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A Computer Probe of the Circular Dichroic Bands of Nucleic Acids in the Ultraviolet Region. I. Transfer Ribonucleic Acid†

Barbara D. Wells‡ and Jen Tsi Yang*

ABSTRACT: The circular dichroism (CD) and optical rotatory dispersion (ORD) between 181 and 300 nm were measured for native and heat-denatured yeast tRNA (unfractionated), yeast $tRNA^{Phe}$, and *Escherichia coli* $tRNA^{Val}$. The CD spectra can be resolved into five Gaussian bands, including a new band at 185 nm that has not been reported previously ($tRNA^{Phe}$ also shows an extra, small band near 224 nm). The ORD computed from the experimental CD by means of the Kronig–Kramers transform is compared with the observed ORD. Native tRNA (unfractionated) and $tRNA^{Val}$ require the introduction of a CD band at 165 nm to give a good ORD fit ($tRNA^{Phe}$ lacks this band for reasons unknown). This extra band seems to disappear completely when tRNAs are denatured at elevated temperatures. Both this 165-nm band and the 185-nm band are conformation dependent; they are probably related to the secondary structure of the RNAs.

Optical rotatory dispersion (ORD) and circular dichroism (CD) have now been used extensively for the study of biopolymers. In nucleic acids, the sugar, ribose or deoxyribose, is optically active; it also induces optical activity in the base chromophores which accounts for the CD between 200 and 300 nm of the mono- and polynucleotides. But CD bands that are not present for mononucleotides may arise from the interactions between monomer residues (mostly between the bases) occasioned by the geometry of the polynucleotide chains. It is just this aspect that can provide information about the secondary and tertiary structure of nucleic acids. With recent theoretical advances, notably by Tinoco and his coworkers (Tinoco, 1968; Johnson and Tinoco, 1969), we now have a better understanding of the origin of optical activity of nucleic acids. To fully explain the CD spectra in relation to conformation, we must take into account all the observed CD bands that are conformation dependent. Until recently, the CD and ORD spectra of nucleic acids have been confined to above 200 nm. Since the vacuum ultraviolet circular dichrometer is being developed, no results between 140 and 180 nm are as yet readily available. In this work we propose a computer probe of the CD bands of nucleic acids in this region. We are able to predict a CD band below 180 nm which accounts for the observed ORD. Our results should complement the corresponding vacuum CD measurements that are difficult to perform at present.

CD is an absorptive property; it is encountered only in the region of the absorpton of the chromophores and it is zero everywhere else. ORD is a dispersive property; optical rotation at any wavelength is a sum of the contributions from all optically active absorption bands and is therefore influenced by electronic transitions quite distant from the region in question, although the contributions from these distant bands could be small. The interdependence of CD and ORD can be evaluated by the Kronig–Kramers transform (Moffitt and Moscowitz, 1959; Moscowitz, 1960). Any disagreement between the ORD computed from experimental CD and the observed ORD indicates that additional CD bands must be taken into consideration. In this case extra CD bands are generated by trial and error until a good ORD fit is obtained.

In this work we study the CD and ORD of three tRNAs, the unfractionated yeast tRNA, representing an average tRNA, yeast $tRNA^{Phe}$, and *E. coli* $tRNA^{Val}$ as two specific molecules with different primary structures. Unlike DNAs and other RNAs, tRNAs are relatively small molecules. The X-ray diffraction study of $tRNA^{Phe}$ at 5-Å resolution has now been completed; this tRNA has a unique tertiary structure that is quite different from any of the proposed models of the cloverleaf folding based on chemical and hydrodynamic data (Cramer, 1971; Kim *et al.*, 1973). A variety of physical methods including CD and ORD have been applied to the elucidation of tRNA conformation in solution. Together with the

† From the Department of Biochemistry and Biophysics and Cardiovascular Research Institute, University of California, San Francisco, California 94143. *Received October 26, 1973.* This work was aided by U. S. Public Health Service Grants GM-10880 and HL-06285.

‡ Taken in part from a Ph.D. dissertation by Barbara D. Wells, University of California, San Francisco, 1973. Present address: Institute of Molecular Biophysics, The Florida State University, Tallahassee, Fla. 32306.

