*Biophysical Chemistry*, 46 (1993) 283–294
Elsevier Science Publishers B.V., Amsterdam



BIOCHE 01746

# Structure and dynamics of peptide–polynucleotide complexes

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(Received 17 August 1992; accepted 17 November 1992)

**Abstract**

The mode and the dynamics of LysTrpLys-binding to double helical DNA and to single stranded poly(A) has been analyzed by measurements of the chemical relaxation detected by fluorescence and of the rotational diffusion using the electric dichroism. The chemical relaxation, induced by electric field pulses, requires two exponentials for a satisfactory representation, indicating a two step reaction mechanism. The data are consistent with a bimolecular reaction step followed by a relatively slow intramolecular transition, which is expected to reflect "insertion" of the Trp-indole residues between the nucleic acid bases. The experimental data are analyzed quantitatively by global fitting with exact correction of the convolution due to the experimental device. In this procedure a complete set of relaxation curves is fitted directly to the reaction model and, thus artifacts resulting from erroneous assignments of coupled modes are avoided. According to this analysis the bimolecular reaction step is controlled by diffusion. The intramolecular transition in adenylate chains is found to be dependent on the chain length and on the ionic strength $I$: at $I = 2.5$ m$M$ the "insertion" rate constant is $3 \times 10^4$ s$^{-1}$ for the polymer and $2 \times 10^5$ s$^{-1}$ for A(pA)$_{19}$; the rate constant for poly(A) increases with increasing salt concentration. The corresponding "insertion" rate constant for DNA double helices with 30 kbp is $2.5 \times 10^4$ s$^{-1}$. For DNA double helices we find again an increase of the "insertion" rate with increasing salt concentration and with decreasing chain length. The mode of LysTrpLys-binding to double helical DNA is compared with that of LysTyrLys, LysLeuLys and LysGlyLys by measurements of the rotational diffusion of complexes with restriction fragments of different chain lengths. The persistence lengths derived from these measurements do not reveal any special effects resulting from insertion of aromatic residues. Apparently "insertion" of indole rings into double helical DNA does not increase the length of the double helix, which may be attributed to a special form of insertion, e.g. partial insertion. According to these results the interaction of the indole residues of LysTrpLys with DNA double helices is not equivalent to e.g. intercalation of aromatic residues like ethidium–neither with respect to structure nor to dynamics.

*Keywords:* Chemical relaxation; Electric dichroism; Global fitting; Intercalation mechanism; Persistence length

## 1. Introduction

Interactions between proteins and nucleic acids are based on various types of molecular contacts. Usually, natural protein–nucleic acid complexes are maintained by very special combinations of these types of contacts and, thus, these complexes can hardly be used to get quantitative information on the individual contributions. The characterization of individual contributions requires the analysis of model complexes formed from compo-

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nents with selected residues. Previous investigations of such model complexes [1–3] demonstrated that the contributions of single residues to protein–nucleic acid interactions are rather small and, thus, the quantitative characterization requires a particularly high accuracy.

Among the peptides used previously as model compounds, LysTrpLys and its binding to various polynucleotides has been characterized most intensively. The efforts were partly motivated by the expectation that the aromatic side chain of tryptophan would lead to relatively strong interactions due to stacking. Of course, another reason for the choice of tryptophan was its fluorescence, which was used as a convenient label for the experimental analysis. The lysine residues served to enhance the affinity to polynucleotides due to electrostatic interactions. Although LysTrpLys is a very simple peptide, its interactions with polynucleotides are already relatively complex: from measurement of the fluorescence it has been concluded that at least two different complexes are formed, one with stacked and the other one with unstacked indole ring. These conclusions were partly based on the assignment that the quantum yield of stacked indole rings is zero, whereas the quantum yield in complexes with unstacked indole rings remains as in the "free" peptide. The quantum yields were derived from measurement of fluorescence intensities of other simple stacked complexes and from measurements of fluorescence lifetimes [1,2,4].

If the aromatic indole rings are partly unstacked and partly stacked between nucleic acid bases, it should be possible to detect two separate reaction steps: one corresponding to the bimolecular step of binding and another one reflecting "insertion" or "intercalation" of the aromatic residue. Measurements of the chemical relaxation by the electric field jump method provided evidence for the existence of such separate reaction steps [5,6]. However, some problems remained, because part of the parameters obtained by the different procedures [1,2,5,6] are not consistent. In our present investigation we have analyzed the chemical relaxation in more detail by numerical procedures, which have been advanced compared to those used in previous investigations with respect to averaging, deconvolution and fitting of complete ensembles of relaxation data ("global fitting"). The conventional fit of time constants to individual relaxation curves has been avoided, because this procedure may introduce artifacts for the case of closely coupled relaxation processes. We have also studied the rotational diffusion of various peptide–DNA complexes, in order to get more information on the structure of these complexes in solution. The set of parameters derived from this analysis now provides a more complete view of the reactions and the complexes in solution.

## 2. Materials and methods

LysTrpLys, LysTyrLys, LysValLys and LysGlyLys were obtained from Bachem Feinchemikalien AG (Switzerland). The peptides were purified by DEAE cellulose chromatography. The concentrations were determined after acidic hydrolysis by ninhydrin according to the method of Moore and Stein [7]. Concentrations of the peptides are given in units of mol oligomers/l, whereas the concentrations of the polynucleotides are given in units of mol nucleotide residues/l.

