Chiral and Structural Discrimination in Binding of Polypeptides with Condensed Nucleic Acid Structures*

(Received for publication, April 17, 1989)

Ziv Reich, Yitzhak Ittah, Sarah Weinberger, and Abraham Minsky†

From the Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot 76100, Israel

In biological systems nucleic acids are invariably found in highly compact forms. These rather intricate forms raise questions of basic importance which are related to the various factors involved in the condensation processes, the chemical, physical, and structural features revealed by the packed species, and the effects of the extremely tight packaging upon interactions of the DNA molecules with proteins and drugs. A means for addressing these questions on a molecular level is provided by various procedures known to induce in vitro condensation of DNA molecules into highly compact species which, in turn, may serve as a model for the in vivo physical organization of nucleic acids. A study of the optical properties of the tightly packed DNA molecules indicates that the interactions of these species with polypeptides are characterized by distinct, hitherto unobserved, chiral and structural discrimination. Specifically, the polypeptides found to be selected against are composed of those amino acids that are not normally used in protein biosynthesis, such as D-lysine or ornithine. These findings provide new clues to long debated topics such as the specific universal chirality of amino acids in proteins or the correlation between conformational flexibility of polypeptides and their ability to form stable compact complexes with nucleic acids.

The extremely tight packaging of genomic nucleic acid molecules, characterizing all living systems, raises many unanswered questions of fundamental importance. The encapsulation of nucleic acids into preassembled phage proheads, the factors that control and affect DNA organization in sperm cells, and the condensation processes leading to the formation of eukaryotic metaphase chromosomes are all far from being fully understood. Equally unclear are issues related to the chemical, physical, and structural features revealed by the condensed DNA phases or topics concerned with the effects of the extremely tight packaging on interactions with proteins and drugs. Systematic studies of these intricate structures and processes are intrinsically difficult, being complicated by the myriad factors involved in the organization of the in vivo complexes as well as by the necessity to isolate the species without introducing unknown uncontrolled artifacts.

A means to overcome these problems is provided by various procedures known to induce in vitro condensation of nucleic acids into highly compact forms (1-6). The resulting structures, obtained under well defined conditions and relatively easily studied, may serve as a basic general model for the condensed biological systems.

The current study was motivated by the question of how are the interactions between DNA molecules and proteins affected by the tight packaging of nucleic acids. We find that interactions involving condensed DNA species and basic positively charged polypeptides are characterized by distinct chiral and structural discrimination. The findings suggest hitherto unconsidered clues to long debated topics such as the specific chirality of amino acids in proteins, the correlation between conformational flexibility of polypeptides and their ability to form stable compact complexes with nucleic acids, or the origin for the handedness found to characterize the higher order states of DNA organization, i.e. nucleosomal structures and chromatin fibers.

MATERIALS AND METHODS

Poly-L-lysines (degree of polymerization = 60, 110, 153), poly-D-lysines (degree of polymerization = 48, 72, 123, 210), poly-L-ornithine (degree of polymerization = 93), and highly polymerized calf thymus DNA were purchased from Sigma. DNA was dissolved in 20 mM Tris buffer, pH 7.5, and sonicated for 2 × 30 s using an Ultratip Labsonic sonicator. The DNA fragments were loaded on a Sephacryl S-400 (Pharmacia LKB Biotechnology Inc.) column and eluted with 20 mM Tris buffer, pH 7.5. Fractions of 5 ml were collected, and the size distribution of the DNA fragments in each fraction was determined by 0.75% agarose gel electrophoresis. The samples were extensively dialyzed against 5 mM Tris buffer, 5 mM EDTA and concentrated by ultrafiltration. DNA concentrations were determined by measuring the absorption at 260 nm, using an extinction coefficient of 6600 M⁻¹ cm⁻¹ on a Hewlett-Packard 8460A diode array spectrophotometer. DNA molecules used in the present study were of mean length of 6000 base pairs; longer nucleic acid segments were found to form precipitates shortly after condensation was induced.

