OPTICAL ROTATORY DISPERSION OF NUCLEOCLUPEINE AND OTHER MODEL NUCLEOPROTEINS

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Optical rotatory dispersion (ORD) is a powerful physical method in conformational studies of molecules in solution. The technique also provides much information on the specific association of molecules of biological interest. We have found that the ORD is useful in conformational characterizations of complexes formed between DNA and nuclear basic proteins as well as synthetic basic amino acid polymers in solutions.

High molecular weight DNA (average M. W. $= 6 \times 10^6$, viscosity) prepared from herring testes was subjected to 10 Kc sonic oscillation for 20 minutes at 2 - 4°. The molecular weight of the sonicate was found to be 3.7×10^5 (viscosity). The electron microscopic observations show that the fragmentation was satisfactory and the average M. W. was determined to be 3.4×10^5 by the measurement of the length and number distribution of the fragments. No "denaturation" has occurred in the double-helical structure during the sonic treatment as judged from the spectrophotometric thermal denaturation profile of the sonicate. Clupeine was prepared from the acetone-dried herring sperm heads (Ando et al., 1957). Poly-L-ornithine and poly-L-arginine were chemically synthesized and kindly given by Mr. S. Kawashima. Complexes were prepared by titrating the solutions of DNA in 0.01 M NaCl-0.001 M Tris-HCl, pH 7.0 with equal volume solutions of polypeptides in the same buffered saline under constant stirring. By using sonicated DNA, complexes were kept "soluble" up to the polyanion-polycation charge ratio of unity. The concentration of DNA was adjusted to 1.53×10^{-4} mole P/liter ($A_{260} = 1.1$) for all solutions used in

the ORD measurements. The concentration of polypeptide was determined by the amino acid analysis of the solution and expressed as mole cation/liter. All complexes reported in this communication had peptide cation/DNA-P ratio of unity. ORD measurements were made at 23° on a JASCO Model ORD/UV-5 spectropolarimeter with a 1-cm quartz cell. Blanks were determined just before and after measurement of each sample. Measurements were repeated at least twice for each sample. Rotations were expressed in molar rotation per DNA-P, $[\phi] = [\alpha] \cdot M_o/100$, where $[\alpha]$ is the specific rotation and M_o is mean residue molecular weight which is correlated to molar concentration of DNA as expressed in mole P/liter.

Fig. 1 and 2 represent typical ORD curves of sonicated DNA and complexes. The ORD spectrum of sonicated DNA is essentially identical with that of intact DNA. Each complex has a characteristic ORD curve which is obtainable with a perfect reproducibility when the complexes are prepared under constant conditions. The rotation of peptides was very small above 230 mμ and the sum of that of DNA and peptide does not much differ from the dispersion curve of DNA. Since ultraviolet absorption of the solutions of DNA and various complexes do not show characteristic difference or, if any, the subtle difference is obscured by the presence of light scattering in the solutions of complexes, the ORD technique is more advantageous for the detection of conformational changes in these systems. The contribution of light scattering to the observed absorbancy at the maximum (260 mμ) was estimated for the solution of each complex by an extrapolation method (Leach and Scheraga, 1960). The value T_{260} = (absorbancy at 260 mμ due to light scattering)/(absorbancy at 260 mμ measured) was used as a parameter for the estimation of turbidity. The values of T_{260} thus obtained were 0.49, 0.19, 0.20 and 0.17 for DNA-clupeine, DNA-$(Arg)_{20}$, DNA-$(Lys)_{100}$ and DNA-$(Orn)_{19}$, respectively. It has also been found that the sizes of the light scattering particles are in the comparable order for all kinds of complexes as judged from the data based on the slopes of logarithm of the absorbancies vs. log λ curves. From these evidence it is now concluded that the observed changes in the ORD spectrum of DNA are not due to light scattering but these changes are directly related to conformational changes of DNA molecules by the formation of complexes.

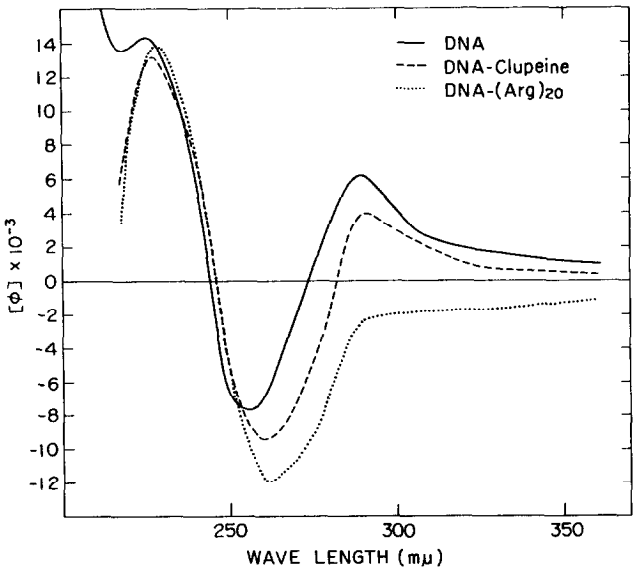

Fig. 1. Optical rotatory dispersion of DNA, DNA-clupeine and DNA-$(Arg)_{20}$.

