Maniatis, T., Ptashne, M., and Maurer, R. (1973), *Cold Spring Harbor Symp. Quant. Biol. 37,* 857.
Mertz, J. E., and Berg, P. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 4879.
Middleton, J., Edgell, M. H., and Hutchison, C. A. (1972), *J. Virol. 10,* 42.
Noll, M. (1974), *Nucleic Acids Res. 1,* 1573.
Peacock, A. C., and Dingman, C. W. (1969), *Biochemistry 8,* 608.
Pinder, J. C., Staynov, D. Z., and Gratzer, W. B. (1974a), *Biochemistry 13,* 5367.
Pinder, J. C., Staynov, D. Z., and Gratzer, W. B. (1974b), *Biochemistry 13,* 5373.
Pirrotta, V., Ptashne, M., Chadwick, P., and Steinberg, R. (1971), *Proc. Nucleic Acid Res. 2,* 703.
Pribnow, D. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 784.
Prunell, A., and Bernardi, G. (1973), *J. Biol. Chem. 248,* 3433.
Richardson, C. C. (1965), *Proc. Natl. Acad. Sci. U.S.A. 54,* 158.
Sharp, P. A., Sugden, B., and Sambrook, J. (1973), *Biochemistry 12,* 3055.
Sinsheimer, R. L. (1959), *J. Mol. Biol. 1,* 43.
Staynov, D. Z., Pinder, J. C., and Gratzer, W. B. (1972), *Nature (London), New Biol. 285,* 108.
Van deSande, J. H., Caruthers, M. H., Sgaramella, V., Yamada, T., and Khorana, H. G. (1972a), *J. Mol. Biol. 72,* 457.
Van deSande, J. H., Loewen, P. C., and Khorana, H. G. (1972b), *J. Biol. Chem. 247,* 6140.
Zeiger, R. S., Salomon, R., Dingman, C. W., and Peacock, A. C. (1972), *Nature (London), New Biol. 238,* 65.

Fluorescence Detected Circular Dichroism Study of the Anticodon Loop of Yeast $tRNA^{Phe}$ †

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ABSTRACT: Fluorescence detected circular dichroism (FDCD) measurements have been used to study the conformations of the anticodon loop of yeast phenylalanine tRNA. To our knowledge this is the first application of fluorescence detected circular dichroism. Much smaller amounts of tRNA are needed for the measurement of FDCD than for the conventionally measured circular dichroism. Furthermore, FDCD is specific for conformational changes near the anticodon loop. The FDCD measurements suggest a transition in the anticodon loop near 20° in 0.01 *M* $MgCl_2$–0.1 *M* NaCl (pH 7). This is followed by a broad transition from 30 to 60° and finally a sharp melting at 75° consistent with the absorbance detected melting of the entire tRNA. Removal of Mg^{2+} from the tRNA at 1° causes nearly a factor of two decrease in the FDCD near 230 nm. This indicates a decrease in conformational rigidity in the anticodon loop on removal of Mg^{2+}.

Many biological processes depend upon very localized structural features or conformational changes, and there is, therefore, a great demand for probes of local conformation. We recently reported a new technique, fluorescence detected circular dichroism (FDCD), which combines the conformational sensitivity of circular dichroism (CD) with the specificity of fluorescence (Turner et al., 1974). This technique is thus capable of providing unique structural information for proteins and nucleic acids. In this paper, the method is used to study the conformations of the anticodon loop of yeast $tRNA^{Phe}$.

The anticodon loop of tRNA is responsible for reading the genetic code during protein synthesis. This important function has generated great interest in its conformation. The structure of $tRNA^{Phe}$ in crystals (Kim et al., 1974; Robertus et al., 1974) provides a starting point for the interpretation of solution studies. It is of course important to determine the conformations in solution and to understand how they depend on the environment.

Several studies have taken advantage of the fact that in yeast $tRNA^{Phe}$ the base immediately adjacent to the 3′ end of the anticocon, called Y base, is moderately fluorescent (RajBhandary and Chang, 1968). Thus the fluorescence intensity has been used to monitor conformational changes of the loop (Beardsley et al., 1970; Eisinger et al., 1970; Robison and Zimmerman, 1971). The fluorescence properties of fragments derived from the loop have also been studied (Maelicke et al., 1973). Phosphorescence of the bases in the loop at low temperature has proved to be another useful probe (Hoover et al., 1974). Another technique which has provided important information is binding of oligonucleotides (Eisinger et al., 1970; Uhlenbeck et al., 1970, Högenauer, 1970; Uhlenbeck, 1972; Pongs et al., 1973; Eisinger and Spahr, 1973; Eisinger, 1971; Eisinger and Gross, 1974.)

