(1965b), *Biochemistry* 4, 876.
Goldstein, I. J., and Iyer, R. N. (1966), *Biochim. Biophys. Acta 121*, 197.
Goldstein, I. J., Iyer, R. N., Smith, E. E., and So, L. L. (1967), *Biochemistry 6*, 2373.
Grabar, P., and Burtin, P. (1960), Analyse Immunoelectrophoretique, Paris, Masson et Cie.
Kabat, E. A., and Mayer, M. (1961), Experimental Immunochemistry, 2nd ed, Springfield, Ill., C. C Thomas, pp 72, 85.
Kabat, E. A., and Schiffman, G. (1962), *J. Immunol. 88*, 782.
Karush, F. (1956), *J. Am. Chem. Soc. 78*, 5519.
Karush, F. (1957), *J. Am. Chem. Soc. 79*, 3380.
Landsteiner, K. (1945), The Specificity of Serological Reactions, Cambridge, Harvard University.
Leskowitz, S., and Waksman, B. H. (1960), *J. Immunol. 84*, 58.
Luderitz, O., Westphal, O., Staub, A. M., and Le Minor, L. (1960), *Nature 188*, 556.
Mage, M., Bassett, E. W., Tanenbaum, S. W., and Beiser, S. M. (1963), *J. Immunol. 90*, 318.
Marrack, J. R., and Orlans, E. S. (1958), *Progr. Stereochem. 2*, 228.
Rude, E., Westphal, O., Hurwitz, E., Fuchs, S., and Sela, M. (1966), *Immunochemistry 3*, 137.
Sandford, P. A., and Conrad, H. E. (1966), *Biochemistry 5*, 1508.
Tanenbaum, S. W., Burke, G. C., and Beiser, S. M. (1961), *Biochim. Biophys. Acta 54*, 439.
Westphal, O., and Feier, H. (1956), *Chem. Ber. 89*, 582.
Yarif, J., Rapport, M. M., and Graf, L. (1962), *Biochem. J. 85*, 383.

Optical Rotatory Dispersion and RNA Base Pairing in Ribosomes and in Tobacco Mosaic Virus*

C. Allen Bush† and Harold A. Scheraga‡

ABSTRACT: A method for distinguishing base-paired double-stranded ribonucleic acid (RNA) from the single-stranded stacked conformation is discussed. The technique is based on a comparison of the optical rotatory dispersion, in the absorbing region, of RNA in salt-free solutions, where it is single stranded, with that in solutions of moderate or high salt concentration, where it is base paired. The optical rotatory dispersion of the single-stranded stacked conformation is calculated from that of dinucleoside phosphates while the influence of base pairing is estimated from the curves for double-stranded polyadenylic acid (poly A) plus polyuridylic acid (poly U) and polyguanylic acid (poly G) plus polycytidylic acid (poly C). The method is used to demonstrate that the RNA in *Escherichia coli* ribosomes is in a largely base-paired conformation with mainly G–C base pairs. This conformation is found in both the 30S and 50S subunits as well as in the 70S particle, and base pairing is not changed between 10^{-2} and 10^{-4} M Mg^{2+} ion concentration. The results are applicable to ribosomes from other sources, and are at variance with recent proposals that the RNA of ribosomes is in the single-stranded stacked conformation. The technique is also applied to existing optical rotatory dispersion data on tobacco mosaic virus (TMV) where base pairing is impossible in the known virus structure. We conclude that the RNA in TMV is in a rigid single-stranded stacked conformation of the same geometry as dinucleoside phosphates and single-stranded stacked RNA in salt-free solution.

For an ultimate understanding of the mechanism by which ribosomes participate in protein synthesis, it is of interest to know, among other things, the conformation of the RNA in the ribosome. While studies of this question have been reported, they are not all in agreement. From the results of X-ray scattering from wet gels of ribosomes (Klug *et al.*, 1961) and of hypochromicity (Schlessinger, 1960), the RNA of ribosomes has been thought to be in a largely double-stranded base-paired conformation, much the same as RNA is found in solution under appropriate conditions. The base pairing in solution arises from the folding back of the single chain into hairpin loops (Cox, 1966). Also, optical rotatory dispersion studies show a similarity between RNA and ribosomes (Sarkar *et al.*, 1967; McPhie and Gratzer, 1966). However, recent experiments on the binding of acridine orange dyes to ribo-

* From the Department of Chemistry, Cornell University, Ithaca, New York 14850. *Received June 12, 1967.* This work was supported by a research grant (GB-4766) from the National Science Foundation, and by a research grant (GM-14312) from the National Institute of General Medical Sciences of the National Institutes of Health, U. S. Public Health Service.

