HYPOCHROMICITY OF SHORT DOUBLE HELICES

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1. Introduction

Experiments on the melting of natural and synthetic nucleic acids with short chain length – by following the change of the ultraviolet spectrum as function of temperature, ionic strength etc. – have become an important tool for investigating the stability and the structural features of their secondary and tertiary structure [1]. A potential source of information is usually not kept in mind with the appropriate weight in such experiments, namely the chain length dependence of the hypochromicity, which is the relative magnitude of the absorbance change upon going from the double helical to the single stranded conformation.

Such a dependence was predicted for example on the basis of a simple electrostatic model, involving the interactions of dipoles induced by light in neighbouring chromophores of a polymer [2–4]. Oligo (dG–dC), with its strong tendency of forming base pairs, offers the opportunity to study the melting of double helices with four (or more) base pairs, providing a nice experimental system for a comparison with theoretical predictions.

2. Materials and methods

Oligo (dG–dC) of different, homogeneous, chain lengths were obtained by separating a partial digest of poly(dG–dC) on a DEAE-column, using a NaCl-gradient in 90% formamide. The oligomers were characterized by paper chromatography, end group determination and equilibrium centrifugation. (Experimental details are given elsewhere [14]). Melting experiments were performed in thermostatted cuvettes in a Zeiss PMQ II spectrophotometer. Most measurements were made at 280 nm, where the absorbance of single stranded molecules at high temperature is close to that of the constituting nucleotides [5].

The hypochromicity $h_\chi(N)$ of an oligomer with chain length N is defined as

$$h_\chi(N) = 1 - \epsilon^h_\chi / \epsilon^r_\chi \qquad (1)$$

where ϵ^h_χ is the extinction coefficient in the double helical state at the wavelength χ and ϵ^r_χ the one of the constituting nucleotides. (If ϵ^r_χ is close to that of the randomly coiled single strands, one can use also the absorbance of the denatured molecules at high temperature.)

3. Results

The melting of oligo (dG–dC) with different chain lengths was measured at various oligomer concentration as a function of temperature in 1.0 M NaCl, pH 7.2. The results, together with kinetic experiments and the data of equilibrium centrifugation, agree with a dissociation of a double stranded helix into two single strands. The values determined for the hypochromicity at 280 nm are shown in fig. 1 as function of the reciprocal chain length.

This kind of plot is very convenient for a phenomenological presentation of the optical properties of oligomers with different lengths. Introducing an 'effective length' $(N–x)$ gives for the hypochromicity of a particular oligomer: $h(N) = h(\infty). (N–x)/N$, with $h(\infty)$ denoting the hypochromicity of the corresponding polymer. A simple transformation leads to

$$h_{\mathrm{rel}} = [h(N)/h(\infty)] = 1 - x\ [1/N] \qquad (2)$$

Thus by plotting the relative hypochromicity against the reciprocal chain length gives a line with slope x, from which the 'effective length' is determined.

Simple interpretations of eq. 2 are for example: a) Assuming that the base pair at the end of a double helix does not contribute to the hypochromicity (because it is in a different environment compared to the internal ones) will give $x = 2$; b) If e.g., the interaction of induced dipoles with the third nearest neighbor is of primary importance for the hypochromicity, this lead to $x = 3$, but the value of x has not necessarely to be an integer.

The experimental data for oligo (dG–dC) fit very well a value of $x = 2$. They are furthermore consistent with the theoretical calculation for DNA in the B-form by Rich and Tinoco, as shown by the broken lines in fig. 1, redrawn from [6]. Taking into account the direction of the transition moments of guanosine and cytosine for the first absorbance band around 270 nm [7, 8], the experimental data are in full agreement with the theoretical prediction. That poly (dG–dC) under these solvent conditions is in the B-form of DNA can be concluded from the circular dichroism and the Raman-spectrum.

The high stability of the dG • dC base pair in the double helix at low temperature and high ionic strength, as deduced from the thermodynamic analyses of the melting curves, makes it very unlikely, that bases at the end of $(\mathrm{dG{-}dC})_n \cdot (\mathrm{dG{-}dC})_n$ will be unpaired to a significant amount [14].

4. Discussion

According to theories of the hypochromicity, the chain length dependence will be influenced by the kind of base pairs, the geometry of the double helix and the wave length of observation. For these reasons the following estimates can only be regarded as semiquantitative. (Results obtained on the melting of $(\mathrm{A}_n\mathrm{U}_n) \cdot (\mathrm{A}_n\mathrm{U}_n)$ are compatible with $x = 2$ for these RNA-oligomers [9].)

