficiently high signal to noise ratio. In the model system poly(U) + Lys_3 relaxation time constants were measured at various degrees of perturbation. Any systematic variation of the relaxation time as predicted by the model calculations described above has not been detected although a rather wide range of perturbations (estimated to be from 5 to 50%) was covered in these experiments. For comparison Runge Kutta integrations were also performed for the simple reaction model (corresponding to a usual second order reaction) described in sect. 3.1. In this case a perturbation of 20% led to a time constant, which was only 3% lower than that calculated from the linearised relaxation equation. For the same conditions ($c_L^0 = 30\ \mu M$, $c_P^0 = 30\ \mu M$; $K = 10^6\ M^{-1}$, $n = 5$, $k_R = 2 \times 10^9\ M^{-1}\ s^{-1}$) the time constant of excluded binding at 20% perturbation is lower by a factor of 2.2 than that obtained from eq. (9). Thus the calculated time constant of excluded binding at high ligand mobility is extremely sensitive to the extent of perturbation, whereas no comparable sensitivity is found for the simple model. In this respect the experimental data are consistent with the simple model.

Another problem has to be considered, when the excluded binding model is used in the form described by Jovin and Striker. If we consider the reaction of a ligand with a naked lattice, the theory predicts a relatively high probability of reaction due to the great number of possible attachment sites. The statistical factor resulting from these different reaction pathways is directly introduced into the recombination rate. This may lead to an overestimate of the recombination rate in the case of a diffusion controlled reaction in which the rate of complex formation is determined by the rate of diffusional encounter between the ligand and the sphere of the polymer. As soon as the ligand enters the sphere of the "naked" polymer lattice, complex formation will almost certainly occur. Thus in this case the size of the polymer sphere will be a more important factor for the recombination rate than the number of reaction sites in the sphere (cf. ref. [13]).

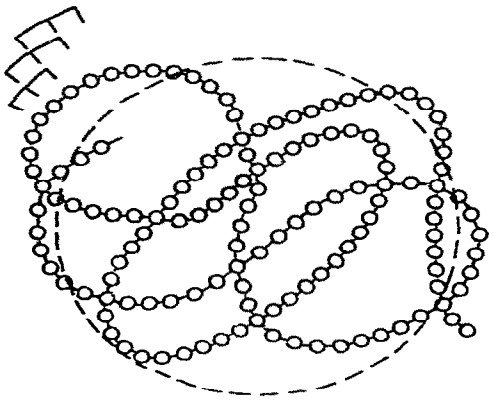

Fig. 4. Scheme of reaction sites according to the sphere model.

3.2.3. Ligand binding to a polymer sphere – an appropriate model

The rate of diffusion controlled reactions is determined by the frequency of diffusional encounters between the reaction partners. When electrostatic effects can be excluded the rate constant is according to Smoluchowsky [14] given by

$$k_R = 4\pi N_A r_D (D_A + D_B) \qquad (13)$$

where N_A represents Avogadro's number, r_D the reaction distance (closest approach at which spontaneous reaction occurs) and $D_A(D_B)$ are the diffusion coefficients of the reactants. Let us now consider the reaction of a ligand with a polymer chain containing N identical subunits, each of them being capable of binding one ligand without any interactions between adjacent sites. Usually the kinetics of such reactions is treated by defining a (uniform) concentration of binding sites without any consideration of the fact that these sites are tied together into polymer blocks. According to this simple model the binding rate is proportional to the concentration of binding sites and the degree of polymerisation does not appear in the late law. Using the equation derived by Smoluchowsky the simple model would correctly describe the rate

of recombination if the term $r_D(D_A + D_B)$ would linearly be related to the chain length N. It is obvious, however, that the diffusion coefficient decreases upon polymerisation. The reaction distance r_D will increase with the degree of polymerisation, but usually not directly with N. If we consider a polymer in a random coil conformation, the mean radius increases with $N^{0.5}$ in the case of a Gaussian chain or with $N^{0.6}$ if excluded volume effects are considered [8,15]. These relations demonstrate that the models usually applied for ligand binding to polymers may lead to erroneous results. Such effects will be especially important in the case of excluded binding, where the high number of binding sites predicted at low degrees of binding may lead to an overestimate of the forward reaction rate.

In the following a model is proposed, which considers the effect of polymerisation as well as excluded binding effects in a simplified manner. The polymer is represented by a reaction sphere (cf. fig. 4). At low degrees of binding practically every ligand approaching the polymer sphere will be bound. When many of the binding sites are occupied already, the probability of binding will decrease. The critical step is now to find an appropriate weighting function. In terms of eq. (13) the reaction distance r_D has to be defined as a function of the degree of binding. A completely satisfactory solution of this problem is not yet available. It seems that in any case some simplification has to be introduced. A simple assumption would be, that r_D is proportional to the number of free binding places as defined by the excluded binding model. However, this will certainly overestimate the variation of r_D with the degree of binding. In this respect the fraction of free polymer residues $(1 - \theta)$ seems to be a more satisfactory weighting function. In the case of excluded binding $(1 - \theta)$ does not decrease as much as the relative number of free binding places, when the lattice is being occupied by ligands. This gradual decrease is expected to reflect the change in the reaction distance more accurately. According to the weighting function $(1 - \theta)$ the recombination rate is proportional to the probability that a ligand hits any free lattice residue. This function seems to be appropriate for the case of high ligand mobility along the polymer chain. Using this simple approach the rate of complex formation is given by