† Postdoctoral Fellow of the National Institute of General Medical Sciences, National Institutes of Health, 1966–1968.

‡ To whom requests for reprints should be addressed.

somes and to RNA have led Furano *et al.* (1966) to propose that RNA in ribosomes is in a single-stranded stacked conformation with little base pairing. They correctly point out that the single-stranded stacked conformation is hypochromic; thus, their proposal is not inconsistent with hypochromicity data. Since the X-ray and dye binding studies give inconsistent results, they proposed that their nonbase-paired model be tested by other experiments.

In this paper, we report optical rotatory dispersion experiments on ribosomes which strongly support the base-paired double-helical model over the single-stranded stacked one. The method of analysis of the data is also applied to intact TMV whose structure is known from X-ray scattering to be such that the RNA cannot be in the base-paired double-helical conformation. The technique, which can be applied to other RNA–protein complexes, relies on the extensive studies of the optical rotatory dispersion of RNA models carried out by Tinoco and his co-workers (Cantor and Tinoco, 1965; Warshaw and Tinoco, 1966; Cantor *et al.*, 1966). It is based on the fact that the optical rotatory dispersion of single-stranded RNA is determined primarily by nearest neighbor interactions between bases; if base pairing occurs, in addition to stacking, the optical rotatory dispersion pattern acquires some of the features of double-stranded poly A plus poly U and poly G plus poly C, respectively. This method has been applied[1] to the RNA extracted from TMV, where the conformation was controlled by variation of the salt concentration (Cantor *et al.*, 1966). At low salt concentration, where the unshielded charges of the phosphate groups cause the strands to repel each other, the RNA is in the single-stranded stacked conformation. The phosphate repulsion does not cause the bases to unstack, as shown by the fact that the optical rotatory dispersion of di- and trinucleotides is the same in the presence and absence of salt (Cantor *et al.*, 1966). At higher salt concentration (0.01 M), the charges become shielded and hydrogen-bonded base pairing occurs. The optical rotatory dispersion pattern of TMV–RNA was found to change, upon addition of salt, as the conformation changed from a single-stranded to a double-stranded one. The changes observed were shifts in the wavelengths of the peaks and troughs, but not a large change in the magnitude of the Cotton effects. We propose to utilize this difference in optical rotatory dispersion to distinguish the single-stranded conformation from the base-paired double-helical one in rRNA and in ribosomes, respectively, from *Escherichia coli*.

Experimental Section

Ribosomes and Ribosomal Subunits. E. coli B was purchased frozen from Grain Products Corp. of Muscatine, Iowa. The cells were ground with alumina, and ribosomes were prepared by differential centrifugation in 0.01 M Tris–0.01 M magnesium acetate (pH 7.4) buffer by the method of Tissieres *et al.* (1959). The crude ribosome pellet was suspended with a Teflon tissue homogenizer, and the solution was clarified by centrifugation for 30 min at 15,000 rpm (Spinco SW-50 rotor). The ribosomes were washed by layering this suspension on a 10% sucrose solution and spinning at 50,000 rpm in the SW-50 rotor for 3 hr to form a pellet. The pellets were stored frozen at −30°. Suspensions of 70S ribosomes were made by taking up the pellet in 0.01 M Tris–0.01 M magnesium acetate buffer at pH 7.4.