Poly(A) and DNA from calf thymus of high molecular weight ($\approx 30 \times 10^3$ base pairs) were obtained from Boehringer Mannheim. $A(pA)_{19}$ was prepared as described by Porschke and Jung [8]. The DNA samples with average chain lengths of 150, 500 and 1000 base pairs were prepared from calf thymus DNA of high molecular weight by digestion with the restriction nucleases HaeIII and AluI (from Boehringer Mannheim) and subsequent separation by gel chromatography on sepharose 4B. The average chain lengths were assigned by comparison with fragments of defined sequence using gel electrophoresis. The plasmid DNA's pSP64 (2999 bp), pBR322 (4363 bp), pHC79 ($\sim$ 6524 bp), pBT I-10 (7750 bp) and PM2 ($\sim$ 9800 bp) were from Bochringer Mannheim. The restriction fragments used for the measurements of rotational diffusion were prepared by HaeIII digestion of the plasmid DNA pWH802

[9] and separation by high performance liquid chromatography on Nucleogen (Macherey-Nagel, Düren, Germany) according to the procedure of Colpan and Riesner [10]; the fragments were shown to be homogeneous by acrylamide gel electrophoresis. All samples were dialyzed extensively first against a high salt buffer containing 1 *M* NaCl, 10 m*M* Na-cacodylate pH 7.0, 1 m*M* EDTA and finally against several changes of 1 m*M* NaCl, 1 m*M* Na-cacodylate pH 7.0, 0.2 m*M* EDTA (standard buffer B). For some measurements we used different concentrations of this buffer, which are denoted by the corresponding factors: e.g. 2 × B corresponds to 2 m*M* NaCl, 2 m*M* Na-cacodylate pH 7.0, 0.4 m*M* EDTA.

For the measurements of both chemical relaxation and rotational diffusion we used an electric field jump apparatus with a pulse generator constructed by Grünhagen [11] and with an optical detection system described previously [5]. The fluorescence was excited at 290 nm and collected behind cutoff filters WG320 from Schott & Gen., Mainz, Germany. The electric field pulses used for perturbation and the resulting fluorescence changes were recorded by the dual channel transient recorder 7612AD from Tektronix. The times corresponding to the start and the end of the electric field pulses were determined automatically by an efficient algorithm from the recorded electric pulse [12]. This procedure provided a safe basis for averaging of relaxation curves measured after pulse termination in spite of variations of the pulse length. The reference signal used for deconvolution of the fluorescence signal was obtained by a fast diode, which was inserted at the position of the measuring cell and driven by a fast pulse generator. Reference signals for deconvolution of electrodichroism transients were obtained by application of electric field pulses to aqueous solutions and recording of the birefringence. In both cases, the signals were recorded and processed in complete analogy to standard experimental data. All the experimental data were first stored on a LSI-11/23 and then were transfered to the facilities of the Gesellschaft für wissenschaftliche Datenverarbeitung mbH Göttingen for the final evaluation by various fitting procedures.

