Nucleic Acids Research

Circular dichroism calculations for polyinosinic acid in proposed multi-stranded geometries.

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ABSTRACT

Circular dichroism spectra have been calculated for multi-stranded polyinosinic acid using three different right-handed structures proposed from X-ray diffraction studies. Agreement between calculated spectra and spectra measured at high salt concentration is best for a four strand structure in which the bases are tilted with respect to the helix axis, as proposed by Arnott et al. (1974). For structures in which the bases are perpendicular to the helix axis, the characteristic negative circular dichroism of polyinosinic acid at long wavelength no longer appears in the calculated spectra. It is clear that a negative circular dichroism at long wavelength does not indicate a left-handed polynucleotide helix.

Guanine is unique among the four common nucleic acid bases in that it is capable of base pairing with other guanine molecules at neutral pH. Under appropriate conditions, guanylic acid in aqueous solution is known to form viscous gels of a tetrameric structure in which each base forms two hydrogen bonds (N1-O6 and N2-N7) to two other bases (Gellert et al., 1962; Tougard et al., 1973; Sasisekaran et al., 1975). Polyguanylic acid (poly G) and polyinosinic acid (poly I) have been studied by a variety of physical techniques, reviewed by Rice et al. (1973) and Thiele and Guschlbauer (1973). The overall evidence suggests that in low salt solution these polymers are in an ordered single strand conformation of unknown form. At higher ionic strength, a stable multi-strand structure appears, probably composed of four parallel strands connected by base pairing of the same type observed in guanylic acid gels. Base pairing in poly I (which lacks the NH_2 group of guanine at the C2 position) is thought to be the same as in poly G, but with

one less hydrogen bond per base pair, i.e. with only the O6 and N1 atoms hydrogen bonded.

On the basis of circular dichroism (CD) measurements of the high-salt form which show a long wavelength CD spectrum opposite in sign to that usually seen for polynucleotides, Thiele and Guschlbauer (1973) suggested a left handed helix for the strands. X-ray diffraction studies on the high-salt form of poly I have led to different conclusions. In 1958, Rich proposed a structure composed of three parallel strands connected by base pairing as described above. More recently, two independent X-ray studies (Arnott et al., 1974; Zimmerman et al., 1975) have concluded that poly I forms a complex of four parallel strands. The proposed structures are similar, but not identical. One important difference is that Arnott et al. determined that the bases are negatively tilted, with the perpendicular to the plane of the base oriented 8.9° away from the helix axis, whereas Zimmerman et al. found the bases to be essentially perpendicular to the helix axis. The X-ray structures all contain right handed helices.

We have made calculations of the circular dichroism of the high-salt form of poly I to see if the measured CD in solution is consistent with any of the proposed X-ray structures and perhaps to allow a choice between the two most recent structures. Calculations were carried out using an all-order coupled oscillator polarizability theory assuming an effective dielectric constant of 2. This method and the type of monomer parameters that are required for its use have been discussed in detail (Cech, 1975; Cech et al., 1975).

For poly I calculations, the absorption spectrum of inosine (Beaven et al., 1955) was resolved into two absorption bands, one of ε_{max} = 3.8 x 10^3 ℓ mole^{-1}cm^{-1} at 266 nm and a larger band of ε_{max} = 11.38 x 10^3 at 246.5 nm. Transition monopoles for hypoxanthine calculated by Hug and Tinoco (1973) using a CNDO-CI method were assigned to these transitions. Set I as defined by Hug and Tinoco was assigned to the 266 nm transition and set II to the 246.5 nm transition, in accordance with other work (Cech and Tinoco, 1975) in which a similar assignment for guanine gave best overall results. Crystal

or film measurements of transition angles have not been done
for hypoxanthine, so the experimentally measured values for
the corresponding guanine transitions were used. Since CNDO
calculations predict very similar electronic properties for
guanine and hypoxanthine, the use of guanine data when neces-
sary was considered justifiable.

The calculated CD spectrum for four stranded poly I using
the coordinates for the bases of Arnott et al. with 11 bases
per strand (about one turn of the helix) is shown in Figure 1
(solid line). Bases in any one strand are related by an axial
rise, Δz, of 3.41 Å and an angle θ of 31.3°. For comparison,
the CD spectrum of poly I as measured by Thiele and
Guschlbauer (1973) at 3°C in 1.0 M NaCl, after gradual addition
of NaCl, is also shown. This was considered representative of the
CD spectra measured for poly I in high salt, but published spectra
all differ slightly depending on exact solution conditions and
method of preparation. Agreement is remarkably good above 240 nm.
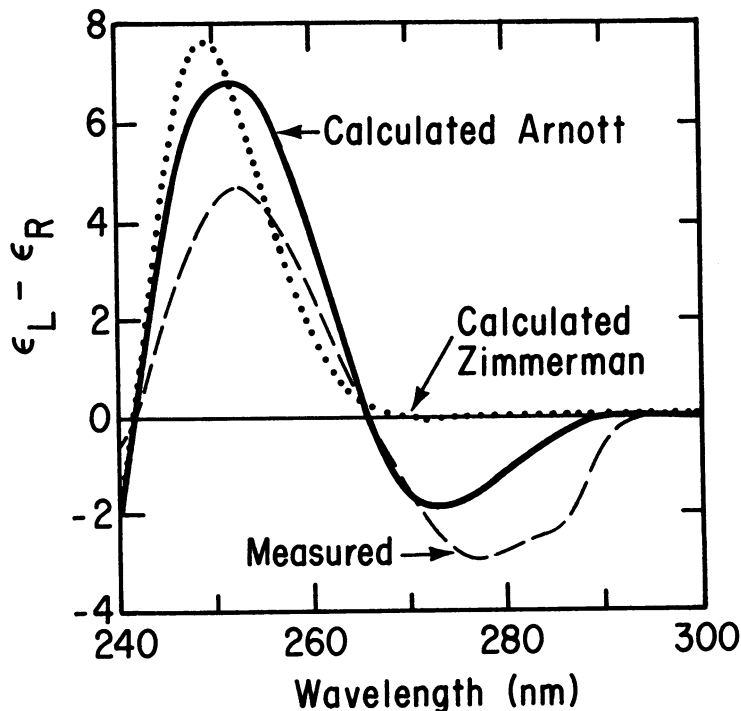

