THE STUDY OF THE FIRST AND SECOND ABSORPTION BAND HYPOCHROMISM IN NATURAL DNA

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1. Introduction

The application of the consistent theoretical treatment [1] to the investigation of the hypochromic effect in the first u.v. absorption band of double-stranded homopolynucleotides made it possible to obtain, for the first time, the quantitative agreement between theory and experiment [2,3]. The hypochromism investigation of natural DNA seems extremely difficult because of the aperiodic primary structure and the lack of data on the base sequence. On the other hand, the investigation on the origin of the hypochromic effect of such an important biological object as DNA is of immediate significance in considering such processes as the renaturation and denaturation of nucleic acids. The point is that without knowledge of intra- and interstrand interaction contributions to double-helix hypochromism the correct interpretation of experimental data is impossible.

It was shown [4] that in studies of homopolynucleotide hypochromism the nearest neighbour approximation may be used. This approach does not require a knowledge of the polymer primary structure and, as was recently shown by us [5], could be generalized for natural DNA.

The u.v. absorption study of natural DNA is also highly desirable in connection with the problem of the relative intensity change of the second absorption band because the experimental data available for natural DNA [6–9] are insufficient to determine the hypochromism of this band.

In recent years, due to the development of electron microspectroscopy, studies of the far ultraviolet absorption in DNA solid thin films could be performed [10–12]. Unfortunately, it is impossible to use these spectra for the direct calculation of hypochromism because during preparation of the solid film the secondary DNA structure is destroyed [8]. However, the absorption band maxima and minima displacements are negligible under conformation transitions [8]. This allows one to determine the shortwavelength border of the native DNA second absorption band and to proceed to the theoretical study of its hypochromism.

In this work we present the results of theoretical investigations on the origin of the intensity change which takes place in the first two long-wavelength absorption bands during DNA formation.

2. Method

As follows from the treatment developed in [5], one should know the nearest neighbour frequencies as well as the hypochromism value of the Watson–Crick pairs and intrastrand and cross dimers of bases for calculation of the DNA absorption band hypochromism.

The hypochromism of base dimers and base pairs was calculated on the basis of the monomer electronic characteristics which were computed by the CNDO/S CI method where all valence electrons were considered [13]. This choice of monomer characteristics allowed us to take into account a large number of different vacuum electronic states. This is significant when using a consistent theoretical treatment and important for the evaluation of intra- and interstrand component contributions to the hypochromic effect in double-

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stranded polynucleotides. Moreover, the good agreement between the calculated [13] and experimentally obtained electronic characteristics provided an opportunity to make an unambiguous assignment of theoretically calculated electronic transitions in monomers to the first and second absorption bands of the DNA studied. The geometry of the DNA B form was used for the hypochromism calculation of dimers and pairs.

In our work *E. coli* DNA and calf thymus DNA were chosen for investigation. These have been studied best of all from the spectroscopic view-point. For the calculation of their hypochromism we used the nearest neighbour frequencies obtained by Kornberg et al. [14,15].

3. Results and discussion

The results of calculations are presented in table 1. In the same table we show the hypochromism values computed by us from experimental absorption spectra of the DNA studied and corresponding equivalent mixtures of nucleotides [9,16,17].

One may see that the theoretically calculated hypochromism values of the first DNA absorption band are in good accordance with the experimental data.

Theory also predicts a significant hypochromism for the second absorption band. In this case the quantitative comparison of theory with experiment is impossible because, as one can see from the analysis of the solid DNA film absorption spectra [11,12], only a part of the second u.v. absorption band was studied for native DNA. Nevertheless, in the whole spectral range studied (185–195 nm $\leqslant \lambda \leqslant$ 230 nm) of the native DNA second absorption band [6–9] there exists a point hypochromism; this is in qualitative agreement with the results obtained by us.

It should be noted that the early work of Rhodes [18] and DeVoe and Tinoco [19] predicted hyperchromism for the second polynucleotide absorption band. They considered only interactions of the first two polynucleotide absorption bands in their calculations of the hypochromism, but such treatment automatically gives a hyperchromism for the second absorption band.

The contributions of intra- and interstrand interactions in the first band hypochromic effect for double-stranded polynucleotides have been discussed in a number of theoretical and experimental papers (see [2]). The theoretical results for homopolynucleotides [2–4] showed the necessity of taking into account cross-interactions for the interpretation of the absorption intensity changes.

The agreement obtained between theory and experiment allowed us to turn to the investigation of the origin of the hypochromic effect in DNA.

We calculated the hypochromism components of *E. coli* and calf thymus DNA first and second absorption bands caused by intrastrand interactions (H_1) and cross-interactions (H_2) of bases and interactions of bases in Watson–Crick pairs (H_3). One may see from the data in table 1, that the most important contribution to the hypochromism of the first and

Table 1

The values of hypochromism (H) and its components (H_1, H_2, H_3) of the first and second DNA absorption bands (%)

DNA	Band	Experiment[a]		Theory			
		H	Source	H	H_1	H_2	H_3
Calf thymus	I	38 37	[16] [17]	40	30	6	4
	II	Point hypochromism	[8]	44	26	9	9
E. coli	I	36	[9]	38	30	4	4
	II	Point hypochromism	[7,9]	44	27	8	9

[a]Calculated as described in [3].

second absorption band which arises during the DNA formation is caused by the intrastrand during the DNA formation is caused by the intrastrand interactions bases. The contribution of cross-interactions is comparable with that of H-bonds. Consequently, such interactions should be necessarily taken into account when interpreting of the spectral changes observed in the helix-coil type conformation transitions in DNA. The importance of consideration of cross-interaction was emphasized in a pioneering work by Spirin, Gavrilova and Belozersky in 1959 [16].

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