Circular dichroism spectra were recorded on a Jasco J-600C spectropolarimeter equipped with a DP-500N data processor. Spectra were taken at room temperature, using 1-cm light path cells. The conditions used to prepare condensed DNA polypeptide species are described in the figure legends. As CD spectra of compact nucleic acid structures are usually hard to reproduce accurately (for a discussion on the theoretical aspects of this phenomenon, see Ref. 10), each experiment was repeated 3-5 times. Each curve in all figures is, consequently, a representative CD spectrum obtained from a number of independent experiments. It should be emphasized, however, that for identical mixtures only the absolute magnitude of the CD spectra exhibits a narrow variability, whereas the shape and the sign of the signals are fully reproducible.

RESULTS AND DISCUSSION

Under appropriate conditions DNA molecules undergo a cooperative compaction process. The resulting structures are characterized by unusual chemical and physical properties among which the most remarkable is the presence of prominent optical anomalies. These anomalies are observed as nonconservative CD spectra whose magnitudes are significantly larger than those revealed by dispersed DNA molecules.
associated with a well defined long range asymmetry that is exhibited by polypeptides exhibit extremely large optical properties obtained upon the interactions of compact nucleic acid species. DNA segments, result in the CD phenomena depicted in Fig. 1A. Negative ellipticities are observed at all salt concentrations, whereas condensation in the presence of poly-D-lysine results in invariably positive CD signals (Fig. 1 B and C), in clear contrast to the systems devoid of polypeptides. The modified CD bands indicate that the condensation process is accompanied by the formation of ordered tertiary species in which long range couplings between nucleotide chromophores are induced (7–10). Of particular interest are the phenomena observed upon DNA condensation by dehydrating agents at high ionic strength conditions (11, 12). DNA molecules undergo, under such conditions, a cooperative transition into compact forms that exhibit nonconservative CD spectra whose sign and magnitude are determined solely by the ionic strength. It has been suggested that the condensed dsDNA molecule is organized in parallel arrays which fold back and forth, thus forming rod-shaped tertiary structures (11, 13). These arrays are packed tightly enough to allow efficient interhelical chromophore couplings which, combined with a salt concentration-dependent twist in between the contiguous dsDNA segments, result in the CD phenomena depicted in Fig. 1A.

In a previous study we have shown that the complexes obtained upon the interactions of compact nucleic acid species with polypeptides exhibit extremely large optical properties associated with a well defined long range asymmetry that is determined by the polypeptide conformation (12). When DNA condensation is induced in the presence of poly-L-lysine, large negative ellipticities are observed at all salt concentrations, whereas condensation in the presence of poly-D-lysine results in invariably positive CD signals (Fig. 1 B and C), in clear contrast to the systems devoid of polypeptides. The modified chiral properties revealed by the DNA-polypeptide complexes were related to the particular handedness exhibited by the polv-L- or poly-D-lysines; such handedness, defined in turn by the uniform chirality of the amino acids in each polymer, stabilizes specifically one sense of the interhelical twist of the rod-shaped condensed DNA molecules. Theoretical considerations have indicated that for asymmetric particles of similar size, chromophore density, and twist angle, the signs of the nonconservative CD spectra are determined solely by the sense of the twist; species of opposite handedness reveal CD signals which are mirror images of each other. Specifically, right-handed helical arrangements display positive CD signals, whereas left-handed conformations result in negative spectra (14). This particular assignment of the absolute configuration of highly packed asymmetric particles, such as nucleic acid condensates, is supported by the observations according to which the association of H1 histone with linker DNA segments results in the superhelical left-handed chromatin structure, as evidenced by electron microscopy (23); CD measurements of DNA-H1 complexes reveal nonconservative negative signals (15, 16). Thus, we suggest that upon interaction with packed DNA species, poly-L-lysine induces and specifically stabilizes a clockwise left-handed tertiary structure; DNA-poly-D-lysine complexes assume, on the other hand, a right-handed long range asymmetry, characterized by positive nonconservative CD signals.

