THERMAL DENATURATION OF COMPLEXES OF DNA WITH SEQUENTIAL AND RANDOM LYSINE CONTAINING POLYPEPTIDES

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Complexes of DNA with lysine containing sequential polypeptides and random amino-acid copolymers were studied using the method of thermal melting. The effect of lysine content, lysine distribution along the polypeptide chain and of the lysine/nucleotide input ratio on the melting pattern was determined. Polypeptide redistribution on DNA and reversibility of the DNA melting in individual transitions were also tested. It was shown that the behavior of DNA complexes of polypeptides with relatively low density of lysine differed from that of lysine rich polypeptides due to a low degree of charge neutralization and to the possibility of polypeptide redistribution during melting in the former case. In the latter case the binding was found to be essentially irreversible. Higher thermal stability and lower redistribution rate were obtained using complexes prepared by gradient dialysis as compared with those prepared by direct mixing.

Studies of the binding of basic polypeptides with DNA represent one of the approaches which can be used to investigate the structural factors governing protein–DNA interactions. Several binding studies using homopolypeptides and amino-acid random copolymers were described[1–4]. Recently the synthesis of sequential basic polypeptides was successfully performed in order to obtain polypeptide segments with controlled amino-acid composition and sequence[5–9]. Binding studies using sequential polypeptides can yield information not only about the effect of the kind and amount of amino-acid residues present in the polypeptide chain, but also about specific effects of the sequence.

In order to characterize DNA–polypeptide complexes, the method of thermal denaturation has been used by several authors. Using this method, Pinkston and coworkers[2] studied complexes of DNA with random copolymers of Lys and Ala. Schwartz and Fasman[10] reported comparison of two polypeptides, random and sequential, containing Lys, Ala, and Gly*. In our laboratory two series of polypeptides, random and sequential, extending over a broad range of lysine contents and with varying lysine distribution have been synthesized[4–7]. In a previous paper results concerning

* All the amino acids in the present paper are in L configuration.

the effect of the chain length of the polymers $(Lys\text{-}Ala\text{-}Ala)_n$ on the melting behavior as well as on circular dichroism of complexes have been reported[11]. In the present paper, our main interest was devoted to the effect of the amount and distribution of lysine residues on the melting pattern of complexes. In addition, the effect of the method of preparation of complexes (either by dialysis or by direct mixing) and the redistribution of the ligand on DNA were studied.

EXPERIMENTAL

Calf thymus DNA was isolated according to Kay and coworkers[12]. A fraction containing no satellite components[13] was used for preparation of complexes. Synthetic poly(dA-dT).poly(dA-dT) was purchased from Boehringer (Mannheim). DNA concentration was checked spectrophotometrically using the value of $\varepsilon_{260} = 6\,600$ l . . $mol^{-1}\,cm^{-1}$.

Polypeptides $(Lys^x,Ala^y)_n$, $(Lys\text{-}Ala\text{-}Gly)_n$ ($\bar{M}_w \sim 5\,550$, $n \sim 23$), $(Ala\text{-}Lys\text{-}Pro)_n$ ($\bar{M}_w \sim 10\,000$, $n \sim 34$), $(Ala\text{-}Lys\text{-}Pro\text{-}Lys)_n$ ($\bar{M}_w \sim 5\,800$, $n \sim 14$), $(Ala\text{-}Lys\text{-}Lys\text{-}Pro\text{-}Lys)_n$ ($\bar{M}_w \sim 14\,000$, $n \sim 25$), $(Lys\text{-}Lys\text{-}Gly)_n$ ($\bar{M}_w \sim 15\,000$, $n \sim 32$) and $(Lys\text{-}Lys\text{-}Gly\text{-}Ala\text{-}Ala\text{-}Ala)_n$ ($n \geqq 10$) were synthesized and characterized as described elsewhere[4-6]. Appropriate amounts of polypeptides were dissolved in deionized water in a concentration of 1 mg/cm^3.

