THE UNIQUE PROTONATED CONFORMATION OF POLY D (G–C) AS DETECTED BY CIRCULAR DICHROISM STUDIES

Vasantha NARASIMHAN and Ashley M. BRYAN
Department of Chemistry, State University of New York, Albany, New York 12222, USA

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1. Introduction

Several recent studies indicate that conformational changes other than the helix-coil transition take place in the DNA molecule even at temperatures and pH values far removed from those needed to cause the denaturation process [1–4]. These are mainly attributed to the modifications of the helical parameters of the double stranded molecule [5,6]. Such changes are predicted to take place even at physiological conditions inside the cell, prior to transcription and replication processes [7]. The utilization of the synthetic polymers of known base sequences, to study such conformational changes will be very valuable in understanding these phenomena. In this context, we have initiated studies on several synthetic double stranded DNA polymers. Since circular dichroic techniques (CD) are very sensitive in detecting fine structural alterations in a molecule [8,9], we have utilized this technique, besides the ultraviolet (UV) spectrophotometric studies, to investigate the conformational changes in the polymers poly d (G–C) and poly dG:poly dC.

2. Materials and methods

Poly d (G–C) was purchased from Boehringer-Mannheim Corporation as the lyophilised sodium salt. According to the specifications provided, the polymer had a base ratio of G:C as 1:1 in strictly alternating sequence, with no contaminations from salts or low molecular weight nucleotides and was homogeneous in alkaline CsCl gradient. Poly dG:poly dC was purchased from Miles Laboratories. It was authenticated to be a high molecular weight, double stranded polymer with 43% dG and 57% dC. The natural DNA mentioned in these studies was isolated from pig brain nuclei by the method of Mori et al. [10] with some slight modifications. It was a highly polymerous and native sample with practically no contamination from RNA or proteins. The G+C content was 37.8±0.2% as determined from the melting temperature [11].

The UV absorption studies were conducted in a Cary 14 recording spectrophotometer in a 1 cm cell kept at a constant temperature of 25°C. The CD studies were performed in a Jasco spectropolarimeter model J-10, in a 1 cm, jacketed, cylindrical cell, also at 25°C. The pH measurements were taken in the Radiometer pH meter model 26 with the expanded scale.

The concentrations of the polymer used in these studies were between 25 to 30 μg/ml, in unbuffered NaCl solutions. The final ionic strenght of the solutions was adjusted to be 2×10^{-2} M in Na^+. The pH of the solutions were adjusted by the addition of sub microliter quantities of 0.5 N and 1.0 N HCl to 5 ml of the polymer solution from which an aliquot was placed in the cell for taking the spectra. The pH was checked both before and after taking the spectra and did not vary more than 0.01 pH unit.

3. Results

The UV absorption spectrum of poly d (G–C), presented in fig.1, exhibits a maximum at 256 nm over a pH range of 6.5 to 4.5. On further lowering the pH, a distinct shoulder appears at 275 nm with no

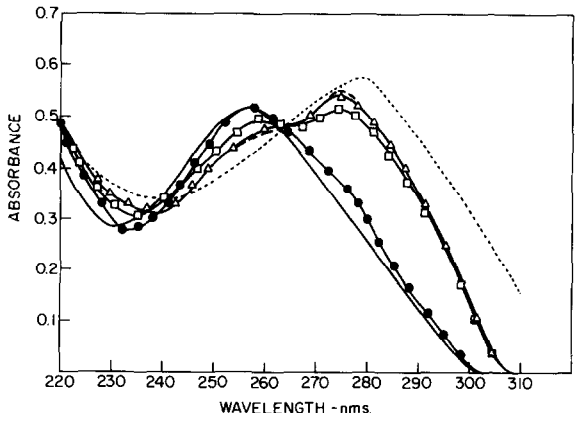

Fig.1. UV absorption spectra of Poly d (G–C) at 25°C in 0.02 M Na^+ ionic strength at different pH's. (——) pH 6.0; (•–•–•) pH 4.3; (□–□–□) pH 3.3; (△–△–△) pH 3.0; (——) pH 2.75; (.....) pH 2.3.