Materials and Methods

Materials. Yeast $tRNA^{Phe}$ was purchased from Boehringer-Mannheim. According to their assay, it has an accep-

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tance activity of 1030 pmol of phenylalanine per optical density unit at 260 nm. The procedure for removal of Mg^{2+} has been described previously (Blum et al., 1972). This procedure leaves a small amount of Mg^{2+} (about 1 Mg^{2+}/tRNA); it is this concentration which we will call "no Mg^{2+}." The melting temperature of tRNA^{Phe} in 0.1 *M* NaCl, 10^{-5} *M* EDTA, and 0.01 *M* cacodylate (pH 7) is 52°, in reasonable agreement with previously reported results (Cramer et al., 1968; Römer et al., 1969; Beardsley et al., 1970). Base hydrolysis was accomplished by incubating the tRNA in 0.3 *M* NaOH at 37° for 24 hr, heating to 60° for 35 min, and incubating at 37° for 12 hr more (Blum, 1971). On removal from the bath, it was neutralized with 5 *M* HCl, and then enough 1 *M* cacodylate buffer at pH 7 was added to make the solution 0.045 *M* in buffer.

Fluorescence Detected Circular Dichroism. FDCD measures the difference in fluorescence intensity for left and right circularly polarized excitation as a function of excitation wavelength. The apparatus for the measurement has been described previously (Turner et al., 1974). For these experiments the bandwidth of the excitation light was held constant at 2 nm. One of the problems associated with the technique is the attainment of a correct base line. This base line is determined by scanning a solution of a fluorescent compound which is not optically active. Unfortunately, shifts and even artifactual peaks are observed in the base line upon changes in the concentration or nature of the fluorescent species. These artifacts appear to be due to scattered excitation light or filter fluorescence which reaches the photomultiplier. There are several clues which indicate this. First of all, a decrease in fluorescence intensity always results in an apparently higher ellipticity. This effect can be seen by simply decreasing the concentration of the base-line fluorophore. This behavior can result in artifactual peaks if the sample has a minimum in its excitation spectrum where the base-line fluorophore does not. Thus proflavine, although optically inactive, can appear to have an FDCD peak at 315 nm when sodium fluorescein is used as a base line. Artifactual shifts can also be produced by the addition of scattering particles, either latex spheres or Al_2O_3, to a solution of sodium fluorescein. The CD spectra of such suspensions show no optical activity in the 550–300-nm region indicating that the scattering particles do not induce any CD in the fluorescein. Nevertheless, a solution of fluorescein and scattering particles exhibits a slowly decreasing FDCD ellipticity which peaks at about 235 nm as the spectrum is scanned toward lower wavelengths. All of these observations are consistent with the suggestion that scattered light reaching the photomultiplier can produce an apparent ellipticity. The measures which have been employed to minimize this problem are listed as follows.

1. Choice of Filter. The filter used for rejection of stray light should, of course, have a high transmittance throughout the spectral region spanned by the fluorescence emission. It should also have as sharp a cut-off as possible on the short wavelength side so that the FDCD can be measured to the longest wavelength possible. Beyond this, colored glass filters vary considerably in the rejection of light outside their transmittance band. Many are also fluorescent (Turner, 1973). It is extremely important to choose a filter which will transmit the minimum amount of extraneous light. This choice of filter is best made empirically by observing the base-line shifts caused by the addition of scattering particles to a fluorescent solution. For the work on tRNA, the smallest shift was achieved with a Corning 4-96 filter, and it was therefore used for all the measurements reported here. In some cases, two filters are better than one. However, the increase in stray light rejection must be weighed against the decrease in fluorescence transmission, which lowers the signal-to-noise ratio. As a conservative precaution FDCD spectra have only been analyzed out to 325 nm, even though the cut off for the 4-96 filter is at 350 nm.

2. Choice of Base-Line Fluorophore. Problems are encountered if the base line and sample solutions produce different ratios of stray light to fluorescent light. To minimize this, the base-line fluorophore is selected to have an excitation spectrum closely approximating that of the sample. The concentration of the base-line solution is then adjusted to provide roughly the same fluorescence intensity as the sample of interest. Because of this matching, any effects due to scattered light should be present in both the base line and sample scans, and should therefore cancel. For tRNA, sodium fluorescein provided an adequate base line.

3. Choice of Photomultiplier. Photomultipliers have slightly different quantum efficiencies for left and right circularly polarized light. This difference is a function of wavelength. The variation in this behavior from tube to tube, within a given model number, can be considerable. We based our choice of photomultiplier on flatness of FDCD base line. Three Hammamatsu R375 tubes were tested. Two provided reasonably flat base lines and the best one was used for the tRNA experiments. Satisfactory performance has also been observed with an RCA 6217 and a DuMont KM2703 tube.

4. Alignment of Photomultiplier. It was found that different, optically inactive fluorophores gave small shifts in the FDCD base line. These shifts are sensitive to the angle of the photomultiplier relative to the axis formed by the phototube and the fluorescence cell. This angle was adjusted until negligible shifts were observed and the phototube was then firmly secured.

Several methods were used to test the effectiveness of the above measures. Base lines were scanned using proflavine, lumiflavine, sodium salicylate, and sodium fluorescein as the fluorophore. All of these were equivalent from 300 to 200 nm except for small regions (extending from at most 20 nm) in which a given fluorophore had a minimum in its excitation spectrum. This problem is minimized with tRNA because there are no deep troughs in its excitation spectrum (see Figure 3). As a further test, sodium fluorescein base lines were run at concentrations of 8×10^{-6}, 4×10^{-7}, and 8×10^{-8} *M*. They were all essentially coincident.