Reconstituted complexes were prepared by mixing the components in 3M–4M-NaCl in an appropriate ratio r (molar ratio of lysine residue to DNA phosphate) followed

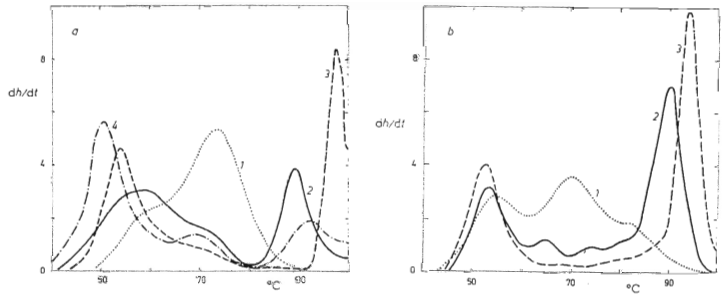

FIG. 1

Melting curves of DNA complexed with sequential lysine-containing polypeptides, $r = 0{\cdot}63$, in 0·002M-Na^+. (*a*) Reconstituted complexes; (*b*) directly mixed complexes. *1* DNA–$(Ala\text{-}Lys\text{-}Pro)_n$; *2* DNA–$(Ala\text{-}Lys\text{-}Pro\text{-}Lys)_n$; *3* DNA–$(Lys\text{-}Lys\text{-}Gly)_n$; *4* DNA–$(Lys\text{-}Lys\text{-}Gly\text{-}Ala\text{-}Ala\text{-}Ala)_n$.

by salt gradient dialysis according to Carroll[14] to 0·15M-NaCl, and finally to EDTA – phosphate buffer ($1 . 10^{-4}$M-Na_2 EDTA, $8 . 10^{-4}$M-Na_2HPO_4, $2 . 10^{-4}$M-NaH_2PO_4; pH = 7·5, $[Na^+] = 0{\cdot}002$) with several changes. To form directly mixed complexes an appropriate volume of polypeptide solution was gradually added from a microsyringe to the solution of DNA in EDTA–phosphate buffer under stirring.

The absorbance A_{260} change during the melting experiment was measured in 1 cm optical path cells using a Hilger-Uvispek spectrophotometer adapted for the measurement of the melting curves. Cell temperature was changed continuously and recorded by means of a thermocouple. The absorbance increase (hyperchromicity) h normalized to the total absorbance increase at 100°C was calculated and the derivatives dh/dt were obtained from cubic spline functions of the integral melting curve as described elsewhere[15,16]. The effect of complex aggregation on absorbance during the melting experiment was checked by measuring the absorbance at 310 nm before and after the experiment. In most cases only a small difference (average value $\Delta A_{1\,cm}^{310} = 0{\cdot}009$) was observed.

RESULTS

Complexes of Sequential Polypeptides

Melting of reconstituted polypeptide–DNA complexes (Fig. 1*a*, Table I) show the presence of at least three distinct melting transitions. In most cases transition I is a band with the peak (T_{mI}) in the region 50–60°C (Table I), which corresponds to the melting of free DNA (ref.[10,11]). Transition II, the T_m of which (T_{mII}) is located within the limits of 70–80°C (Table I), is most pronounced in the case of polymeric tripeptides containing one lysine residue (see also[11]). At constant r this peak decreases with increasing lysine content and in the case of the $(Lys\text{-}Lys\text{-}Gly)_n$–DNA complex it disappears within the limits of experimental error. Above 85°C transition III is usually found, except in the case of above mentioned polymeric tripeptides at lower r values (Fig. 1*a*).

As shown in Table I, T_{mI} values shift to higher temperatures with increasing r, but the values of T_{mII} and T_{mIII} remain fairly independent of r. The values of T_{mIII} clearly follow the increase of the lysine content. In the case of $(Lys\text{-}Lys\text{-}Gly\text{-}Ala\text{-}Ala\text{-}Ala)_n$ transition III is found even at low r ratios in spite of the fact that this polymer has the same average lysine content as the polymeric tripeptides, where transition II strongly prevails (Fig. 1*a*, Table I).

Melting curves of directly mixed complexes (Fig. 1*b*) are rather similar to those of reconstituted complexes. However, the T_{mIII} values are lower, melting bands are better distinguished and more symmetrical, and the region II appears to be less important. The value of T_{mIII} depends on lysine content and distribution in a similar manner as in the case of reconstituted complexes (Table II).

Complexes of Random Copolymers $(Lys^x,Ala^y)_n$

Melting curves of reconstituted complexes DNA–$(Lys^x,Ala^y)_n$ also show the presence of several melting transitions (Fig. 2*a*). In this case, however, only the T_m of transition I, belonging to the free DNA, can be satisfactorily defined. Transition II probably exists in some cases, but is not well separated. Transition III occurs around 100°C and the exact value of the melting temperature cannot be estimated under our experimental conditions.

