The Nature of the Circular-Dichroic Spectra of Complexes between Ribonuclease A and Nucleotides

By SERGEY M. DUDKIN,*§ LEVON V. KARABASHYAN,†
MARAT YA. KARPEISKY,* SERGEY N. MIKHAILOV,*
NELLE SH. PADYUKOVA* and VALENTEEN G. SAKHAROVSKY‡

*Institute of Molecular Biology, U.S.S.R. Academy of Sciences, Moscow,

†Institute of Biochemistry, Armenian S.S.R. Academy of Sciences, Yerevan,

and ‡Institute of Biochemistry and Physiology of Micro-organisms,
U.S.S.R. Academy of Sciences, Pushchino-on-Oka, U.S.S.R.

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The circular-dichroism and proton-magnetic-resonance spectra of complexes of ribonuclease A with dihydrouridine 3'-phosphate, 2'- and 3'-CMP, arabinosyl-3'-CMP, 1-(2-hydroxyethyl)cytosine 2'-phosphate and 1-(3-hydroxypropyl)cytosine 3'-phosphate were studied. Comparison of the results shows that non-additivity of the circular-dichroic spectrum of an enzyme-nucleotide complex may be due to: (a), alteration of the circular-dichroic spectrum of the nucleotide under the influence of the asymmetric protein matrix (induced dichroism), and (b) a change in the nucleotide conformation. The contribution of each of the two factors was estimated to calculate the circular-dichroic spectra of 2'-CMP and 3'-CMP in complex with ribonuclease A. 3'-CMP in this complex was characterized by negative circular dichroism in the long-wavelength absorption band of the nucleotide, whereas 2'-CMP was characterized by positive circular dichroism. Since both nucleotides in the complex are known to be in an *anti* conformation, it follows that even small changes in the conformation considerably modify the circular-dichroic spectrum of the nucleotide in complex with the enzyme.

The structure of complexes between RNAase|| A and substrate-like inhibitors, with particular reference to the conformation of the nucleotide in complex with the protein in solution, was studied by several authors (Meadows *et al.*, 1969; Richards & Wyckoff, 1971; Oshima & Imahori, 1971; Haar *et al.*, 1974; Gorenstein & Wyrwicz, 1974). The techniques of p.m.r., c.d. and dispersion of optical rotation are being widely used to study the conformation of nucleosides, nucleotides and their derivatives in a free state in solution (Milles *et al.*, 1969*a,b*; Rogers & Ulbricht, 1970, 1971). These techniques are highly sensitive to the conformation of nucleoside fragments, which depends on the mutual arrangement of the heterocycle and the sugar ring, and is characterized by the ϕ C-N angle of rotation about the glycosidic bond.

The c.d. technique has been used to determine the conformation of nucleotides bound to RNAase A (Oshima & Imahori, 1971; Simons, 1971). In particu-

lar, an increase in the amplitude of the Cotton effect in the c.d. spectrum of 2'-CMP, as it is bound to RNAase A, has been ascribed to an alteration of the nucleotide ϕ C-N to that characteristic of an *anti* conformation (Oshima & Imahori, 1971). However, a change in ϕ C-N is not the only factor that can affect parameters of the nucleotide spectrum in the complex. Non-additivity of the c.d. spectrum of the enzyme-nucleotide complex can be due to three main factors: (a) alteration of the c.d. spectrum of the protein in the complex, (b) change in the conformation of the nucleotide and, consequently, of its c.d. spectrum, and (c) variation of the c.d. spectrum of the nucleotide under the influence of the asymmetric protein matrix (induced c.d.).

The present work was undertaken to study the contribution of each of the three factors into the c.d. spectrum of the nucleotide that is specifically bound at the active site of RNAase A, and to determine the 'proper' c.d. spectrum of the nucleotide in the complex.