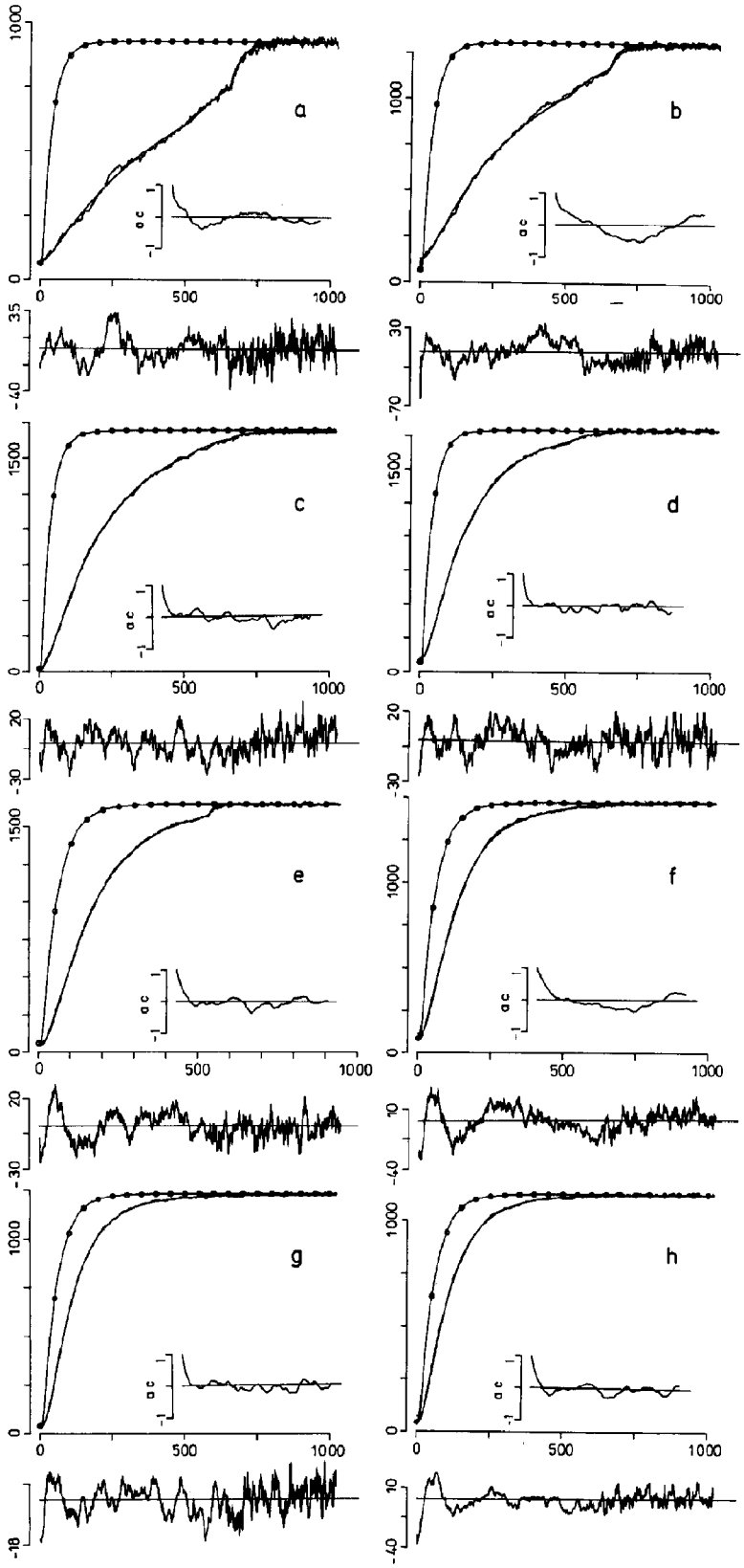
## 3. Results

### 3.1. Reaction dynamics

The binding of LysTrpLys to various polynucleotides was characterized by chemical relaxation induced by the electric field jump technique. We have applied rectangular electric field pulses and measured the relaxation after pulse termination. The electric field induces a net dissociation of the positively charged peptides from the negatively charged polynucleotides by a "Wien" [13] or "dissociation field" [14] effect. After pulse termination the binding equilibrium is restored, reflected by an overall decrease of the fluorescence. The electric field jump technique has been selected for the present investigation, because electric fields induce particularly large perturbations of complexes maintained by electrostatic interactions. Furthermore, the time resolution of the technique is relatively high.

It is known from equilibrium studies [1,2,5,6] that LysTrpLys covers approximately three nucleotide residues and, thus, its binding is influenced by excluded site effects [15]. Because the kinetics of ligand binding in the presence of excluded site effects can be extremely complex [16], we have used conditions, where these are avoided due to a large excess of the polymer over the ligand. All the relaxation curves for the binding of LysTrpLys to the various polymers used for the present analysis were obtained under this condition. Another advantage resulting from the excess polymer concentration is a quasi-first-order reaction, which simplifies the analysis of data recorded after large perturbations of the reaction equilibrium. As shown already in previous investigations [5,6] the relaxation curves both for polyriboadenylates and for double helical DNA require two exponentials for satisfactory fits. Furthermore, the reciprocal time constant associated with the slower relaxation process does not increase linearly with the free reactant concentration, but tends to saturate at high concentrations. These observations demonstrate that the minimal reaction mechanism is as follows:

$$\mathrm{DNA} + \mathrm{Peptide} \underset{k_1^-}{\overset{k_1^+}{\rightleftharpoons}} C_1 \underset{k_2^-}{\overset{k_2^+}{\rightleftharpoons}} C_2. \qquad (1)$$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10

It has been shown already that the experimental data are consistent with this mechanism. However, a conflict exists with respect to the equilibrium constants $K_2 = k_2^+/k_2^-$, which are much larger according to the relaxation data [5,6] than according to the analysis of the fluorescence quenching parameters [1,2].

For an increased accuracy of our experimental data, we have taken advantage of the technical progress in electronics and computation. The signal-to-noise ratio of relaxation curves was increased by an improved averaging procedure (cf. Section 2). The relaxation time constants found at high concentrations are rather small and, thus, deconvolution of the data is essential. We have used a procedure for deconvolution [8], which serves to eliminate perturbations due to the experimental device efficiently and exactly. However, the most important progress is in the final evaluation of the relaxation data. We have omitted the standard evaluation of relaxation time constants from individual relaxation curves and instead fitted the complete set of relaxation curves directly to the reaction model The reaction model is specified within the fitting program and is used to calculate, from a given initial set of rate constants, the relaxation time constants for all the solutions used in the experiments with different concentrations. The best fits for the amplitudes and the baseline of each relaxation curve are evaluated by a generalized linear regression and the sum of residuals is calculated. Then, the rate constants are subjected to a nonlinear fit procedure ("simplex") for evaluation of optimal values. Because the overall binding constant $K = K_1 \times (K_2 + 1) = k_1^+/k_1^- \times (k_2^+/k_2^- + 1)$ has been determined independently by standard fluorescence titrations, three rate constants remain to be fitted. Examples of fits are shown in Figs. 1 and 2. Due to the input of many relaxation curves measured over a relatively wide range of concentrations, usually the output parameters can be obtained at a relatively high accuracy.

The main advantage of the "global" fitting procedure results from the combination of two separate fitting steps, which were required according to the standard procedure used previously, into a single one. It is well known that the assignment of relaxation time constants is very difficult and may be almost impossible, when relaxation processes are closely coupled to each other. Global fitting is a real progress for these cases. A check of the example given for the case of LysTrpLys-binding to poly(A) in Fig. 1 demonstrates that the relaxation time constants are very close to each other: for some reactant concentrations the time constants differ by not more than a factor of 3.3 and, thus, a reliable assignment by direct fitting is very difficult. Global fitting has been applied for several sets of experimental data collected at different conditions. The results are compiled in Table 1.

While the amplitudes of the two relaxation effects measured for poly(A) are always of identical sign, the corresponding amplitudes found for DNA double helices are opposite to each other. As shown in Fig. 2, the data can be fitted at a satisfactory accuracy by the two-step reaction model defined by eq. (1). Although the individual relaxation processes reflect normal modes of the whole system and cannot be assigned to single reaction steps, the main part of the amplitude associated with the fast relaxation effect results from the bimolecular step. Thus, the quantum