$$k_R c_l c_p^\infty (1 - \theta),$$

where c_p^∞ is the total concentration of polymer molecules. When a ligand is bound to the polymer the probability of dissociation at a given site is defined by the intrinsic rate constant of dissociation. However, the probability to escape from the polymer sphere will also depend upon the degree of binding. When the lattice is empty ($\theta = 0$), any ligand dissociated from a given site will be bound to another site with a rather high probability, because the local concentration of free sites is very high. This effect will lead to a relatively low rate of dissociation from the polymer sphere at low θ. On the contrary high θ-values will result in a relatively high probability of ligand dissociation from the polymer sphere, since a ligand dissociation from a given site will rarely hit an empty site in the polymer sphere. This dependence of the overall rate of dissociation upon θ must not be calculated explicitly. For the present simplified approach it is sufficient to define that forward and backward rates at equilibrium should be consistent with the excluded binding model. Since the expression for the forward rate has also been defined already, the term for the backward rate can simply be evaluated from the equilibrium parameters. The net production of complex is given by

$$dc_c/dt = k_R c_l c_p^\infty (1 - \theta) - k_D^\theta c_c, \qquad (14)$$

where k_D^θ is the overall rate constant of dissociation, which is a function of θ. At equilibrium $dc_c/dt = 0$ and thus

$$k_D^\theta = k_R c_p^\infty (\bar{c}_l/\bar{c}_c)(1 - \bar{\theta}), \qquad (15)$$

where the bars indicate equilibrium parameters. For small perturbations k_D^θ can be considered as constant. Using this approximation the relaxation time can be calculated from eq. (14) by application of the standard procedure

$$1/\tau = k_R [\bar{c}_l (n/N) + c_p^\infty (1 - \bar{\theta})] + k_D^\theta. \qquad (16)$$

The calculated dependence of $1/\tau$ upon the ligand concentration is compared for the various models in fig. 5. A Runge Kutta integration using eq. (14) confirmed that the time constant obtained does not exhibit a strong dependence upon the degree of perturbation (cf. discussion in the previous section).

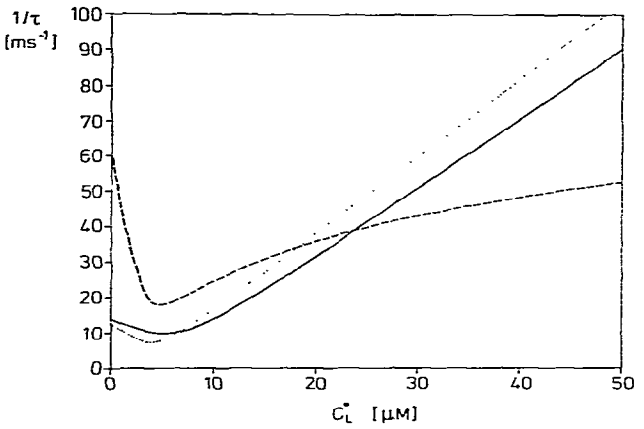

Fig. 5. Reciprocal relaxation time constants as a function of the total ligand concentration according to the simple model (—), to the excluded binding model given by Jovin and Striker (– –) and to the sphere model (. . . .). (Parameters are as defined for fig. 3; in the case of the sphere model $k_R \cdot n/N = 2 \times 10^9\ M^{-1}\ s^{-1}$).

Table 1
Opalescence effect and helix formation induced by Arg_3 and Lys_3 in various polynucleotides. (1 mM Tris pH 8, 50 μM EDTA, polynucleotide concentration ~100 μM).

Poly-nucleo-tide	Oligo-peptide	lowest concen-tration at which opales-cence was ob-served (μM)	opales-cence disappears at tem-perature (°C)	helices induced
poly(A)	Arg_3	200	~78	–
poly(I)	Arg_3	150	~80	–
poly(C)	Arg_3	400	~36	–
poly(U)	Arg_3	400	~44	+
poly(A)	Lys_3	>1000	–	–
poly(I)	Lys_3	500	~35	+
poly(C)	Lys_3	>1000	–	–
poly(U)	Lys_3	>1000	–	+

4. Results

4.1. Opalescence and coacervation effects

Opalescence or coacervation is frequently observed, when polyarginine or polylysine are added to polynucleotide solutions [2,4,16]. As may be expected arginine- and lysine-oligomers do not cause opalescence or coacervation as readily as the corresponding polymers. In general a great excess of oligopeptide relative to the polynucleotide is required to induce opalescence. The most sensitive way to detect opalescence effects is the measurement of absorbance. Addition of peptide up to a concentration above the opalescence threshold leads to a usually slow increase of the absorbance also at wavelengths $\lambda > 300$ nm outside the absorbance range of the polynucleotides or peptides. This increased absorbance reflects the opalescence, which is also directly visible. Usually the opalescent particles coagulate after some time and form coacervates.

The opalescence phenomena were studied at various temperatures by recording absorbance-temperature profiles. These measurements led to a rough characterisation of opalescence domains. The results are compiled in table 1. In the case of Arg_3 the lowest peptide concentration that induced opalescence was between 150 and 400 μM depending upon the polynucleotide. In contrast to Arg_3, Lys_3 was observed to induce opalescence only in poly(I) solutions. Even in this case the threshold concentration is much higher for Lys_3 than for Arg_3. Thus, under our experimental conditions Arg_3 causes opalescence clearly more effectively than Lys_3.

In all the systems investigated the opalescence disappeared upon heating in a defined temperature range, which covered usually 10 to 20°C. The temperatures at the midpoint of the transition opalescent to clear solution are given in table 1. It should be added that the polynucleotides listed in table 1 did not exhibit opalescence effects in the presence of the dipeptides Arg_2 and Lys_2.

4.2. Cooperative helix formation

In some polynucleotides addition of peptides induced the formation of structures, which exhibited cooperative melting transitions. The observation of such a transition in a narrow temperature range was used as an indication for the melting of an ordered, multistranded, helical structure.