In addition to the modification of the DNA tertiary structure, the pollysines seem to induce the formation of aggregates whose asymmetric quaternary conformation is, again, determined by the polypeptide handedness. This is based on the observation that upon increasing the solid angle of CD detection the magnitudes of the CD spectra exhibited by the DNA pollysine species are substantially reduced (Fig. 2, B and C), whereas those revealed by compact DNA structures

---

1 The abbreviation used is: ds, double stranded.
binding of polypeptides are not significantly affected (Fig. 2A). These findings indicate that besides differential absorption which characterizes nucleic acid compact forms, DNA-polypeptide complexes exhibit differential scattering, typical of aggregates of well defined long range asymmetry (7–10, 17). The formation of large asymmetric aggregates is further indicated by the observation that nucleic acid-polypeptide condensates obtained from particularly long dsDNA molecules (mean length of 23,000 base pairs) tend to form precipitates shortly after condensation has been induced; moreover, the CD signals revealed by these condensates exhibit non-zero values outside the absorption band. Such “tails” are known to characterize asymmetric aggregates (10); their magnitudes were shown to correlate with the size of the particles (9). The formation of aggregates is also suggested by a strict length requirement that has to be met by the polypeptides in order to exhibit the CD anomalies shown in Figs. 1 and 2, as well as those to be described. It was found that DNA condensation in the presence of polypeptides shorter than 60 residues (or monomeric lysines) results in optical properties that are identical with those obtained in the absence of the polymer (Fig. 1A); polypeptides composed of 70 amino acids are required to elicit the CD phenomena that characterize the compact DNA-polysine complexes. No significant modifications of the optical properties can be detected upon a further increase of the polypeptide’s length. Thus, it may be suggested that, if long enough, the polypeptides can attach a number of compact DNA molecules in an asymmetric form, resulting in chiral aggregates.

Fig. 3 presents the CD spectra obtained upon DNA condensation performed at 0.8 M NaCl in the presence of either poly-L-lysine, poly-D-lysine, or a racemic mixture composed in equal amounts in terms of amino acid molarity. The deviation of the experimental spectrum from the integrated computed signal is observed at various ionic strengths; the extent of this deviation is, however, gradually diminished as the salt concentrations are increased (Fig. 3, B–D). It should be noted that the optical phenomena depicted in Fig. 3, as well as those shown in Figs. 1 and 2, are observed at DNA concentrations ranging between 10^{-9} and 10^{-4} M in base pair residues. At this range, the molar ellipticities of the various condensed species are virtually constant, provided that the molar ratio between the nucleic acids and polypeptides is maintained. This observation indicates that the crucial factor in determining the structural features of the condensed complexes is the relative concentrations of the interacting species, as opposed to the absolute values. At DNA concentrations that exceed 10^{-4} M (in base pairs) the condensation process results in the appearance of visible translucent particles, suggesting an enhanced formation of large aggregates.

We suggest that the results presented in Fig. 3 amount to a chiral discrimination, where, following the interaction of tightly packed DNA species with a racemic mixture of the two “enantiomeric” polylsines, the properties of the resulting complexes are dominantly determined by the polypeptide composed of the naturally occurring L-amino acid.

Two distinct processes responsible for such a chiral discrimination can be envisaged: either a preferential binding of packed DNA species to the poly-L-lysine or a situation where, upon excess of protein over nucleic acid in terms of amino acid versus nucleotide molarities, both poly-L- and poly-D-lysines interact with a given DNA molecule. The properties of the compact ternary complex thus obtained are, however, determined exclusively by one of the polypeptides. The following procedures appear to indicate that both processes are operative.

Conclusive evidence that interactions between compact nucleic acid species and polypeptides are characterized by a preferential binding is provided by the following experiment. DNA-polysine condensed aggregates, formed in the presence of a racemic mixture of polypeptides at various salt concentrations, were precipitated by centrifugation and the CD spectra of the supernatant solutions, assumed to contain free unbound polysine molecules, were studied. The results, presented in Fig. 4, point unambiguously toward an enrichment of the supernatant solutions with poly-D-lysine, indicating, consequently, a preferential complexation of packed DNA species with the poly-L-lysine.

