Double-Helical Character of Ribonucleic Acid from Virus-Like Particles found in *Penicillium chrysogenum*

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1. RNA was isolated from virus-like particles found in *Penicillium chrysogenum* and resolved into two fractions by gel filtration through agarose columns. 2. Fraction 1 was excluded and had the following properties: 50.9% G+C [AMP 0.246, UMP 0.246, CMP 0.252, GMP 0.255 (mole fraction)]; mol.wt. about 1.2×10^6 daltons; $s_{20,w}$ 12.3 S and 'melting' temperature about 100°C (solvent 0.15 M-sodium chloride–0.015 M-sodium citrate pH 7.2); optical rotation $[\alpha]_{\max}$ 6000° at 278 nm; circular dichroism $(\epsilon_L - \epsilon_R)_{\max} = 8.18 \text{ l mol}^{-1} \text{ cm}^{-1}$ at 260 nm. 3. Properties of fraction 2 include 37.8% G+C [AMP 0.313, UMP 0.312, CMP 0.186, GMP 0.189 (mole fraction)]; mol.wt. about 140 000 daltons; $s_{20,w}$ 7.3 S, T_m about 85°C (solvent 0.15 M-sodium chloride–0.015 M-sodium citrate, pH 7.2); optical rotation $[\alpha]_{\max}$ 6000° at 278 nm; circular dichroism $(\epsilon_L - \epsilon_R)_{\max} = 8.24 \text{ l mol}^{-1} \text{ cm}^{-1}$ at 260 nm. 4. The properties of both fractions were consistent with a double-helical conformation.

Double-helical RNA species are of interest because some compounds of this type have been shown to inhibit the growth of certain viruses and tumours. Since the first report that heterologous nucleic acids appeared to have antiviral activity (Rotem, Cox & Isaacs, 1963) both natural and synthetic double-helical RNA species have been shown to induce the synthesis or release of interferon. The synthetic complex poly rI·poly rC is an active interferon inducer (Field, Tytell, Lampson & Hilleman, 1967) and so are double-helical RNA species isolated from virus-like particles present in certain moulds (see for example Lampson, Tytell, Field, Nemes & Hilleman, 1967; Kleinschmidt, Ellis, Van Frank & Murphy, 1968; Banks, Buck, Chain, Darbyshire & Himmelweit, 1969b). Double-helical RNA with antiviral activity was also isolated from *Penicillium chrysogenum* (Sutherland & Bessell, 1969) and virus-like particles have been demonstrated in this mould (Banks, Buck, Chain, Darbyshire & Himmelweit, 1969a). The aim of this work is to describe some properties of this material. Optical properties were studied in detail because of the value of double-helical RNA as reference compounds in studies of the conformation of single-stranded RNA species.

EXPERIMENTAL

Materials. Guanidinium chloride was prepared by neutralizing AnalaR guanidinium carbonate with hydrochloric acid. Chemicals utilized in measurements of

optical properties of RNA were of AnalaR quality. Sodium dodecyl sulphate was obtained from Marchon Products Ltd., Whitehaven, Cumberland, U.K., under the trade name Empicol LZ/BP. All chemicals used subsequent to phenol treatment were of AnalaR quality. Column chromatography was carried out on the 200–400 mesh grades of Bio-Gel, A-1.5 m, Bio-Gel A-15 m, and Bio-Gel A-50 M, 6, 4 and 2% sphere-beaded agarose respectively, obtained from Bio-Rad Laboratories, Richmond, Calif., U.S.A. Pancreatic ribonuclease was obtained from the Boehringer Corp. (London) Ltd., London W.5, U.K.

Growth of *P. chrysogenum* and the isolation of RNA. The nucleic acids whose properties are described were extracted from mycelium produced in the course of commercial penicillin production by large-scale fermentation of *P. chrysogenum*. Nucleic acids that possessed the same properties were produced when spores of *P. chrysogenum* A.T.C.C. 10002 were used to inoculate medium [sucrose 3%, NaNO₃ 0.2%, KH₂PO₄ 0.1%, KCl 0.05%, MgSO₄ 0.05%, yeast extract (Difco) 0.5%, FeSO₄ 0.001%; pH adjusted to 6.8] and the mycelium was harvested after 3–5 days' shaking at 26°C. The mycelium was separated by filtration, washed with water or 0.1 M-NaCl–0.01 M-sodium citrate, pH 7, and stored frozen.