The method of preparation has a greater effect upon the character of the melting curves than in the case of DNA complexes with sequential polypeptides. Transitions I and III are well defined with little DNA melting in the region of transition II (Fig. 2*b*). T_{mIII} values are lower than in the case of reconstituted complexes and they increase with increasing content of lysine in the polypeptide (Fig. 2*b*).

TABLE I

Melting temperatures of reconstituted complexes DNA–sequential polypeptides

Polypeptide	r	T_{mI}	T_{mII}	T_{mIII}
$(Ala\text{-}Lys\text{-}Pro)_n$	0·32	55·6	72·9	—
	0·63	—	73·2	—
	0·95	—	69·9	81·9
$(Lys\text{-}Ala\text{-}Gly)_n$	0·3	56·6	76·6	—
	0·6	—	76·1	—
	0·9	—	73·4	88·4
$(Lys\text{-}Lys\text{-}Gly\text{-}Ala\text{-}Ala\text{-}Ala)_n$	0·32	51·9	70	91·6
	0·63	50·4	68·8	92·5
	0·95	55·0	70·7	93·4
	1·23	56·6	71·7	93·5
$(Ala\text{-}Lys\text{-}Pro\text{-}Lys)_n$	0·32	54·3	76·1	90
	0·63	58·6	71	88·8
	0·95	63·9	72	89·4
$(Ala\text{-}Lys\text{-}Lys\text{-}Pro\text{-}Lys)_n$	0·32	54·3	76·6	92·5
	0·63	60·0	76	93·7
	0·95	62·0	72	93·2
$(Lys\text{-}Lys\text{-}Gly)_n$	0·32	53·2	—	98·8
	0·65	53·8	—	97·1
	0·95	60·4	—	97·4

Redistribution of Polypeptides on DNA

Complexes of calf thymus DNA with polypeptides $(Lys\text{-}Ala\text{-}Gly)_n$, $(Ala\text{-}Lys\text{-}Pro)_n$, $(Ala\text{-}Lys\text{-}Pro\text{-}Lys)_n$ and $(Ala\text{-}Lys\text{-}Lys\text{-}Pro\text{-}Lys)_n$ have been prepared by gradient dialysis and in some cases also by direct mixing (complex of $(Lys\text{-}Ala\text{-}Gly)_n$ and $(Ala\text{-}Lys\text{-}Pro\text{-}Lys)_n$). The ratio r of these complexes was 0·6. In the next step an appropriate amount of poly(dA-dT).poly(dA-dT) was added to the complexes to achieve a final ratio $r = 0{\cdot}3$. An aliquot of the mixture was measured immediately after addition of the synthetic polynucleotide. Another aliquot was kept at 4°C and measured 7 days after mixing (with $(Ala\text{-}Lys\text{-}Lys\text{-}Pro\text{-}Lys)_n$ complex also 3 weeks after mixing).

TABLE II

Melting temperatures of directly mixed complexes DNA–sequential polypeptides, $r = 0{\cdot}63$

Polypeptide	T_{mI}	T_{mII}	T_{mIII}
$(Ala\text{-}Lys\text{-}Pro)_n$	55·0	70·3	81
$(Lys\text{-}Lys\text{-}Gly\text{-}Ala\text{-}Ala\text{-}Ala)_n$	51·0	74·7	87·1
$(Ala\text{-}Lys\text{-}Pro\text{-}Lys)_n$	53·4	—	89·6
$(Ala\text{-}Lys\text{-}Lys\text{-}Pro\text{-}Lys)_n$	53·2	—	91·4
$(Lys\text{-}Lys\text{-}Gly)_n$	52·9	—	93·6

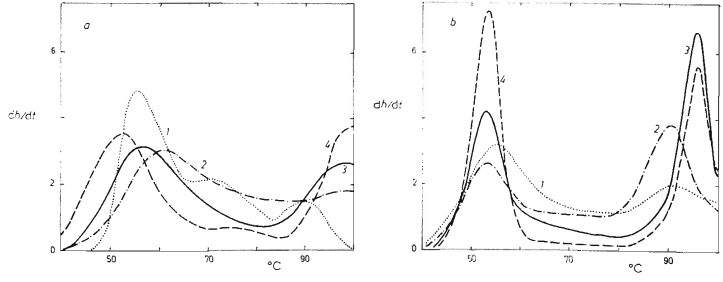

FIG. 2

Melting curves of DNA complexed with random copolymers $(Lys^x, Ala^y)_n$, $r = 0{\cdot}63$, in 0·002M-Na^+. (*a*) Reconstituted complexes; (*b*) directly mixed complexes; *1* DNA–$(Lys^{30},Ala^{70})_n$; *2* DNA–$(Lys^{47},Ala^{53})_n$; *3* DNA–$(Lys^{68},Ala^{32})_n$; *4* DNA–$(Lys^{78},,Ala^{22})_n$.