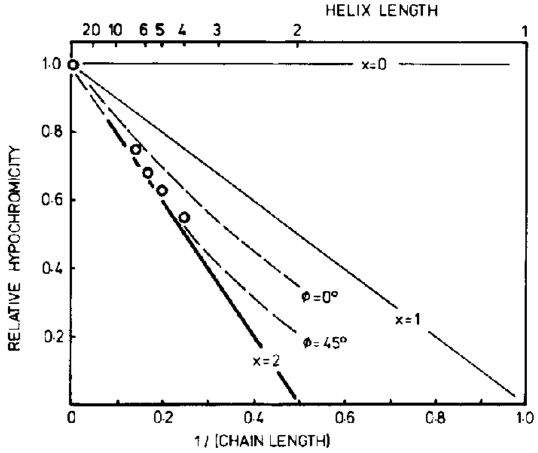

Fig. 1. Chain length dependence of the relative hypochromicity $h(N)/h(\infty)$ for the melting of oligo(dG–dC)•oligo(dG–dC) in 1.0 M NaCl, pH 7.2 at 280 nm (o–o–o). The data are plotted as a function of the reciprocal chain length – $1/N$. According to eq. 2 an effective chain length of (N-2) describes these results. They are in agreement with theoretical prediction, shown by the broken lines for two different directions of the transition moments with respect to the geometry of the DNA-double helix [6].

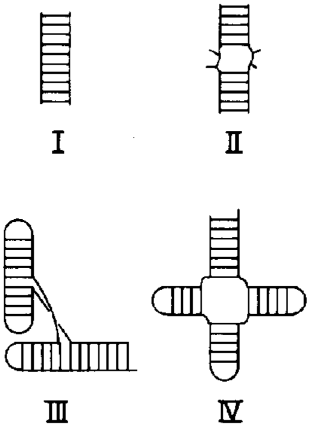

Fig. 2. Schematic drawings of double helical molecules discussed in the text. (Base pairs are shown as rungs of a ladder) While I and II (III and IV) respectively) have the same number of base pairs, their change in absorbance upon melting is expected to differ by about 30% due to the chain length dependence of hypochromicity.

A value of $x = 2$ is used in estimating the difference in hypochromicity for the molecules shown schematically in fig. 2. Molecule I and II respectively contain 10 base pairs each, but in II they are interrupted in the middle by mismatching nucleotides, so that no spatial correlation exists between the two halves of the oligomer. Upon changing to a single stranded conformation, and using eq. 2 with $x = 2$, the hypochromicity of I will be about 33% larger than for II, although the same number of base pairs will be disrupted. Such an estimate is in reasonable agreement with experimental data on the melting of $(A_4GC_nU_4)\cdot(A_4GC_nU_4)$ [10].

An other example is tRNA, shown in fig. 2 in two different conformations III and IV. In III – according to the X-ray structure of phenylalanine–tRNA [11] – two base paired parts of the molecule form a continous helix, being one 'bar' of the L-like structure. Molecule IV has the same base paired regions, but without spatial correlation. Simply on the basis of the chain length dependence of the hypochromicity – without taking into account any other spectral contributions – one expects for III about 30% larger hypochromicity than for IV [12, 13]. Thus molecules with the same number of base pairs but different conformations *may* show a considerable difference in their spectral properties.

For a more quantitative correlation between structural features and hypochromicity, it would be very desirable to have more comparisons between theoretical calculations and experimental results, using other synthetic oligomers and polymers of defined sequence.

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References

[1] Riesner, D. and Römer, R. (1973) in: Physical Properties of Nucleic Acids, Vol. 2, (Duchess, J., ed.), Academic Press, New York.

[2] Tinico, Jr., I. (1960) J. Amer. Chem. Soc. 82, 4785–4791.

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

[4] Weissbluth, M. (1971) Quart. Rev. Biophysics 4, 1–34.

[5] Pohl, F.M. and Jovin, T.M. (1972) J. Mol. Biol. 67, 375–396.

[6] Rich, A. and Tinoco, Jr., I (1960) J. Amer. Chem. Soc. 82, 6409–6411.

[7] Johnson, Jr. W.C. and Tinoco, Jr., I. (1969) Biopolymers 8, 715–731.

[8] Fucaloro, A.F. and Forster, L.S. (1971) J. Amer. Chem. Soc. 93, 6443–6448.

[9] Martin, F.H., Uhlenbeck, O.C. and Doty, P. (1971) J. Mol. Biol. 57, 201–215.

[10] Gralla, J. and Crothers, D.M. (1973) J. Mol. Biol. 78, 301–319.

[11] Kim, S.H., Quigley, G.J., Suddath, F.L., McPherson, A., Sneden, D., Kim, J.J., Weinzierl, J. and Rich, A. (1973) Science 179, 285–288.

[12] Romer, R., Riesner, D., Coutts, S.M. and Maass, G. (1970) Eur. J. Biochem. 15, 77–84.

[13] Cole, P.E., Yang, S.K. and Crothers, D.M. (1972) Biochemistry 11, 4359–4368.

[14] Pohl, F.M. (1973) Eur. J. Biochem. (submitted).