Frozen mycelium (1 kg) was suspended in a solution containing 4% (w/v) NaCl and 1% (w/v) sodium dodecyl sulphate (101) and stirred for 20 h at approx. 25°C. The suspension was filtered and an equal volume of industrial methylated spirits added to the filtrate. After being left overnight at 5°C the precipitate was separated, redissolved in 0.3 M-sodium acetate (2 litres) and reprecipitated with aq. 95% ethanol (2 litres). The reprecipitated material was spun down (2000g, 15 min), redissolved in 0.3 M-sodium acetate (1 litre) and treated with an equal volume of aq. 90% phenol containing 0.1% (w/v) of

8-hydroxyquinoline. After thorough mixing (15 min, 25°C) the mixture was centrifuged (2500g, 60 min, 5°C), the aqueous phase separated, and the nucleic acids were freed from phenol by repeated precipitation from 0.3M-sodium acetate with an equal volume of ethanol. The precipitate was dissolved in a small volume of water and residual ethanol removed under vacuum. The concentration of nucleic acid was adjusted to about 1% (i.e. $E_{260} = 200$) and the solution treated with 2.5M-potassium phosphate buffer, pH 7, and methyl Cellosolve as described by Kirby (1956) to remove polysaccharide. The upper phase, containing nucleic acid, was dialysed.

The dialysed solution was concentrated until $\epsilon_{260} =$ approx. 400; an equal volume of 4M-NaCl was then added and the solution kept at 5°C for at least 18 h. The precipitated material was removed by centrifuging (23000g, 30 min, 5°C). The supernatant was allowed to warm to room temperature (about 25°C), when one-fifth of its volume of ethanol could be added without causing any permanent precipitation. After being left overnight at 5°C the precipitate was spun down (23000g, 30 min, 5°C), redissolved in a small volume of water, dialysed at 5°C and freeze-dried.

Column chromatography. Nucleic acid samples (15–45 mg in 10–20 ml) prepared as described above were applied to columns (8 cm² × 90 cm) of Bio-Gel A-15m packed in 0.2M-sodium acetate, and developed by upward flow with the same salt solution. The flow rate was 30–40 ml/h. The effluent was monitored through an LKB Uvicord, and 10 ml fractions were collected. Two main components were separated: fraction 1, which was completely excluded, and fraction 2, for which $V_e = 1.8 \times V_0$.

Samples of fraction 1 were combined, concentrated and further purified by chromatography on Bio-Gel A-50m, when the main component was apparently still excluded ($V_e = 38\%$ column volume). The peak fractions were dialysed and freeze-dried. Bio-Gel A-1.5m was used for the final purification of fraction 2. On elution with 0.2M-sodium acetate V_e was found to be $1.3 \times V_0$.

METHODS

U.v. spectrophotometry. A Unicam SP.800 or a Unicam SP.700 spectrophotometer was used for all u.v. measurements. The sample cell holder of the Unicam SP.700 was a copper block that could be heated electrically to temperatures up to 95°C and maintained at a temperature within this range to better than 0.1°C. Values of extinction were corrected for changes in RNA concentration owing to the thermal expansion of water.

Optical rotation. Optical rotatory dispersions over the range 220–500 nm were measured with a Bellingham and Stanley Polaromatic spectropolarimeter. Cells of 1 cm path length were used. The instrument was calibrated with standard sucrose solutions. The extinction at 260 nm was never more than 1.7 and the measured rotations were directly proportional to concentration. Readings were independent of slit width (usually 1 mm). The results are expressed as specific rotation $[\alpha] = \alpha^\circ/lc$, where α° is the measured rotation, l dm is the path length and c (g/100 ml) is the concentration of RNA, and the mean residue rotation $[m] = [\alpha] \times \text{mean residue wt.}/100$.

Circular dichroism. The circular dichroism of dilute solutions of RNA (about 50 mg/l) was measured in 1 cm cells at 25°C with a Roussel-Jouan Dichrograph model CD 185. The results are expressed as circular dichroism $[CD] = (\epsilon_L - \epsilon_R)$ where ϵ_L and ϵ_R are respectively the extinctions per g-atom of P for left and right circularly polarized light, and as residue ellipticity $[\theta_M] = 3300[CD]$.