The final test method involved adding scattering particles to sodium fluorescein solutions to increase the intensity of scattered excitation. Two types of particles were used, Al_2O_3 which was centrifuged for 20 min at 2000 rpm to provide a homogeneous suspension, and latex spheres of 0.1 μ diameter which were obtained from Polysciences, Inc., Warrington, Pa. (cat. 0876). Both suspensions showed ν^4 scattering behavior when their apparent absorbance was measured in a Cary 14. The scattering corresponded to apparent absorbances at 235 nm of 0.13 and 0.07 for the Al_2O_3 and the latex spheres, respectively. Dye was added to these solutions to give a concentration of 4×10^{-7} *M* sodium fluorescein, and the FDCD spectra were measured. Base-line shifts of approximately 0.005° were observed. This is sufficient discrimination for work with tRNA since the nucleic acid is not expected to be a strong scatterer, and low concentrations were used (OD_{260} ~0.2–0.8, OD_{235} ~0.1–0.4). However, it is clear that caution will be required

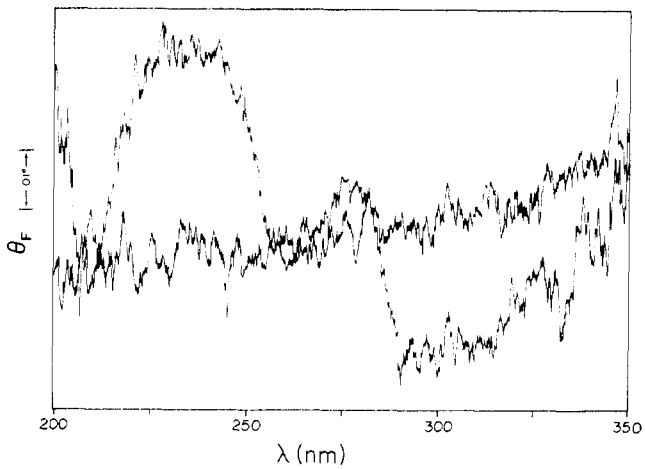

FIGURE 1: Chart recording of FDCD spectrum of 0.2 A_{260} $tRNA^{Phe}$ in 0.01 M $MgCl_2$, 0.1 M NaCl, 0.01 M cacodylate (pH 7). Base line is 2.5×10^{-7} M sodium fluorescein. Full scale is 0.1°.

in extending the technique to larger biological assemblies.

Other Spectra. Absorption spectra were measured with a Cary 14 spectrophotometer. CD spectra were measured with a Cary 60 spectropolarimeter with Model 6001 CD attachment. Fluorescence spectra were obtained with a Perkin-Elmer MPF-3L fluorimeter equipped with an accessory for correction of excitation spectra. For the excitation spectra the fluorescence was monitored at 450 nm with a 30-nm bandwidth. A 5-nm bandwidth was used for the excitation monochromator. The optical densities of the samples used for fluorescence measurements ranged from 0.05 to 0.07 at 260 nm. No correction factors were applied for this absorption. Excitation spectra were only measured to 240 nm because of the low signal-to-noise ratios at shorter wavelengths.

Results

FDCD spectra of $tRNA^{Phe}$ were measured in 0.1 M NaCl, 0.01 M cacodylate buffer at pH 7, and either with 0.01 M $MgCl_2$ or with no Mg^{2+}. The spectrum of $tRNA^{Phe}$ hydrolyzed with base to the component nucleotides was also measured. All spectra were measured at 27° unless otherwise noted. A typical chart recording of a spectrum is shown in Figure 1. The spectra were digitized and smoothed in a manner described previously (Blum et al., 1972). The smoothed spectra are shown in Figure 2. Before FDCD spectra can be interpreted, the effect of the differential absorption by the sample of left and right circularly polarized light must be subtracted. The necessity for this becomes clear if one considers the fluorescence of nonoptically active fluorophores at the rear of a cell containing optically active material. If the material in the cell exhibits positive CD, for example, these molecules will experience a higher intensity of light relative to left circularly polarized light due to the preferential absorption of left circularly polarized light throughout the cell. Thus the fluorescence intensity will be greater for right than for left circularly polarized light, even though the fluorophore is not optically active. The measured fluorescence-detected ellipticity, θ_F, as read directly from the chart paper in units of degrees is related to other parameters by (Tinoco et al., manuscript in preparation)

$$\theta_F = \left(\frac{-1}{0.0349}\right)\left(\frac{\Delta\epsilon_F - 2\epsilon_F R}{2\epsilon_F - \Delta\epsilon_F R}\right) \qquad (1)$$