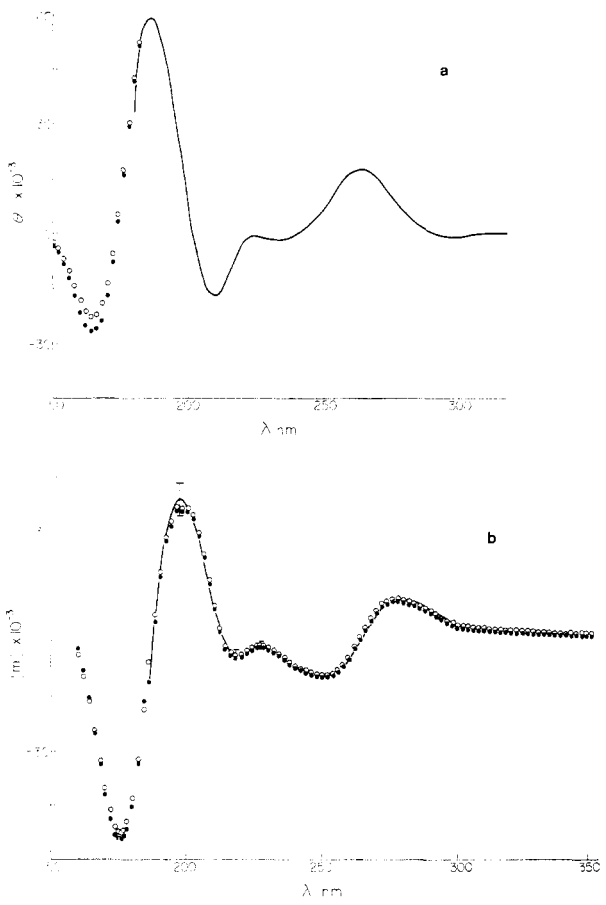

FIGURE 1: (a) The CD spectrum of unfractionated yeast tRNA in 0.15 M KF at 25°. Solid line, experimental; points, extrapolated according to items 2 (○) and 3 (●) of Table II. (b) The ORD spectrum: solid line, experimental; points, computed from (a) including the extra band of items 2 and 3

study of double-stranded RNAs and DNAs (Wells and Yang, 1974), we will in time have a better understanding of the secondary and tertiary structure of these tRNAs in solution.

Experimental Section

The unfractionated yeast tRNA (batch No. 11) and *E. coli* $tRNA^{Val}$ (control No. 23-4-406)(Miles) and yeast $tRNA^{Phe}$ (Boeringer Mannheim; control No. 719-410) were used without further purification. All samples were dissolved in 0.15 M KF (pH 7.3) and exhaustively dialyzed against the same solvent. Phosphate analyses (Chen *et al.*, 1956) were performed after spectral measurements. The molar extinction coefficients, ϵ(p), based on phosphorus, were 7.46×10^3 for the unfractionated tRNA, 7.79×10^3 for $tRNA^{Phe}$, and 7.48×10^3 for $tRNA^{Val}$. The concentrations used varied between 10^{-3} and 10^{-5} M (nucleotide); the mean residue weights are 322 for the unfractionated tRNA, 325 for $tRNA^{Phe}$, and 324 for $tRNA^{Val}$. The melting temperatures of the three samples were 54, 53, and 63°, respectively.

The CD was measured in a Durrum-Jasco J-5, SS 10 modified circular dichrometer and the ORD in a Cary 60 spectropolarimeter. The sample cell was placed in a temperature-controlled block mounted within the sample compartment; the temperature of the cell was monitored by a copper–constantin thermocouple.

The circular dichrometer was calibrated with a solution of *d*-10-camphorsulfonic acid (Cassim and Yang, 1969). The xenon lamps for both instruments were frequently adjusted and, if necessary, replaced to ensure ultraviolet penetration down to at least 181–182 nm. The cells were always checked for cleanliness by requiring exactly the same instrument base line on the most sensitive setting with and without the cell. The cells used varied in path length from 1 cm to 0.05 mm. The data points were taken at 1-nm intervals; each point was an average of 3–5 separate measurements. The data could be reproduced to within 5% at 25°, but were worse at high temperatures because of poor signal to noise ratios resulting from the hyperchromicity and reduced optical activity of the samples. The CD data were expressed in terms of mean residue ellipticity, $[\theta]$, and the ORD mean residue rotation, $[m]$.