Fig. 1. "Global" fit of relaxation curves measured for poly(A) + LysTrpLys in buffer B at 20°C after electric field pulses of 31 kV/cm. The eight panels show the individual relaxation curves (data with noise), the fitted relaxation curves (without noise, in most cases indistinguishable from the measured data) and the reference curve used for deconvolution (marked by circles); the ordinates give the change in the fluorescence light intensity in mV; the time scale is given in "channels" with three different ranges of time intervals; the lower part of each panel shows the difference between the measured and the fitted relaxation curve; the insert shows the autocorrelation of the residuals. The parameters of the individual panels are listed below in the following sequence: concentrations of polymer and of LysTrpLys in $\mu M$; first time interval in $\mu$s, last "channel" with first interval, second time interval in $\mu$s, last channel with second interval, third time interval in $\mu$s: (a) 20, 1.021; 0.3, 512, 0.6 649, 4; (b) 40, 1.5; 0.3, 512, 0.6, 644, 4; (c) 80, 2.46; 0.3, 512, 0.6, 663, 2; (d) 120, 3.41; 0.3, 512, 0.6, 666, 2; (e) 200, 5.33, 0.2, 511, 0.4, 530, 2; (f) 300, 7.72, 0.2, 511, 0.4, 630, 2; (g) 400, 10.12; 0.2, 511, 0.4, 640, 2; (h) 500, 12,51, 0.2, 511, 0.4, 621, 2. The fitted parameters are compiled in Table 1.

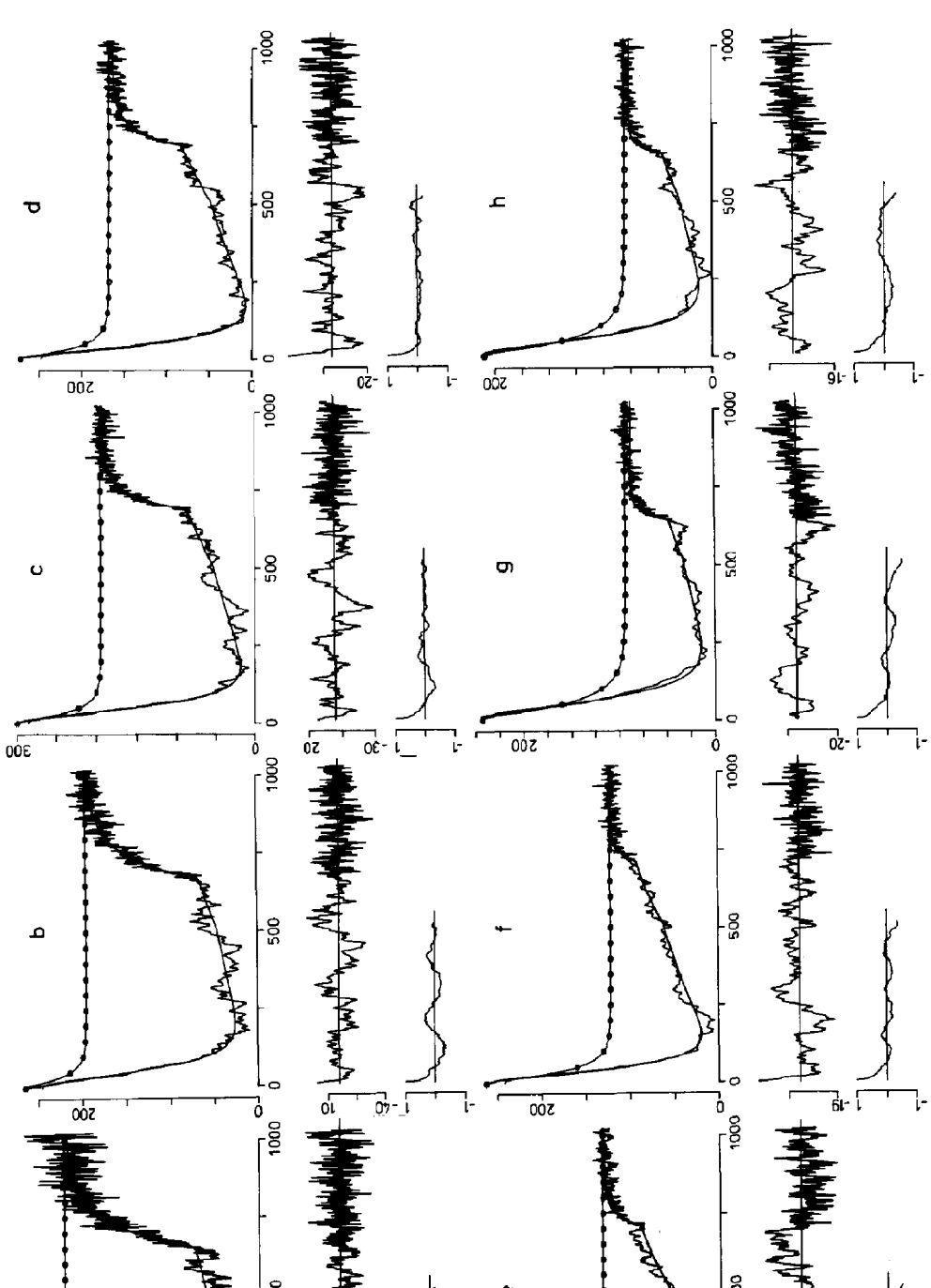


Fig. 2. "Global" fit of relaxation curves measured for DNA (calf thymus, 30 kbp) + LysTrpLys in buffer B at 20°C after electric field pulses of 11 kV/cm. The data are given in the same form as in in Fig. 1, however, the autocorrelation of the residuals is presented below the time dependence of the residuals. (a) 20, 0.72; 0.3, 511, 0.6, 653, 12; (b) 40, 1.146; 0.3, 511, 0.6 670, 8; (c) 80, 1.997; 0.3, 511, 0.6, 687, 8; (d) 120, 2.848; 0.3, 511, 0.6, 686, 6; (e) 200, 4.55; 0.3, 511, 0.6, 707, 6; (f) 300, 6.68; 0.3, 511, 0.6, 710, 6; (g) 400, 8.81; 0.2, 511, 0.4, 641, 6; (h) 500, 10.93; 0.2, 511, 0.4, 651, 6. The fitted parameters are compiled in Table 1.

yield of the complex $C_1$ for the case of DNA seems to be higher than that of the free peptide.

