The Circular Dichroism of a *t*-Ribonucleic Acid and its Iodinated Derivative in Aqueous Solution

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The circular dichroism (c.d.) of a *t*-ribonucleic acid (*t*-RNA) and its iodinated derivative indicate that the iodination procedure does not significantly alter the helical conformation of the polynucleotide. This is further confirmed by c.d. studies of the effects of pH and the addition of ethylene glycol (a known destacking agent) upon the helical conformation of the *t*-RNA and the iodinated RNA.

It has been known for a number of years[1] that circular dichroism (c.d.) is an extremely sensitive probe of conformational changes in polynucleotides and that the optical activity of oligonucleotides results largely from an interaction between adjacent bases, *i.e.* nearest neighbours in the polymeric chain which are stacked one above the other.[2] Furthermore, second base neighbours do not make a significant contribution to the optical activity. The relationship between the c.d. and base-stacking–conformation of various ribonucleic acids (RNA) have been studied from time to time[3–8] but the c.d. of an iodinated RNA does not seem to have been measured. We were particularly interested in the effects of chemical modification of a *t*-RNA to give its iodinated derivative, in relation to possible changes in its conformation, since we have been using a radioactively labelled iodinated *t*-RNA as a macromolecular substrate in *in vitro* pinocytosis studies. These studies are analogous to the *in vitro* pinocytosis studies on a similarly radioactively labelled bovine serum albumin where o.r.d. and c.d. studies were used to demonstrate that the chemically treated protein retained its native conformation.[9] It is important to know whether such chemically treated pinocytic substrates retain their native conformation since large differences in rates of pinocytic uptake are known to arise, in the case of proteins, depending on whether the molecules are in a native or denatured conformation.[9]

Experimental

The *t*-RNA sample, generously donated by Dr. R. Walker, Department of Chemistry, University of Birmingham, was a heterogeneous mixture of *t*-RNAs with a range of 78—85 nucleotides per molecule and an approximate molecular weight of 25 000.

The Iodination Method.—The iodination (using $K^{127}I$) of the *t*-RNA was carried out in a scaled-up procedure based upon our adaptation of the radioiodination procedure (using $Na^{125}I$) for nucleic acids devised by Commerford.[10] *t*-RNA (10 mg) was dissolved in iodination buffer (10 cm^3; 0.1M-sodium ethanoate–0.04M-ethanoic acid; pH 4.7). To this solution was added, at 0 °C, KI solution (5 cm^3; 2.5×10^{-4}M in iodination buffer) followed by thallium(III) chloride solution (5 cm^3; 9.2×10^{-3}M in iodination buffer). This mixture was then heated at 60 °C for 20 min and then cooled on ice. The iodinated *t*-RNA was then recovered by precipitation by adding a tenth volume of 20% potassium ethanoate solution and then two volumes of absolute ethanol, and the suspension centrifuged at 10 000 r.p.m. for 25 min.

For the pinocytosis studies using radioactively iodinated *t*-RNA the normal procedure is to remove unstable radioactive label on the uracil residues prior to use following a procedure adapted from that of Browning *et al.*[11] This procedure was also followed for the ^{127}I derivative obtained from the iodination described above. The iodinated *t*-RNA pellet obtained after centrifuging was dissolved in 20mM-Tris–sodium ethanoate buffer (10 cm^3) at pH 7.6 which contained 2mM-EDTA and 0.5% sodium dodecyl sulphate. The solution was then heated for 20 min at 60 °C and subsequently cooled on ice. The iodinated *t*-RNA was recovered in exactly the same manner as in the first stage of the procedure.

The *t*-RNA pellet was resuspended in absolute ethanol and recentrifuged.

Aqueous solutions of the original *t*-RNA and the iodinated derivative prepared by the above procedure were made up separately to contain 1 mg cm^{-3} and these were diluted ten times prior to the measurement of the c.d. spectra in a 5 mm pathlength silica cell. Experiments were also conducted on the effects of added solutes, such as HCl, NaOH, ethylene glycol, urea, phosphate buffer, upon the c.d. spectra and these were added during the dilution stage prior to measurement of the spectra.

In addition to the above iodination treatment, a sample of *t*-RNA was put through the iodination procedure but *without* any KI present in order to judge the effects of chemical treatment of the *t*-RNA other than iodination upon its conformation.

The complete and partial hydrolysis of the original *t*-RNA was also carried out with, respectively, 2M-NaOH at 100 °C for 4 h and with pancreatic ribonuclease (Sigma R7003) for 4 h at 37 °C.