Method of Analysis

All CD spectra were resolved into a sum of Gaussian bands using the Du Pont curve resolver. Each band can be expressed as

$$[\theta_i] = [\theta_i^0] \exp\{-(\lambda - \lambda_i)^2/\Delta_i^2\} \quad (1)$$

where $[\theta_i]$ and $[\theta_i^0]$ are the mean residue ellipticities at wavelengths λ and λ_i (the extremum) and Δ_i is the half-bandwidth of the ith band. The rotational strength R_i can be calculated from the relation (Moscowitz, 1960)

$$R_i = 1.233 \times 10^{-42}[\theta_i^0]\Delta_i/\lambda_i \quad (2)$$

The experimental CD spectrum can be converted to a computed ORD spectrum through the use of the Kronig–Kramers transform (Moscowitz, 1960)

$$[m(\lambda')] = (2/\pi)\, \mathrm{P}\int_0^\infty \{[\theta(\lambda)]\lambda/(\lambda'^2 - \lambda^2)\}\, d\lambda \quad (3)$$

where the symbol P before the integral indicates that the Cauchy principal value has to be taken, that is, for a single CD band

$$\mathrm{P}\int_0^\infty = \lim_{\delta \to 0}\left[\int_0^{\lambda'-\delta} + \int_{\lambda'-\delta}^\infty\right]$$

A computer program for the CDC 6400 has been written by J. Thiéry (for details, see Thiéry (1969)). This program assumes that the CD is zero outside the wavelength range computed. If the experimental spectrum terminates before a band is complete, the remainder of this band is extrapolated to zero on the basis of $[\theta_i^0]$, Δ_i, and λ_i found for this band. If the computed ORD differs from the experimental spectrum, we introduce a Gaussian band below 185 nm (see Results), whose inclusion would give a good fit between the measured and computed ORD.

We used a normalized mean square deviation and denote it as "fit" to test the agreement between the calculated and observed ORD (Blum *et al.*, 1972), eq 4, where E_j and

$$\text{fit} = \{\textstyle\sum(E_j - C_j)^2/\sum E_j^2\}^{1/2} \quad (4)$$

C_j are the experimental and calculated rotation at wavelength j. Fit was calculated over the entire measured ORD spectrum at 1-nm intervals. In our case, the quantity "fit" was usually less than 0.1. For measurements at 25° the fit between replicate experiments of a sample was less than 0.05.

Results

Figures 1–3 show the CD and ORD at 25° for unfractionated tRNA, $tRNA^{Phe}$, and $tRNA^{Val}$. Table I itemizes the resolved Gaussian bands of the CD spectra of the three tRNAs. In general, the band positions and bandwidths of each of the Gaussian bands do not vary much among the

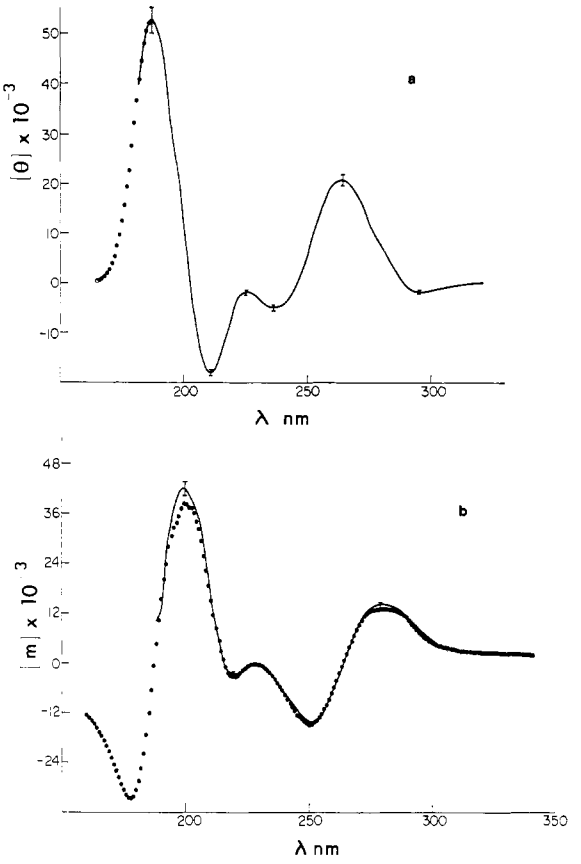

FIGURE 2: (a) The CD spectrum of yeast $tRNA^{Phe}$ in 0.15 M KF at 25°: solid line, experimental; points, extrapolated (see item 5 of Table II). (b) The ORD spectrum: solid line, experimental; points, computed from (a).