In the case of $(Lys\text{-}Ala\text{-}Gly)_n$ ($n \sim 23$) complex (Fig. 3) a slight shift and a broadening of poly(dA-dT).poly(dA-dT) peak was observed immediately after mixing. After 7 days the redistribution has proceeded significantly. Complex of $(Ala\text{-}Lys\text{-}Pro)_n$ ($n \sim 34$) (not shown) behaves similarly but the equilibrium establishment is a little slower and similar to that of $(Lys\text{-}Ala\text{-}Ala)_n$ of about the same molecular weight[11]. In the case of $(Ala\text{-}Lys\text{-}Pro\text{-}Lys)_n$ (molecular weight similar to that of $(Lys\text{-}Ala\text{-}Gly)_n$) the redistribution is still lower (not shown). Only a very slight broadening of poly(dA-dT).poly(dA-dT) melting transition was observed after 7 days at 4°C. No redistribution of $(Ala\text{-}Lys\text{-}Lys\text{-}Pro\text{-}Lys)_n$ was observed even after 3 weeks at 4°C.

Directly mixed complexes behave essentially in the same manner as the reconstituted ones, but in all cases the redistribution rate is higher (Fig. 3).

Reversibility of DNA Melting in the Complexes

The DNA complexes of $(Lys\text{-}Ala\text{-}Gly)_n$ and $(Ala\text{-}Lys\text{-}Lys\text{-}Pro\text{-}Lys)_n$ were tested for melting reversibility. In the case of DNA–$(Lys\text{-}Ala\text{-}Gly)_n$ two types of experiments have been carried out. In the first one ($r = 0{\cdot}3$) the reversibility of transition I was tested (Fig. 4*a*). In spite of some hysteresis on decreasing the temperature after the melting of transition I, the second melting of transition I cannot be distinguished from the first one suggesting a total melting reversibility. In the second experiment with a complex of $r = 0{\cdot}9$ (where transition I is practically lacking) after the first melting of transition II only partial reversibility was found on cooling (Fig. 4*b*). In the case of DNA–$(Ala\text{-}Lys\text{-}Lys\text{-}Pro\text{-}Lys)_n$ ($r = 0{\cdot}9$) the reversibility of melting of transition II was tested and partial reversibility was observed.

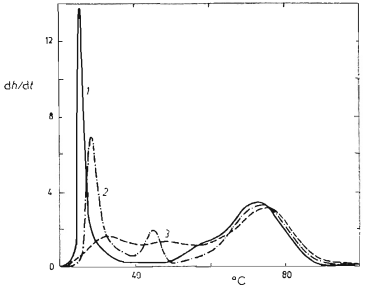

FIG. 3

Derivative melting curves of mixture of synthetic poly(dA-dT).poly(dA-dT) and reconstituted (*1*, *3*) or directly mixed (*2*) complex of calf thymus DNA with $(Lys\text{-}Ala\text{-}Gly)_n$. Lys/DNA = 0·6 measured in 0·002M-Na^+ immediately after mixing (*1*, *2*) and 7 days after mixing (*3*).

DISCUSSION

The evaluation of the melting pattern is rather difficult, because the melting temperatures and relative areas under the derivative melting curves are not connected in a simple way to the properties of respective complexes. Theoretically two limiting cases have been considered. McGhee[17] treated the case of DNA melting in the presence of large reversibly bound ligands. On the other hand, Li and coworkers[2,18] interpreted their results using the assumption that no redistribution of ligand occurs during melting of DNA–polypeptide complexes. Since in the case of our DNA–polypeptide complexes in low ionic strength the electrostatic forces provide the main contribution to the binding free energy it seems reasonable to suppose that the amount and distribution of the positively charged lysine residues will be of primary importance.