C.d. Measurements.—The c.d. spectrometer is a home-built single-beam instrument which uses the monochromator of a Hilger Uvispek spectrophotometer which has been fitted with a motorised wavelength drive. Light from the monochromator is linearly polarised by a Rochon prism and then modulated at 50 kHz into right- and left-circularly polarised light by a Morvue photoelastic modulator. The light beam, after passing through the sample, falls onto a photomultiplier (EMI 9789Q/B) the output from which is fed to a simple circuit to separate the a.c. component (arising from the c.d. of the sample) from the d.c. component. The a.c. component is fed to a Brookdeal 9501 lock-in amplifier and the processed signal is recorded on a potentiometric chart-recorder. The d.c. component of the photomultiplier signal (which is proportional to the overall level of transmitted light) is led to earth *via* a resistor, the voltage drop across which is monitored by a voltmeter. While the c.d. spectrum is being measured this d.c. signal is maintained at a constant value by adjusting the EHT voltage to the photomultiplier (or, exceptionally, by changing the spectrometer slit widths). This procedure is necessary to compensate for the changes with wavelength of the light source output and the photomultiplier sensitivity.

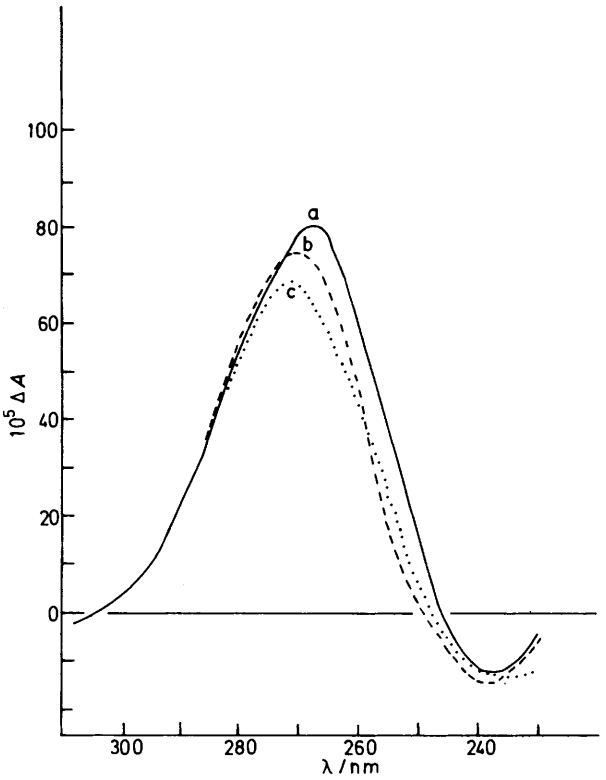

Figure 1. C.d. of various RNA samples in H_2O (0.1 mg cm^{-3}): —— = *t*-RNA; — — — = chemically processed *t*-RNA but *not* iodinated; · · · · = I-RNA

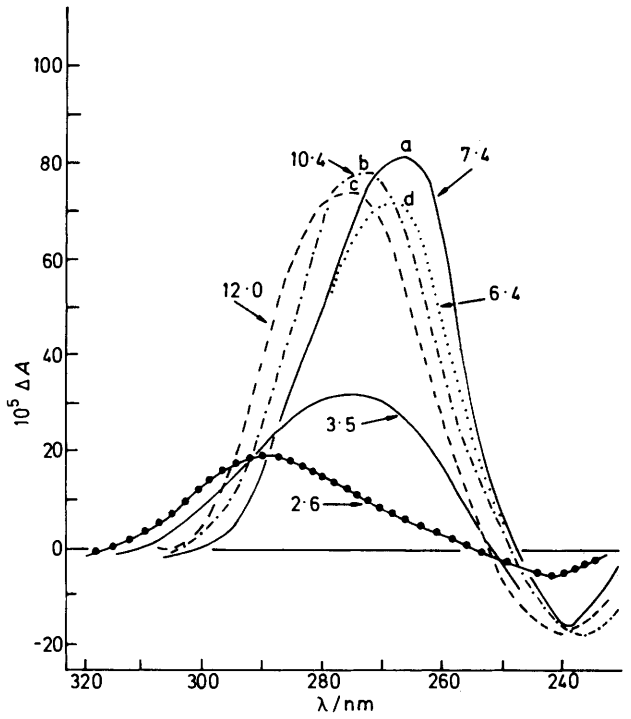

Figure 2. C.d. of *t*-RNA at various pH (adjusted by HCl and NaOH), concn. = 0.1 mg cm^{-3}