Surprisingly, DNA condensation processes, induced in the presence of both polypeptides at higher salt concentrations, result in larger amounts of free poly-D-lysine (Fig. 4, spectra 5 and 6), apparently contradicting the observation according to which the chiral discrimination is more pronounced at lower ionic strengths. A plausible explanation for this discrepancy is offered by a set of experiments in which DNA condensation was induced in the presence of the two polysine species; the concentration of one enantiomeric polypeptide was kept constant (at equal molar ratio with the dsDNA molecules in terms of amino acid and base pair molarities) whereas the quantities of the other enantiomeric polypeptide species were gradually increased. CD signals maxima, plotted as function of the molar ratio between the two enantiomeric polysines (Fig. 5), point toward a strikingly different effect.
exerted by the excess of protein on the properties of the DNA-poly-L- and DNA-poly-D-peptide complexes. The optical, and hence structural, properties of the condensed DNA-poly-L-lysine species are not altered by the presence of the poly-D-peptide in increasing quantities when the condensation process is induced at low ionic strength, small but salient structural effects are observed, however, when the titration is conducted at high salt concentration. Specifically, these effects are rather conspicuous following the increase of the poly-D-lysine concentrations up to a poly-D-versus poly-L-lysines molar ratio of 0.5:1; addition of larger amounts of the poly-D-lysine does not affect the structural parameters of the resulting compact complexes (Fig. 5B). In clear contrast, nucleic acid-polypeptide condensates prepared from mixtures containing DNA, poly-D-lysine, and increasing amounts of the poly-L-peptide exhibit extremely large structural modifications as a function of the poly-L-versus poly-D-lysine molar ratios. At both low and high salt concentrations, the large positive nonconservative CD signals that characterize the DNA-poly-D-lysine condensed species are converted into negative bands. It should be noted, however, that the optical parameters revealed at low ionic strength at the end of the titration coincide with the parameters that characterize the DNA-poly-L-lysine condensates devoid of the poly-D-peptide; such is not the case at high ionic strength, as the CD values revealed under these conditions by the DNA-poly-L-lysine compact forms are modified during the titration with the poly-D-peptide.

Based on the limited quantity of free poly-D-lysine observed at low ionic strengths, we suggest that under these conditions the overall excess of protein over nucleic acids leads to the formation of ternary complexes where both poly-L- and poly-D-lysines are bound to a single condensed DNA molecule. The nonconservative negative ellipticities revealed by these complexes, assuming corresponding to a long range left-handed conformation, as well as the lack of any optical effects exerted at these conditions by the increasing amount of the poly-D-peptide (Fig. 5A), point toward a predominant structural effect of the poly-L-lysine upon the compact ternary species. At high ionic strengths, the larger amount of unbound poly-D-lysine (Fig. 4) implies an enhanced preferential complexation of the poly-L-lysine with packed DNA. The apparent limited chiral discrimination observed under these conditions (Fig. 3, C and D) suggests, however, a significant contribution of the poly-D-peptide to the structural features of the condensed DNA-polypeptide complexes. The results presented in Fig. 5B corroborate this assumption by indicating that only a limited quantity of poly-D-lysine, apparently up to half the molar amount of the poly-L-lysine, is incorporated in the compact ternary complexes obtained at high salt concentration, yet this quantity is sufficient to substantially modify their structural patterns. Consequently, the structural effect of the poly-L-lysine that predominates at relatively low ionic strength is substantially negated at high salt concentrations by the enhanced effect of the poly-D-peptide exhibited under these conditions. Thus, both a preferential binding effect and a preferential structural effect seem to be involved in processes revealed by condensed nucleic acid species; the relative extent of these two distinct phenomena depends, however, on the ionic strength.