To effect a separation into the 50S and 30S subunits, the washed pellets were suspended in 10^{-4} M magnesium acetate–0.01 M Tris at pH 7.4 and fractionated in 5–20% exponential gradients of sucrose in the SW-50 swinging-bucket rotor. For this purpose, the Model L2 Spinco ultracentrifuge was operated at 50,000 rpm for 90 min. The fractions containing the 50S and 30S particles were dialyzed for 1 hr against 10^{-4} M magnesium acetate–0.01 M Tris to reduce the sucrose concentration, and were then layered on new sucrose gradients for a second fractionation. With two fractionations, it was possible to obtain 30S particles free from any contamination by the heavier subunits. Pure 50S particles could be obtained from a single fractionation. The plastic tubes were punctured in a Büchler tube piercer, and fractions were collected. The ultraviolet absorption was monitored in a flow cell of 0.1-ml volume with a Canalco ultraviolet flow analyzer. With this apparatus, the 50S and 30S particles could be easily distinguished as separated peaks on the diagram from the flow analyzer. The sucrose was removed by dialysis for 20 hr against 10^{-4} M magnesium acetate–0.01 M Tris (pH 7.4) buffer at 4°. Sedimentation rates were measured in the Spinco Model E analytical ultracentrifuge, using the ultraviolet absorption optics, both before and after the measurements of the optical rotatory dispersion to confirm the presence of 70S, 50S, and 30S particles, respectively. Sedimentation runs on the subunits were carried out in 0.01 M Tris–10^{-4} M magnesium acetate and those of the 70S ribosome in 0.01 M Tris–0.01 M magnesium acetate, both at pH 7.4.

rRNA was prepared according to the method of Stanley and Bock (1965) using sodium dodecyl sulfate–phenol and Macaloid[2] adsorbant. The RNA was sufficiently free of nuclease activity so that sharp boundaries of 16S and 23S RNA could be identified in the Spinco Model E analytical ultracentrifuge even after measurement of the optical rotatory dispersion of the sample.

In order to carry out measurements at low salt concentration, the RNA was dialyzed against 10^{-4} M EDTA (pH 6.8) as suggetsed by Cantor *et al.* (1966). By removing all salt, and especially divalent cations, the polyelectrolyte stretches out and has little hydrogen-

[1] The optical rotatory dispersion method has also been applied recently to a study of the conformation of alanine and tyrosine tRNAs (Vournakis and Scheraga, 1966).

[2] A gift of Baroid Division, National Lead Co., Houston, Texas 77001.

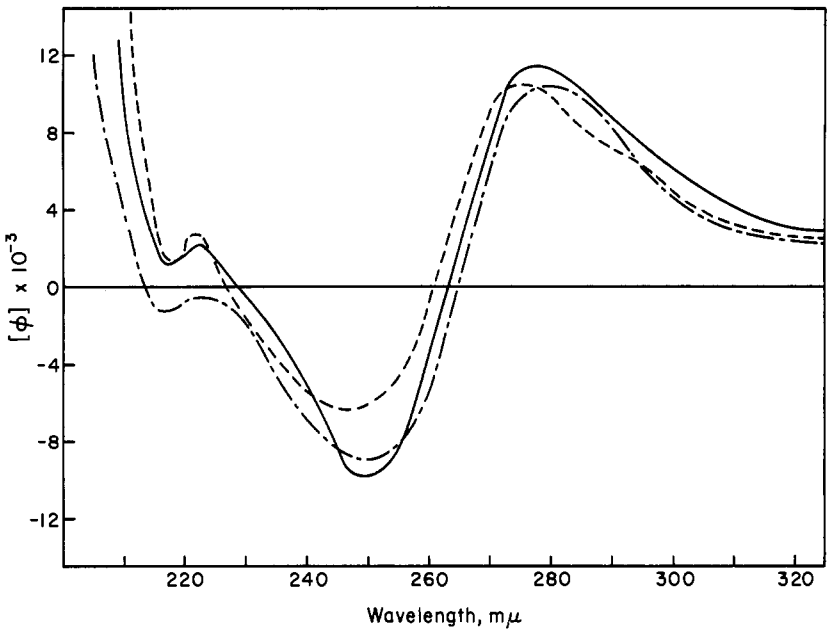

FIGURE 1: Molar rotation per residue of RNA of 30S and 50S ribosomes in 10^{-4} M magnesium acetate–0.01 M Tris and of 70S ribosomes in 0.01 M magnesium acetate–0.01 M Tris (all at pH 7.4); temperature, 8°. (– – – –) 30S, (— — — —) 50S, and (———) 70S.

