THE INTERACTION OF β-CYCLODEXTRIN WITH NUCLEIC ACID MONOMER UNITS

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Summary

β-Cyclodextrin is examined as a potentially useful probe of nucleic acid structure. From circular dichroism (CD) data the binding constant and the enthalpy and entropy of binding to 5'-AMP are determined. The CD spectrum of the bound complex is calculated. The binding to 5'-dAMP, 3',5'-cyclic AMP, adenosine and adenine is also examined. No evidence is seen for the involvement of hydrophobic forces. CD data for 5'-GMP, 5'-UMP, and 5'-CMP in 0.01 M β-cyclodextrin show that the binding is not as specific as previously reported.

Introduction

β-Cyclodextrin is one of the Schardinger dextrins and consists of seven residues of D-glucose attached by α 1-4 linkages to form a toroidal molecule. Many compounds interact with cyclodextrins and an inclusion-type complex is thought to be formed with the central cavity (1,2). Hoffman and Bock have previously studied the interaction of cyclodextrins with nucleic acids by ultraviolet (UV) absorption difference spectroscopy (3). They found that β-cyclodextrin would only interact with adenosine or inosine compounds. Other bases or the six membered cyclodextrin did not produce an observable interaction.

Since the binding of β-cyclodextrin produced a fairly large change in the UV spectrum, and β-cyclodextrin is itself optically active, it was felt that a large change in the circular dichroism (CD) spectrum would occur on binding, and that CD would be an additional and perhaps more sensitive way to study cyclodextrin binding. From the previous work (3) there appeared to be advantages to studying β-cyclodextrin binding as a probe of nucleic acid structure. The binding is weak, therefore not much energy is available for deformation of the structure studied. The large change in UV properties

compensates for this weak binding so that low levels of binding can be observed. The binding appeared to be specific for adenosine and inosine; thus the localized structure where these nucleosides occur in RNA could be studied. Since the electronic transitions of adenosine and inosine are perturbed by the binding, this may be a way of gaining information on their electronic structure. Admittedly it may not be simple to derive this information, but this is at least more evidence with which any understanding of the electronic structure will have to be consistent.

In this paper the interaction with the adenine moiety is the primary concern; the interaction with inosine was not investigated.

Materials & Methods

β-Cyclodextrin was obtained from Pierce Chemical Co. Nucleic acid materials were obtained from Calbiochem, Sigma, or Schwarz. All solutions contained 0.01 M Na^+ pH 7 phosphate buffer, 0.10 M NaCl. Circular dichroism and UV absorption data were obtained as described elsewhere (4). There is no contribution from β-cyclodextrin to the CD above 210 nm, and thus no correction was necessary in the reported spectra.

Results & Discussion

The data given in Figure 1 show that binding of β-cyclodextrin to 5'-AMP produces a large effect on the CD spectrum. From these data, and similar data not shown, it is possible to determine a binding constant for the interaction from the equation

$$\frac{1}{\Delta[\theta]} = \frac{1}{[\Delta\theta_{AB}]K(C)} + \frac{1}{[\Delta\theta_{AB}]} \qquad (1)$$

where $\Delta[\theta]$ is the observed molar difference CD, $[\Delta\theta_{AB}]$ is the difference in molar ellipticity between the free and complexed nucleotide, K is the binding constant, and (C) is the concentration of β-cyclodextrin. The plot shown in Figure 2 yields the values K = 90 ± 18 and $[\Delta\theta_{AB}]_{255}$ = 13,400 ± 2600 from a least squares analysis. The application of equation (1) assumes that a 1:1 complex is formed. This is justified by the isosbestic point at 223 nm

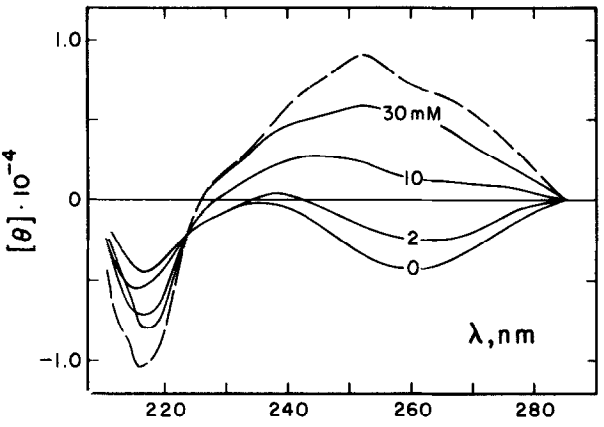

Fig. 1.