Materials and Methods

Preparation of compounds

RNAase A (Reanal) was purified by a technique described elsewhere (Crestfield *et al.*, 1963). Ara-3'-

§ To whom reprint requests should be addressed.

|| Abbreviations: RNAase, ribonuclease; p.m.r., proton magnetic resonance; c.d., circular dichroism; H₂-3'-UMP, dihydrouridine 3'-phosphate; ara-3'-CMP, β -D-arabinofuranosylcytosine 3'-phosphate; HOPr-3'-CMP, 1-(3-hydroxypropyl)cytosine 3'-phosphate; HOEt-2'-CMP, 1-(2-hydroxyethyl)cytosine 2'-phosphate.

CMP (Nagyvary & Provenzale, 1969), HOPr-3'-CMP and HOEt-3'-CMP (Kritzyn *et al.*, 1975), and 2'-CMP and 3'-CMP (Cohn, 1950) were prepared in a chromatographically pure state. H₂-3'-UMP (Raj Bhandary, 1968) was synthesized by directly reducing 3'-UMP. All other reagents were commercial preparations of the highest grade available, and deionized double-distilled water was used throughout.

Spectrophotometry and circular dichroism

Absorption spectra were recorded by using a Cary 15 spectrophotometer. The following molar absorption coefficients were taken for spectrophotometric studies: RNAase A, $\epsilon_{278} = 9800 \text{ litre} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$, pH 7.0 (Sela & Anfinsen, 1957); 2'- and 3'-CMP, $\epsilon_{260} = 7600 \text{ litre} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$, pH 7.0 (Beaven *et al.*, 1955); ara-3'-CMP, $\epsilon_{272} = 9200 \text{ litre} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$, pH 7.0 (Nagyvary & Provenzale, 1969); HOPr-3'-CMP, HOEt-2'-CMP, $\epsilon_{273} = 7000 \text{ litre} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$, pH 7.0 (Kritzyn *et al.*, 1975); H₂-3'-UMP, $\epsilon_{235} = 6100 \text{ litre} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$, in 0.1 M-KOH (Venkstern & Bayev, 1967).

Difference absorption spectra (Hummel *et al.*, 1961) were recorded directly with the Cary 15 spectrophotometer, by using a tandem scheme of installing cuvettes with a light-path 0.2 or 0.1 cm. In one set, the first cuvette contained RNAase A, and the second cuvette, the nucleotide; in the other set, solutions having the same concentrations of the enzyme and the nucleotide were placed in the first cuvette, whereas a buffer solution was poured in the second cuvette. The slit of the spectrophotometer was maintained at 0.5 mm. The concentration of RNAase A was about 50 μM , and the concentration of the nucleotides was from 20 μM to 2 mM.

C.d. spectra were measured in cuvettes with a path-length of 0.1–1.0 cm, with a Jonan-Roussel model KD 185 dichrograph. The formation of enzyme-inhibitor complexes in c.d. experiments was controlled through changes in the amplitude of difference absorption spectra of the complexes. In all cases, the amplitude of the Cotton effect in the c.d. spectra of the complexes was extrapolated to a complete saturation of the enzyme with a ligand. Binding constants for ligands studied were estimated by us using a microcalorimetry technique, and all were more than $1 \times 10^4 \text{ M}^{-1}$ (I. V. Antonov & S. M. Dudkin, unpublished work). The same concentrations of RNAase A and nucleotides were used as when recording absorption spectra. The experimental error for difference c.d. spectra amplitudes was determined to be $\pm 10\%$. Throughout spectral measurements 0.05 M-Tris/HCl buffer, 0.05 M-sodium acetate and 0.1 M-NaCl were used. The pH of a buffer solution was adjusted with concentrated acetic acid, and measured at 25°C with Radiometer model PHM-64 pH-meter.

Proton magnetic resonance

All spectra were recorded at 100 MHz with a Varian model HA 100 D spectrometer operated with an internal-lock system and equipped with a Varian V 4315 digital-readout frequency counter and a V 6040 variable-temperature controller. The spectrometer was interfaced with a Varian C 1024 time-averaging computer to improve the signal-to-noise ratio.