Because this result appears to be in contrast with measurements of fluorescence lifetimes, we have checked for potential artifacts resulting from the electric field jump technique. Application of high electric field pulses may induce denaturation of DNA double helices, which may cause unusual relaxation effects. We know from independent investigations that the denaturation of DNA double helices is induced, when the electric field strength exceeds a threshold value, which is defined by the DNA chain length and the ion concentrations in the solution [17]. In order to avoid this reaction, we have measured the binding of LysTrpLys to DNA by field pulses far below the threshold.

In a previous investigation [6] the special relaxation effect with amplitudes of opposite sign has not been found for sonicated DNA with ~500 bp. For a more detailed characterization of the special effect, we have measured the chemical relaxation resulting from LysTrpLys-binding to DNA over a wide range of different chain lengths. In order to avoid "damaged" parts of double helices resulting from sonication, DNA samples of low chain length were prepared by restriction enzymes, which are known to produce blunt helix ends. Furthermore, we have used several plasmid DNA's of different chain length. The main result obtained from these measurements is in contrast to the previously published conclusion [6]: relaxation effects with inverted amplitudes do exist also for short DNA double helices. The existence of this effect has not been detected previously, because the "apparent" amplitude associated with the fast relaxation effect decreases with decreasing chain length and was hidden due to superposition and convolution. Application of an efficient deconvolution technique demonstrates the existence of two relaxation effects with amplitudes of opposite sign for all chain lengths including a DNA sample with 150 bp. Deconvolution demonstrates that the real amplitude $A_1$ associated with the fast relaxation effect for short DNA's is larger than the one judged from the appearance of the relaxation curves. The difference mainly comes from the fact that the time constant of the second relaxation process decreases with decreasing chain length (Fig. 3) and, thus, the apparent amplitude of the first relaxation effect decreases with decreasing chain length due to superposition. We have compiled the chain length dependence of the two amplitudes induced by pulses of high electric field strength (31 kV/cm) in Fig. 4.. At this field strength and at the given salt concentration we expect the onset of field induced denaturation at a chain length of about 5000 bp, according to independent measurements obtained previously [17]. In agreement with the expectation we find a clear change of the relaxation response at this chain length: the "slow" amplitude $A_2$ decreases, whereas the "fast" amplitude $A_1$ increases to particularly high values. Thus, unusually high values of $A_1$ combined with very low values of $A_2$ reflect field induced denaturation of double helices. However, the "special" relaxation effect with a clearly detectable amplitude $A_1$ remains for chain lengths far below the "critical" one, where field induced denaturation has not been detected. The measurements used for the analysis of the LysTrpLys binding mechanism were only taken in the range far below the critical one. The parameters obtained from this analysis are compiled in Table 1.

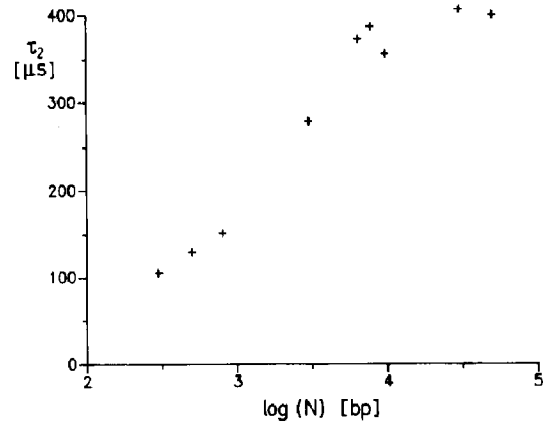


Fig. 3. Relaxation time constant $\tau_2$ in $\mu$s associated with the second relaxation effect of LysTrpLys-binding to DNA double helices as a function of the logarithm of the chain length (buffer B, 20°C, after electric field pulses of 11 kV/cm, 2 $\mu M$ LysTrpLys, 87 $\mu M$ DNA nucleotide residues).

As shown in Fig. 3, the time constant $\tau_2$ associated with the slow relaxation process clearly

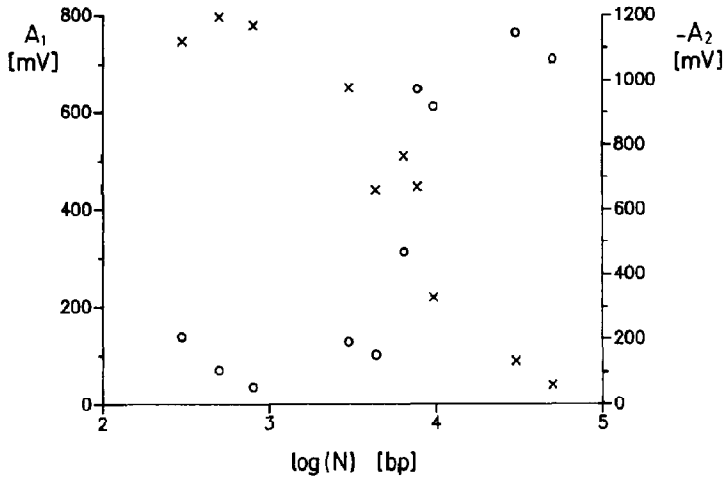


Fig. 4. Amplitudes $A_1$ (○) and $A_2$ (×) in mV associated with the first and the second relaxation effect of LysTrpLys-binding, respectively, as a function of the logarithm of the DNA chain length (buffer B, 20°C, after electric field pulses of 31 kV/cm, 2 $\mu M$ LysTrpLys, 87 $\mu M$ DNA nucleotide residues).

increases with increasing chain length. Such a result would be expected for a diffusion controlled reaction, because the data were collected at constant residue concentrations, which implies a decreasing concentration of helices with increasing chain length $N$ and thus a decrease of $\tau_2$ with increasing $N$. However, the time constants $\tau_2$ are too large for a diffusion controlled association of LysTrpLys to DNA. We have to conclude that it is the rate of the *intramolecular* step which decreases with increasing chain length. The chain length dependence found for the case of LysTrpLys-binding to DNA is in contrast with data obtained recently for intercalation of ethidium into DNA (Meyer-Almes and Porschke, submitted). In the latter case the time constants measured at given DNA residue concentrations are independent of the chain length. Apparently intercalation of ethidium and "insertion" of the LysTrpLys-indole rings into DNA double helices are not equivalent.