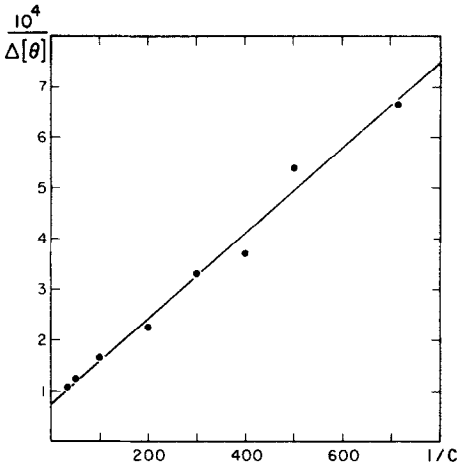

Fig. 2.

Figure 1. The CD of 5'-AMP at 5°C. The solid curves were obtained in β-cyclodextrin solutions whose concentration in mM is indicated on the curves. 30mM cyclodextrin is a supersaturated solution at 5°C. The dashed curve is the CD of the bound complex calculated from equation (2).

Figure 2. A plot of equation (1) for 5'-AMP which yields K = 90 ± 18 and $[\Delta\theta]$ = 13,400 ± 2600. The first two points were obtained in 0.03M and 0.02M β-cyclodextrin which are supersaturated solutions at 5°C.

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shown in Figure 1, and by the linearity of the plot in Figure 2. With the equilibrium constant, the CD spectrum of the bound complex can be calculated:

$$[\theta_B] = \frac{1 + K(C)}{K(C)} [\theta] - \frac{1}{K(C)} [\theta_A] \qquad (2)$$

where $[\theta_B]$ is the CD of the bound complex, $[\theta]$ is the perturbed molar CD, and $[\theta_A]$ is the CD of 5'-AMP. $[\theta_B]$ calculated using this equation, K = 90, and the CD spectrum in 0.01 M β-cyclodextrin is shown in Figure 1. The calculated CD using the observed CD at different concentrations of β-cyclodextrin were similar. The calculated CD for the bound complex shows a broad positive peak near 255 nm with a magnitude of 9,000 to 10,000, and a sharp negative peak near 215 nm with a magnitude of -9,000 to -10,000. The detailed features such as the shoulder near 270 nm cannot be relied upon for energy and intensity. Though all calculated spectra showed the same features there was considerable variation in magnitude and position. However, it can be seen from a comparison

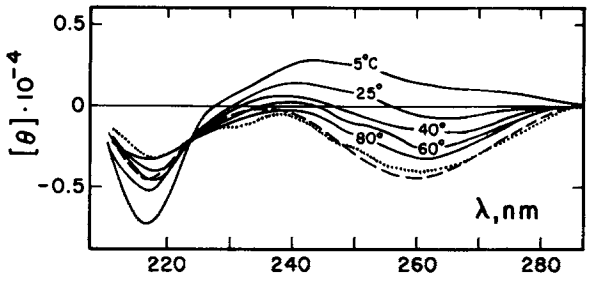

Figure 3. The variation of CD with temperature. 5'-AMP at 5°C (--); 5'-AMP at 80°C (....); solid curves are 5'-AMP in 0.01 M β-cyclodextrin at the indicated temperatures.

of the CD of 5'-AMP (Fig. 1) that the CD of the bound complex indicates more spectral detail. An analysis in terms of the electronic transitions of 5'-AMP has not been attempted.

The binding is sensitive to temperature as shown in Figure 3. Since the CD spectrum of the bound complex is known from above, the binding constants at the various temperatures can be calculated, and the enthalpy and entropy of binding can be determined from the van't Hoff equation. This assumes that the CD of the bound complex does not change with temperature, but since the CD of 5' AMP in the 255 nm region does not change appreciably in this temperature range, the assumption is probably good within the limits of accuracy. The variation at higher temperatures in the CD below 220 nm (Fig. 3) is similar to variation in the CD of 5' AMP. The K, ΔH° and ΔS° values derived from this analysis are given in Table 1. The binding constant at 25°C is larger than that previously reported for 3'-AMP (3) but the difference may not be significant.

Figure 4 shows the results of β-cyclodextrin binding to 5'-dAMP, 3', 5'-cyclic AMP, adenine, and adenosine. Adenine by itself, of course, is not optically active. 5'-AMP, adenosine, and adenine show the same trend in binding as previously seen (3). The results with 5'-dAMP indicate that cyclodextrin may also be a useful probe of DNA structure. The similarity of these results with those for 5'-AMP is evidence that the mode of binding is similar in these cases.