The pD of each sample was checked before and after each spectrum, and the spectrum was only accepted if the measurements agreed to within 0.02 pH unit. The temperature was maintained at 32°C, and pD of samples was adjusted to 5.5. Precision-bore p.m.r. tubes with coaxial inserts containing hexamethyldisiloxane were used. Hexamethyldisiloxane served as a reference and provided the lock signal. All chemical shifts are reported downfield from hexamethyldisiloxane.

Spectra were fed into the computer memory with an electric pantograph, constructed on the basis of a spectrometer recorder, directly from the chart record. The concentration of RNAase A was 5 mM. In all cases, the complex was obtained by titrating the enzyme with an appropriate nucleotide to attain complete saturation, and changes in the position of signals were registered for C2-H of histidine residues, the protons of aromatic amino acid residues, and C5-H, C6-H and C1-H of the nucleotide. The enzyme and the nucleotide were dissolved in 0.2 M-NaCl in ²H₂O and the pD was adjusted by titrating the solutions with 0.1 M-²HCl or 0.1 M-NaO²H. No corrections were introduced for readings of the pH-meter, and pH values thus obtained were used in the study.

Results and Discussion

Evaluation of the individual contributions made by different factors that are responsible for non-additivity of the c.d. spectra of complexes between RNAase A and nucleotides is a prerequisite for studying the structure of these complexes by means of c.d. Optical activity of RNAase A within the range 240–320 nm is known to depend on the interaction between tyrosine and cysteine chromophores, and also between them and their local environment (Horwitz & Strickland, 1971; Strickland, 1972). The character of this interaction changes when the complexes are being formed, and this may affect the c.d. spectra at 240–320 nm. For this reason, non-additivity of the c.d. spectrum of the complex between RNAase A and 3'-CMP at 240–250 nm was assumed to result from a conformational transition in the protein molecule which involved cysteine residues (Simons, 1971). However, the alteration of optical activity in the region of 240–250 nm may be

brought about by the different environment of the chromophore of the nucleotide when the latter is in a free state or is bound to the enzyme; similar changes are found in the c.d. spectra of nucleotides when an organic solvent replaces water (Milles *et al.*, 1969a).

The formation of complexes between RNAase and pyrimidine nucleotides is not accompanied by noticeable changes in the spectral properties of the protein at 240–320 nm, as was shown in our previous work done with the complexes of RNAase A and 4-thiouridine 3'-phosphate, and the complexes of RNAase S and des-Asp¹²¹-Ala¹²²-Ser¹²³-Val¹²⁴-RNAase A with 2'- and 3'-CMP (Karabashyan *et al.*, 1973; Sakharovsky *et al.*, 1973). To support this conclusion, we studied spectra of the complex between RNAase A and H₂-3'-UMP (Fig. 1). The corresponding cyclophosphate (H₂-U>p) is a good substrate for RNAase A (Richards & Wyckoff, 1971), and therefore H₂-3'-UMP, which shows neither absorption nor optical activity within the range 260–320 nm, can be used as model compound. The c.d. spectrum of the complex between RNAase A and H₂-3'-UMP is entirely additive at 255–320 nm (see Fig. 1). Non-additivity of the spectrum of this complex at shorter wavelengths is due to a small Cotton effect in the c.d. spectrum of the nucleotide. This evidence proves our earlier conclusion that non-additivity of the c.d. spectra of complexes between

RNAase A and pyrimidine nucleotides at 240 nm is not caused by changes in the spectrum of the enzyme (Karabashyan *et al.*, 1973). Consequently, conformational changes in the molecule of RNAase A that occur when it binds nucleotides do not affect the c.d. of the protein in the region of long-wavelength absorption of aromatic amino acid residues.