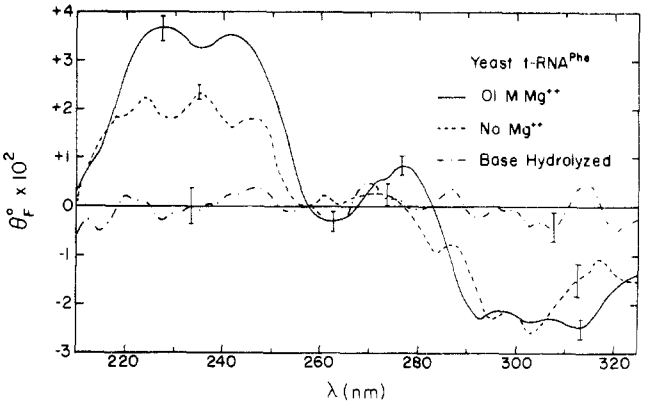

FIGURE 2: Smoothed FDCD spectra of $tRNA^{Phe}$, 0.1 M NaCl, 0.01 M cacodylate (pH 7); (—) 0.01 M $MgCl_2$, 0.2 A_{260}; (- - -) 10^{-5} M EDTA, no Mg^{2+}, 0.3 A_{260}; (- · - ·) base hydrolyzed, 0.3 A_{260}.

where

$$R = \frac{A_L(1 - 10^{-A_R}) - A_R(1 - 10^{-A_L})}{A_L(1 - 10^{-A_R}) + A_R(1 - 10^{-A_L})}$$

The difference in extinction coefficients for left and right circularly polarized light for the fluorophore is $\Delta\epsilon_F = \epsilon_{FL} - \epsilon_{FR}$, ϵ_F is the extinction coefficient of the fluorophore for unpolarized light, and A_L and A_R are the absorbances of the solution for left and right circularly polarized light. The factor 0.0349 = ln 10/65.96. Two important characteristics of the above equation should be noted. The value of θ_F can be nonzero even if $\Delta\epsilon_F$ is zero as noted before, and θ_F is independent of the quantum yield of the fluorophore. Essentially the magnitude of θ_F is related to the asymmetry ($g_F = \Delta\epsilon_F/\epsilon_F$) of the fluorescent transition not its absolute magnitude. Quenching of the fluorescence does not effect θ_F directly.

Solving for $\Delta\epsilon_F$ we obtain an expression for the FDCD:

$$\Delta\epsilon_F = 2\epsilon_F\left[\frac{R - 0.0349\theta_F}{1 - 0.0349R}\right] \qquad (2)$$

R will be order of magnitude 10^{-3} or smaller for most solutions; therefore the denominator can generally be equated to 1. A good approximation to R can be obtained by keeping only linear terms in $\Delta A = A_L - A_R$. The linearized R is

$$R_l = \frac{\Delta A}{2A} - \frac{2.303\Delta A 10^{-A}}{2(1 - 10^{-A})} \qquad (3)$$

In order to apply these equations, it is necessary to measure ΔA, A, and ϵ_F. The CD spectra measured agreed well with those reported previously (Blum et al., 1972; Beardsley et al., 1970). The values of ϵ_F can be obtained by measuring the corrected excitation spectrum for the sample. Figure 3 shows corrected excitation spectra measured for $tRNA^{Phe}$. It also shows the corrected excitation spectrum measured for the Y base by Maelicke et al. (1973). All the spectra have been normalized to give the same height for the largest peak. The excitation spectra of our base-hydrolyzed samples followed the general trend of the Y base spectrum reported by Maelicke et al., but with considerably reduced signal-to-noise ratios. It is clear from Figure 3 that the $tRNA^{Phe}$ containing the Y base has a very different corrected excitation spectrum from the isolated Y base. This change is larger than would be expected for a change in shape and magnitude due to interaction with adjacent bases. We therefore suspect that energy transfer may be occurring in this system.

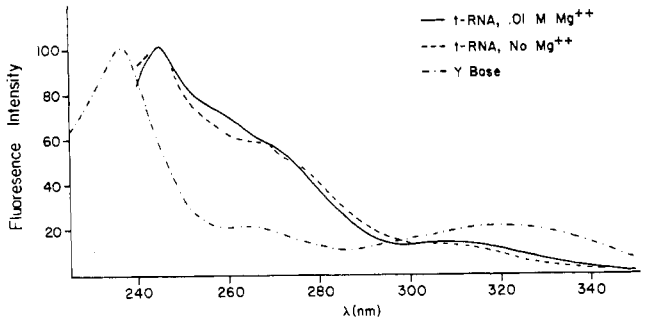

FIGURE 3: Corrected excitation spectra. (—) 0.07 A_{260} tRNA^{Phe}, 0.01 M $MgCl_2$, 0.1 M NaCl, and 0.01 M cacodylate (pH 7); (- - -) 0.05 A_{260} tRNA^{Phe}, 10^{-5} M EDTA, 0.1 M NaCl, and 0.01 M cacodylate (pH 7); (- · · ·) 0.1 A_{237} Y base, 0.01 M Mg^{2+}, 0.01 M Tris-acetate (pH 7.5) (Maelicke et al., 1973).