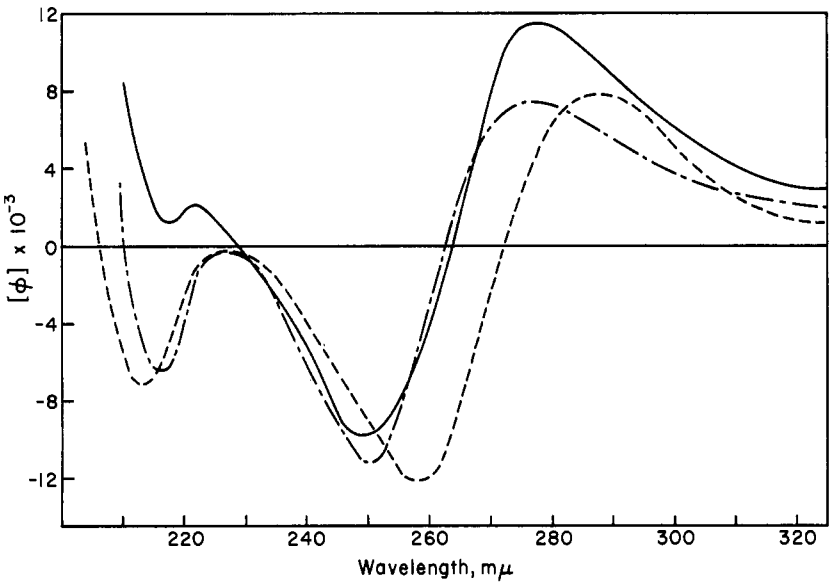

FIGURE 2: Molar rotation per residue of RNA of 70S *E. coli* ribosomes in 0.01 M magnesium acetate–0.01 M Tris (pH 7.4) (temperature, 8°), of *E. coli* rRNA in 10^{-4} M EDTA (pH 6.8) (temperature, 24°), and of *E. coli* rRNA in 0.1 M KCl–0.01 M Tris (pH 7.4) (temperature, 6°). (———) Ribosome, (– – – –) RNA in H_2O, and (— — — —) RNA in 0.1 M KCl.

bonded base pairing. Local order persists as a result of base stacking which is not disrupted by the lack of shielding of the phosphates. Measurements were also made on RNA which was prepared in 0.1 M KCl where base pairing is present. Identical optical rotatory dispersion was obtained when the desalted RNA solution was made 0.1 M in KCl by the addition of salt.

Determination of Concentration. Concentrations were measured by ultraviolet spectrophotometry on the Zeiss QII and the Cary 14 spectrophotometers. The optical density at 260 mμ of a 1-mg/ml solution in 0.1 M KCl

(pH 7.4)–Tris buffer in a 1-cm cell was taken to be 22.3 for RNA (Stanley and Bock, 1965) and that of ribosomes to be 15.1 in 0.01 M Tris–0.01 M magnesium acetate buffer (pH 7.4) (Kurland, 1966).

Optical Rotation Measurements. The optical rotatory dispersion was measured in the Cary 60 spectropolarimeter with a Xenon arc lamp (Osram type XBO-450 W/P) light source. Measurements on ribosomes and the RNA in salt solution were made in a water-jacketed cell at 7°. For measurements in salt-free solution, the water jacket was held at 25°.[3] The cell path length was 1 cm and the optical density at 260 mμ of the samples was in the range of 0.8–1.0.

Results

The rotation is given in units of molar rotation per residue of RNA in both the RNA and in the ribosome. This quantity is given by $[\phi] = \alpha M_{res}/10c$, where α is the rotation in degrees per decimeter, M_{res} is the average molecular weight of a residue, and c is the concentration in grams per milliliter. The data are reported in these units in order to focus attention on the RNA in the ribosome. The protein, which makes up 35% of the ribosome weight, gives rise to a Cotton effect in the region of 225 mμ, but the effect is not large, apparently because of the low per cent of α-helix in the ribosomal protein. Several recent studies have described the optical rotatory dispersion of ribosomal proteins (McPhie and Gratzer, 1966; Sarkar *et al.*, 1967). In this work, we will ignore the rotation introduced by the protein. From a comparison of the ribosome optical rotatory dispersion to that of the free RNA, one can see that, in the region of wavelengths longer than 240 mμ, the Cotton effects can be ascribed to RNA alone, allowing us to compare the ribosome Cotton effects with those in free RNA, ignoring the protein.

