ON THE ORIGIN OF THE HYPOCHROMIC EFFECT IN DOUBLE-STRANDED POLYNUCLEOTIDES

V. I. DANILOV

Department of Quantum Biophysics, Institute for Theoretical Physics, Academy of Sciences of the Ukrainian SSR, Kiev, USSR

Received 10 June 1974

1. Introduction

The theoretical study of the hypochromic effect in polynucleotides has been the subject of several papers [1–6]. However, these investigations [1–3,5,6] had some significant shortcomings: 1) the investigation was inconsistent–electronic transition energies and moments in monomers were taken from experiments, whereas polarization directions and matrix elements of the monomer-monomer interaction operator were calculated theoretically; 2) only those electronic transitions from which the first two absorption bands arise were considered in the calculation of polynucleotide hypochromism; 3) approximations used for the interaction operator matrix elements were often chosen arbitrarily.

These shortcomings, along with the absence of quantitative agreement between theoretical and experimental results, considerably diminish the importance of these quantum-mechanical investigations. Moreover, the question of the contribution of intra- and interstrand monomer–monomer interactions to the absorption intensity change for a double-stranded polymer has not been studied theoretically. The elucidation of this problem is important for the understanding of the role of H-bonds and stacking interactions between the bases in the UV absorption change observed in the processes of denaturation and renaturation of polynucleotides and nucleic acids [7–15].

The aim of this work is both to reconcile quantitatively the results of a systematic theoretical study of the hypochromic effect of the simplest polynucleotides, poly(dA)·poly(dT) and poly(dG)·poly(dC) with the experimental data and the elucidation of the origin of this phenomenon.

2. Method

For this study of the first absorption band of polynucleotides the perturbation theory was used. When calculating the hypochromism the formula which included only the dispersion term was used, as in [1–3, 5].

The characteristics of monomers necessary for the calculation polynucleotide hypochromism with the help of perturbation theory were computed theoretically by Pariser–Parr–Pople method with all singly excited configurations taken into account. A detail description of the results of the calculation was given in [16]. All the π-electronic transitions in monomers were considered in the calculations of polynucleotide hypochromism. In studying polynucleotide systems which are rather complicated theoretically the consideration of π-electronic states only is justified, because the absorption bands at present observed experimentally are due to π–π^* transitions [17–19].

In accordance with the consistent theoretical treatment proposed by us in [16], the matrix elements of monomer–monomer interaction operator were calculated to the same approximation which was used for the calculation of monomers. As shown in [16] this approximation is the best for studies of hypochromism by the perturbation theory method.

As a model for polynucleotides, the fragment of DNA in the B form, consisting of ten Watson–Crick pairs was used.

3. Results and discussion

The calculated hypochromism values for poly(dA)·

Table 1
The hypochromism values (%) of polynucleotides

Polynucleotide	Theory				Experiment**
	[3]	[4]*	[5]	Our results	[20]
poly(dA)·poly(dT)	33	8	42	46	40 39
poly(dG)·poly(dC)	45	-2	9	43	35 36

* Hypochromism values for the first absorption band were obtained by us from theoretical results [4].

** These values were calculated by us in [20] from existent experimental data on absorption spectra of corresponding poly- and mononucleotides.

poly(dT) and poly(dG)·poly(dC) and the experimental hypochromism values, necessary for the comparison with theory are adduced in table 1.

From table 1 one can see, first of all, good agreement between theoretical and experimental results. Further, the experimental fact that poly(dG)·poly(dC) is characterized by a lower hypochromism than poly(dA)·poly(dT) is confirmed. It is essential that the differences between the hypochromism values of these polynucleotides given by theory and by experiment are very close.

Comparing the hypochromism values obtained by us and by other authors (table 1), one should note that good agreement of theory with experiment is achieved in the present work for the first time. One may also see that the data of DeVoe and Tinoco [3] do not reproduce the experimental dependence of hypochromism upon the base composition. Although, in the work of Brown and Pysh [5] this dependence was represented in a correct way, the hypochromism value for poly(dG)·poly(dC) is in poor agreement with experiment. The scheme suggested by Miyata and Yomosa [4] where the hypochromism is treated as an effect of interaction between different exciton bands does not show any agreement with experiment; moreover, it does not lead to hypochromism for poly(dG)·poly(dC) at all.

The elucidation of the degree of participation of intra- and interstrand interactions in producing the hypochromic effect for double-helical polynucleotides is of special interest. The hypochromic effect of double-stranded polynucleotides is usually considered to be caused by stacking interactions of bases inside the strands. At the same time different views are expressed in literature about the contribution of the interactions between the bases in complementary pairs.