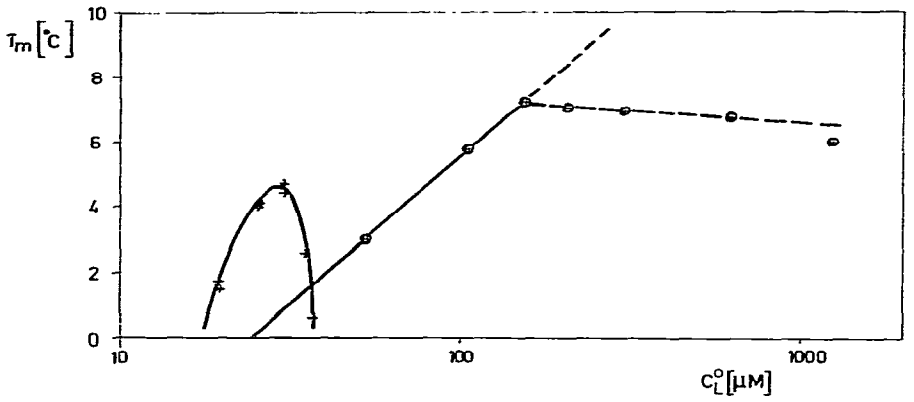

Fig. 6. T_m of melting transitions observed in poly(U) induced by Arg_3 (+) and by Lys_3 (o) as a function of the peptide concentration (in 1 mM Tris, 50 μM EDTA at pH 8).

4.2.1. poly(U)

A strongly cooperative transition in poly(U) was observed both in the presence of Arg_3 and Lys_3, under conditions where opalescence effects were not detected. Apparently the ordered structure of poly(U) is equivalent to the one formed at high salt concentration [17,18]. This conclusion is supported by the close similarity of the CD-spectra with respect to shape and peak heights observed at the low temperature end of the transitions in the presence of Arg_3, Lys_3 and high salt like 0.5 M CsCl [18]. Although the helical structures induced by Arg_3 and Lys_3 appear to be similar, the dependence of the melting temperature upon peptide concentration is quite different. With Arg_3 a peptide concentration of 20 μM is sufficient to induce helix formation at low temperatures ($T_m \approx 2°C$). A maximum in T_m of about 46°C is observed at a peptide/nucleotide ratio P/N ≈ 0.33. The T_m is again below 0°C at P/N ≈ 0.5 (cf. fig. 6). In the case of Lys_3 a higher peptide concentration is required to induce helix formation. Upon increase of the Lys_3 concentration the T_m approaches a limit value around 7°C, which remains almost constant, when the peptide concentration is increased further (cf. fig. 6).

Melting experiments were also conducted in a buffer containing 10 mM NaCl, 1 mM Tris pH 8.0, 50 μM EDTA. Under these conditions of increased ionic strength higher peptide concentrations were required to induce helix formation: e.g. at 250 μM Lys_3 the T_m was 1.3°C in the presence of 10 mM NaCl, whereas the T_m was 6.9°C in the absence of NaCl. Thus an increase of the ionic strength lead to a decrease of the T_m values. This unusual result may be explained on the basis of the polyelectrolyte theory developed by Manning [19].

4.2.2. poly(I)

Cooperative transitions were also observed in poly(I), when Lys_3 was added in a concentration range between 100 and 1000 μM. The midpoint of the transitions were found in the temperature range between 32 and 28°C, with a slight decrease of the melting temperature upon increase of the Lys_3-concentration. The CD spectrum of the ordered form (at 0°C minimum at 247 nm: $\epsilon_L - \epsilon_R = -4.3\ M^{-1}\ cm^{-1}$; maximum at 252.5 nm: $\epsilon_L - \epsilon_R = 3.9\ M^{-1}\ cm^{-1}$) is similar to that reported for the multistranded helix form observed at high salt concentration [20]. The formation of the ordered structure upon addition of peptide is a rather slow process, which takes hours under the conditions of our experiments. Relatively low rates of helix formation in poly(I) were also observed at high ionic strengths [20]. The low rates may be partly responsible for the fact that the melting transition is not reversible. The main reason for this irreversibility seems to be a precipitation reaction which is observed in the temperature range of 30 to 40°C. Thus mixing of poly(I) and Lys_3 at low temperatures preferentially leads to the formation of a multistranded helix structure, whereas cooling of the same mixture from temperatures above the melting transi-

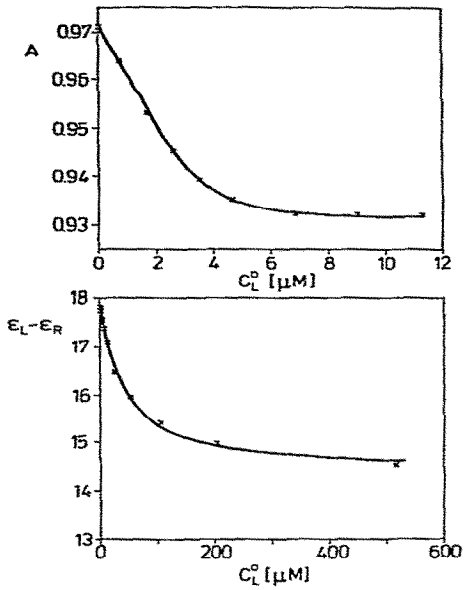

Fig. 7. a) Absorbance at 260 nm, b) CD amplitude [M^{-1} cm^{-1}] at 264 nm of poly(A) as a function of the Lys_3 concentration in 1 mM Tris, 50 μM EDTA pH 8 (c_P^0 = 97 μM). The solid line represents a fit according to the simple model.