As RNAase A binds 2'- or 3'-CMP, a difference c.d. spectrum is observed at 240–320 nm (Fig. 2). Difference c.d. spectra of RNAase A complexes with HOPr-3'-CMP, HOEt-2'-CMP and ara-3'-CMP, i.e. compounds similar in structure to 2'- and 3'-CMP, have been studied to evaluate the contribution of induced c.d. in the difference c.d. spectra. The first two compounds possess no chiral properties, and changes in the ϕ C-N angle of ara-3'-CMP are within the range typical of an *anti* conformation; therefore these analogues can be considered suitable for studying induced c.d.

The c.d. spectra of the complexes of RNAase A with HOPr-3'-CMP, HOEt-2'-CMP and ara-3'-CMP, as well as the spectra of the complexes with 2'- and 3'-CMP, are not additive (Figs. 3 and 4). As follows from Figs. 3 and 4, the difference c.d. spectra of the three analogues are quite similar in the amplitude and the position of the maximum; therefore one is bound to conclude that the effect is brought about by the protein. The position of the maximum of the induced c.d. spectrum at 270 nm, which corresponds to the location of the B_{2u} transition of cytosine, suggests presumably that c.d. is induced in the long-wavelength band of absorption of the nucleotide. As shown in Figs. 3 and 4, the induced effect hardly depends either on the mutual orientation of cytosine and a ribose residue or on the nature of a sugar residue. The amplitude of the induced Cotton effect does not change for all the analogues studied in the pH range of 4.5–7.5. Thus the difference c.d. spectra of the complexes of RNAase A with 2'- or 3'-CMP comprise a significant contribution of c.d. induced by the asymmetric field of the protein matrix.

To discuss further the origin of non-additivity of the c.d. spectra for complexes of RNAase A with cytosine derivatives, one must be certain that all the nucleotides studied in the complexes are similarly oriented with respect to the groups of the enzyme active site. To this end, the p.m.r. spectra of the complexes have been studied. As was found previously (Meadows *et al.*, 1969), the binding of pyrimidine nucleotides in the active site of RNAase A affects the position of signals of the imidazole C2-H of three of the four histidine residues (His-12, His-48, His-119) of the enzyme, as well as the position of proton signals of aromatic amino acids. The value of changes in the chemical shift of C2-H in His-12 and His-119 is determined by the position and orientation of the inhibitor phosphate residue in the catalytic area of the enzyme active site, whereas the shift of

Fig. 1. C.d. spectra of RNAase A and its complex with H₂-3'-UMP

(1) C.d. spectrum of RNAase A; (2) c.d. spectrum of RNAase A in complex with H₂-3'-UMP. The concentration of RNAase A is 46 μ M and that of H₂-3'-UMP is 3 mM. Other conditions are as indicated in the Materials and Methods section.

Fig. 2. *C.d. spectra of 2'- and 3'-CMP in complex with RNAase A, and difference c.d. spectra originating when the nucleotides are bound to the enzyme*

The spectra of the RNAase A complexes with the nucleotides were calculated by using difference absorption spectra (see the Materials and Methods section). (a) (1) C.d. spectrum of 3'-CMP; (2) c.d. spectrum of RNAase A; (3) c.d. spectrum of the complex of RNAase A and 3'-CMP. (b) (1) C.d. spectrum of 2'-CMP; (2) c.d. spectrum of RNAase A; (3), c.d. spectrum of the complex of RNAase A and 2'-CMP. (c) Difference c.d. spectrum of the complex of RNAase A and 3'-CMP. (d) Difference c.d. spectrum of the complex of RNAase A and 2'-CMP. Other conditions are described in the Materials and Methods section.