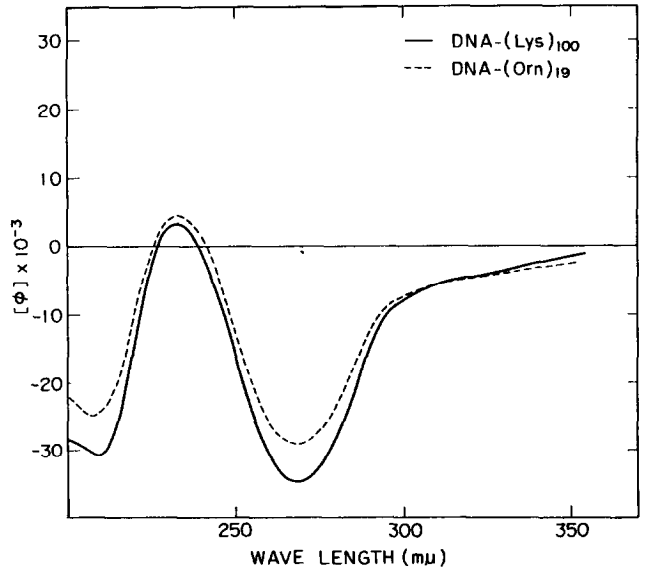

Fig. 2. Optical rotatory dispersion of DNA-$(Lys)_{100}$ and DNA-$(Orn)_{19}$

The differences between the ORD spectra of DNA-$(Arg)_{20}$ and DNA-$(Lys)_{100}$ or DNA-$(Orn)_{19}$ clearly show that the nature of the basic groups (amino or guanidino) is more important in exerting the effects on con-

formational changes in DNA upon complex formation than the length of the side chain (ornithine or lysine). The similarity of the ORD curves of DNA-$(Lys)_{100}$ and DNA-$(Orn)_{19}$ as well as DNA-$(Orn)_8$---not included in the figure---also indicates that the difference in degree of polymerization of a peptide does not affect the ORD spectrum of the complex formed by DNA and these sizes of oligo- and polypeptides.

We are especially interested in the difference found in the ORD of DNA-clupeine and DNA-$(Arg)_{20}$. Presently clupeine is only naturally occurring nuclear basic protein for which the complete chemical structure is elucidated. It has been fractionated into 3 pure molecular species and all of their amino acid sequences have been determined in our laboratory (Ando et al., 1962; Ando and Suzuki, 1966, 1967). The chemical structure of clupeine Z is representatively shown: H-Ala·Arg·Arg·Arg·Arg·Ser·Arg·Arg·Ala·Ser·Arg·Pro·Val·Arg·Arg·Arg·Arg·Pro·Arg·Arg·Val·Ser·Arg·Arg·Arg·Arg·Ala·Arg·Arg·Arg·Arg-OH. It should be important to study the exact molecular structure of nucleoprotamines and their biological consequences with chemically pure molecular species of protamine. The present study was undertaken with such an objective, and the presently obtained ORD data indicate that, in nucleoclupeine prepared under the conditions described, the molecular geometry of DNA is rather close to free DNA in solution and quite different from that in DNA-$(Arg)_{20}$. The following interpretation is conceivable as possible origin of the observed difference in ORD phenomena:

[1] The molecular structures of DNA-clupeine and DNA-polyarginine are quite different. In the nucleoprotamine structure based on the X-ray diffraction studies, the fully extended polypeptide chain was placed in the small groove and wrapped around the nucleic acid helix (Feughelman et al., 1955). However, since no X-ray data are available for DNA-polyarginine complex, we cannot draw any definite conclusion on its structure.

[2] The structures of DNA-clupeine and DNA-polyarginine are essentially same as suggested by Feughelman et al. (1955) except for the placement of neutral amino acid residues which occur singly. Our sequence analysis revealed that there are 1 to 3 single neutral amino acid residues in each clupeine molecule. Since a single residue cannot make a loop, some phosphate groups should be left not combined with guanidinium groups.

The presence of such free phosphate anionic segments is accounted to be responsible for the retainment of free DNA like conformation in aqueous solution.

[3] DNA double helix is distorted by the consecutive binding of phosphate groups with guanidinium groups. The distance between the successive side-chains pointing in the opposite directions is not exactly equal to the distance between two phosphate groups (one phosphate on each nucleotide chain). The cumulative "unfitness" would cause the distortion of normal double helical structure of DNA. In the case of nucleoclupeine, the consecutive arginine residues are at most 4. Thus a small number of consecutive arginine residues would not upset the double helical form of DNA and instead the "unfitness" can be adjusted at the position of neutral amino acid residues.

The difference between DNA-polyarginine and DNA-polylysine or DNA-polyornithine can also be explained if one considers that differences in the distance between charged groups and in the strength of the binding and most probably the degree of hydration around charged groups are the origin of the conformational dissimilarity of DNA in the complexes.

Although the exact nature of the mechanism of the ORD phenomena found in the present study needs to await further study, the presently found ORD dependence of nucleoproteins on the structure of the basic proteins used must be regarded as a useful criterion in the studies of not only nucleoprotamines but also nucleohistones. It is also interesting to note that Maestre and Tinoco (1967) reported similar conformational changes of DNA in viruses. The pattern of their ORD spectra of some viruses is apparently quite similar to our ORD spectra of complexes. Both in viruses and in sperm heads DNA is tightly packed although no definite evidence has been presented to show that DNA in viruses is present in associated form with polyamines or peptides.

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