change at 256 nm. At pH 3.3 and below, a major peak is present at 275 nm but hypochromicity is observed at 256 nm. At pH 2.3 and below, aggregation seems to occur with increase in absorption around 310 nm. The hyperchromicity at pH 3.0 at 275 nm is about 60% to 62%. The homopolymer poly dG:poly dC exhibits a similar behavior in the UV adsorption spectra on protonation, except that the hyperchromicity at 275 nm was reduced to 50% to 52%.

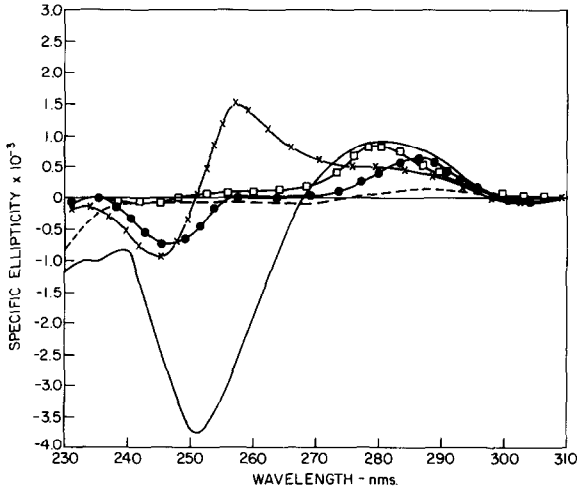

Fig.2. CD spectra of poly d (G–C) at 25°C and 2 × 10^{-2} M Na^+. (——) pH 6.0; (•–•–•) pH 3.8; (x–x–x) pH 3.5; (□–□–□) pH 3.0; (——) pH 2.5.

The CD spectra of poly d (G–C) at different pH values at 25°C are presented in fig.2. The spectrum at pH 6.5 to 6.0 has a rather broad and weak positive band at 278 to 284 nm and a relatively sharp and intense negative band at 251 nm. Between the pH values of 3.6 and 3.1, it is seen that, the intensities of the positive and the negative bands are reduced while an entirely new, sharp positive band appears at 257 nm. This new band, however, disappears below pH 3.0, and while at this pH, the original positive band at 284 nm is restored to the initial intensity and the negative band at 251 nm is completely lost. At pH 2.5 and below, the spectrum resembles that of a disordered structure. However, on titrating back to the initial pH of 6.5, the spectrum returns to the original position even though the negative band does not completely regain its initial intensity. The CD spectral changes of this polymer, interestingly enough, bear a close similarity to that of natural DNA on protonation (see fig.3).

The CD spectra of the homopolymer duplex poly dG:poly dC at different pH values are presented in fig.4. Very much unlike the alternating polymer, the CD spectrum of this polymer shows considerable variations depending on the dG percentage as reported by Green and Mahler [12]. The polymer used in our studies, with a dG content of 43%, exhibits a small negative band at 274–275 nm and a sharp positive band at 252 nm. On decreasing the pH, a gradual reduction in the intensities is noticed and the spectrum at pH 2.2 shows evidence of a disordered structure. It

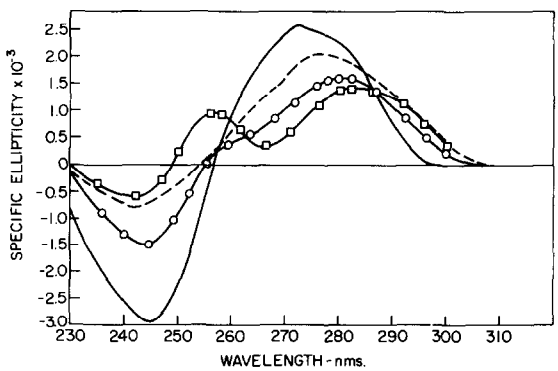

Fig.3. CD spectra of brain nuclear DNA at 25°C in 0.02 M NaCl at different pH values. (——) pH 6.1; (○–○–○) pH 3.9; (□–□–□) pH 3.6; (——) pH 3.1.