Ultracentrifugation. A Spinco model E ultracentrifuge fitted with u.v. optics was used for analytical work. In the optical path the camera was replaced by a photoelectric cell that scanned the slit, and the movement of the sedimenting species was followed by means of an X-Y recorder. Cells of 12 mm path length were used in all cases. Measurements were made at temperatures in the range 10–25°C. RNA solutions were dilute (25 mg/l).

Viscometry. A Cannon-Fenske viscometer (Griffin and George Ltd., Wembley, U.K.) with a flow time for water (8 ml) of about 290 s at 25°C was used for all measurements. The temperature was controlled to within 0.01°C.

Determination of $\epsilon_{(P)}$ by chemical analysis of phosphorus. The phosphorus concentration of RNA solutions (about 0.8 mg/ml) of known extinction (measured in triplicate) was determined (duplicate samples) by the method of Chen, Toribara & Warner (1956). Values of $\epsilon_{(P)}$ were then calculated.

Determination of $\epsilon_{(P)}$ by alkaline hydrolysis. The spectrum of RNA (ϵ_{260} about 1.0) in 1M-sodium phosphate buffer, pH 7.2, was recorded (light-path length 1 cm) and 10M-KOH (10 μ l/ml of RNA solution) was added to bring the solution to pH 12.3. The spectrum was measured immediately at 25°C and later at 37°C over a period of 24 h. No further change was observed after 10 h at 37°C when ϵ_{260} increased to 151% of the value at pH 7 (fraction 1) or 154% of the initial value at pH 7 (fraction 2). The concentration of nucleotides was measured from the spectrum at pH 12.5 on the basis of the molar extinction coefficients of the nucleotides (Pabst Circular OR-10) and the known nucleotide composition of fractions 1 and 2.

Determination of base composition. The procedure was described by Smith & Markham (1950). RNA (1–3 mg) was hydrolysed by acid [0.1–0.3 ml of M-HCl (AnalaR), at 100°C for 1 h] to pyrimidine nucleotides and purine bases, which were then separated by paper chromatography as described by Smith & Markham (1950) or by Wyatt (1951). Samples (20 or 25 μ l) of the hydrolysate were spotted on to a sheet (30 cm × 50 cm) of paper (Whatman no. 1). The solvent systems used were: (1) 2-methyl propan-2-ol-conc. HCl-water (35:4:11, by vol.) (chromatography was carried out at 25°C); (2) propan-2-ol-conc. HCl-water (39:10:11, by vol.) (chromatography was carried out at 20°C). After the paper was equilibrated with vapour phase, chromatography was begun and continued until the solvent approached the end of the paper (about 40 h for solvent 1 and about 25 h for solvent 2). The papers were then dried at about 20°C, the spots were located under u.v. light and marked. The spots were cut out together with blank samples of equivalent size taken from either side of each spot, and eluted with 0.1M-HCl (AnalaR) for 18 h. The spectrum of each sample was measured against the appropriate blank and the concentration of base or nucleotide was obtained on the basis of the known molar extinction coefficients (Beaven, Holiday & Johnson, 1955; Pabst Circular OR-10). Good agreement was obtained between the analyses of different

Fig. 1. Sedimentation profiles of fraction (1) and fraction (2) in different solvents: (a) 1 mM-EDTA-0.01 M-sodium phosphate buffer, pH 7.4; (b) 0.15 M-NaCl-0.015 M-sodium citrate, pH 7.2.

Fig. 2. 'Melting' profile of (a) fraction 1 and (b) fraction 2 in 1% formaldehyde-0.1 M-sodium phosphate buffer, pH 7.2. In both (a) and (b) $E_{260} = 1.0$ at 25°C. Curves (1)(1') were obtained when RNA was heated to 95°C for the first time and curves (2)(2') were followed on subsequent heating cycles. ○, $\Delta\epsilon_{260}$; ●, $\Delta\epsilon_{280}$.