All the data described above were measured at a high excess of polymer binding sites with respect to the LysTrpLys-concentration. For one set of data obtained for the oligomer $A(pA)_{19}$, this condition could not be maintained, because this oligomer was not available in a sufficiently large quantity. In order to get relaxation effects up to high reactant concentrations, we have used in this case not only small but also large LysTrp-

Table 1

Parameters of LysTrpLys-binding obtained by "global" fitting of relaxation data (20°C; estimated accuracy ±20%). The overall binding constants are from refs. [5] and [6]

| Polymer | Buffer | $K_1(K_2+1)$ | $k_1^+$ | $k_2^+$ | $k_2^-$ | $K_2$ |
|---|---|---|---|---|---|---|
| Poly(A) | B | $5\cdot10^4$ | $2.2\cdot10^8$ | $2.9\cdot10^4$ | $8.9\cdot10^3$ | 3.3 |
| Poly(A) | 2×B | $1.25\cdot10^4$ | $1.5\cdot10^8$ | $4.2\cdot10^4$ | $1.7\cdot10^4$ | 2.5 |
| Poly(A) | 4×B | $7\cdot10^3$ | $4\cdot10^8$ | $9.1\cdot10^4$ | $2.7\cdot10^4$ | 3.4 |
| $A(pA)_{19}$ | B | $3.9\cdot10^4$ | $1\cdot10^{11}$ | $2\cdot10^5$ | $7\cdot10^4$ | 3 |
| DNA 30 kbp | ½×B | $3.2\cdot10^5$ | $2\cdot10^8$ | $7.3\cdot10^3$ | 63 | 120 |
| DNA 30 kbp | B | $8\cdot10^4$ | $1.4\cdot10^8$ | $2.5\cdot10^4$ | 420 | 60 |
| DNA 30 kbp | 2×B | $2\cdot10^4$ | $1.6\cdot10^8$ | $8.1\cdot10^4$ | $2.1\cdot10^3$ | 40 |
| DNA 1000 bp | B | $8\cdot10^4$ | $5\cdot10^8$ | $1\cdot10^5$ | $1\cdot10^3$ | 100 |
| 500 bp | B | $8\cdot10^4$ | $1\cdot10^9$ | $1\cdot10^5$ | $1\cdot10^3$ | 100 |

Lys-concentrations. Fluorescence titration experiments demonstrated that the number of LysTrpLys-peptides bound to one oligomer $A(pA)_{19}$ under our experimental conditions is restricted to $3 \pm 0.5$. We have used this stoichiometric number for the global fitting, but only to calculate the concentration of binding sites; the different complex species with one, two and three ligands and the multiplicity of the ligand distribution were not considered explicitly. The global fit obtained with this simplified model is satisfactory. The resulting parameters are included in Table 1. The insertion rate constant $k_2^+$ is found to be larger for $A(pA)_{19}$ than for poly(A). This is consistent with the result of a previous analysis of LysTrpLys-binding to $A(pA)_5$, where a separate slow step could not be identified anymore [18].

### 3.2. Rotational diffusion

If the indole rings of LysTrpLys intercalate between the nucleic acid bases of double helices, the effective length of the resulting complex should be increased. In the case of single stranded adenylate chains, the length increase due to the intercalation of the indole rings of LysTrpLys could be clearly detected by measurements of dichroism decay time constants [8]. We have now used the same electrooptical procedure for the analysis of LysTrpLys-complexes with double helical DNA fragments. The time constants reflecting the rotational diffusion of various restriction fragments did not change very much upon addition of LysTrpLys. It is known that binding of the "classical" intercalator ethidium bromide induces changes of the dichroism decay time constants, which are clearly detectable already, when a single ethidium is bound to a fragment with 95 bp [19, cf. also 20]. Because LysTrpLys does not only bind to double helical DNA by stacking of the indole ring but also by electrostatic interactions via its lysine residues, we have measured the effect of LysGlyLys, LysValLys and LysTyrLys for comparison. In all cases the changes of the dichroism decay time constants remained relatively small, even though the degree of peptide binding was considerable. For a quantitative assignment of the effects induced by the various peptides we have measured dichroism decay times at constant concentrations of peptides and of DNA phosphate residues with DNA restriction fragments of different chain length. For example, data have been collected at 2.5, 5, 40 and 100 $\mu M$ LysTrpLys together with 10 $\mu M$ DNA nucleotide residues in buffer B, which leads to binding degrees of 24.7, 38.1, 72.2 and 80.5%, respectively (according to the excluded site binding model using a binding constant $8 \cdot 10^4\ M^{-1}$ and a number of three nucleotide residues covered by one LysTrpLys-peptide [6]). The binding degrees for the other peptides are expected to be somewhat lower. In all cases the dichroism decay time constants $\tau_d$ have been measured as a function of the electric field strength $E$ down to low $E$-values and the resulting $\tau_d$-values have been extrapolated down to $E = 0$, in order to eliminate any influence from field induced dissociation of ligands. The resulting sets of time constants have been used to evaluate the persistence length of double helical DNA in the presence of the different peptides according to a hydrodynamic model [21] combined from the equation for rigid rods of Tirado and Garcia de la Torre [22] and the correction terms for wormlike chains of Hagerman and Zimm [23]. As shown in Table 2, addition of peptides induces some decrease of the persis-