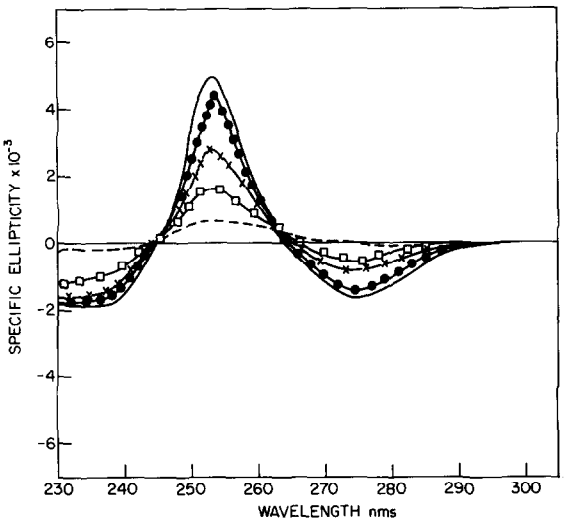

Fig.4. CD spectrum of poly dG:poly dC at 25°C and 2×10^{-2} M Na^+. (——) pH 6.0; (•–•–•) pH 4.5; (x–x–x) pH 3.3; (□–□–□) pH 2.7; (.....) pH 2.2.

may also be noticed that on bringing back the pH from 2.2 to 6.5 only partial recovery of the bands occurred, whereas, the reversal of the pH from 3.2 to 6.5 produced 90% reversibility.

4. Discussion

The phenomenon of hyperchromism around the 275 nm region and hypochromism around 255 nm region in the UV absorption spectra, has been reported previously during the acid titration of dGMP [13] and poly G [14]. The UV spectral changes observed for the polymers poly d (G–C) as well as poly dG:poly dC are in agreement with such observations. Hence these changes may be accounted for by the partial protonation of the deoxy guanosine residues followed by opening up of the double helix and on further completion of protonation leading to denaturation.

However, the CD spectral changes that occur on protonation of these polymers are vastly different and present a more complex picture. The appearance of a small second positive band at 257 nm on protonation of many natural DNA samples have been reported in several studies [15,17]. Guschlbauer et al. [17] attribute this observation to the conformational change of the deoxy guanosine residues from the 'anti' to the 'syn' from on protonation of DNA, based on their pH studies of deoxyguanosine by optical rotatory disperion and CD [18]. Our present results on the polymer poly d (G–C) seem to suggest a similar type of conformational transition in this polymer. On the other hand the protonation of poly dG:poly dC does not exhibit such transition and simply indicates a gradual denaturation process. Guschlbauer et al. [17] have suggested that the dG strand in this polymer may already exist in the 'syn' conformation which might account for the absence of the transient second positive band in this polymer on protonation.

The CD spectrum of the neutral form of poly d (G–C) is comparable to that expected for the 'C' form of a double stranded polynucleotide [19], and hence might differ only in the magnitude of the tilt of the bases while the conformation of the bases themselves are the same as in DNA. Thus the same kind of conformational transition responsible for the second positive band of DNA on protonation can be expected for poly d (G–C) as well. The initial differences in the helical parameters and the unique base sequence and composition of the polymer as compared to natural DNA, may be the reasons for the increased intensity of the 257 nm band in this polymer. While only tentative suggestions can be made at present from these results, the fact that a close similarity exists between the protonation behavior of DNA and this polymer is self evident from the results presented here. A thorough understanding of the exact nature of this conformational change in this polymer of known sequence and composition should be able to provide important information regarding the transitions in natural DNA molecules. Further detailed studies for such an understanding are currently in progress.

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