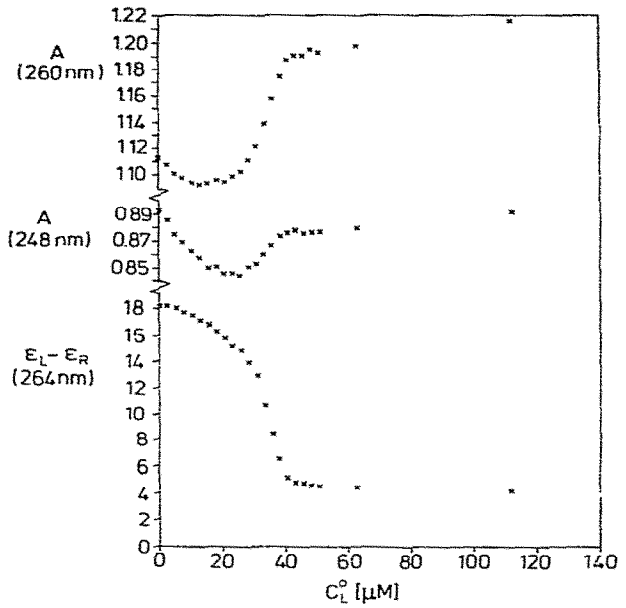

Fig. 8. Absorbance (248 and 260 nm) and CD amplitude (in M^{-1} cm^{-1} at 264 nm) of poly(A) as a function of the Arg_3 concentration in 1 mM Tris, 50 μM EDTA pH 8 (c_P^0 = 111 μM).

tion preferentially leads to precipitation. None of the experiments performed with mixtures of poly(I) and Arg_3 at various Arg_3 concentrations ranging from 10 to 1000 μM provided any evidence for the formation of a multistranded helical structure. Thus the two peptides Arg_3 and Lys_3 exhibit quite different properties with respect to helix formation in poly(I).

4.3. Equilibrium titrations at low peptide concentrations

The experiments described in the following sections were designed to evaluate quantitative parameters associated with the binding of basic peptides to single stranded polynucleotides in a peptide concentration range where complications due to opalescence or multistrand helix formation can be excluded. A relatively simple approach for the evaluation of binding constants is the measurement of absorbance- or CD-spectra as a function of the ligand concentration. This approach provided useful quantitative information in the case of Lys_3. An example for a UV-titration curve (polyA + Lys_3) is given in fig. 7a.

The absorbance changes are relatively high and can be measured accurately. Furthermore a rather well defined limit value of the absorbance is approached at high ligand concentrations. In this case it is possible to evaluate binding parameters from the titration curve (for a procedure cf. ref. [7]).

When the poly(A) + Lys_3 titration is carried out under the same conditions, but the process is followed by measurements of the circular dichroism (cf. fig. 7b), quite a different result is obtained: the concentration of Lys_3 required to induce changes in the CD spectrum of poly(A) is clearly higher than that required for the changes in the UV spectrum. If the variation of the CD spectrum as a function of the Lys_3 concentration is fitted according to the simple binding model, an association constant of 2×10^4 M^{-1} is derived. The corresponding constant obtained from UV measurements is around 6×10^6 M^{-1}. Thus there appear to be two different binding processes, one of them being reflected mainly in the UV absorbance and the other one reflected by the CD spectrum.

Even more complex titration data were obtained

Table 2
Binding constants K and number of nucleotide residues per binding site n obtained from UV-titration curves for the interaction of Lys_3 with various polynucleotides. The data are mean values obtained from 3 titrations evaluated according to both the simple and the excluded binding model (1 mM Tris pH 8, 50 μM EDTA); accuracy ±20%.

	$K[M^{-1}]$ "simple"	$K[M^{-1}]$ "excluded"	n "simple"	n "excluded"
poly(A)	6.0×10^6	7.6×10^6	5.9	4.8
poly(I)	1.4×10^6	1.8×10^6	2.9	2.4
poly(C)	3.0×10^6	2.3×10^6	6.4	5.1

when Arg_3 was added to single stranded polynucleotides. The titration curves measured in the case of poly(A), for example, clearly demonstrate that there is more than one binding phase (cf. fig. 8): 1) the first phase is characterised by a decrease of the absorbance at 248 nm, yet very little change in the CD-spectrum. 2) in the second phase the absorbance at 248 nm increases again, whereas the CD amplitude of the first peak around 265 nm decreases considerably. 3) Further increase of the Arg_3 concentration finally leads to opalescence (not shown in fig. 8). Obviously it is not simple to extract quantitative information from these titration data. The sigmoid shape of the CD titration curve seems to indicate some cooperativity in the binding process. Apparently the first phase of binding is not yet accompanied by gross changes of the poly(A) conformation, whereas a considerable change of conformation seems to be induced in the second phase.

Unfortunately it is not possible to compare UV and CD titration curves for all the other polynucleotide–oligopeptide combinations. The changes in the CD spectra of poly(C), poly(I) and poly(U) upon addition of the basic peptides are rather small and cannot be measured accurately enough. The UV titration curves obtained with Arg_3 and various polynucleotides did not yield a well defined limit value of the absorbance at high peptide concentrations and thus could not be used to extract quantitative information. UV titration curves performed with Lys_3 were more useful: the results of their quantitative analysis are given in table 2.

The ligands used in the present investigation cover more than one site of the polymer lattice. Thus the simple model used in the evaluation of Lys_3 titration curves may lead to erroneous results, since excluded binding phenomena (cf. ref. [10]) may have some influence on the parameters evaluated. In order to test for the influence of the model the titration curves were also evaluated according to the excluded binding model. In both cases the evaluation is based upon the assumption that the change in absorbance is linearly related to the degree of binding. Any cooperative effects were not considered. The parameters obtained according to the two models are only slightly different. (cf. table 2). Thus the influence of excluded binding phenomena is rather small in the case of the present equilibrium titrations.

Titration experiments were also conducted with the dipeptides Arg_2 and Lys_2. However, the results could not be used for any quantitative evaluation, since the limit value of the absorbances at high concentrations could not be defined with sufficient accuracy.