In Figure 1 we see the similarity in the Cotton effects of the 30S and 50S subunits of the ribosome. The differences in the curves are not great and can probably be ascribed to slight differences in average base composition. Sarkar and Yang (1967) have recently reported optical rotatory dispersion data on the separated subunits of *E. coli* ribosomes which are in general agreement with our data.

We cannot make a detailed analysis of the slight differences in the subunits for reasons which will be mentioned in the discussion below. However, we are able to conclude that there are no major structural differences between the two subunits. Furthermore, the optical rotatory dispersion per residue of RNA of the 70S ribosome is observed to be the average of the two subunits. Other workers have reported that there is no change in the optical rotatory dispersion as a result of dissociating the 70S ribosome into its subunits at low Mg^{2+} ion concentration (Sarkar *et al.*, 1967).

[3] The measurements on RNA in *salt-free* solution were carried out at 25° since they will be compared below to the optical rotatory dispersion of dinucleoside phosphates which were measured at 25° (Warshaw and Tinoco, 1966).

In Figure 2 we compare the data for the 70S ribosome with those for the RNA in 0.1 M KCl solution. The similarity of their optical rotatory dispersion behavior has been pointed out (Sarkar *et al.*, 1967). We also include the optical rotatory dispersion data of the RNA in water where we believe it to be in the single-stranded stacked conformation. By simply adding KCl to a concentration of 0.1 M to this solution, one can regain the optical rotatory dispersion curve characteristic of the base-paired RNA. The low-salt curve is shifted by about 8 mμ to longer wavelength from that of the base-paired RNA in salt solution. We feel that this shift is significant and that it indicates that the RNA in the ribosome is in the base-paired double-helical conformation, and not in the single-stranded stacked conformation as proposed by Furano *et al.* (1966).

Discussion

Calculation of the Optical Rotatory Dispersion. In order to support the contention that the conformation of RNA in water is the single-stranded stacked form, we have calculated the optical rotatory dispersion from the optical rotatory dispersion curves of dinucleoside phosphates, according to the method of Cantor *et al.* (1966). In this method, it is assumed that the optical rotatory dispersion of a single-stranded polymer depends primarily on the nearest neighbor base-stacking interactions, which are obtainable from the optical rotatory dispersion data on dinucleoside phosphates. The validity of this method was demonstrated by Cantor and Tinoco (1965) who studied the optical rotatory dispersion of dinucleoside phosphates and trinucleoside diphosphates and concluded that the optical rotatory dispersion of a trinucleoside diphosphate could be predicted from that of its component dinucleoside phosphates. This property results from the fact that it is largely the interaction between neighboring aromatic bases that gives rise to the large Cotton effects observed (Bush and Tinoco, 1967). The formula successfully used to predict the molar rotation $[\phi]_{ijk}$ of a trinucleoside, I-p-J-p-K, from that of the component dinucleosides I-p-J and J-p-K is

$$[\phi]_{ijk} = [\phi]_{ij} + [\phi]_{jk} - [\phi]_j \qquad (1)$$

This formula is based on the assumption that only nearest neighbor interactions contribute to the trimer rotation. The optical rotatory dispersion ($[\phi]_j$) of the monomers is small but must also be taken into account.

In order to extend this method of calculation to a single-stranded polymer, it is necessary to know the sequence. Since this information is not available, we assume that the bases are distributed randomly along the RNA. Hence

$$[\phi] = 2\sum_{i=1}^{4}\sum_{j=1}^{4} x_i x_j [\phi]_{ij} - \sum_{i=1}^{4} x_i [\phi]_i \qquad (2)$$