A question of basic importance is concerned with the factors responsible for the observed chiral discrimination. The CD spectra shown in Fig. 1 (parts B, C, and D), as well as those shown in Figs. 2 and 3, are obtained whether the polypeptide is present during DNA condensation or added after the compaction process has been induced by the dehydrating agent. This observation indicates that the structural effects of the
polypeptides result from the interaction between the polylysine and DNA molecules which are already condensed into ordered compact species. This finding is further supported by the observations according to which in the absence of a dehydrating condensing agent DNA-polylysine complexes dissociate at salt concentrations exceeding 0.8 M (18). However, addition of poly-L-lysine to preformed DNA-poly-L-lysine compact species fails to alter the optical properties that characterize the DNA-poly-L-peptide complexes; similarly, treatment of condensed DNA-poly-D-lysine complexes with the poly-L-peptide does not affect the positive CD signals revealed by these species. The inert behavior exhibited by condensed DNA-polylysine complexes (but not by compact nucleic acid structures devoid of polypeptides) may be assigned to the formation of large highly condensed and, hence, kinetically stable aggregates following DNA-polylysine interactions. Accordingly, the selective binding observed upon treatment of compact DNA species with a racemic mixture of polylysines is kinetically controlled; it must originate from a higher complexation rate between nucleic acids and the poly-L-peptides.

Enantiomeric selectivity was reported to characterize interactions between dsDNA molecules, in their extended secondary conformation, and various organometallic species containing a chiral center (19-21). The interactions leading to the chiral discrimination described in the present study involves a higher structural order, namely tertiary conformation of nucleic acids and long range structural secondary structure assumed by the polylysine upon complexation with tightly packed DNA molecules (12).

In addition to the chiral discrimination, processes involving compact DNA species exhibit strict structural preferences. When poly-L-ornithine is substituted for poly-L-lysine in the condensation systems, the resulting DNA-poly-L-ornithine complexes reveal exceedingly small CD spectra (Fig. 1D). These small ellipticities indicate that, in clear contrast to polylysines, polyornithines fail to induce a specific long range ordering upon packed DNA forms. Significantly, the magnitudes of the ellipticities that characterize the DNA-polyornithine complexes are substantially smaller than those exhibited by the system devoid of polypeptides (Fig. 1A). As the intensities of the ellipticities that characterize the DNA-polyornithine complexes are substantially smaller than those exhibited by protamines in sperms and to be responsible for the extreme compactness encountered in these cells (22).

A gratifying result of this study is related to the observation that in both cases where chiral discrimination or structural preference is obtained, namely poly-L-versus poly-D-lysine and polylysine versus polyornithine, the polypeptide composed of amino acids not normally used in biosynthesis is discriminated against. Left-handed tertiary conformations of compact nucleic acids-polypeptide complexes, specifically induced by proteins constructed from L amino acids, are shown to predominate over long range right-handed structures associated with the D residues. This distinct conformational predisposition might have led to an enrichment of the first functional DNA-polypeptide species with proteins containing the L enantiomer. Such a preference could have propagated to include various DNA-binding proteins, thus triggering a process that finally resulted in the universality of the amino acids L configuration. The suggestion that the chirality of protein residues had been committed once the configurational isomer of nucleic acids was defined by the D-ribose moiety is further buttressed by the following observations. First, long range left-handedness seems to be a general characteristic of biological DNA-structural protein tertiary complexes such as nucleosomes (22), chromatin (23), and sperm cells (24). Second, the polymerization of a racemic solution of D and L amino acids was shown to be stereoregulated even in the absence of a chiral initiator. Such a regulation, associated with secondary conformational constraints, results in the formation of a mixture which, though optically inactive, is composed of polypeptides highly enriched in a given enantiomer, as opposed to random polymers containing both L and D residues in a 1:1 ratio (25).

The pronounced difference in the structural effects exerted by polylysines and polyornithines, ascribed to the higher flexibility of the lysine-containing peptide, suggests a correlation between the tendency of a polypeptide to undergo facile conformational changes and its ability to form stable compact complexes with nucleic acids (12). The requirement for conformational change from a random coil to an ordered structural motif is reminiscent of processes believed to be exhibited by protamines in sperms and to be responsible for the extreme compactness encountered in these cells (22).