The c.d. spectrometer was calibrated with an aqueous solution of camphor-10-sulphonic [^{2}H]acid [$\Delta\varepsilon_{291}$ 2.20 (ref. 12)], and also with a solution of *epi*-androsterone in dioxane at 304 nm. At the concentrations used the ratio of $\Delta A_{max.}$ for the two calibration substances should be 1.50, and in fact the measured ΔA ratio was 1.51. The signal-to-noise ratio for the c.d. measurements on the *t*-RNA solutions was *ca.* 25:1 with a value of ΔA of 1 × 10^{-5} per mm of chart paper.

Results and Discussion

Since the exact molecular weight of the iodinated *t*-RNA and its extent of iodination are not known, the c.d. results are presented as ΔA (= $A_l - A_r$), *i.e.* differential dichroic absorbance, rather than converting the results into $\Delta\varepsilon$ or molar ellipticity. The original *t*-RNA and its iodinated derivative were compared in three ways using c.d.: (a) a direct comparison of the c.d. in aqueous solution before and after iodination; (b) a comparison of the c.d. in phosphate buffer of varying pH; (c) a comparison of the c.d. when ethylene glycol was added.

In addition, a comparison was made between the complete (using NaOH) and partial (using ribonuclease) hydrolysates of the original *t*-RNA. The c.d. present in the original aqueous *t*-RNA was completely absent in the nucleotide mixture produced by complete hydrolysis with NaOH, while the partial enzymic hydrolysis resulted in a mixture of oligo- and mononucleotides and showed a small residual c.d. maximum, thus showing that the large positive c.d. maximum in the original *t*-RNA arises only from the presence of the intact polynucleotide chain.

In Figure 1 are presented the c.d. curves of (a) a freshly made-up *t*-RNA, (b) *t*-RNA chemically treated as for iodination but with no KI added, and (c) the iodinated *t*-RNA, all solutions being at 0.1 mg cm^{-3} concentration and at their natural pH. Comparison of Figures 1a and 1b shows that the chemical treatment has only slightly changed the CD spectrum. The spectrum for the iodinated RNA (Figure 1c) is slightly lower than Figure 1b, but the iodinated derivative has a higher molecular weight and thus for the same weight of material gives a lower molar concentration and hence a lower ΔA. A more quantitative comparison between the samples is by calculation of their '*g*-factor', which is independent of the path length and concentration of the solution, and is defined as in equation (1).[13]

$$g = \frac{\Delta\varepsilon_{(c.d.\text{-}max.)}}{\varepsilon_{(abs\text{-}max.)}} = \frac{\Delta A_{(c.d.\text{-}max.)}}{A_{(abs\text{-}max.)}} \quad (1)$$

The c.d.-max was at *ca.* 266—270 nm whereas the absorbance maximum (measured on a Varian DMS 100 spectrophotometer) was at 258 nm. The values of *g* for the untreated, chemically treated, and iodinated *t*-RNA were 8.7 × 10^{-5}, 8.0 × 10^{-5}, and 7.7 × 10^{-5}, respectively. These data indicate, that within experimental error, the iodination and its associated chemical process does not materially affect the c.d. spectrum and hence the conformation of the RNA.

The c.d. of the *t*-RNA is markedly affected by changes in pH, carried out by additions of HCl or NaOH, this being due to protonation of the bases and also ionisation of the phosphate groups (Figure 2). The effect of pH upon the helicity of the iodinated RNA and the original *t*-RNA was compared in phosphate buffer solutions (from 0.2M-NaH_2PO_4–Na_2HPO_4 mixtures) and the results are shown in Figures 3 and 4. Comparison of Figures 4a and 4b shows that the acid–base–c.d. relationship of the *t*-RNA and its iodinated derivative is essentially the same, the curve for the latter showing a slightly lower level of c.d. arising from the higher molecular weight and hence lower molar concentration. The c.d. maximum was enhanced in the phosphate buffer solution compared with the