signals of the aromatic amino acid residues upfield is induced by the binding of the inhibitor heterocyclic base in the region of the active site where the side group of Phe-120 is located (Meadows *et al.*, 1969; Haar *et al.*, 1974; Gorenstein & Wyrwicz, 1974). At the same time as the complex is being formed, signals of C5-H and C6-H in the heterocyclic base and of C1'-H in the ribose residue of the nucleotide shift downfield (Meadows *et al.*, 1969). Apparently, the chemical shift of the aforementioned groups of the enzyme-inhibitor complex may provide information about the relative orientation of the inhibitor at the active site of RNAase A. To determine the position of shifted signals of the enzyme, we used the technique of difference p.m.r. spectra, which considerably improved the accuracy of measurements. Such spectra of the complexes are shown in Fig. 5. In all cases, as inhibitors are being bound, signals of histidine C2-H and of protons in aromatic amino acids of the protein shift in the same direction, and

the magnitude of these shifts is similar. As follows from Fig. 5, the shift of signals in the absorption region of the aromatic amino acids is caused by the binding of the heterocyclic base of the inhibitor rather than of the whole nucleoside fragment.

As was found by comparing changes in the position of signals of C6-H, C5-H and C1'-H of nucleotides resulting from the complex-formation (Table 1), the character of changes is the same in all complexes; this is also true of signals of the enzyme groups. Consequently, analogues under study are bound in the same region of the active site of RNAase A, and their orientation with respect to the groups of the active site should also be almost identical. At the same time, as shown in Table 1, these changes differ in absolute value. Therefore the mode of interaction between the base and the protein in the complex of RNAase A with the inhibitor depends on the structure of a fragment that connects the base with the phosphate in the nucleotide molecule. However, these

Fig. 3. C.d. spectrum of RNAase A in complex with ara-3'-CMP and difference c.d. spectrum for the complex of the nucleotide with the enzyme

The spectrum of the complex was calculated as in Fig. 2. (a) (1) C.d. spectrum of ara-3'-CMP; (2) c.d. spectrum of RNAase A; (3) c.d. spectrum of the complex between RNAase A and ara-3'-CMP. (b) Difference c.d. spectrum for binding RNAase A with ara-3'-CMP. For other conditions, see the Materials and Methods section.

changes have no effect, within the accuracy limits of the experiment, on the parameters of induced c.d. spectra, as follows from comparing the data in Table 1 and the c.d. spectra given in Figs. 3 and 4.

This analysis led to the conclusion that the contribution of induced c.d. to the spectra of the complexes of RNAase A with 2'- and 3'-CMP is similar in shape and amplitude to the difference c.d. spectra of complexes between RNAase A and model inhibitors. In consistency with this conclusion and the fact that the c.d. spectrum of the protein in the complex does not change within the range 260–300 nm, the amplitude of the c.d. spectrum of the nucleotide bound to RNAase A can be expressed as follows:

$$[\theta]_{\text{exp.}} = [\theta]_{\text{sol.}} + [\theta]_{\text{ind.}} + \Delta[\theta]_{\text{con.}} \quad (1)$$

$$[\theta]_{\text{prop.}} = [\theta]_{\text{exp.}} - [\theta]_{\text{ind.}} \quad (2)$$

where $[\theta]_{\text{exp.}}$ = amplitude of experimentally observed c.d. spectrum of the nucleotide in complex with RNAase A; $[\theta]_{\text{sol.}}$ = amplitude of the c.d. spectrum of a free nucleotide in aqueous solution; $[\theta]_{\text{ind.}}$ = amplitude of the c.d. spectrum induced in the absorption band of the nucleotide by the asymmetric field of the protein; $[\theta]_{\text{con.}}$ = contribution to the c.d. spectrum of the nucleotide in complex with RNAase A caused by a change in the nucleotide conformation; $[\theta]_{\text{prop.}}$ = amplitude of the c.d. spectrum of the nucleotide in complex with RNAase A determined by its conformation in the complex.

Our analysis and eqns. (1) and (2) were used to calculate 'conformational' contributions ($\Delta[\theta]_{\text{con.}}$) to the c.d. spectra of nucleotides in the complexes and 'proper' c.d. spectra of these nucleotides in complex with RNAase A (Fig. 6). Comparison of the spectra given in Fig. 6 with the c.d. spectra of the nucleotides in solution in the region of B_{2u} transition of a heterocyclic base has shown that c.d. of 3'-CMP in complex with RNAase A differs in sign and amplitude from c.d. of this nucleotide in solution.