Table 1. Nucleotide composition and sedimentation properties of fractions 1 and 2

Property	Solvent	Fraction 1	Fraction 2
Nucleotide composition (mol fraction)	—	AMP 0.246, UMP 0.246 CMP 0.252, GMP 0.255	AMP 0.313, UMP 0.312 CMP 0.186, GMP 0.189
% G+C	—	50.9	37.8
$s_{20,w}$	0.15 M-NaCl-0.015 M-sodium citrate, pH 7.2	12.3 S	7.3 S
η_{sp}/c		5.22 dl/g ($c = 85 \mu\text{g}$ of RNA/ml)	0.42 dl/g ($c = 83 \mu\text{g}$ of RNA/ml)
$\dagger M_w$ (cf. Eigner & Doty, 1965)		1.2×10^6 daltons	0.14×10^6 daltons

† The weight-average molecular weight M_w was calculated from the eqn. of Mandelkern & Flory (1952):

$$M_w = \left[\frac{s_{20,w}^0 \cdot [\eta]^{1/3} \eta_0 \cdot N 10^{-13}}{\beta(1-\bar{v}\rho)} \right]^{3/2}$$

η_0 is the viscosity of the solvent, N is Avogadro's number, $\bar{v}$ is the partial specific volume of double-helical RNA and ρ is solvent density; $\beta = 2.4 \times 10^6$ is a coefficient defining the relation between M_w and sedimentation-viscosity $s_{20,w}$ (expressed in Svedberg units) is the sedimentation coefficient at zero concentration (the median value of $s_{20,w}$ obtained in dilute solutions, e.g. 0.025 g of RNA/l, is approx. $s_{20,w}^0$); $[\eta]$ is the intrinsic viscosity (dl/g) at zero shear. In calculating M_w it is assumed that $\eta_{sp}/c \approx [\eta]$ in dilute RNA solutions and that η_{sp}/c is scarcely dependent on the rate of shear when $[\eta] < 10$ dl/g (Littauer & Eisenberg, 1959).

Table 2. *Summary of u.v.-absorption spectra of fractions 1 and 2*

		Fraction 1	Fraction 2
ϵ_{260} ($1\text{mol}^{-1}\text{cm}^{-1}$) at 25°C	1 mM- or 10 mM-Sodium phosphate buffer, pH 7.2	6920 $\pm$ 50	6890 $\pm$ 60
ϵ_{260} ($1\text{mol}^{-1}\text{cm}^{-1}$) at 25°C	1 mM-Sodium phosphate buffer, pH 12.6 (before hydrolysis)	9200	9160
ϵ_{260} ($1\text{mol}^{-1}\text{cm}^{-1}$) at 95°C	1 mM- or 10 mM-Sodium phosphate buffer, pH 7.2	10000	10500
ϵ_{260} ($1\text{mol}^{-1}\text{cm}^{-1}$) of hydrolysate at pH 7	Calculated (Pabst circular OR 10)	11050	11450
ϵ_{260} ($1\text{mol}^{-1}\text{cm}^{-1}$)	25°C, 8 M-Urea-0.01 M-sodium phosphate buffer, pH 7.2	6920	6890
	95°C, pH 7.2	9700	9950
	0.15 M NaCl-0.015 M-sodium citrate, pH 7.2	approx. 100°C (cf. 90°C for DNA analogue)	approx. 85°C (cf. 85°C for DNA analogue)
T_m (°C)	1 mM-EDTA-0.01 M-sodium phosphate buffer, pH 7.4	76°C (cf. 70°C for DNA analogue)	64°C (cf. 64°C for DNA analogue)
	0.1 M-Sodium phosphate buffer, pH 7.2	89°C	75°C
$(\epsilon_{260}(95^\circ\text{C}) - \epsilon_{260}(25^\circ\text{C}))$	1 mM- or 10 mM-Sodium phosphate buffer, pH 7.2	1.85	2.87
$(\epsilon_{280}(95^\circ\text{C}) - \epsilon_{260}(25^\circ\text{C}))$

Fig. 3. 'Melting' profile of RNA in 0.01 M-sodium phosphate buffer, pH 7.2 ($E_{260} = 1.0$ at 25°C). (a) Fraction 1. (b) Fraction 2. $\circ$, $\Delta\epsilon_{260}$; $\bullet$, $\Delta\epsilon_{280}$.