Thus, the authors of [7,9,21,22] on the basis of results obtained concluded that the complementary interactions made a considerable contribution to the hypochromic effect of the double-stranded polynucleotides. At the same time it was concluded that H-bonds played an insignificant role [10,11].

Unfortunately, the authors of [7,9–11,21,22] did not take into account number of factors essential for hypochromism: change in configuration of single-stranded polynucleotides in the transition from the free to the bonded state presence of interactions between the bases, which were not H-bonded and which belonged to a different strand.

In the present work the hypochromism components for double-stranded polynucleotides caused by intrastrand base interactions (H_1), interstrand interactions in base pairs (H_2) and cross-interactions of bases (H_3) are calculated.

The results of theoretical calculations (table 2) show that the maximal contribution to the hypochromic effect is made by intrastrand interactions between monomers of the same chain (60–76%). Interstrand interactions between the bases ($H_2 + H_3$) also make a considerable contribution. The formation of the H-bonds makes a small contribution 2–12% to the total value of hypochromism. The fact that the pairing of bases lead to inconsiderable hypochromism of the pairs was earlier mentioned by us in [23,24] where the isolated pairs had been computed. One can come to the same conclusion on the basis of the analysis of experimental data of Spirin et al. (see [8] and discussion in [20]).

Table 2
The hypochromism components (%) for double-stranded polynucleotides

Polynucleotide	H_1	H_2	H_3
poly(dA)·poly(dT)	35	1	10
poly(dG)·poly(dC)	26	5	12

References

[1] Tinoco Jr., I. (1960) J. Am. Chem. Soc. 82, 4785–4790; (1961) J. Am. Chem. Soc. 83, 5047.

[2] Rhodes, W. (1961) J. Am. Chem. Soc. 83, 3609–3617.

[3] DeVoe, H. and Tinoco Jr. I. (1962) J. Mol. Biol. 4, 518–527.

[4] Miyata, T. and Yomosa, S. (1969) J. Phys. Soc. Japan 27, 727–735.

[5] Brown, E. and Pysh, E. S. (1972) J. Chem. Phys. 56, 31–37.

[6] Pysh, E. S. and Richards, J. L. (1972) J. Chem. Phys. 57, 3680–3685.

[7] Warner, R. C. (1957) J. Biol. Chem. 229, 711–724.

[8] Spirin, A. S., Gavrilova, L. P. and Belozersky, A. N. (1959) Biokhimiya 24, 600–611.

[9] Shugar, D. (1960) in: The Nucleic Acid (Chargaff, E. and Davidson, I. N. eds), Vol. 2, p. 56, Academic Press, New York and London.

[10] Michelson, A. M. (1963) in: The Chemistry of nucleosides and nucleotides, Ch. VIII, Academic Press, London and New York.

[11] Smith, K. C. and Hanawalt, P. C. (1969) in: Molecular photobiology, p. 52, Academic Press, New York and London.

[12] Inman, R. B. and Baldwin, R. L. (1964) J. Mol. Biol. 8, 452–469.

[13] Riley, M., Maling, B. and Chamberlin, M. J. (1966) J. Mol. Biol. 20, 359–389.

[14] Michelson, A. M. and Pochon, F. (1969) Biochim. Biophys. Acta 174, 604–613.

[15] Green, G. and Mahler, H. R. (1970) Biochemistry 9, 368–387.

[16] Danilov, V. I. and Pechenaya, V. I. (1974) Studia biophysica, in press.

[17] Yamada, T. and Fukutome, H. (1968) Biopolymers 6, 43–54.

[18] Vinogradov, I. P., Zemskih, V. V. and Dodonova, N. Ya. (1974) Opt. Spektrosk. 36, 596–599.

[19] Zheltovsky, N. V. and Danilov, V. I. (1974) Biofizika in press.

[20] Volkov, S. N., Danilov, V. I. and Pechenaya, V. I. (1975) Molekul. Biol. in press.

[21] Thomas, G. J., Jr. and Kyogoku, Y. (1967) J. Amer. Chem. Soc. 89, 4170–4175.

[22] Gratzer, W. B. and McClare, C. W. F. (1967) J. Amer. Chem. Soc. 4224–4225.

[23] Kuprievich, V. A., Danilov, V. I. and Shramko, O. V. (1967) Molekul. Biol. 1, 343–352.

[24] Danilov, V. I., Zheltovsky, N. V., Ogloblin, V. V. and Pechenaya, V. I. (1971) J. Theor. Biol. 30, 559–571.