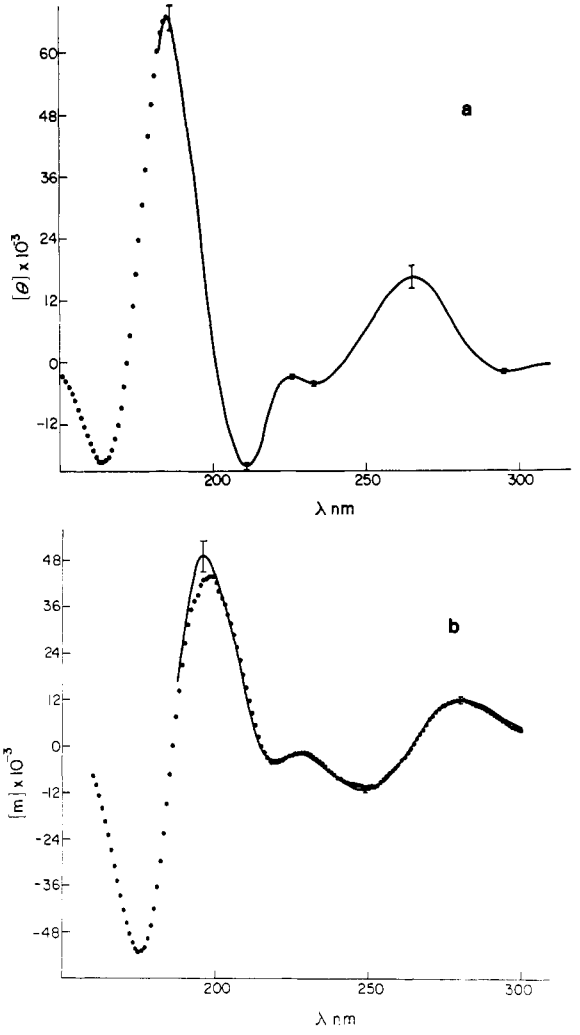

FIGURE 3: (a) The CD spectrum of *E. coli* $tRNA^{Val}$ in 0.15 M KF at 25°. Solid line, experimental; points, extrapolated (see item 10 of Table II). (b) The ORD spectrum. Solid line, experimental; points, computed from (a).

three tRNAs, except Δ_i for band 3. The extrema of these bands fall quite close to the experimental. The only exception is that $tRNA^{Phe}$ requires a small positive band near 224 nm (band 4) to give a good fit with the measured spectrum.

For the CD to ORD computation, we first extrapolated band 6 to zero ellipticity. This can be easily accomplished since the experimental measurements terminate around 181 nm which is below the extremum at 185–187 nm. If the measured and calculated ORD do not fit, we extended the extrapolation of band 6 beyond the zero ellipticity by following the contour line of band 6. This procedure immediately eliminated a negative extremum above 170 nm. On the other hand, the introduction of a negative band below 160 nm would overcorrect the computed ORD resulting in a poor ORD fit. Thus, our search was narrowed to a wavelength range between 160 and 170 nm. To further simplify our task, we arbitrarily chose a Δ_i of 10 nm for the extra band. Next, we guessed a rotational strength for this band and determined the band position by the ORD fit ($[\theta_i{}^0]$ is automatically fixed once R_i and λ_i are chosen, according to eq 2). This process was then repeated by using a larger or smaller rotational strength until a best fit was obtained. By trial and error the extrapolation is presumed the best estimate when the ORD fit gives the lowest possible numerical value. Table II summarizes the results of such a computer probe for the three tRNAs. In all cases the best ORD fit has values <0.1.

Unfractionated Yeast tRNA. The CD spectrum above 200 nm has been reported by Sarkar *et al.* (1967), but we have now observed a maximum at 186 nm (Figure 1a). The corresponding ORD also shows a maximum near 197 nm (Figure 1b). Our computer probe indicates that the five CD bands could not completely account for the observed ORD. The necessity of adding an extra band is seen from item 1 in Table II, which contains no negative band below 170 nm and shows a poor fit. Item 2 in Table II appears to be the best estimate of this extra band; the computed ORD, including this extra band (open circles), agrees well with the experimental curve (solid line). Increasing the rotational strength (item 3) or shifting the band position (item 4) of this extra band results in a worsening fit.