Fig. 4. Hypochromism of (a) fraction 1 and (b) fraction 2 as a function of temperature. Solvent: 8 M-urea-0.01 M-sodium phosphate buffer, pH 7.2. In each case $E_{260} = 1.0$ at 25°C. $\circ$, $\Delta\epsilon_{260}$; $\bullet$, $\Delta\epsilon_{280}$.

preparations and between replicates. In all four samples of fraction 1 and five samples of fraction 2 were hydrolysed. On average two replicates of each sample were analysed.

Although small losses of bases and nucleotides occur no correction was made except for CMP, for which a recovery of 95% was assumed. This nucleotide is known to be

Fig. 5. 'Melting' profiles of fraction 1 in buffers of various ionic strengths. Solvents: (1) 0.01M-Sodium phosphate buffer, pH 7.2; (2), 0.1M-sodium phosphate buffer, pH 7.2; (3), 0.15M-NaCl-0.015M-sodium citrate, pH 7.2; (4) 8M-urea-0.01M-sodium phosphate buffer, pH 7.2; (5) 4M-guanidinium chloride-0.01M-sodium phosphate buffer, pH 6.5; (6) the profile of the entirely single-stranded form obtained by heating RNA for the second and third time in 1% formaldehyde-0.1M-sodium phosphate buffer, pH 7.2 (data from Fig. 2a), corrected for the contribution to E owing to chemical modification of the base residues by subtracting $\Delta E = 0.020$ (or $\Delta \epsilon_{(P)260} = 140$) from the ordinate.

Fig. 6. Effect of ionic strength and temperature on the spectrum of fraction 2. Solvents: (1) 1M-sodium phosphate buffer, pH 7.2; (2), 0.1M-sodium phosphate buffer, pH 7.2; (3) 0.15M-NaCl-0.015M-sodium citrate, pH 7.2; (4) 8M-urea-0.01M-sodium phosphate buffer, pH 7.2; (5) 4M-guanidinium chloride-0.01M-sodium phosphate buffer, pH 6.5; (6) fraction 2 in 1% formaldehyde-0.1M-sodium phosphate buffer, pH 7.2, second and subsequent heating cycles (data from Fig. 2b) corrected for contribution to E owing to chemical modification of the base-residues by subtracting $\Delta E = 0.036$ (or $\Delta \epsilon_{260} = 250$) from the ordinate.

prone to hydrolysis under the conditions used: Schein & d'Angelo (1969) reported losses of up to 9%, whereas they recovered guanine quantitatively from the hydrolysis mixture. The maximum differences observed were 1.9 mol % G, 2.8 mol % A, 1.8 mol % CMP and 2.5 mol % UMP.

RESULTS

The isolation of RNA with antiviral activity from the mycelium of *P. chrysogenum* was described by Sutherland & Bessell (1969). Evidence that the RNA component was double-helical was obtained from its solubility in 2M-sodium chloride and its resistance to ribonuclease. For example the RNA fraction soluble in 2M-sodium chloride was resolved into two fractions by gel filtration through a column

of agarose (solvent 0.1M-sodium chloride). Although treatment with 10 μ g of ribonuclease/2.5mg of RNA per ml at 5°C for 25h (solvent 0.15M-sodium chloride-0.015M-sodium citrate, pH 7.2) is sufficient to degrade single-stranded RNA to small fragments, the elution pattern of the RNA fraction soluble in 2M-sodium chloride was not affected and T_m (Table 2), $s_{20,w}$ and η_{sp}/c (Table 1) were scarcely altered.

The sedimentation profile (Fig. 1) of the first fraction (fraction 1) to be eluted was broad, indicating heterogeneity in molecular weight. However, a spread of s values could arise from minor changes in conformation such as occasional breaks in one or other of the strands of a double-helix. A value of $s_{20,w} = 12.2S$ was found for this fraction when

Fig. 7. Denaturation and renaturation of fraction 1 in 8M-urea-0.01M-sodium phosphate buffer, pH 7.2. ○, $\Delta\epsilon_{260}$, and ●, $\Delta\epsilon_{280}$ observed when RNA was heated to 95°C for the first time. The solution was cooled slowly to 25°C and again heated to 95°C (Δ, $\Delta\epsilon_{260}$ and ▲, $\Delta\epsilon_{280}$). ($E_{260} = 1.0$ at 25°C before heating.)