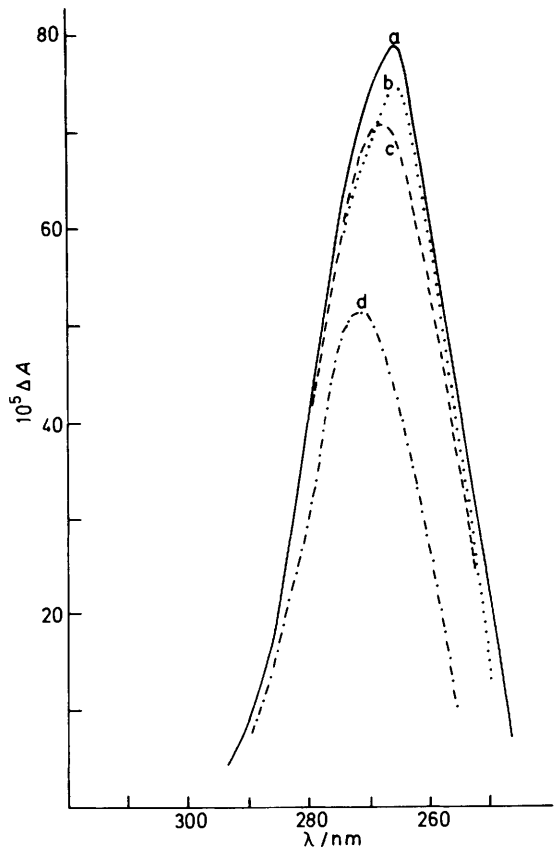

Figure 3. C.d. of I-RNA in phosphate buffer (0.1 mg cm^{-3}); a = pH 9.1; b = pH 6.8; c = pH 5.7; d = pH 4.4

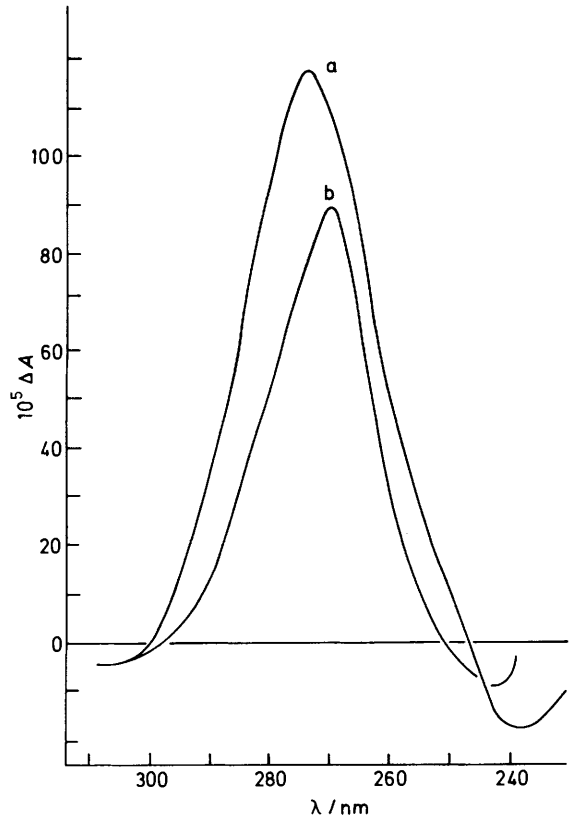

Figure 5. C.d. of *t*-RNA (approx. 0.15 mg cm^{-3}) and effect of urea; a = soln. in water; b = soln. in 80% saturated urea solution

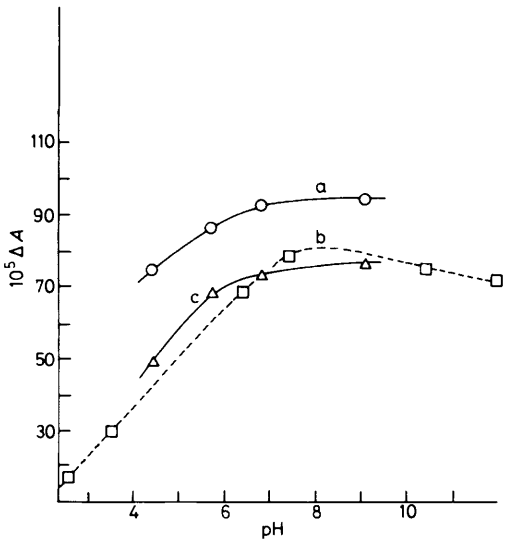

Figure 4. C.d. maxima as a function of pH. ○ = *t*-RNA in phosphate buffer; △ = I-RNA in phosphate buffer; □ = *t*-RNA in H_2O with pH adjusted with HCl/NaOH