Yeast $tRNA^{Phe}$. Our CD spectrum (Figure 2a) resembles that obtained by Blum *et al.* (1972); the ellipticities at 295 and 263 nm also agree well with the literature values, but those below 250 nm differ markedly from the findings of Blum *et al.* (1972). Surprisingly, the observed CD spectrum, including the remainder of the incomplete 187-nm band through extrapolation, gave the best ORD fit (Figure 2b). Extrapolations 6, 7, and 8 in Table II indicate a trend of worsening fit with increasing rotational strength of an extra band below 170 nm. Among the RNAs and DNAs (Wells and Yang, 1974) studied, yeast $tRNA^{Phe}$ alone shows no evidence of a CD band around 165 nm. The reason for the absence of this band is not clear.

E. coli $tRNA^{Val}$. The CD spectrum (Figure 3a) is similar to that reported by Adler and Fasman (1970), Willick and Kay (1971), and Blum *et al.* (1972), but the magnitudes of

TABLE I: Resolved CD Spectra of tRNAs at 25°.[a]

Band[b]	Unfractionated Yeast tRNA	Yeast $tRNA^{Phe}$	*E. coli* $tRNA^{Val}$
(1) λ_1 (nm)	295	293	295
$[\theta_1^0]$	−1400	−2400	−2500
Δ_1 (nm)	7.5	9	9
$R_1 \times 10^{40}$	−0.44	−0.91	−0.96
(2) λ_2 (nm)	265	264	265
$[\theta_2^0]$	17600	20800	17000
Δ_2 (nm)	14	15	15
$R_2 \times 10^{40}$	6.5	14.6	11.9
(3) λ_3 (nm)	233	239	233.5
$[\theta_3^0]$	−2200	−6300	−4950
Δ_3 (nm)	6.5	12	8
$R_3 \times 10^{40}$	−0.75	−3.9	−2.1
(4) λ_4 (nm)		224	
$[\theta_4^0]$		2000	
Δ_4 (nm)		5	
$R_4 \times 10^{40}$		0.55	
(5) λ_5 (nm)	209	211	210.5
$[\theta_5^0]$	−17800	−18400	−20800
Δ_5 (nm)	8	8	8
$R \times 10^{40}$	−8.41	−8.6	−9.7
(6) λ_6 (nm)	185	187	185
$[\theta_6^0]$	59600	52600	67300
Δ_6 (nm)	9.5	10	10
$R_6 \times 10^{40}$	37.8	34.7	44.8
Total $R \times 10^{40}$	39.7	36.4	43.9

[a] Solvent, 0.15 M KF. [b] Dimensions, $[\theta^0]$ in deg cm² dmol⁻¹; R in erg cm³ rad.

our extrema differ from theirs probably because of different experimental conditions used (salts as well as temperature). Like the unfractionated tRNA, $tRNA^{Val}$ has a CD spectrum composed of five Gaussian bands and requires an additional band around 165 nm for a good ORD fit (Figure 3b) (item 10 in Table II appears to be the best estimate of the extra band).

TABLE II: An Extrapolated CD Band and the Resultant ORD Fit of tRNAs.

	Extrapolated Band[a] λ_t (nm)	$R_t \times 10^{40}$	Total[b] $R \times 10^{40}$	ORD[c] Fit
Unfractionated tRNA				
1			39.7	0.38
2	165	−17.6	22.1	0.08
3	165	−20.7	19.0	0.09
4	163	−17.6	22.1	0.10
$tRNA^{Phe}$				
5			36.4	0.09
6	165	−2.4	34.0	0.10
7	165	−4.4	32.0	0.12
8	165	−6.4	30.0	0.15
$tRNA^{Val}$				
9			43.9	0.17
10	165	−15.2	28.7	0.09
11	165	−22.9	21.0	0.13

[a] $R_t = 1.233 \times 10^{-42}[\theta_t^0]\Delta_t/\lambda_t$ in erg cm³ rad; Δ_t is preset at 10 nm. [b] Sum of total R in Table I and R_t of the extrapolated band. [c] Equation 4; see text for detail.