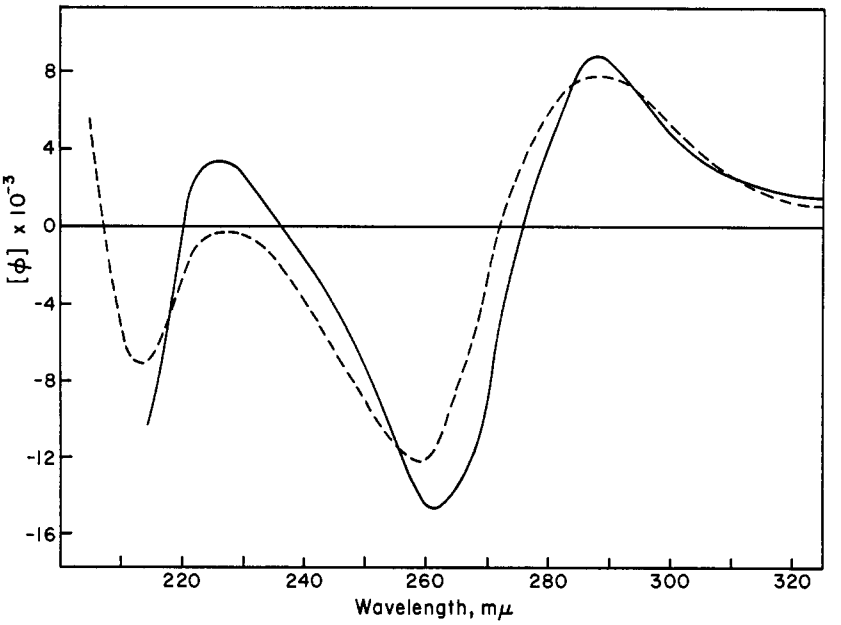

FIGURE 3: Molar rotation per residue of RNA of *E. coli* rRNA, calculated by eq 2, and measured in 10^{-4} M EDTA (pH 6.8) at 24° (same curve as in Figure 2). (———) Calculated and (– – – –) RNA in H_2O.

where x_i is the fraction of nucleoside I in the RNA and $[\phi]_{ij}$ is the molar rotation per residue of the dinucleoside phosphate I-p-J. $[\phi]_i$ is the molar rotation of the nucleoside I. The assumption of randomness of the sequence in *E. coli* rRNA is supported by the data of Bautz and Hedding (1964) on the mono-, di-, and trinucleotides present in the pancreatic ribonuclease hydrolysate. The oligonucleotides found agree well with the assumption of randomness. The base composition used is A:U:C:G = 0.246:0.205:0.225:0.324 (Bautz and Hedding, 1964), and the optical rotatory dispersion data on the mono- and dinucleosides are from Warshaw and Tinoco (1966).

In a previous application of this method, Cantor *et al.* (1966) found that the optical rotatory dispersion of TMV–RNA at low salt concentration, where the conformation is single stranded, could be predicted accurately from the nearest neighbor frequencies and the optical rotatory dispersion of the component dinucleoside phosphates. They also observed that, in 0.1 M salt solutions, where base pairing occurs, the optical rotatory dispersion of TMV–RNA changed. The change which they observed could be accounted for by including the effects of A–U and G–C base pairs as computed from the optical rotatory dispersion of double-strand poly A plus poly U and poly G plus poly C (Sarkar and Yang, 1965a,b).

In Figure 3 we compare the calculated curve with that measured for rRNA in water. The agreement is good, giving maxima, minima, and crossings somewhat like those found for nonbase-paired TMV–RNA (Cantor *et al.*, 1966). The agreement at the short-wavelength side of the Cotton effect is not so good as throughout the rest of the curve, much as found by Cantor *et al.* (1966) for TMV–RNA. This discrepancy might be due to the greater experimental uncertainty of the rotation in this wavelength region or possibly to the importance of interactions other than those of the nearest neighbors among the short-wavelength electronic transitions.

Structure of RNA in Ribosomes. In summary, it appears that the differences between the 30S and the 50S ribosomal subunits are sufficiently small to be accounted for by small differences in base composition and base pairing. There are small observed differences in base composition of the RNAs in the two subunits (Stanley and Bock, 1965). In addition there may be some differences in the amount and kind of base pairing which could account for the small differences which we observe in the optical rotatory dispersion. Since the differences in base composition and pairing are small and uncertain, we do not feel it is meaningful to attempt to interpret the small differences in optical rotatory dispersion between the 30S and 50S ribosomes at this time.

TABLE I: Summary of Optical Rotatory Dispersion Data (Maxima, Crossings, and Minima, in mμ).

	λ_{max}	$\lambda_{crossing}$	λ_{min}
Ribosome	278	264	250
RNA in KCl	278	263	251
RNA in H_2O	288	272	258
RNA calculated	288	276	262

TABLE II: Optical Rotatory Dispersion for TMV–RNA under Various Conditions.