Polytripeptides $(Lys\text{-}Ala\text{-}Gly)_n$ and $(Ala\text{-}Lys\text{-}Pro)_n$ *i.e.* polypeptides with relatively low amount of uniformly distributed isolated lysine residues represent one extreme in our series. It was shown[11] that the melting pattern of the $(Lys\text{-}Ala\text{-}Ala)_n$–DNA complexes depend on the molecular weight of the polypeptide. Polymer $(Lys\text{-}Ala\text{-}Ala)_{10}$ binds reversibly to DNA and the theory of McGhee[17] can be applied at least qualitatively. The melting pattern of DNA–$(Lys\text{-}Ala\text{-}Ala)_{34}$ is, however, more complex and cannot be interpreted on the basis of this theory, Polymerization degrees of the polymeric tripeptides studied in this paper are about the same (for $(Ala\text{-}Lys\text{-}Pro)_n$) or slightly lower (for $(Lys\text{-}Ala\text{-}Gly)_n$). In all these cases the same type of melting patterns was observed. Moreover, similar melting patterns were found

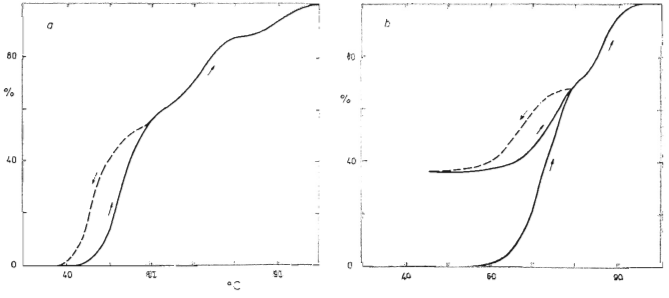

FIG. 4

(*a*) Integral melting curve of complex DNA–$(Lys\text{-}Ala\text{-}Gly)_n$, Lys/DNA = 0·3, in 0·002M-Na^+; ——— heating, - - - - - cooling. (*b*) Integral melting curve of complex DNA-$(Lys\text{-}Ala\text{-}Gly)_n$, Lys/DNA = 0·9, in 0·002M-Na^+; ——— heating, - - - - cooling

for DNA complexes of $(Lys\text{-}Val\text{-}Ala)_n$ and $(Lys\text{-}Nva\text{-}Ala)_n$ (ref.[19]). These melting curves are characterized by the presence of three well separated melting transitions. The first one is due to melting of free DNA segments as can be concluded from T_{m1} value and its ionic strength dependence[11], the other two transitions (II and III) belong to complexed DNA. According to Li and coworkers[18,20], the parameter $\beta = r/[(A_{II} + A_{III})$ (where A_{II} and A_{III} are the areas under the second and third transition relative to the total area under the derivative melting curve) defined the stoichiometric ratio of basic lysine residues of the polypeptide to acidic phosphate groups of the polynucleotide in the complexed part of DNA. As demonstrated in Table III, this parameter for polytripeptides with one lysine residue significantly differs from 1 at lower input ratios r which indicates an incomplete charge neutralization in the

TABLE III

Values r and β for reconstituted (*a*) and directly mixed (*b*) complexes of DNA with sequential polypeptides

Polypeptide	r	β	
		(*a*)	(*b*)
$(Ala\text{-}Lys\text{-}Pro)_n$	0·32	0·65	—
	0·63	0·79	0·92
	0·95	1·01	—
$(Lys\text{-}Ala\text{-}Gly)_n$	0·3	0·63	—
	0·6	0·70	—
	0·9	0·94	—
$(Lys\text{-}Ala\text{-}Ala)_n$[a]	0·3	0·66	—
	0·6	0·75	—
	0·9	0·90	—
$(Ala\text{-}Lys\text{-}Pro\text{-}Lys)_n$	0·32	0·70	—
	0·63	0·97	0·83
	0·95	1	—
$(Ala\text{-}Lys\text{-}Lys\text{-}Pro\text{-}Lys)_n$	0·32	0·85	—
	0·63	0·94	0·94
	0·95	1·09	—
$(Ly_s\text{-}Lys\text{-}Gly)_n$	0·32	0·97	—
	0·63	1·03	0·92
	0·95	1·03	—

[a] See ref.[11].

complexes. Increasing the ratio r, parameter β approaches 1 reflecting a full charge neutralization.