Table 2

Persistence length $p$ and hydrodynamic diameter $d$ from dichroism decay time constants according to a hydrodynamic model combined from the equation for rigid rods by Tirado and Garcia de la Torre [22] and the wormlike chain correction of Hagerman and Zimm [23] (20°C; buffer B; estimated accuracy ±10%; the data in buffer B without added ligand are from [21]; the data for 100$\mu M$ $Mg^{2+}$ (from [21]) were measured in a buffer corresponding to B, but without EDTA)

| Peptide | $c$ ($\mu M$) | $p$ (nm) | $d$ (nm) |
|---|---|---|---|
| LysTrpLys | 2.5 | 79 | 3.1 |
| LysTrpLys | 5 | 88 | 3.0 |
| LysTrpLys | 40 | 68 | 3.1 |
| LysTrpLys | 100 | 61 | 3.2 |
| LysTyrLys | 5 | 87 | 2.7 |
| LysLeuLys | 5 | 98 | 2.6 |
| LysGlyLys | 5 | 98 | 2.5 |
| LysGlyLys | 100 | 72 | 2.8 |
| – | – | 87 | 2.6 |
| Mg | 100 | 50 | 2.6 |

tence length, which is hardly influenced by the presence of the aromatic residues in the cases of LysTrpLys or LysTyrLys. A decrease of the persistence length resulting from binding of ligands with the general structure Lys *X* Lys must be expected because of electrostatic effects (cf. example of $Mg^{2+}$ in Table 2; [21] and references cited therein). The standard form of intercalation should result in an increase of the effective length and should be reflected by an increase of the persistence length. Thus, our data indicate that the aromatic residues of LysTrpLys and of LysTyrLys do not interact with DNA in a mode corresponding to standard intercalation.

## 4. Discussion

The quantitative analysis of exponential decay curves with closely coupled components is known to be a difficult task. It is likely that this problem will remain at least to some extent, in spite of the development of increasingly sophisticated algorithms for numerical evaluations. In our present investigation we have reduced the difficulty by using a procedure, which is particularly attractive for the assignment of reaction mechanisms from experimental data obtained by chemical relaxation procedures. The "global" fitting procedure reduces two fitting steps required according to standard procedures to a single one, and, thus, clearly reduces the danger of errors. However, the global fitting procedure should not be used without appropriate precautions. For all applications it will be necessary to screen the available experimental data for potential alternative mechanisms, before global fitting can be applied. This part of the analysis has been performed for our present system in previous investigations [5,6] and has shown that the most simple mechanism consistent with the experimental data requires two reaction steps. As usual, there are more complex mechanisms, which are also consistent with the available experimental data.

The results obtained for the binding of LysTrpLys to poly(A) are now in agreement with conclusions obtained from the analysis of fluorescence quenching parameters [1,2] with respect to the equilibrium constant $K_2$. The main reason for the disagreement in the set of parameters obtained previously [5] appears to be the superposition of two relaxation effects and problems in their separation.

Problems may still be suspected for the case of LysTrpLys-binding to double helical DNA. The equilibrium constant found for the "insertion" step is higher than expected. It may also be questioned, how the existence of a slow reaction step, which is a clear candidate for an insertion step, can be related to the absence of a change in the persistence length. Thus, it may be argued that part of the observed relaxation effects is due to field induced denaturation of the double helix.

The experimental chain length dependence of the amplitudes $A_1$ and $A_2$ (cf. Fig. 4) clearly shows the conditions, where the electric field pulses induce denaturation of the double helices. These results are consistent with those obtained in previous investigations [17], which demonstrate that the field induced denaturation is a cooperative process and that a threshold field strength must be exceeded to induce denaturation. All the measurements used for the evaluation of LysTrpLys-binding parameters were performed under conditions far below the denaturation threshold. Thus, field induced denaturation should not be expected under our experimental conditions.

The intramolecular transition constants $K_2$ obtained from our relaxation measurements for DNA are much larger than those assigned previously from relative fluorescence intensities and fluorescence lifetimes (1,2,4]. It is not clear, where this difference comes from. It cannot be excluded that the process characterized by the field jump measurements is not exactly equivalent to that characterized by the relative fluorescence intensities. For example, binding of the peptide may induce some conformation change of the DNA, which is closely coupled to insertion of the indole ring; this coupled process may be detected by the chemical relaxation analysis as one step, whereas the analysis of the relative fluorescence intensity may respond to insertion exclusively. According to our relaxation data there is an intermediate with a higher quantum yield than that of the free peptide. Such an intermediate was not detected

by measurements of the fluorescence lifetimes, which may be explained by the fact that this intermediate—according to our binding constant $K_2$—exists at a rather low concentration. In summary, the data obtained by analysis of the chemical relaxation and of the relative fluorescence intensities are not necessarily in contrast to each other.