Fig. 8. Denaturation and renaturation of fraction 2 in 8M-urea-0.01M-sodium phosphate buffer, pH 7.2. ○, $\Delta\epsilon_{260}$, and ●, $\Delta\epsilon_{280}$ observed when RNA was heated to 95°C for the first time. The solution was cooled slowly to 25°C and again heated to 95°C (Δ, $\Delta\epsilon_{260}$ and ▲, $\Delta\epsilon_{280}$). ($E_{260} = 1.0$ at 25°C before heating.)

1mM-EDTA-0.01M-sodium phosphate buffer, pH 7.4, was the solvent. A similar value, $s_{20,w} = 12.3S$, was obtained for fraction 1 in 0.15M-sodium chloride-0.015M-sodium citrate, pH 7.2, solution. The insensitivity of $s_{20,w}$ to electrolyte concentration is a characteristic of double-helical nucleic acids. Fraction 2 sedimented as a single boundary that moved at similar rates ($s_{20,w} = 7.0S$ and $7.3S$ respectively) in the two solvents.

Values of η_{sp}/c (Table 1) observed for the two fractions (solvent 0.15M-sodium chloride-0.015M-sodium citrate, pH 7.2) were considerably higher than those reported for single-stranded RNA species of comparable $s_{20,w}$ value (for example, see Littauer & Eisenberg, 1959).

The nucleotide composition of both fractions was characteristic of double-helical RNA since AMP and UMP were present in equivalent amounts, as were CMP and GMP (see Table 1).

The optical properties of both fractions agree with a double-helical conformation. The extinction per g-atom of P at 260nm was found to be close to

$6900l\text{mol}^{-1}\text{cm}^{-1}$ for both fractions, compared with about $7600l\text{mol}^{-1}\text{cm}^{-1}$ for ribosomal RNA (see, for example, Cox, 1970a). Moreover, when either fraction was heated in 1% formaldehyde-0.1M-sodium phosphate buffer, pH 7.2, there was scarcely any reaction between the base residues and formaldehyde at 55°C or below, in marked contrast with ribosomal RNA (cf. Cox, 1967, 1969, 1970a). The abrupt increase in extinction over a narrow temperature range (Fig. 2) and on increasing the pH to 12.5 (Table 2) are typical of double-helical nucleic acids. The reaction of the base-residues with formaldehyde prevents the formation of base-pairs and the hypochromism found on cooling from 95 to 25°C is attributed to the ability of unpaired residues to stack one-upon-another along a single strand. The 'melting' behaviour in other solvents, such as 0.01M-sodium phosphate buffer, pH 7.2 (Fig. 3), and 8M-urea-0.01M-sodium phosphate buffer, pH 7.2 (Fig. 4), particularly the dependence on ionic strength (see Figs. 5 and 6) of T_m (the temperature at which 50% of the total increase in E is

Fig. 9. Optical rotatory dispersion of (a) fraction 1 and (b) fraction 2. Solvent: 1% formaldehyde-0.1M-sodium phosphate buffer, pH 7.2; ○, 25°C, unheated solution; ●, 25°C, after heating to 95°C.

Fig. 10. Circular dichroism of (a) fraction 1 and (b) fraction 2. Solvent: 1% formaldehyde-0.1M-sodium phosphate buffer, pH 7.2; ○, 25°C, unheated solution; ●, 25°C, after heating to 95°C.

found), are all typical of double-helical nucleic acids. The spectrum of neither fraction 1 nor fraction 2 was affected when the solution was made 8 M in urea at 25°C but a red shift was noticed after heating to 95°C, leading to a lower value of ϵ_{260} (Table 2). A

similar effect was found for DNA (Cox & Kanagalingam, 1968). Fraction 1 is richer in G+C and 'melts' at higher temperatures than fraction 2, and also at a higher temperature than its DNA analogue (see Fig. 2). The increase in T_m of about 11°C that was