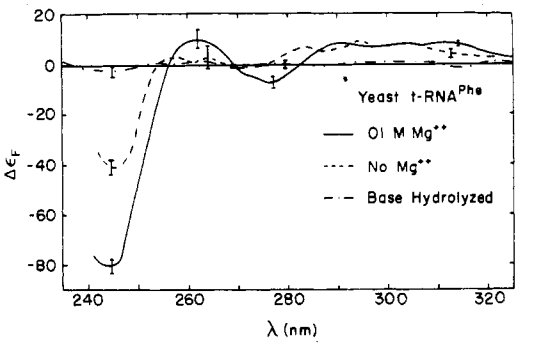

FIGURE 4: Analyzed FDCD of tRNA^{Phe}, 0.1 M NaCl, and 0.01 M cacodylate (pH 7); (—) 0.01 M $MgCl_2$; (- - -) 10^{-5} M EDTA, no Mg^{2+}; (- · · ·) base hydrolyzed. Similar spectra were also obtained from 0.8 A_{260} tRNA^{Phe}, 0.01 M $MgCl_2$, and 0.4 A_{260} tRNA^{Phe}, no Mg^{2+}.

If energy transfer is important in the system, the previous equations must be modified by replacing $\Delta\epsilon_F$ and ϵ_F by the following expressions:

$$\Delta\epsilon_F = \epsilon_{FL} - \epsilon_{FR} + \sum_i a_i(\epsilon_{iL} - \epsilon_{iR})$$

$$\epsilon_F = \epsilon_F + \sum_i a_i\epsilon_i \qquad (4)$$

where a_i is the fraction of energy absorbed by base i which is transferred to Y, and ϵ_{iL}, ϵ_{iR}, and ϵ_i are the extinction coefficients of base i for left circularly polarized, right circularly polarized, and unpolarized light, respectively. This equation requires knowledge of the absolute magnitude of the term ($\epsilon_F + \Sigma a_i\epsilon_i$). This has been obtained by measuring the extinction coefficient of tRNA^{Phe} at 335 nm where only the Y base is expected to absorb, and scaling the corrected excitation spectrum from this value. The extinction coefficient used for 335 nm was 1700 M^{-1} cm^{-1}. Because of experimental problems associated with measuring the low optical density at 335 nm, and the high optical density at 258 nm, this number may only be accurate to ±25%. Thus the absolute magnitudes of CD peaks are only good to ±25%. However, the shapes are not affected. Using the corrected excitation spectrum for tRNA with Mg^{2+} shown in Figure 3 and the value of 1700 M^{-1} cm^{-1}, the value of ($\epsilon_F + \Sigma a_i\epsilon_i$) calculated for the 245-nm peak is 34,300 M^{-1} cm^{-1}. This can be compared with the extinction coefficient of 32,000 reported for the 235-nm peak of free base Y (Funamizu et al., 1971).

The CD spectra calculated from eq 2 are shown in Figure 4. These spectra were derived using the raw, unsmoothed FDCD data for eq 2, and then applying the smoothing routine to the calculated results. The error bars represent the standard deviation between raw and smoothed points in a given band. As can be seen from eq 4, these spectra represent the CD of the Y base plus a fraction of the CD of bases transferring energy to the Y base. The most striking feature of the spectrum for tRNA in 0.01 M Mg^{2+} is the large magnitude of the negative peak at 245 nm. The $\Delta\epsilon_F$ of approximately −80 M^{-1} cm^{-1} contrasts sharply with the average $\Delta\epsilon$ per nucleotide for the 260-nm peak of RNA^{Phe}, which is about −7 M^{-1} cm^{-1}. Perhaps a better comparison is the so called g factor or $\Delta\epsilon/\epsilon$. The average of this quantity for tRNA at 260 nm is about 10^{-3} per nucleotide, whereas for the spectrum derived from FDCD measurements it is 2.3×10^{-3}. While such a large magnitude is unusual, it is not unprecedented. Nelson et al. (1971) have measured a

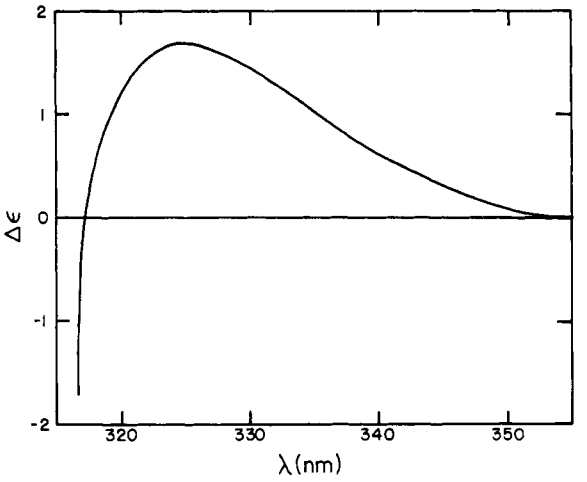

FIGURE 5: CD spectrum of 33 A_{260} tRNA, 0.01 M $MgCl_2$, 0.1 M NaCl, and 0.01 M cacodylate (pH 7).

$\Delta\epsilon$ at 260 nm of approximately 60 M^{-1} cm^{-1} for ApG modified by N-2-acetylaminofluorene. In this respect, it is interesting that both N-2-acetylaminofluorene and the Y base are comprised of three rings which are not fully conjugated.