The insertion of aromatic residues into DNA double helices has been analysed in various systems and by many different authors. In spite of the large number of investigations, the views on this type of reaction are not converging yet. With respect to the mechanism and the rate of insertion reactions into double helices, two limit cases are possible in analogy to the classical $S_N1$ and $S_N2$ mechanisms: the rate of the reaction may be determined by unstacking of base pairs and, in this case, would be independent of the aromatic residue to be inserted; in the other limit unstacking and insertion of the aromatic residues may be closely coupled to each other and, then, the rate could be dependent on the type of the aromatic residue. Unfortunately there are no well established references on the rate of insertion reactions. Even for the case of ethidium intercalation into DNA, different authors [24–27] arrived at completely different conclusions on the mechanism and the rate constants. Thus, a comparison is hardly possible. In a recent investigation (Meyer-Almes and Porschke, submitted) the rate constant for insertion of ethidium into DNA double helices was found in the range around $1 \times 10^3$ $s^{-1}$. According to Ramstein et al. [28] the rate constant of proflavin intercalation into poly[d(AT)] is $3.6 \times 10^3$ $s^{-1}$ (at 17°C). However, Ramstein et al. did not find a separate slow step for the reaction of proflavin with poly[d(GC)]. In summary, a final consistent view on the dynamics of intercalation reactions has not been established yet. Nevertheless, evidence has been accumulated for several systems that intercalation into double helices proceeds with rate constants in the range of $10^2$ to $10^3$ $s^{-1}$. According to results obtained by various methods [29–32], the time constant for unstacking of base pairs is in the same time range. Thus, the insertion rate of aromatic residues into DNA double helices appears to be determined by the rate of base pair unstacking at least in some cases.

Although the rate constants found for the intramolecular binding steps of LysTrpLys and ethidium are of similar magnitude, there seems to be a major difference indicated by the dependence of the time constants $\tau_2$ on the chain length $N$: in the case of LysTrpLys the $\tau_2$-values decrease with increasing $N$, whereas the corresponding time constant found in the case of ethidium is independent of $N$ (Meyer-Almes and Porschke, submitted). Another difference is found by the hydrodynamic analysis of the complexes. Intercalation of ethidium leads to a clear increase of the effective hydrodynamic length of DNA double helices [19,20]. According to our present results, the "insertion" of indole rings into double helices does not increase the effective hydrodynamic length of the double helix. This may be due to a special type of insertion, which does not correspond to the "classical" form of intercalation. It is conceivable that small aromatic systems like indole rings induce some rearrangement of the stacking between nucleic acid bases, which allows for partial insertion. It is also possible that insertion of indole rings induces some bending of the double helix, which has not been detected in our electrooptical experiments because bending at many binding sites combined with some increase of the contour length may compensate each other. These problems require further experimental analysis.

## Acknowledgements

We are grateful to Manfred Jung for assembling of the global fit program with deconvolution and to Jörg Ronnenberg for technical assistance. For our computations we used the facilities of the Gesellschaft für wissenschaftliche Datenverarbeitung m.b.H., Göttingen.

## References

1 C. Hélène and J.C. Maurizot, CRC Crit. Rev. Biochem. 10 (1981) 213.

2 C. Hélène and G. Lancelot, Prog. Biophys. Mol. Biol. 39 (1982) 1.
3 D. Porschke, Landolt–Börnstein, Neue Serie VII, Band 1d, ed. W. Saenger (Springer, Berlin, 1990) p. 264.
4 T. Montenay-Garestier, J. Fidy, J.C. Brochon and C. Hélène, Biochimie 63 (1981) 937.
5 D. Porschke, Nucleic Acids Res. 8 (1980) 1591.
6 D. Porschke and J. Ronnenberg, Biophys. Chem. 13 (1981) 283.
7 S. Moore and W.H. Stein, J. Biol. Chem. 211 (1954) 907.
8 D. Porschke and M. Jung, J. Biomol. Struct. Dyn. 2 (1985) 1173.
9 B. Unger, G. Klock and W. Hillen, Nucleic Acids Res. 12 (1984) 7693.
10 M. Colpan and D. Riesner, J. Chromatogr. 296 (1984) 339.
11 H.H. Grünhagen, Ph.D. Thesis, Universität Braunschweig (1974).
12 D. Porschke, H.J. Meier and J. Ronnenberg, Biophys. Chem. 20 (1984) 225.
13 M. Wien, Physik. Z. 32 (1931) 545.
14 L. Onsager, J. Chem. Phys. 2 (1934) 599.
15 J.D. McGhee and P.H. von Hippel, J. Mol. Biol. 86 (1974) 486.
16 I.R. Epstein, Biopolymers 18 (1979) 2037.
17 S. Diekmann and D. Porschke, Biophys. Chem. 16 (1982) 261.
18 D. Porschke, Eur. J. Biochem. 86 (1978) 291.
19 D. Porschke, N. Geissler and W. Hillen, Nucleic Acids Res. 10 (1982) 3791.
20 M. Hogan, N. Dattagupta and D.M. Crothers, Biochemistry 18 (1979) 280.
21 D. Porschke, Biophys. Chem. 40 (1991) 169.
22 M.M. Tirado and J. Garcia de la Torre, J. Chem. Phys. 73 (1980) 1986.
23 P.J. Hagerman and B.H. Zimm, Biopolymers 20 (1981) 1481.
24 J.L. Bresloff and D.M. Crothers, J. Mol. Biol. 95 (1975) 103.
25 L.P.G. Wakelin and M.J. Waring, J. Mol. Biol. 144 (1980) 183.
26 W.D. Wilson, C.R. Krishnamoorthy, Y.H. Wang and J.C. Smith, Biopolymers 24 (1985) 1941.
27 R.B. Macgregor, R.M. Clegg and T.M. Jovin, Biochemistry 26 (1987) 4008.
28 J. Ramstein, M. Ehrenberg and R. Rigler, Biochemistry 19 (1980) 3938.
29 D. Porschke, Mol. Biol. Biochem. Biophys. 24 (1977) 191.
30 D.J. Patel, L. Shapiro and D. Hare, Q. Rev. Biophys. 20 (1987) 35.
31 J.L. Leroy, M. Kochoyan, T. Huynh-Dinh and M. Gueron, J. Mol. Biol. 200 (1988) 223.
32 M. Frank-Kamenetskii, Nature 328 (1987) 17.