	λ_{max} (mμ)	([ϕ] × 10^{-4})$_{max}$ (deg)	$\lambda_{crossing}$ (mμ)	λ_{min} (mμ)	([ϕ] × 10^{-4})$_{min}$ (deg)
Simmons and Blout (1960) (RNA in TMV)	288	3.93	276	264	−3.82
Cantor *et al.* (1966) (RNA, no salt, pH 6.9)	285.5	1.12	271.5	258	−1.84
Cantor *et al.* (1966) (RNA calculated)	288.5	0.88	276	261.5	−1.66
Cantor *et al.* (1966) (RNA, in salt)	282	1.38	264	252.5	−1.34

It is probable that both A–U and G–C base pairs are present but that G–C pairs predominate. The optical rotatory dispersion curves for ribosomes and rRNA in salt are similar to those for yeast alanine and tyrosine tRNAs in which it is proposed that G–C base pairing dominates (Vournakis and Scheraga, 1966). The first maximum for the rRNA curve occurs at 278 mμ. This is near the λ_{max} of double-stranded poly G plus poly C at 276 mμ (Sarkar and Yang, 1965b). Doubly stranded poly A plus poly U has its λ_{max} at 286 mμ (Sarkar and Yang, 1965a). Therefore, if A–U base pairs are present in amounts approximately equal to G–C pairs, as in salt solutions of the RNA isolated from TMV, the λ_{max} is shifted to 282 mμ (Cantor *et al.*, 1966). A similar difference between the optical rotatory dispersion curves of yeast alanine and tyrosine tRNA was interpreted as evidence for more A–U base pairs in the tyrosine tRNA than in the alanine tRNA (Vournakis and Scheraga, 1966).

We conclude that the optical rotatory dispersion curves in the range of 240–300 mμ can be grouped into two classes as shown in Table I. The ribosomes and RNA in salt show base pairing while the curves for RNA measured in water and the calculated curve are shifted to longer wavelength and indicate a lack of base pairing. The presence of base pairs in RNA in salt solutions is well documented (Cox, 1966; Golub and Nazarenko, 1967).

Tobacco Mosaic Virus. The approach which we have used can also be applied to other RNA-containing biological structures such as tobacco mosaic virus (TMV). In the case of this virus, X-ray scattering has shown that the RNA is constrained by the coat protein into a helix of 23-A pitch so that base pairing is impossible (Klug and Caspar, 1960). Therefore, we would expect the optical rotatory dispersion of the RNA in the virus to agree with that measured by Cantor *et al.* (1966) for TMV–RNA in salt-free solution.

TMV has only 5% RNA content, so that the protein dominates its optical rotatory dispersion and must be subtracted away. This requires that we use a different approach than in the case of ribosomes, where we ignored the contribution of the protein to the optical rotatory dispersion. For the case of TMV, we subtract the rotation of a protein blank from that of the intact virus to obtain the optical rotatory dispersion of the RNA as it is found in the virus. Under appropriate conditions, the TMV protein alone is known to aggregate into rod forms with a structure like that of the native virus. Simmons and Blout (1960) have measured the optical rotatory dispersion of the native virus and of its RNA-free protein rods, and subtracted them to give the optical rotatory dispersion of the RNA as it is found in the virus. To convert specific rotation, which they report, to molar rotation per residue, we use the average residue molecular weight for the salt-free RNA, which is 322.

In Table II we compare the maxima, minima, and crossings of that curve with those found by Cantor *et al.* (1966) for TMV–RNA with salt, without salt, and that calculated by eq 2. It is seen that the wavelengths of the maximum, minimum, and crossing of the RNA in TMV agree very well with the calculated wavelengths, fairly well with the salt-free values, but not well with the values found in salt solution, where base pairing is present. There is a great disparity in magnitude of the molar rotation which we will discuss below. We feel that this agreement is sufficient to indicate the generality of this method of distinguishing single-stranded stacked from doubly helical base-paired RNA in complexes with protein. We obtain reasonable results in structures that differ greatly in base pairing and in RNA content.