Fig. 11. Comparison of T_m (solvent 0.15M-NaCl-0.015M-sodium citrate, pH 7-8, or equivalent) of fractions 1 and 2 and other double-helical polynucleotides. (1) RNA and model polynucleotides. Δ , Fractions 1 and 2; $\circ$, other double-helical RNA species; $\bullet$, poly rA·poly rU. Data for poly (rA·rU), T_m 71.7°C; poly rA·poly rU, T_m 62.5°C; wound tumour virus 38% G+C, T_m 91°C; tobacco mosaic virus RNA (replicative form), 44% G+C, T_m 97°C; reovirus RNA, 44.3% G+C, T_m 94°C; encephalomyocarditis virus RNA (replicative form), 47% G+C, T_m 96°C; Newcastle disease virus RNA (replicative form), 48.6% G+C, T_m 100°C; phage fr RNA (replicative form), 50.7% G+C, T_m 102°C; phage β RNA (replicative form), 51.6% G+C, T_m 99°C; phage MS2 RNA (replicative form), 52% G+C, T_m 103°C; poly rG·poly rC, T_m 140°C, were taken from Kallenbach (1968). Samejima *et al.* (1968) report 44.2% G+C, T_m 92.5°C for rice dwarf virus RNA. Data for turnip yellow mosaic virus RNA (replicative form), 57.5% G+C, T_m 101°C, were taken from Bockstahler (1967); cf. Guschlbauer, Courtois, Bove & Bove (1968), who report T_m 112°C. (2) Relation between % G+C and T_m of DNA given by Marmur & Doty (1962). $\blacksquare$, poly dA·poly dT, T_m 65°C, and $\square$, poly (dA·dT), T_m 78°C, are shown for comparison with poly rA·poly rU and poly

found on increasing the sodium phosphate buffer concentration from 0.01 to 0.1M is comparable with the increase in T_m found for rat liver DNA (Cox & Kanagalingam, 1968).

The $\Delta\epsilon_{260}/\Delta\epsilon_{280}$ ratio found on denaturation reflects the nucleotide composition of the double-helical species involved in the transition (for review see Cox, 1970b). Increments in ϵ_{280} reflect principally the 'melting' of rG·rC base-pairs, whereas both rA·rU and rG·rC base-pairs contribute to $\Delta\epsilon_{260}$. Indeed $\Delta\epsilon_{260}/\Delta\epsilon_{280}$ was related to $f_{rA\cdot rU}/f_{rG\cdot rC}$ ($f_{rA\cdot rU}$ and $f_{rG\cdot rC}$ respectively are the mole fractions of rA·rU and rG·rC base-pairs) on the basis of the 'melting' properties of polynucleotides (Cox, 1966a,b, 1967, 1970b). The values $f_{rA\cdot rU}/f_{rG\cdot rC}$ calculated from $\Delta\epsilon_{260}/\Delta\epsilon_{280}$ agree with the chemical composition of fractions 1 and 2. The values of T_m agree fairly well with those obtained from the relation for double-helical RNA (see Fig. 11 and also Kallenbach, 1968):

$$1/T_m = 2.95 \times 10^{-3} - 0.53 \times 10^{-3} f_{rG\cdot rC}$$

However, fraction 2 in 0.15M-sodium chloride-0.015M-sodium citrate, pH 7.2, 'melts' at about 85°C, compared with 90°C reported (Gomatos & Tamm, 1963) for wound tumour virus RNA (38% G+C).

It appears that the T_m of poly rA·poly rU is not the same as that of poly (rA·rU) because the single-stranded forms are not equivalent, owing to differences in the extent to which residues are 'stacked' along the single strand at the 'melting' temperature (Richards & Simpkins, 1968). The difference in the T_m of poly dA·poly dT and poly (dA·dT) may be explained in the same way. Thus nucleotide sequence might also affect the T_m of RNA and so it is possible that different species of RNA might have the same % (G+C) but differ in T_m . However, the magnitude of this effect is not known. Other factors such as the integrity of the double helix might also affect T_m .