Similar melting curves were found by Schwartz and Fasman[10] in the case of complexes DNA–(Lys-Ala-Pro)$_n$ and DNA–(Lys-Ala-Gly)$_n$. The authors[10] interpret the presence of two well separated melting bands with $T_{m II}$ and $T_{m III}$ as a result of moderate cooperativity of polypeptide binding to DNA, one of the bands ($T_{m II}$) corresponding to non-cooperatively and the other ($T_{m II}$) to cooperatively bound DNA. Our results show that at low r values the part of the DNA covered by the polypeptide melts in transition II. Under these conditions transition I is reversible (Fig. 4*a*) indicating that DNA strands were not separated during melting due to the stabilization of parts of the DNA double helices by the polypeptides. This result confirms the noncooperative character of polypeptide binding when only transition II is present. Schwartz and Fasman[10] do not take into account the possibility of redistribution of the polypeptide during melting. The results presented in this paper and elsewhere[11] suggest that in the case of polytripeptide containing one lysine residue in the monomeric unit partial redistribution of polypeptide may take place. If such polypeptide transfer occurs among native DNA molecules, it should also occur[11] at elevated temperature (probably with a higher rate) between the melted complex II and the unmelted complex and partly accounts for the presence of transition III. The process of polypeptide redistribution from partly-melted-complex molecules to the unmelted molecules can be considered as cooperative, since newly added polypeptides tend to stabilize the complex and make it more resistant to melting Hence, with regard to the mechanism of melting following from our experimental results, the intuitive explanation given by Schwartz and Fasman[10] seems to be essentially correct.

Schwartz and Fasman[10] found that complexes DNA–(Lys-Ala-Pro)$_n$ and DNA–(Lys-Ala-Gly)$_n$ differ in the amount of DNA melted as a complex at the same input ratio r and they conclude that this difference is due to polypeptide sequence. Our results show only minor differences in melting pattern of complexes DNA–(Lys-Ala-Gly)$_n$ and DNA–(Lys-Ala-Pro)$_n$ at similar r values and these patterns are comparable to those of Schwartz and Fasman[10] for the complex DNA–(Lys-Ala-Pro)$_n$. In view of the pronounced dependence of melting pattern of complexes on the polypeptide chain length[11] we feel that the differences observed by Schwartz and Fasman[10] could be rather due to differences in the chain length and/or chain length distribution than to the effect of the sequence. However, the presence of certain amino-acid residues with hydrophobic side chains was found to affect considerably the melting pattern of complexes[19].

In both polypeptide series, random and sequential, transition II disappears with increasing lysine content. Our results with complexes of random copolymers of Lys and Ala are in general agreement with the findings of Pinkston and coworkers[2,18]. In complexes with lysine rich sequential polypeptides the parameter β approaches 1 even

at lower ratios reflecting a full charge neutralization of the complex. The T_{mIII} values of complexes increase with increasing lysine content in both polypeptide series, indicating a better DNA stabilization by higher polypeptide charge density. In the case of polymers containing comparable amount of lysine but differing in its distribution, the T_{mIII} values are higher when the polypeptide contains lysine clusters. Complexes of $(Lys\text{-}Lys\text{-}Gly\text{-}Ala\text{-}Ala\text{-}Ala)_n$ or $(Lys_{30},Ala_{70})_n$ melt at higher temperatures compared with those of $(Lys\text{-}Ala\text{-}Gly)_n$ or $(Ala\text{-}Lys\text{-}Pro)_n$. Complexes of random and sequential polypeptides were also studied by means of specific probes[21]. The more lysine a polypeptide contains the stronger is its competition with actinomycin binding to DNA, the effect being greater in the case of random polypeptides, probably because of lysine clusters present. Thus the resistance of the complex against actinomycin D intercalation roughly parallels its thermal stability, suggesting an important effect of lysine clusters on binding affinity.

In both series of polypeptides, higher T_m values, indicating stronger interaction, were observed in reconstituted complexes formed by thermodynamically reversible binding process as compared to complexes resulting from kinetically controlled direct mixing.

Polypeptide redistribution among polynucleotide molecules was used as an indicator of the reversibility of binding. The redistribution rate is a parameter highly sensitive to the degree of polymerization for polypeptides of the same type[11]. In addition, it is also affected by the method of preparation of complexes. Under conditions of comparable chain length, the redistribution rate is higher for polypeptides containing lower percentage of lysine residues (Fig. 3). In the same case of lysine rich polypeptides (more than 50% of lysine) almost no redistribution was detected. Therefore, a partial reversibility of binding must be considered in melting studies of complexes of DNA with polypeptides of a relatively low charge density. Even a slight modification in the total charge and in the distribution and availability of charged groups within the DNA binding polypeptide segment can appreaciably modify the ability of the respective polypeptide (protein) to change its position along the DNA chain during melting.

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