4.4. Relaxation measurements (at low peptide concentrations)

The most direct source of information on the peptide–polynucleotide interaction is provided by the field jump method. The typical relaxation effects induced by field jumps in single stranded polynucleotides have been described previously [7]. In the present investigation all experiments were conducted in 1 mM Tris-HCl-buffer pH 8.0. As shown previously [7] single stranded polynucleotides exhibit a relaxation effect with a time constant $\tau \leqslant 1$ μs in the presence of this buffer. When peptides like Lys_2 or Lys_3 are added to the polynucleotide solutions, an extra relaxation effect is observed, with a time constant $\tau \gg 1$ μs (as long as the peptide concentration remains low). In all cases studied in the present investigation the amplitude of the extra relaxation effect was big enough for an accurate evaluation of the time constant τ. A typical relaxation curve is given in fig. 9. In all cases the relaxation effect associated with peptide binding could be represented by single exponentials. Measurements at various peptide concentrations revealed a characteristic concentration dependence of the relaxation time constants (cf. figs. 10,11).

For a quantitative evaluation of these data we have to consider in a first step all the components of the systems and their reactions. The solutions contain

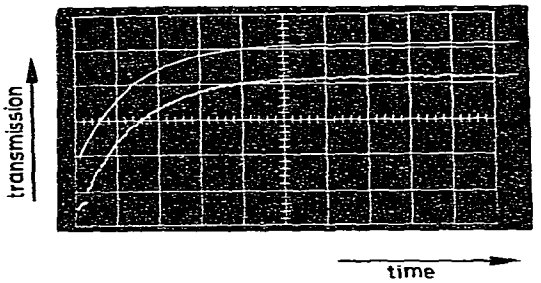

Fig. 9. Field jump relaxation in poly(I) + Arg_3 observed at the end of the field pulse (28 kV, 110 μs, $c_P^0 = 40.4$ μM, $c_\ell^0 = 8.6$ μM, $\lambda = 248$ nm, time scale: 200 μs per big scale unit, transmission scale: 20 mV per big scale unit at 2750 mV total signal). The lower curve is the measured signal, the upper curve is simulated to fit the lower one.

three different components: 1) polynucleotides, 2) peptides and 3) buffer ions. Various reactions between these components are induced by electric fields: a) the tris-ions of the buffer dissociate from the negatively charged polynucleotide chain; b) reaction a increases the electrostatic repulsion between phosphate residues and thus leads to unstacking of the bases; c) the positively charged peptides are also removed from the polynucleotides; d) reaction c increases electrostatic repulsion and leads to unstacking. The reaction a and c are induced by a dissociation field effect [21]. The net reactions a to d are observed, when electric fields are turned on, whereas the opposite net reactions proceed when the field is turned off. The parameters of reaction d need not be equivalent to those of reaction b, since the mode of peptide binding may be different from the mode of monovalent ion association, which may result in a different stacking reaction.

For the purpose of the present investigation it is not necessary to consider all the different reaction steps explicitly. It is obvious from the experiments that the reactions a and b are fast relative to reaction c, as long as the peptide concentration remains below a certain limit. Furthermore it is rather unlikely that binding of the peptide ligands leads to changes of the stacking rate parameters by an order of magnitude. Thus it can be safely assumed that at low peptide concentrations reaction d is also fast with respect to reaction c. Under these conditions all the reactions a, b and d are fast preequilibra and the slow relaxation process associated with reaction c may be described according to the simple reaction scheme (3) or according to the sphere model. Thus the stacking reaction is used as an inherent fast indication system. The shift in the stacking equilibrium due to peptide binding is not considered explicitly in the reaction scheme (3) and also not in the sphere model. Any contribution due to a change of the stacking equilibrium upon peptide binding will be reflected in the k_D-value, as described in sect. 3.1.

All the parameters required, the rate constants k_R and k_D as well as the number of nucleotide residues per binding place n, can be determined from the concentration dependence of the relaxation time. A typical concentration dependence of the reciprocal relaxation time is given in fig. 10. The total polynucleotide concentration c_P^0 is kept constant and the relaxation time measured at various peptide concentrations c_ℓ^0. At low peptide concentrations the relaxation time first remains at an almost constant level. The peptide concentration has to be increased above a certain limit, before a further increase in the peptide concentration leads to a clear rise of $1/\tau$. The basis for these results is as follows:

At low peptide concentrations an increase in c_ℓ^0 does not lead to an increase of the free reactant concentration $c_\ell + c_s$, because most of the peptide is

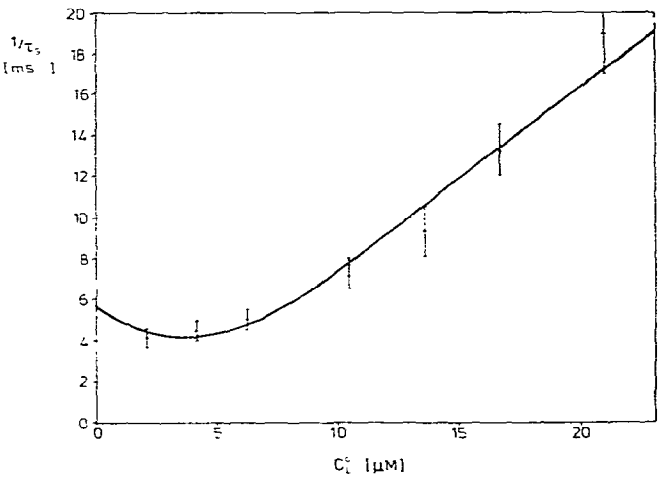

Fig. 10. Reciprocal relaxation time $1/\tau$ in poly(U) as a function of the total Lys_3 concentration c_ℓ^0 ($c_P^0 = 21.0$ μM, 1 mM Tris pH 8.0, 50 μM EDTA). The solid line represents the fit according to the "simple" model.