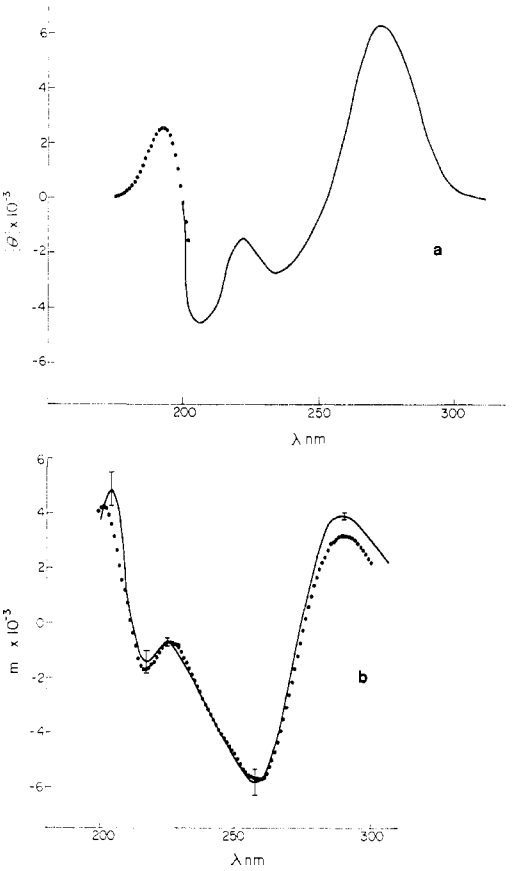

FIGURE 4: (a) The CD spectrum of unfractionated yeast tRNA at 88°. Solid line, experimental; points, extrapolated. (b) The ORD spectrum. Solid line, experimental; points, computed from (a).

Denatured tRNAs. The CD and ORD spectra of all three melted tRNAs are quite similar in shape; Figure 4 illustrates the CD and ORD of the unfractionated tRNA at 88°. Table III lists the resolved Gaussian bands of the CD spectra of the three tRNAs. Since the measurements were limited to about 200 nm, we were compelled to guess band 5 around 185–195 nm. Table IV lists the experimental and computed values of the ORD extrema of the tRNAs. The curve resolving of $tRNA^{Phe}$ still requires a small positive band around 220 nm, even though its rotational strength was small at all temperatures. Whether this band is an artifact of the resolving process is not known. In the case of the unfractionated tRNA and $tRNA^{Val}$, the region around 220 nm was approximated quite well by the two overlapping bands at 235 and 210 nm.

The spectral measurements at high temperatures were not as precise as those at 25° because of the low signal to noise ratios plus the hyperchromic effect of the nucleic acids under our experimental conditions. Accordingly, the ORD fits for the denatured tRNAs are not as good as those for native tRNAs. The best fit we obtained was 0.17 for the unfractionated tRNA and $tRNA^{Phe}$ and 0.30 for $tRNA^{Val}$, which are much higher than those for native tRNAs (*cf.* Table II). The most noticeable finding of the CD spectra of denatured tRNAs is the absence of an extra band at 165 nm. However, this band cannot be completely ruled out for the melted tRNAs because of the uncertainty of the position and magnitude of band 5 at 185–195 nm. If the maximum of band 5 is slightly blue-shifted and its ellipticities are increased, we

TABLE III: Resolved CD Spectra of Denatured tRNAs.

Band[a]	Unfractionated Yeast tRNA at 88°	Yeast $tRNA^{Phe}$ at 80°	*E. coli* $tRNA^{Val}$ at 80°
(1) λ_1 (nm)	275	273	274.5
$[\theta_1^0]$	6350	6800	7900
Δ_1 (nm)	14.5	16	14.5
$R_1 \times 10^{40}$	4.14	4.90	5.15
(2) λ_2 (nm)	236	240	235
$[\theta_2^0]$	−3000	−3300	−3900
Δ_2 (nm)	13	15	16
$R_2 \times 10^{40}$	−2.04	−2.50	−3.27
(3) λ_3 (nm)		219.5	
$[\theta_3^0]$		900	
Δ_3 (nm)		5	
$R_3 \times 10^{40}$		0.25	
(4) λ_4 (nm)	207	205	207
$[\theta_4^0]$	−4650	−4000	−5200
Δ_4 (nm)	9.5	12	9.5
$R_4 \times 10^{40}$	−2.63	−2.90	−2.94
(5) λ_5 (nm)	195	185	190
$[\theta_5^0]$	3230	510	4600
Δ_5 (nm)	10	10	10
$R_5 \times 10^{40}$	2.03	0.34	2.98
Total $R \times 10^{40}$	1.50	0.07	1.91

[a] $[\theta^0]$ in deg cm^2 dmol^{-1}; R in erg cm^3 rad.

would have introduced a negative band at lower wavelengths to compensate for the additional rotatory contributions of band 5. In the present calculations the CD spectra of all three tRNAs are sufficient enough to yield computed ORD spectra that are close to the experimental curves.