Figure 1. Calculated and measured circular dichroism
for polyinosinic acid in high salt.

Arnott et al. proposed that the bases must be tilted with respect to the helix axis in order for a negative CD band to appear at long wavelength. A calculation was therefore done using Arnott et al. coordinates in which the bases were made perpendicular to the helix axis by setting all z values for the X-ray coordinates equal to zero. Since z coordinates for the atoms are all small, from 0.00 to 0.66 Å, this was not considered a serious distortion. The resulting calculated CD spectrum was indeed missing the long wavelength negative band. The calculated CD was essentially zero to 265 nm; the 250 nm peak was virtually unchanged.

A CD calculation for four stranded poly I with 11 bases per strand but using the X-ray coordinates for the bases of Zimmerman et al. (1975) is shown by the dotted line in Figure 1. In these coordinates, the bases in one strand are approximately perpendicular to the helix axis and are related by $\Delta z = 3.36$ Å and $\theta = 31.2°$. Most striking is the fact that the calculated negative band at 272 nm is extremely small, with a maximum value of about $\epsilon_L - \epsilon_R = -0.10$. In addition, the position of the positive maximum is too far to the blue.

Calculations were also done using estimated coordinates for poly I in three strand conformations. With helical parameters proposed by Rich (1958), i.e. $\Delta z = 3.40$ Å and $\theta = 41.6°$, and with bases tilted as in the Arnott et al. model, the CD spectrum was very similar to the four strand calculation using Arnott et al. coordinates except that the positive band was of larger magnitude (e.g. $\epsilon_L - \epsilon_R = +11.2$ at 251 nm).

The same three strand base coordinates coupled with the helical parameters of Arnott et al. rather than of Rich gave a calculated CD pattern which was almost identical to the solid line in Figure 1. Making the bases perpendicular to the helix axis in either of these three strand calculations resulted in the loss of the long wavelength negative band.

Guanosine monomer properties are very similar to those of inosine except that the two long wavelength absorption bands are located further to the red. As might be expected, therefore, calculations predict that four stranded poly G would have a CD spectrum very similar to that of poly I but with maxima and minima at longer wavelength.

Calculations were also done for poly I in the four strand coordinates of Arnott et al. using only one strand, two adjacent strands, 2 strands related by 180° (opposite strands) or 3 strands. The 1 strand and the 2 adjacent strand calculations gave positive CD at long wavelength. For poly G calculated in the same manner, only the 1 strand calculation gave a positive CD pattern. It is apparent that an array of more than one strand is necessary to obtain negative CD at long wavelength, but the necessary number and arrangement of strands is sensitive to oscillator parameters.

Agreement between calculated and measured CD spectra could probably be further improved by empirically adjusting the monomer properties. For example, trial calculations were done with inosine absorption spectrum resolutions in which the first band was chosen to be smaller but further to the red. Such calculations gave better agreement in band position for the first negative band but less intensity. Addition of 200 nm region and background transitions might also improve agreement. It is, however, rather difficult to critically judge the calculations when the measured CD spectra vary so much with solution conditions. Very likely the measured spectra represent a mixture of species in solution, so exact agreement cannot be expected.

Regardless of accuracy, it is clear that the observed CD spectrum for poly I in high salt can originate from a parallel arrangement of four right hand helices. The calculated CD is quite consistent with the proposed 4 strand structure with tilted bases of Arnott et al., but the calculations are not yet reliable enough to rule out a similar 3 stranded structure. The calculations do, however, shed doubt on the proposal by Zimmerman et al., that the bases are perpendicular to the helix axis, since a large negative CD band at long wavelength was calculated only when the bases were tilted. These calculations also prove that CD spectra in which a negative band appears first at long wavelength cannot be taken as indication of a particular helix sense. In the case of poly I, the calculated CD pattern is apparently a complicated function of exciton-type interactions between identical transitions on two or more parallel strands in symmetrical arrays.

Since calculations of either poly I or poly G using only 1 of the 4 strands gave a large positive band at long wavelength, it

seems likely that the helical parameters of the single strands in low salt are different than the parameters of the base paired strands at high salt. Perhaps this is a factor in the complex annealing process observed experimentally as salt concentration is increased.

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