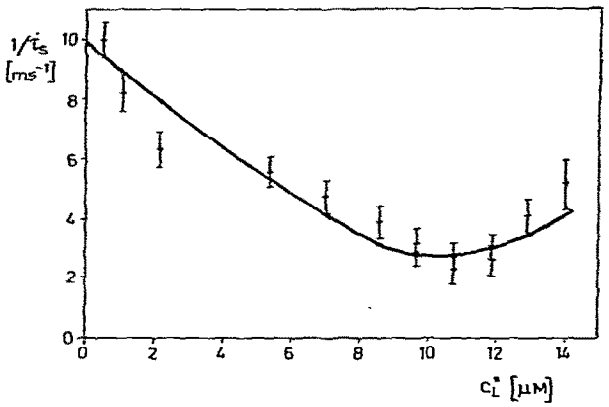

Fig. 11. Reciprocal relaxation time $1/\tau$ in poly(I) as a function of the total Arg_3 concentration c_Q^0 (c_P^0 = 40.4 μM; 1 mM Tris pH 8.0, 50 μM EDTA). The solid line represents the fit according to the "simple" model.

bound to the polymer, resulting in a decrease of the free site concentration c_s. However, as soon as c_Q^0 exceeds the concentration of binding places, an increase of c_Q^0 induces an increase in $1/\tau$. If the binding affinity of the peptide to the polynucleotide is sufficiently high, plots of $1/\tau$ as a function of c_Q^0 show a clear minimum. An example for such a case is given in fig. 11.

Thus the experimental data (cf. figs. 10 and 11) may be used for the evaluation of parameters in the following manner: 1) the number of binding places per nucleotide residue n is defined by the plateau range or by the position of the minimum in $1/\tau$; 2) the rate constant of recombination k_R can be obtained from the slope of $1/\tau$ at high c_Q^0 values and 3) the rate constant of dissociation k_D can be obtained from the low level of $1/\tau$. In the present investigation these parameters were fitted to the experimental data by a least squares procedure using both the simple and the sphere model. The accuracy of the fits for the various data sets were quite similar for the two models. The results are presented in terms of $K = k_R/k_D$, n and k_R (cf. tables 3 and 4).

4.5. Stopped flow experiments

The field jump experiments revealed only single relaxation processes associated with peptide binding in almost all systems. Due to the limited pulse lengths applicable in field jump experiments it cannot be excluded that some slow processes may have escaped our notion. Thus stopped flow experiments were performed to look for slow processes that may be associated with ligand rearrangement on the polymer. In these experiments the polynucleotides were mixed with the peptides, such that a high degree of binding was achieved. Slow effects were not observed in the system poly(A), poly(U) and poly(C) with the ligands Arg_3 and Lys_3. Some indication for the existence of a slow process in the time range around 50 s was found for the reaction of poly(I) with Arg_3 and Lys_3. Since

Table 3
Thermodynamic and kinetic parameters for peptide-polynucleotide interactions. Fitting of relaxation data according to the simple model. (in 1 mM Tris pH 8, 50 μM EDTA; estimated accuracy K: ±20%, n: ±30%, k_R: ±15%).

		K (M^{-1})	n	k_R ($M^{-1}\,s^{-1}$)
poly(A)	Arg_2	4.0×10^5	7	1.6×10^9
poly(A)	Lys_2	2.5×10^5	5.6	1.5×10^9
poly(A)	Arg_3	1.0×10^7	9.7	4.1×10^9
poly(A)	Lys_3	8.0×10^6	8.4	2.5×10^9
poly(C)	Arg_2	5.0×10^5	5.1	1.6×10^9
poly(C)	Lys_2	3.0×10^5	2.7	1.9×10^9
poly(C)	Arg_3	4.4×10^6	10.0	4.5×10^9
poly(C)	Lys_3	3.5×10^6	6.0	3.7×10^9
poly(I)	Arg_3	5.0×10^6	3.8	0.91×10^9
poly(I)	Lys_3	1.1×10^6	5.1	0.94×10^9
poly(U)	Arg_3	1.4×10^6	6.2	1.5×10^9
poly(U)	Lys_3	1.1×10^6	4.4	0.97×10^9

Table 4
Thermodynamic and kinetic parameters for peptide polynucleotide interactions. Fitting of relaxation data according to the sphere-model (conditions and accuracy cf. table 3).

		K (M^{-1})	n	k_R ($M^{-1}\,s^{-1}$)
poly(A)	Arg_2	1.5×10^5	4.2	3.2×10^{11}
poly(A)	Lys_2	1.4×10^5	3.1	4.2×10^{11}
poly(A)	Arg_3	5.4×10^6	7.7	4.4×10^{11}
poly(A)	Lys_3	4.1×10^6	6.6	2.6×10^{11}
poly(C)	Arg_2	3.3×10^5	3.7	2.9×10^{11}
poly(C)	Lys_2	2.8×10^5	2.2	6.1×10^{11}
poly(C)	Arg_3	6.3×10^6	6.5	5.1×10^{11}
poly(C)	Lys_3	2.2×10^6	4.9	4.5×10^{11}
poly(I)	Arg_3	1.0×10^7	3.3	4.1×10^{11}
poly(I)	Lys_3	6.4×10^5	3.9	3.4×10^{11}
poly(U)	Arg_3	6.5×10^5	4.5	2.8×10^{11}
poly(U)	Lys_3	6.4×10^5	3.3	2.6×10^{11}

the amplitude of this process was very small, it was not possible to characterise it in detail. Apparently this process is associated with some conformation change in poly(I), since a similar reaction was found upon mixing of poly(I) with NaCl solutions. In summary, the stopped flow data do not provide evidence for a a slow rearrangement of the ligands on the polymer lattice of poly(A), poly(U) and poly(C). Further experiments are required in the case of poly(I).