Fraction 2 (but not fraction 1, see Fig. 7) in 8M-urea-0.01M-sodium phosphate buffer, pH 7.2, or in 0.15M-sodium chloride-0.015M-sodium citrate, pH 7.2, renatured readily on cooling (see Fig. 8). No particular precautions were necessary since a high degree of renaturation was obtained after the solution was removed from the heating block and then left to cool at room temperature. Both the native and the renatured material had the same sedimentation profile (cf. Figs. 1a and 1b). The optical rotatory dispersion and circular dichroism of fractions 1 and 2 are given in Figs. 9 and 10 and the information is summarized in Table 3. The specific rotation at the maximum of about 280nm is substantially higher than values reported for rRNA [e.g. yeast rRNA, $[\alpha]_{\max}$, 3700° at 280-

Table 3. *Values of optical rotation and circular dichroism of fractions 1 and 2*

Solvent used was 1% formaldehyde-0.1 M-sodium phosphate buffer, pH 7.2.

	Fraction 1	Fraction 2
O.R.D. measured at 25°C	$[\alpha]_{\max}$. 6000° at 278 nm	$[\alpha]_{\max}$. 6000° at 278 nm
before heating to 95°C	$[\alpha]_{\min}$. -5100° at 250 nm	$[\alpha]_{\min}$. -5300° at 247 nm
	Crossover approx. 258 nm	Crossover approx. 257 nm
O.R.D. measured at 25°C	$[\alpha]_{\max}$. 3000° at 286 nm	$[\alpha]_{\max}$. 4300° at 286 nm
after heating to 95°C	$[\alpha]_{\min}$. -4800° at 260 nm	$[\alpha]_{\min}$. -4800° at 256 nm
	Crossover approx. 274 nm	Crossover approx. 269 nm
C.D. measured at 25°C	$(\epsilon_L - \epsilon_R)_{\max}$. 8.18 l mol ⁻¹ cm ⁻¹ at 260 nm	$(\epsilon_L - \epsilon_R)_{\max}$. 8.24 l mol ⁻¹ cm ⁻¹ at 260 nm
before heating to 95°C	$(\epsilon_L - \epsilon_R)_{\min}$. -8.8 l mol ⁻¹ cm ⁻¹ at 210 nm	$(\epsilon_L - \epsilon_R)_{\min}$. -7.4 l mol ⁻¹ cm ⁻¹ at 210 nm
C.D. measured at 25°C	$(\epsilon_L - \epsilon_R)_{\max}$. 5.6 l mol ⁻¹ cm ⁻¹ at 273 nm	$(\epsilon_L - \epsilon_R)_{\max}$. 6.1 l mol ⁻¹ cm ⁻¹ at 273 nm
after heating to 95°C	$(\epsilon_L - \epsilon_R)$. -0.56 l mol ⁻¹ cm ⁻¹ at 210 nm	$(\epsilon_L - \epsilon_R)$. -2.7 l mol ⁻¹ cm ⁻¹ at 210 nm

282 nm, $[\alpha]_{\min}$. -2800° at 241-253 nm, cross-over at 263 nm (McPhie & Gratzer, 1966); *E. coli* rRNA, $[\alpha]_{\max}$. 4000° at 280 nm (Sarkar, Yang & Doty, 1967); rabbit reticulocyte rRNA, $[\alpha]_{\max}$. 4700° at 280 nm, $[\alpha]_{\min}$. -4300° at about 252 nm, cross-over about 265 nm (Cox, 1969)] and in line with values found for other species of double-helical RNA. Over the range 220-300 nm the specific rotations of fractions 1 and 2 are very similar, as are the values of ellipticity. The circular dichroism of fractions 1 and 2 is similar to that found for rice dwarf virus RNA (Samejima, Hashizume, Imahori, Fujii & Miura, 1968), which is known to have a double-helical structure.

DISCUSSION

The difference in the nucleotide composition of fractions 1 and 2 suggest that these fractions are not artifacts of the isolation procedure but are present as distinct species within the virus-like particles (cf. RNA of reo-virus; Bellamy, Shapiro, August & Joklik, 1967).

Fractions 1 and 2 are clearly double-helical RNA species and fewer than 5% of residues are unpaired, as judged by the reaction with formaldehyde (Fig. 2), and 'melting' properties in 0.15 M-sodium chloride-0.015 M-sodium citrate, pH 7.2. The ready renaturation of fraction 2 agrees with the notion that this fraction is homogeneous. It is possible that more stringent extraction conditions might increase the proportion of fraction 1 that renatures. The lower rate of renaturation of this fraction is consistent with the broad sedimentation profile and supports the view that it is more heterogeneous than fraction 2.

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