In Table II, we notice that the magnitudes of the rotation peaks in the RNA in TMV are much greater than those calculated or those measured for salt-free RNA in solution. This is due to the increased rigidity of stacking of the residues under the constraint of the virus structure. The calculated curves are taken from dinucleoside rotations at room temperature where only a portion of the bases are stacked (Warshaw and Tinoco, 1966). The large rotation of the RNA as it is found in TMV indicates that it is in the *fully stacked* or *low-temperature* form. Rotations of this order of magnitude are calculated from molecular theories which assume a rigidly stacked model (Bush and Tinoco, 1967). We conclude that the geometry of the base stacking of the RNA in TMV is the same as that of dinucleosides in solution and of single-stranded stacked RNA in salt-free solution.

Conclusion

We have described a method, based on the results of Cantor *et al.* (1966), for distinguishing single-stranded stacked from base-paired RNA, which can be applied

to RNA in protein complexes. The method gives results consistent with the known structure of TMV, where base pairing is not possible. In the case of *E. coli* ribosomes, we conclude that the RNA is base paired in both subunits of the ribosome as well as in the whole ribosome, and that the base pairs are mostly of the G–C type.

In addition, these results appear to hold for other ribosomes than those of *E. coli*. Optical rotatory dispersion curves similar to those reported here have been found for yeast ribosomes (McPhie and Gratzer, 1966) and also for reticulocyte ribosomes (Blake and Peacocke, 1965). Thus, our statements about base pairing in *E. coli* ribosomes are apparently correct for ribosomes from other organisms.

For native TMV, on the other hand, we conclude that the RNA is in a single-stranded stacked conformation having the same geometry as that of dinucleoside phosphates. The stacking is, however, very rigid giving much higher values of rotation than that of dinucleoside phosphates at room temperature.

Acknowledgment

We wish to thank Mr. John Vournakis for helpful discussions and Dr. Elizabeth Keller for advice on preparation and fractionation of *E. coli* ribosomes.

References

Bautz, E. K. F., and Hedding, L. (1964), *Biochemistry 3*, 1010.

Blake, A., and Peacocke, A. R. (1965), *Nature 208*, 1319.

Bush, C. A., and Tinoco, I., Jr. (1967), *J. Mol. Biol. 23*, 601.

Cantor, C. R., Jaskunis, S. R., and Tinoco, I., Jr. (1966), *J. Mol. Biol. 20*, 39.

Cantor, C. R., and Tinoco, I., Jr. (1965), *J. Mol. Biol. 13*, 65.

Cox, R. A. (1966), *Biochem. J. 98*, 841.

Furano, A. V., Bradley, D. F., and Childers, L. G. (1966), *Biochemistry 5*, 3044.

Golub, E. I., and Nazarenko, V. G. (1967), *Biophys. J. 7*, 13.

Klug, A., and Caspar, D. L. D. (1960), *Advan. Virus Res. 7*, 225.

Klug, A., Holmes, K. C., and Finch, J. T. (1961), *J. Mol. Biol. 3*, 87.

Kurland, C. G. (1966), *J. Mol. Biol. 18*, 90.

McPhie, P., and Gratzer, W. B. (1966), *Biochemistry 5*, 1310.

Sarkar, P. K., and Yang, J. T. (1965a), *J. Biol. Chem. 240*, 2088.

Sarkar, P. K., and Yang, J. T. (1965b), *Biochemistry 4*, 1238.

Sarkar, P. K., and Yang, J. T. (1967), Conformation of Biopolymers, Ramachandran, G. N., Ed., London, Academic, p 197.

Sarkar, P. K., Yang, J. T., and Doty, P. (1967), *Biopolymers 5*, 1.

Schlessinger, D. (1960), *J. Mol. Biol. 2*, 92.

Simmons, N. S., and Blout, E. R. (1960), *Biophys. J. 1*, 55.

Stanley, W. M., Jr., and Bock, R. M. (1965), *Biochemistry 4*, 1302.

Tissières, A., Watson, J. D.,Schl essinger, D., and Hollingworth, B. R. (1959), *J. Mol. Biol. 1*, 221.

Vournakis, J. N., and Scheraga, H. A. (1966), *Biochemistry 5*, 2997.

Warshaw, M. M., and Tinoco, I., Jr. (1966), *J. Mol. Biol. 19*, 29.