While it is not possible to independently check our spectra at short wavelengths, it is possible to make a comparison with conventionally measured CD spectra at long wavelengths. This is because only the Y base is expected to absorb strongly at wavelengths above 320 nm. Thus the spectrum shown in Figure 4 predicts that at long wavelengths a positive CD band should be observable in concentrated solutions of tRNA^{Phe}. Figure 5 shows the conventionally measured CD spectrum of a 33.3 OD (260) solution of tRNA^{Phe} in 0.1 M NaCl, 0.01 M $MgCl_2$, and 0.01 M cacodylate buffer at pH 7. This spectrum is similar to one reported earlier (Takasaki and Imahori, 1973). The spectrum was only measured to roughly 315 nm because of the high optical density below this wavelength. As expected, the region from 320 to 350 nm shows a small, positive CD peak. The magnitude of this peak, however, is only about 1.65 M^{-1} cm^{-1} (per mole of tRNA^{Phe}) whereas the magnitude predicted from Figure 4 is 2.6 M^{-1} cm^{-1}. This is probably due to a contribution from the negative CD peak of tRNA^{Phe} at 295 nm. The magnitude of this peak is −34.1 M^{-1} cm^{-1} (per mole of tRNA) (Blum et al., (1972), so that a residual negative component of −1 M^{-1} cm^{-1} at 325

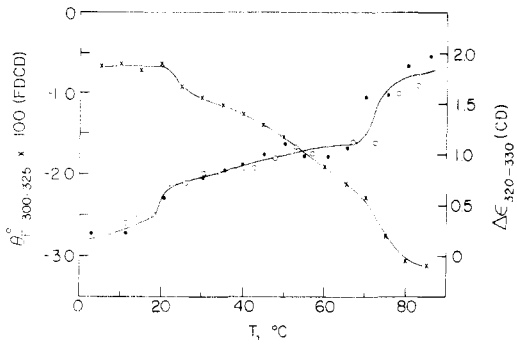

FIGURE 6: Melting curves of $tRNA^{Phe}$ in 0.01 *M* $MgCl_2$, 0.1 *M* NaCl, and 0.01 *M* cacodylate (pH 7). (● and ○) FDCD of 0.2 A_{260} $tRNA^{Phe}$. (+) CD of 33.3 A_{260} $tRNA^{Phe}$.

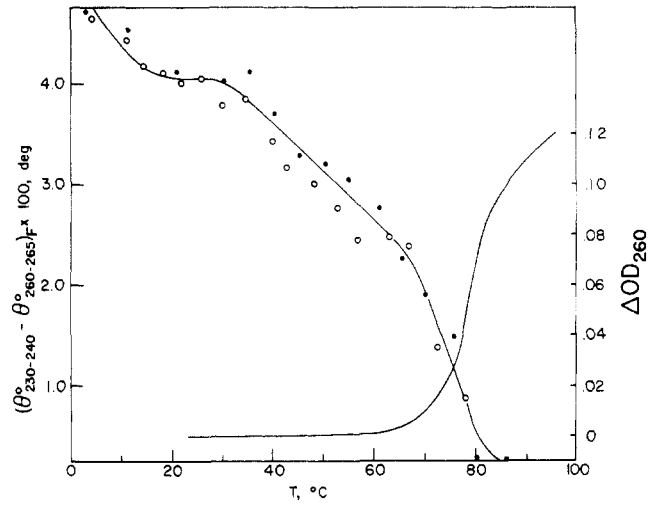

FIGURE 7: Melting curves of $tRNA^{Phe}$ in 0.01 *M* $MgCl_2$, 0.1 *M* NaCl, and 0.01 *M* cacodylate (pH 7). (● and ○) FDCD of 0.2 A_{260} $tRNA^{Phe}$. (—) Absorption at 260 nm.

nm would not be surprising. It thus seems that for both sign and magnitude, the spectrum calculated from the FDCD measurements is in reasonable agreement with that determined conventionally.

Effect of Mg^{2+}. Fluorescence measurements have shown a large change in the quantum yield of the Y base in $tRNA^{Phe}$ upon removal of Mg^{2+} (Beardsley et al., 1970; Eisinger et al., 1970; Robison and Zimmerman, 1971). This has been interpreted as a conformational change resulting in increased exposure of Y to the solvent. Such a shift should produce a change in the CD spectrum of the Y base. Figure 4 shows the CD spectrum calculated from the FDCD spectrum of $tRNA^{Phe}$ without Mg^{2+}. The values for $(\epsilon_F + \Sigma a_i \epsilon_i)$ used in deriving this spectrum were calculated from the excitation spectrum shown in Figure 3, assuming an extinction coefficient of 34,300 M^{-1} cm^{-1} at the 245-nm peak. This assumption is based on the expectation that the extinction coefficient of the Y base in $tRNA^{Phe}$ will not change much with Mg^{2+} concentration. It can be seen from Figure 4 that the removal of Mg^{2+} results in a factor of two decrease in the magnitude of the peak at 245 nm, but only a small change in the magnitude of the peak around 300 nm. At present, it is not possible to interpret these changes in terms of absolute conformation. However, it is clear that the removal of Mg^{2+} results in a structural change around the Y base.