A general meaningful correlation between the in vitro and in vivo condensed nucleic acid forms is pointed out. It may be hoped that a detailed understanding of the chemical an structural features of the compact DNA species described in this study will allow a deeper insight into the tight organization of nucleic acids in vivo.

REFERENCES
1. Jordan, C. F., Lerman, L. S., and Venable, J. H. (1972) Nature 236, 67-77
2. Gosule, L. C., and Shellman, J. A. (1976) Nature 259, 333-335
3. Cheng, S. P., and Mohr, S. C. (1975) Biopolymers 14, 663-674
4. Lenz, K. U. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 4266-4269
5. Yvedhokimov, Y. M., and Salyanov, V. I. (1981) Nucleic Acids Res. 9, 355-373
6. Shin, Y. A., and Eichborn, G. L. (1984) Biopolymers 23, 325-335
7. Shin, Y. A., and Eichborn, G. L. (1977) Biopolymers 16, 375-376
8. Reich, C., Maestas, M. F., Edmondson, S., and Gray, D. M. (1980) Biochemistry 19, 5209-5219
9. Maestas, M. F., and Reich, C. (1980) Biochemistry 19, 5214-5223
10. Keller, D., and Bustamante, C. (1996) J. Chem. Phys. 94, 2973-2990
11. Ruey, R., and Mohr, S. C. (1980) Biopolymers 20, 2533-2552
12. Weinberger, S., Berman, C., and Minsky, A. (1986) J. Am. Chem. Soc. 108, 8231-8232
13. Rechnitz, T. H., and Moustakas, R. N. (1974) Cell 13, 250-256
14. Kim, M. H., Ulbricht, L., Keeler, D., Maestas, M. F., and Bustamante, C. (1986) J. Chem. Phys. 94, 2891-2898
15. Sponar, J., and Fric, I. (1971) Biopolymers 10, 2117-2132
16. Tinto, L., Bustamante, C., and Maestas, M. F. (1980) Annu. Rev. Biophys. Bioeng. 9, 107-141
17. Bustamante, C., Tinto, L., and Maestas, M. F. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 3568-3572
18. Granados, E., and Bello, H. (1983) Biochemistry 22, 3521-3532
19. Barton, J. K., Danishefsky, A. T., and Goldberg, J. M. (1984) J. Am. Chem. Soc. 106, 2172-2176
20. Barton, J. K., Goldberg, J. M., Kuzin, C. H., and Turco, N. J. (1986a) J. Am. Chem. Soc. 108, 2061-2068
21. Barton, J. K. (1986) Science 233, 727-734
22. Lenz, K. U., and Shellman, J. A. (1976) J. Am. Chem. Soc. 108, 233-246
23. Williams, S. P., Alten, D. M., and Ting, C. N. (1981) J. Biochem. 90, 5209-5219
24. Maestas, M. F., Bustamante, C., Hayes, T., Subbarao, J. A., and Tinto, L. (1986) Nature 328, 27-74
25. Spach, G. J. (1974) in International Symposium on Generation and Amplification of Asymmetric Chemistry (Thiemann, W., ed) pp. 259-271. Verlag Chemie, New York.
26. Warrant, R. W., and Kim, S. H. (1978) Nature 271, 130-135
27. Dorman, B. F., and Maestas, M. F. (1975) Proc. Natl. Acad. Sci. U.S.A. 70, 255-259
28. Holowarth, G., and Doty, P. (1965) J. Am. Chem. Soc. 87, 214-228
Chiral and structural discrimination in binding of polypeptides with condensed nucleic acid structures.
Z Reich, Y Ittah, S Weinberger and A Minsky

J. Biol. Chem. 1990, 265:5590-5594.

Access the most updated version of this article at http://www.jbc.org/content/265/10/5590

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/265/10/5590.full.html#ref-list-1