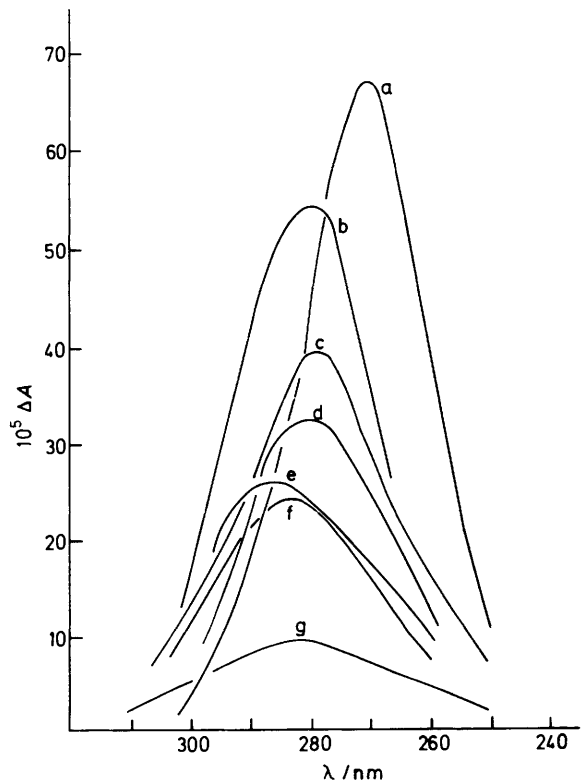

Figure 6. Effect of ethylene glycol on c.d. of I-RNA; a = H_2O; b = 0.5%; c = 1.0%; d = 5.0%; e = 10%; f = 25%; g = 90% v/v.

data in dilute HCl–NaOH (Figure 4c); however, no such enhancement was found in a separate series of experiments with NaCl concentrations up to 1M. The observed effect of an enhancement of c.d. in the phosphate buffer must be due to a

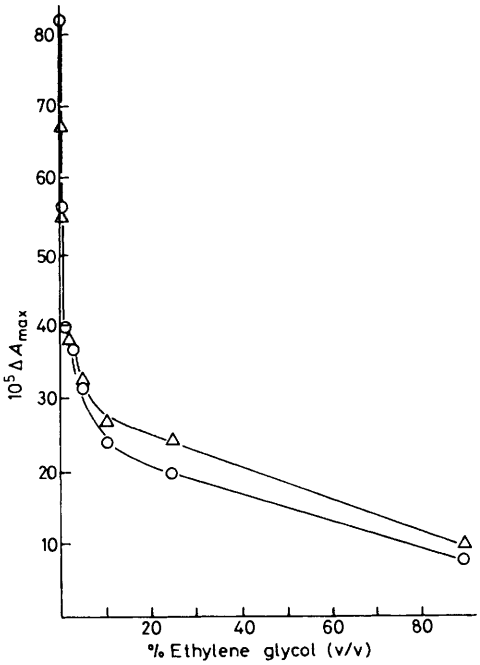

Figure 7. Maximum c.d. as a function of ethylene glycol; ○ = *t*-RNA; △ = I-RNA

specific ionic strength arising from interaction of the buffer with the phosphate groups of the *t*-RNA.

Hydrogen bonding is not a major contributor to stabilisation of the *t*-RNA helicity since addition of concentrated urea solution, a known hydrogen-bond breaker, does not significantly alter the c.d. and hence the helical conformation (Figure 5). It is thought that the helical conformation of polynucleotides is stabilised by hydrophobic forces between the stacked bases, although it is known that uracil does not stack since uracil oligonucleotides show little or no optical activity. The inter-base hydrophobic forces responsible for the stacking can be overcome by ethylene glycol (a reagent known to interfere with hydrophobic forces) leading to a loss of helicity, and this influence on the c.d. is shown for the iodinated RNA in Figure 6. A comparison of the effects of ethylene glycol upon the c.d. maximum for the original *t*-RNA and the iodinated RNA is given in Figure 7. This shows that both molecules have their helicity affected in a similar manner by the addition of the ethylene glycol.

Thus we are fairly confident that the iodination of the *t*-RNA under the conditions used is accompanied by only minimal changes in conformation. This finding is of some relevance to other workers who may wish to use labelled or even unlabelled iodinated RNA as a marker in other studies.

Acknowledgements

The financial assistance of the S.E.R.C. towards the construction of the c.d. spectrometer is gratefully acknowledged. The helpful comments of a referee are gratefully acknowledged.

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Received 25*th February* 1985; *Paper* 5/321