Discussion

According to the sum rule of optical activity, $\Sigma R_i = 0$ over the entire wavelength region (0 to ∞ nm) for an optically active compound. The CD spectrum of denatured tRNAs (Figure 4a) seems to approach this condition (see Table III). However, the total rotational strength of native tRNAs, even including the extrapolated band at 165 nm, is still far from zero. Clearly, there are other CD bands outside the wavelength range studied (140–310 nm), which, if included, will satisfy the sum rule. In our calculations we assume that there is only one Gaussian band below 181 nm which contributes to the ORD above 181 nm. The positive and negative rotatory contributions of all CD bands below 181 nm are embodied in a single CD band. If this assumption fails, the predicted CD band will have the wrong magnitude and to a lesser extent the wrong position. For the Kronig–Kramers transform generating the ORD, the R_i of each of the higher energy bands below 160 nm is weighted in the denominator by the frequency, ν_i, at which it occurs, since $[m_i]_\lambda$ is proportional to $R_i/(\nu_i^2 - \nu^2)$ or, in our term, $R_i\lambda_i^2/(\lambda^2 - \lambda_i^2)$ (Rosenfeld, 1928). These bands which are far away from the wavelengths of interest will have virtually no effect on the observed ORD. Furthermore, their dextro- and levorotatory contributions will tend to cancel to a great extent in the wavelengths studied. Thus, the sum total of these contributions to the ORD spectrum between 160 and 300 nm may approach the uncertainty level of experimental error and will not affect our computation of the ORD fit.

The technique we used has been applied to the optical activity of polypeptides. For instance, the CD of a helical polypeptide shows a double minimum at 222 and 210 nm and a maximum at 191 nm. Evaluation of the Kronig–Kramers transform from CD (above 170 nm) to ORD (between 180 and 600 nm) led to a complete agreement between the measured and computed ORD even though the summation of the observed R_i's does not vanish (Cassim and Yang, 1970). Recent CD measurements in the vacuum ultraviolet region reveals a positive shoulder near 175 nm, a negative band at 160 nm, and another positive maximum below 140 nm that is still experimentally inaccessible (Johnson and Tinoco, 1972). In the present work, additional CD bands may lie below 140 nm which cannot be measured even with the vacuum ultraviolet circular dichrometer.

The conformations of tRNAs have been shown to be quite similar by many physical methods. They have the same CD and ORD profiles (Figures 1–3); the variations in ellipticities and rotations among tRNAs are rather small (Table I). For denatured tRNAs, differences in the CD spectra should essentially reflect the sequence differences, which are indeed fairly small (Table III). Thus, the most important factor governing the CD spectra must be the conformation of the RNA molecules. The guessed band at 165 nm and also the newly observed band at 185–187 nm appear to be conformation dependent and show the most drastic changes upon heat denaturation. The 165-nm band seems to disappear completely at elevated temperatures for unfractionated tRNA and $tRNA^{Val}$, although the ORD fit for the CD and ORD spectra at high temperature was not good enough to rule out any possible residual ellipticities in the region of this extra band. Denaturation of the unfractionated tRNA and $tRNA^{Val}$ was accompanied by a red shift of the 185-nm band by 5–10 nm; the rotational strength of the denatured tRNAs was about $^1/_{20}$th that of the native ones. The reduction of the 187-nm band for $tRNA^{Phe}$ was even more drastic; its rotational strength at 80° was only about $^1/_{100}$th that at 25°. Thus, both bands must be associated with the base stacking of the tRNAs, probably in the double-stranded base-paired region.

The most puzzling finding of this work is the lack of the 165-nm band for yeast $tRNA^{Phe}$. We studied and obtained

TABLE IV: ORD of Denatured tRNAs.[a]

	Unfractionated tRNA		$tRNA^{Phe}$		$tRNA^{Val}$	
Extrema	E	C	E	C	E	C
λ_1 (nm)	290	289	290	289	290	290
$[m_1]$	3950	3190	3020	3450	6490	4230
λ_2 (nm)	257	260	258	258	259	259
$[m_2]$	−5860	−5700	−6150	−6550	−6070	−7430
λ_3 (nm)	225	226	224	226	225	226
$[m_3]$	−683	−720	−1430	−630	−788	−550
λ_4 (nm)	217	217	214	214	217	215
$[m_4]$	−1430	−1730	−2410	−2460	−21	−1520
λ_5 (nm)	204	201		195	196	196
$[m_5]$	4880	4280		2640	8400	6650
λ_6 (nm)		185				
$[m_6]$		−890				