Base-Hydrolyzed $tRNA^{Phe}$. The free Y base is only slightly optically active. In 10% methanol, it displays a $\Delta\epsilon_{235\ nm}$ of -2.2 ± 0.3 M^{-1} cm^{-1} (Nakanishi et al., 1970). The Y nucleotide produced by base hydrolysis of $tRNA^{Phe}$ is expected to have only a small CD. The FDCD spectrum of base-hydrolyzed $tRNA^{Phe}$ was analyzed using the absorption spectrum of Maelicke et al. (1973) assuming an extinction coefficient of 32,000 at 235 nm (Funamizu et al., 1971). The result is shown in Figure 4. As predicted, the calculated CD spectrum has essentially negligible magnitude within the error of the experiment.

Temperature Dependence of FDCD. It is well known that temperature will alter the conformation of tRNA. Previously, however, it has been difficult to study the effect of temperature on the anticodon loop. This is because the quantum yield depends on temperature-dependent quenching mechanisms, as well as conformation (Beardsley et al., 1970; Robison and Zimmerman, 1971; Eisinger et al., 1970). For conformational changes in the microsecond region, the two processes have been separated by using the time dependence of the fluorescence changes (Riesner et al., 1973). FDCD spectra should be sensitive to conformational changes, but not to quenching. The temperature dependence of the uncorrected FDCD ellipticity of $tRNA^{Phe}$ between 300 and 325 nm is shown in Figure 6. For this plot the average of the FDCD ellipticity from 300 to 325 nm was calculated in order to decrease the scatter. Two separate runs are displayed. Also shown is the temperature dependence of the conventionally determined ellipticity. This has been averaged from 320 to 330 nm. Both curves show similar behavior. There is a rapid decrease in magnitude in the region around 75°. This is the region in which tRNA secondary structure begins to disappear, as shown by the optical density melting curve in Figure 7. However, the CD curves also show changes in regions where the optical density is constant. There appears to be a small, but abrupt change at about 20°, and also a very gradual decrease from about 25 to 70°. These transitions appear in both the CD and FDCD curves. The transition at 20° is not well established because of its small amplitude and the marginal signal-to-noise ratios. We have attempted to observe a relaxation effect corresponding to this transition using a temperature-jump apparatus with fluorescence detection. No signal was observed within the time range accessible to our instrument, 10^{-4} to 1 sec. We intend to pursue this search with a laser temperature-jump apparatus capable of 30-nsec time resolution (Turner et al., 1972).

The temperature dependence of the FDCD spectrum at shorter wavelengths is shown in Figure 7. In this curve the average of the FDCD ellipticity from 230 to 240 nm minus the average ellipticity from 260 to 265 nm is plotted against temperature. Once again there is a precipitous drop in ellipticity around 75°, and a more gradual decline from about 30 to 60°. In contrast to the long wavelength results, there is no change around 20°, but there does seem to be a shift in the neighborhood of 10°. The origin of these effects awaits further study.

Discussion

This paper presents the first application of FDCD for the detection of conformational changes in a macromolecule. As such it is important to demonstrate that the technique gives reasonable results in situations where comparison with other techniques is possible. Thus we have measured the effect on tRNA's FDCD spectrum of three known ways of producing structural changes. The ultimate conformational change is produced by base hydrolysis of tRNA to its com-

ponent nucleotides. Just as one would hope, this destroys the FDCD spectrum. A less dramatic change is brought about by the removal of Mg^{2+} from the solution. This is known to change the fluorescence quantum yield by a factor of 2 or more, and to have a much smaller effect on the conventionally measured CD spectrum. The FDCD ellipticity at 245 nm decreases a factor of two upon removal of Mg^{2+} demonstrating that the technique is sensitive to subtle, local changes in conformation. Finally, the FDCD spectra are also sensitive to the disruption of structure which occurs at high temperatures. In addition to this qualitative agreement, it is encouraging that the sign and magnitude of the CD spectrum derived from FDCD measurements are in reasonable agreement with that measured conventionally at long wavelengths. The CD spectrum is also consistent with recent data obtained on fragments of the tRNA loop (Maelicke et al., 1975). Thus it appears that the FDCD is a sensitive probe of structure which can be related to the more common measurement of CD. This latter equivalence holds the promise that future calculations will be able to interpret FDCD spectra quantitatively in terms of structure.

The present results also demonstrate certain advantages of FDCD over other techniques. Because it is only sensitive to electronic interactions, it is not complicated by many of the quenching mechanisms affecting quantum yield determinations. Because fluorescence is detected rather than absorption, it requires a much lower concentration than conventional CD. This is demonstrated by the fact that a 33 OD (260 nm) solution was required to obtain the CD spectrum of tRNA^{Phe} beyond 315 nm by conventional CD, whereas the FDCD determination required an OD (260 nm) of only 0.2. Another advantage over conventional CD is the fact that FDCD spectra monitor only the conformation around the fluorescent chromophore, rather than the whole molecule. Thus FDCD spectra should be able to provide information not available with other methods.