Table 1. Thermodynamic Values for 5'-AMP + β-Cyclodextrin

T	5°C	25	40	60	80
K	$90M^{-1}$	41	28	16	12

$$\Delta H^\circ = -4.9 \pm 0.3 \frac{Kcal}{mole} ; \qquad \Delta S^\circ = -8.7 \pm 1 \quad e.u.$$

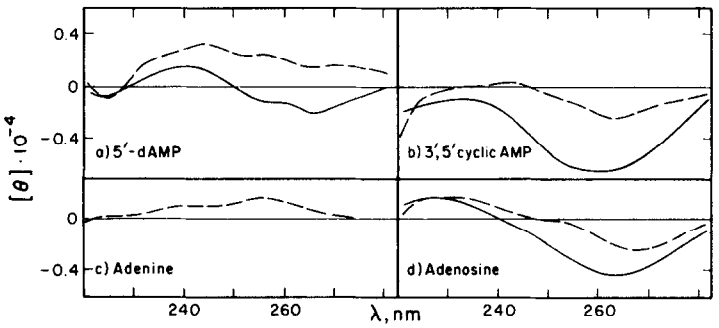

Figure 4. The CD at 5°C of a) 5'-dAMP, b) 3',5'-cyclic AMP, c) adenine, d) adenosine. The solid curve is the CD in buffer and the dashed curve is that in 0.01 M β-cyclodextrin.

While several modes of binding are possible, an inclusion complex has generally been considered to best fit the requirements of the experimental data (1,2,5). There have, however, been indications that other modes of binding can be important (1,6). The hydrophobic character of the cavity has been mentioned by several authors (2,7,8), but the results with 5'-AMP do not indicate a hydrophobic interaction (which should show a favorable entropy and an opposing or indifferent enthalpy (9)). For 5'-dAMP, 3',5'-cyclic AMP, adenosine, and adenine enthalpies and entropies estimated from data at 5° and 25° (not shown) also do not indicate any hydrophobic character to the binding. But, as has been pointed out before (1,10), none of the thermodynamic data available on cyclodextrin complexes (1,7,10) indicates a hydrophobic interaction. There is some fluorescence data which may indicate hydrophobic

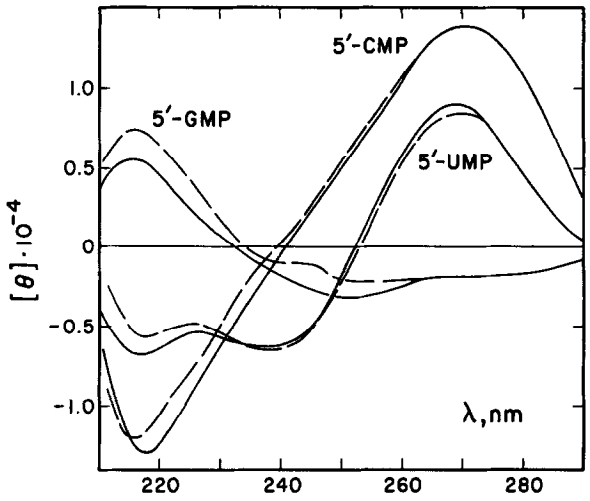

Figure 5. The CD of 5'-UMP, 5'-CMP and 5'-GMP in buffer (——) and in 0.01 M β-cyclodextrin (---). The temperature was 25°C for 5'-UMP and 5°C for the other nucleotides.

character (7), but even this has been interpreted in another manner by Seliskar and Brand (11). It seems likely that an inclusion complex is usually formed, but there is presently no basis for the idea that hydrophobic interactions contribute significantly to the stability of the complex.

Since the CD study of binding of β-cyclodextrin appeared to be more sensitive than the previous UV study, it seemed appropriate to re-check the specificity of the binding. Figure 5 shows that 5'-UMP, 5'-CMP, and 5'-GMP also interact with β-cyclodextrin. The CD differences are sufficiently small that an analysis like that used for 5'-AMP could not produce useful results. However, there are indications from chromatography on cyclodextrin gels (12) that the binding of β-cyclodextrin to 5'-AMP is stronger than binding to 5'-GMP, 5'-UMP or 5'-CMP. While the spectral perturbations of 5'-CMP, 5'-UMP, and 5'-GMP in 0.01 M cyclodextrin are small compared to that of 5'-AMP, this does not mean that they can be neglected. In a polynucleotide chain where cyclodextrin binding could influence stacking, binding to nucleosides other than adenosine could cause larger spectral changes. Conversely, binding to other nucleosides adjacent to A residues could influence the binding to A. These

possibilities are being investigated. There must be reservations about using β-cyclodextrin as a structural probe for nucleic acids until this point is cleared up.

Comparing the CD results in Figure 5 with those in Figure 1 it appears that the binding to 5'-AMP may be structurally different from the binding to 5'-GMP, 5'-UMP, or 5'-CMP, since for the former there is a large effect on the long wavelength transitions, while for the latter there is little or no effect on the long wavelength transitions.

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