[a] Dimension of mean residue rotation: deg cm^2 dmol^{-1}; E, experimental; C, computed from the CD spectra.

the same results of two batches from Boeringer Mannheim. Our CD spectrum is also close to that reported by Blum *et al.* (1972), who prepared their sample from yeast. Thus, the purity of our preparations should not be the cause for concern. The X-ray diffraction study by Kim *et al.* (1973) has revealed significant amount of secondary structure for $tRNA^{Phe}$. If the 165-nm band is also sequence dependent, it seems unlikely that large positive ellipticities in this region for $tRNA^{Phe}$ happen to cancel out the strong negative CD band due to conformation. The fact that all three melted tRNAs do not show this 165-nm band also speaks against any sequence-dependent ellipticities in this region. Thus, this "abnormality" for $tRNA^{Phe}$ is still an unsolved problem.

The 295-nm band has been speculated to be an $n-\pi^*$ transition (Sarkar *et al.*, 1967; Willick *et al.* 1973); it is unique for nucleic acids, especially RNAs, and is not found in polynucleotides. This band is small, melts out quickly, and is therefore considered to be sensitive to conformation. Willick *et al.* (1973) have found that the magnitude of this band increases in the presence of divalent cations, especially Mg^{2+}, and suggested the formation of a more compact tertiary structure under these conditions. The positive band at 265 nm and the negative one near 236 nm have been ascribed to the exciton splitting of the $\pi-\pi^*$ transitions resulting from base stacking (Johnson and Tinoco, 1969; Tinoco, 1968) and are therefore conformation dependent. Upon denaturation the 265-nm band shows a red shift, which has been attributed to the breaking up of the base pairs in RNAs (see, for example, Yang and Samejima (1969)). The CD band at 211 nm shows a blue shift at elevated temperatures; most likely this change also indicates the separation of base pairs and base unstacking.

To summarize, all the observed CD bands of tRNAs are related to their secondary structure. The guessed 165-nm band and newly observed band at 185 nm appear to be most sensitive to the changes in conformation of tRNAs.

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References

Adler, A. J., and Fasman, G. D. (1970), *Biochim. Biophys. Acta 204*, 183.

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

Cassim, J. Y., and Yang, J. T. (1969), *Biochemistry 8*, 1947.

Cassim, J. Y., and Yang, J. T. (1970), *Biopolymers 9*, 1475.

Chen, P. S., Jr., Toribara, T. Y., and Warner, H. (1956), *Anal. Chem. 28*, 1756.

Cramer, F. (1971), *Progr. Nucl. Acid Res. Mol. Biol. 11*, 391.

Johnson, W. C., Jr., and Tinoco, I., Jr. (1969), *Biopolymers 7*, 727.

Johnson, W. C., Jr., and Tinoco, I., Jr. (1972), *J. Amer. Chem. Soc. 94*, 4389.

Kim, S. H., Quigley, G. J., Siddath, F. L., McPherson, A., Smeden, D., Kim, J. J., Weinzeirl, J., and Rich, A. (1973), *Science 179*, 285.

Moffitt, W., and Moscowitz, A. (1959), *J. Chem. Phys. 30*, 648.

Moscowitz, A. (1960), *in* Optical Rotatory Dispersion, C. Djerassi, Ed., New York, N. Y., McGraw-Hill, Chapter 12.

Rosenfeld, V. L. (1928), *Z. Phys. 52*, 161.

Sarkar, P. K., Wells, B., and Yang, J. T. (1967), *J. Mol. Biol. 25*, 563.

Thiéry, J. (1969), Ph.D. Thesis, University of California, Berkeley.

Tinoco, I., Jr. (1968), *J. Chim. Phys. 65*, 91.

Wells, B. D., and Yang, J. T. (1974), *Biochemistry 13*, 1317.

Willick, G. E., and Kay, C. M. (1971), *Biochemistry 10*, 2216.

Willick, G., Oikawa, K., and Kay, C. M. (1973), *Biochemistry 12*, 899.

Yang, J. T., and Samejima, T. (1969), *Progr. Nucl. Acid Res. Mol. Biol. 9*, 223.