There are two ways to apply FDCD spectra to chemical problems. The spectra alone can be used to look for changes. This is especially powerful if the overall absorbance or optical activity is small so that the correction factors are negligible. In this case the FDCD spectrum will only be sensitive to changes near the fluorophore. On the other hand, the FDCD spectrum can be used in conjunction with the absorption, CD, and corrected excitation spectra to calculate a quantitative CD spectrum of the fluorophore. This can then be compared to conventionally measured CD spectra of model compounds.

The FDCD spectra of tRNA^{Phe} presented here provide some interesting results. The large magnitude for the 245-nm peak is very unusual, and probably reflects a highly stacked Y base. A similarly large magnitude in ApG modified by *N*-2-acetylaminofluorene has been shown to arise from the stacking of the fluorene moiety on the adenine ring (Nelson et al., 1971). The removal of Mg^{2+} clearly results in a conformational change and the decrease in the magnitude of the CD probably indicates a less stacked structure. The temperature dependence shows a broad melting from about 30 to 60°; this may be a gradual unstacking similar to that observed for poly(A) (Leng and Felsenfeld, 1966; Van Holde et al., 1965.) Fuller and Hodgson (1967) have proposed a model for the anticodon loop which involves a high degree of stacking for the Y base. Our results are therefore consistent with this model. It also appears that a rather sharp transition may be present at about 20°, and we are attempting to verify this with other techniques.

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References

Beardsley, K., Tao, T. and Cantor, C. R. (1970). *Biochemistry 9,* 3524.

Blum, A. D. (1971). Ph.D. Thesis. University of California Berkeley.

Blum, A. D., Uhlenbeck, O. C. and Tinoco, I. (1972). *Biochemistry 11,* 3248.

Cramer, F., Doepner, H., von der Haar, F., Schlimme, E. and Seidel, H. (1968). *Proc. Nat. Acad. Sci., U.S.A. 61,* 1384.

Eisinger, J. (1971). *Biochem. Biophys. Res. Commun. 43,* 854.

Eisinger, J., Feuer, B. and Yamane, T. (1970). *Proc. Nat. Acad. Sci., U.S.A. 65,* 638.

Eisinger, J. and Gross, N. (1974). *J. Mol. Biol. 88,* 165.

Eisinger, J. and Spahr, P. F. (1973). *J. Mol. Biol. 73,* 131.

Fuller, W. and Hodgson, A. (1967). *Nature 215,* 817.

Funamizu, M., Terahara, A., Feinberg, A. M., and Nakanishi, K. (1971). *J. Amer. Chem. Soc. 93,* 6706.

Högenauer, G. (1970). *Eur. J. Biochem. 12,* 527.

Hoover, R. J., Luk, K. F. S. and Maki, A. H. (1974). *J. Mol. Biol. 89,* 363.

Kim, S. H., Suddath, F. L., Quigley, G. J., McPherson, A., Sussman, J. L., Wang, A. H. J., Seeman, N. C. and Rich, A. (1974), *Science 185,* 435.

Leng, M. and Felsenfeld, G. (1966). *J. Mol. Biol. 15,* 455.

Maelicke, A., von der Haar, F. and Cramer, F. (1973). *Biopolymers 12,* 27.

Maelicke, A., von der Haar, F., Mathias, S. and Cramer, F. (1975). *Biopolymers 14,* 155.

Nakanishi, K., Furutachi, N., Funamizu, M., Grunberger, D. and Weinstein, I. B. (1970). *J. Amer. Chem. Soc. 92,* 7617.

Nelson, J. H., Grunberger, D., Cantor, C. R. and Weinstein, I. B. (1971). *J. Mol. Biol. 62,* 331.

Pongs, O., Bald, R. and Reinwald, E. (1973). *Eur. J. Biochem. 32,* 117.

RajBhandary, U. L. and Chang, S. H. (1968). *J. Biol. Chem. 243,* 598.

Riesner, D., Maass, G., Thiebe, R., Philippsen, P. and Zachau, H. G. (1973). *Eur. J. Biochem. 36,* 76.

Robertus, J. D., Ladner, J. E., Finch, J. T., Rhodes, D., Brown, R. S., Clark, B. F. C. and Klug, A. (1974). *Nature 250,* 546.

Robison, B. and Zimmerman, T. P. (1971). *J. Biol. Chem. 246,* 110.

Römer, R., Riesner, D. and Maass, G. (1969). *FEBS Lett. 5,* 15.

Takasaki, Y. and Imahori, K. (1973). *J. Biochem. 74,* 513.

Turner, D. H., Flynn, G. W., Sutin, N. and Beitz, J. V. (1972). *J. Amer. Chem. Soc. 94,* 1554.

Turner, D. H., Tinoco, I. and Maestre, M. (1974). *J. Amer. Chem. Soc. 96,* 4340.

Turner, W. H. (1973). *Appl. Optics 12,* 480.

Uhlenbeck, O. C. (1972). *J. Mol. Biol. 65,* 25.

Uhlenbeck, O. C., Baller, J. and Doty, P. (1970). *Nature (London) 225,* 508.

Van Holde, K. E., Brahms, J. and Michelson, A. M. (1965). *J. Mol. Biol. 12,* 726.