4.6. pH dependence

Since the peptides used in the presence investigation have a free terminal amino group with pK-values around 7, some pH dependence of the peptide binding reaction is expected in the pH range around 7. The standard pH 8 used in the present investigation was selected in order to prevent any interference by double helix formation in poly(A) and poly(C) expected at lower pH values. Such complications do not exist in poly(U). Thus poly(U) was used as a model system to study the peptide binding reaction at pH 5.9, where the peptide is fully protonated. The procedures used in measurements and data evaluation were identical to those described above. The following results were obtained using the simple model: $K = 7.7 \times 10^6\ M^{-1}$, $n = 5.2$, $k_R = 2.5 \times 10^9\ M^{-1}\ s^{-1}$. Thus the stability constant found at pH 5.9 is higher by a factor of 7 than that obtained at pH 8. This increase is both due to a higher recombination rate and also due to a decrease of the dissociation rate.

5. Discussion

5.1. Experimental methods

The titration experiments described in sect. 4.3. demonstrate that the usual titration procedure does not provide satisfactory results for the present model systems in most cases. Thus the field jump data are the major source of information in the present investigation. Two types of information can be obtained from field jump experiments: a) amplitude(s), b) time constant(s). Amplitude data were not evaluated for the present model systems, since the rather slow relaxation observed in many cases would require relatively long field pulses to achieve true "equilibrium". These long field pulses cause some technical problems like heating of the solution and decay of the electric field. Thus most of the data presented are obtained from relaxation time constants. This procedure has the advantage that any interference by spectroscopic parameters is excluded, since the time constants are only dependent upon rate constants (i.e. also thermodynamic parameters) and concentrations.

5.2. Equilibrium parameters

In most cases the interaction between proteins and nucleic acids is mainly driven by strong electrostatic attraction of positively charged Lys- and Arg-residues with the negatively charged phosphate groups of the nucleic acids [1]. The characterisation of both thermodynamic and kinetic parameters for this type of interaction in simple model systems was the main goal of the present investigation. During the investigation the model systems turned out to be more complex than anticipated. The rather confusing titration curves obtained for the system poly(A) + Arg_3 may serve as an example. Various phases of binding are observed. One of them appears to be cooperative. However, there is no indication for the formation of any multistranded helical structure in this case. Some of these observations remain unexplained. Any detailed explanation will have to consider the fact that one of the reaction partners is a polyelectrolyte. Furthermore excluded binding effects [10] have to be accounted. To the knowledge of the author a theory which considers both the electrostatic effects of charged ligand binding to polyelectrolytes and excluded binding effects is not yet available.

In the present investigation the data are evaluated according to two different models. The application of the simple model may be expected to introduce some systematical error. However, the evaluation of the available equilibrium titration curves according to the simple and the excluded binding model do not give very different results (cf. table 2). Furthermore, the equilibrium titration data are quite similar to the results evaluated from the relaxation data according to the simple model. However, some systematic differences become apparent, when the results evaluated from relaxation data according the simple and the sphere model are compared with each other. Most of the stability constants obtained by the sphere model

are lower by roughly a factor of 2 than those obtained by the simple model. This seems to be due to the consideration of excluded binding effects in the sphere model. In two cases the K-values derived by the sphere model are higher than those derived by the simple model. The reason for this deviation is not yet clear. The most interesting result obtained by the simple model – the much higher affinity of Arg_3 to poly(I) than that of Lys_3 to poly(I) – is clearly confirmed by the sphere model. The number of nucleotide residues per binding site obtained from the sphere model is lower than that obtained from the simple model in all cases. This effect is also attributed to the consideration of excluded binding effects in the case of the sphere model.

The question arises, which values are more reliable? It is apparent from sect. 3 that the sphere model should provide a more accurate representation of the binding reaction. Thus the parameters obtained from this model should more closely reflect the true values. In the following discussion, however, the main emphasis will not be on the absolute values obtained for various systems, but more on a comparison of their relative magnitude. Such a comparison of the equilibrium parameters obtained by a given method should not be influenced very much by any of the model assumptions.

The data collected in the present investigation represent the first phase of peptide association, which is characterised by relatively high binding constants. As can be seen from the number of nucleotide residues per binding place n, there is no complete neutralisation of the polynucleotide charge during the first binding phase. A similar effect has been observed recently for the association of Mg^{2+} with single stranded polynucleotides [7]. This effect may be explained on the basis of the polyelectrolyte theory developed by Manning [22], which predicts that only a limited fraction of charges is compensated during the first phase of ion condensation. Further compensation of charges above this limit is expected at higher ligand concentrations. Evidence for corresponding steps of peptide association is found in the titration curves described in sect. 4.3. Peptide binding, which approached complete charge neutralisation, was observed by Latt and Sober in their investigation of oligolysine association with double helical polynucleotides [23].

If electrostatic attractions are the sole type of interaction in the present model systems the association constants for Arg_n and Lys_n peptides should be very similar, since both peptides carry the same number of charges with a very similar distance between the charged groups. This expectation is veryfied in most cases investigated. The magnitude of the association constants is strongly influenced by the electrostatic potential of the polynucleotides. This is illustrated by a comparison of the present data with some association constants obtained previously for peptide binding to oligonucleotides [24]. The constants measured for the interaction of Arg_n and Lys_n peptides with various oligomers of the type $X(pX)_5$ are lower by a factor of about 100 than those obtained for the corresponding polymers. If some differences in the experimental conditions are accounted for, this factor is even higher.

The data obtained for the various polynucleotides seem to indicate some base specific variation in the electrostatic potential. The association constants obtained for Lys_3 decrease in the series poly(A) > poly(C) > poly(U). It is conceivable that the electrostatic potential decreases with a decreasing degree of order in the polynucleotide chain. Thus the above series is in line with the independent observation that stacking interaction lead to the formation of an ordered single stranded helical conformation in poly(A) [25–27]. A similar conformation is also found in poly(C), whereas the conformation of poly(U) is more like that of a random coil [25–27].

The association constant obtained for the interaction of Lys_3 with poly(I) roughly fits into the above series, since poly(I) has been characterised to form a poorly stacked helix [18]. However, the difference in the association constants found for the Arg_3 and Lys_3 interaction with poly(I) cannot be explained any more on the basis of electrostatic interactions. Obviously the Arg_3-poly(I) interaction is stabilised by some additional interaction. Since the guanidino group of arginine is expected to be an excellent hydrogen bond donor and the hypoxanthine base provides complementary acceptor positions [28–30], the particularly strong interaction between Arg_3 and poly(I) can be explained by hydrogen bonding. Evidence for this type of interaction has also been given recently in the case of oligopeptide-oligonucleotide associations [24].

The specific mode of Arg interactions is also reflected in the data obtained for the cooperative formation of multistranded double helices. Lys_3 was found to induce helix formation in poly(I), whereas no indication of a corresponding process was observed with Arg_3. The failure of Arg_3 to induce helix formation will be due to blocking of some groups required for hydrogen bonding in the poly(I) helix as a consequence of site binding of Arg_3 to single stranded poly(I). Thus the difference in the free binding energy of Lys_3 and Arg_3 is amplified by cooperative coupling resulting in completely different secondary structures depending upon the presence of Lys_3 or Arg_3.

A similar effect may explain the strong decrease of the T_m-value of poly(U), when the Arg_3 concentration is increased above a certain concentration limit. Above this limit Arg_3 appears to associate with binding sites, which are required for the formation of poly(U) double helices. The difference in the affinity of Arg and Lys residues appears to be further amplified in the case of the corresponding polypeptides. $(Lys)_\infty$ was found to induce the formation of double helical poly(U) even at relatively high temperatures, whereas $(Arg)_\infty$ did not induce helix formation at all [31].

A further indication for the particular properties of Arg is represented by the coacervation effects. The capability of Arg to provide hydrogen donor groups to more than one ligand at a time may be used as an explanation for its strong coacervation tendency.

5.3. Kinetic parameters

The rate constants obtained according to the simple model are clearly different from those obtained according to the sphere model. The reason for this difference is mainly the different choice of concentration units for the polynucleotide component [cf. sect. 3]. In order to decide, which set of rate constants is reasonable, it will be useful to compare the rate constants for oligopeptide-polynucleotide complexes with those obtained previously for oligopeptide-oligonucleotide complexes. The rate constants of recombination found for the oligomer-oligomer complexes [24] are in the range of 2×10^{10} M^{-1} s^{-1}. For a comparison it will be necessary to discuss the various factors, which influence the rate of recombination upon the change of the nucleotide chain length:

a) First of all the polymers will diffuse more slowly than the oligomers. Since the rate of recombination for a diffusion controlled reaction depends upon the sum of the diffusion coefficients for the reaction partners [14], this effect may in the limit lead to a rate reduction by a factor of 2 in the case of the polymer-systems (i.e. when the diffusion coefficient of the polymer is much smaller than that of the oligomer).

b) The rate constants for the oligopeptide-polynucleotide complexes were obtained at pH 8 and may be influenced by a peptide protonation reaction. The terminal amino groups of the free peptides are mainly in the unprotonated state at pH 8, but become protonated to a large extent upon binding to the polynucleotides [32]. Thus the protonation reaction might influence the recombination rate. It is obvious, however, that the protonation reaction cannot be the rate determining step in the peptide binding. First of all, the protonation reaction is fast compared with the peptide binding under the conditions of the present experiments. Moreover, protonation of the peptide is not required for the recombination step, although it will enhance the recombination rate. The magnitude of this effect is demonstrated in the case of poly(U) + Lys_3, where full protonation of the peptide at pH 5.9 leads to an increase in the recombination rate by a factor of 2.5 compared with that observed at pH 8 (cf. sect. 4.6). In this system a pH-independent measure of the chain length effect is available: $U(pU)_5$ forms a complex with Lys_3 8 times faster than poly(U) (data at pH 5.9; cf. ref. [24]), if the comparison is based upon the result obtained by the simple model.

c) The electrostatic potential of the nucleotides will also influence the recombination rate. Since the electrostatic potential is higher for polymers than for oligomers, this effect should result in a higher rate for the polymers than for the oligomers.

According to a rough estimate the effects a) and c) may cancel each other. Thus, if we base the comparison upon the data obtained from the simple model, a reduction in the rate for the polymers by a factor of about 8 has to be explained. This result illustrates very clearly a deficiency of the simple model: as discussed already in sect. 3.2.3. it does not consider the fact, that the binding sites are tied together into polymer spheres. This effect is considered in the sphere model. The rather high rates of recombination obtained by the sphere model, in the range of 3 to 4×10^{11} M^{-1}

s^{-1} reflect the relatively large reaction distances of the polymer spheres. According to this model the rates of recombination for the polymers are clearly higher than those found previously for the oligomers [24]. An increase in the chain length from 5 to 1000 leads to an increase of the recombination rate by a factor of about 15. Since it is expected that the influence of the diffusion coefficient and of the electrostatic potential at various chain lengths may roughly cancel each other (cf. discussion above), this factor is an approximate measure for the variation in the reaction distance. Thus the experimental data indicate, that the reaction distance is not a linear function of the chain length, but roughly seems to be a function of the corresponding square root ($\sqrt{1000/5} \approx 14$). As mentioned above, the mean square radius of coiled polymers increases with the square root of the chain length (according to polymer statistics [8,15], excluded volume effects will slightly modify this relation). Thus the present results are in agreement with the expectation from polymer statistics.

5.4. Conclusion

In summary the data obtained in the present investigation demonstrate that some specificity of protein nucleic acid interaction can already be observed in very simple model systems. These model systems are useful for the separate characterisation of particular molecular interactions. The results obtained with the present systems will also serve as a background for the investigation of more complex systems involving different types of amino acids.

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