Table 1. Difference chemical shifts (in p.p.m.) of proton signals of RNAase A and nucleotides in the complex with respect to similar signals of free RNAase A and nucleotides (pH 5.5, 0.2M-NaCl, 32°C)

A minus sign preceding a value indicates downfield shift. $\delta f.e.(f.n.)$, chemical shift of protons of the free enzyme (or nucleotide); $\delta com.$, chemical shift of protons of the enzyme (or nucleotide) in the complex. For further details, see the Materials and Methods section.

Complex with	$\Delta\delta = \delta com. - \delta f.e.$				$\Delta\delta = \delta com. - \delta f.n.$		
	His-12 C2-H	His-119 C2-H	His-48 C2-H	Aromatic signal 1	C5-H	C6-H	C1'-H
2'-CMP	-0.71	-0.15	-0.10	0.30	-0.10	-0.28	-0.12
3'-CMP	-0.62	-0.15	-0.07	0.30	-0.08	-0.25	-0.06
HOPr-3'-CMP	-0.01	-0.15	-0.10	0.30	-0.10	-0.28	
HOEt-2'-CMP	-0.42	-0.15	-0.10	0.40	-0.20	-0.14	
Ara-3'-CMP	-0.52	-0.15	-0.07	0.30	-0.07	0	-0.28

Fig. 4. C.d. spectra of the complexes of RNAase A with HOPr-3'-CMP and HOEt-2'-CMP and difference c.d. spectra appearing when the nucleotides are bound to the enzyme

The spectra of the complexes were calculated similarly to those in Fig. 2. (a) (1) C.d. spectrum of RNAase A in complex with HOPr-3'-CMP; (2) c.d. spectrum of RNAase A. (b) (1) C.d. spectrum of RNAase A in complex with HOEt-2'-CMP; (2) c.d. spectrum of RNAase A. (c) Difference c.d. spectrum originating when the complex of RNAase A with HOPr-3'-CMP is being formed. (d) Difference c.d. spectrum originating when the complex of RNAase A with HOEt-2'-CMP is being formed. Other conditions are as described in the Materials and Methods section.

The inversion of the sign of the Cotton effect found as a free 3'-CMP molecule is being bound by the protein fits in well with the fact that, according to the data obtained by p.m.r. (determination of Nuclear Overhauser Effect), this binding is accompanied with a change of the *syn* conformation of the nucleotide to an *anti* conformation (Karpeisky & Yakovlev, 1975, 1976).

A somewhat different situation arises when we consider the c.d. spectra of the complexes between RNAase A and 2'-CMP. As Fig. 6 shows, the conformational contribution of 2'-CMP to the difference c.d. spectra of the complex in the region of the B_{2u} transition of the heterocyclic base is very close to zero. It might be expected therefore that the conformation of 2'-CMP in complex with RNAase A is the same as the preferential conformation of this nucleotide in solution, which constitutes over 90% of *syn* conformers in the population (Karpeisky & Yakovlev, 1975). However, a *syn* conformation has been found to be ruled out for 2'-CMP in complex with RNAase A (Karpeisky & Yakovlev, 1976).

Thus the same c.d. spectrum for 2'-CMP in a free state and in complex with RNAase A stands for two different nucleotide conformations, namely *syn* and *anti* respectively. Since each conformation is characterized by a sufficiently wide range of values of the ϕ_{C-N} torsion angle, it seems plausible that conformers of different type, but having rather close values of ϕ_{C-N} hardly differ in parameters of their c.d. spectra in the region of B_{2u} transition of the nucleotide heterocyclic base. At the same time, though 2'- and 3'-CMP bound at the active site of RNAase A are in an *anti* conformation, the 'proper' c.d. spectra of these nucleotides differ in the amplitude and sign of the Cotton effect in the B_{2u} absorption band of cytosine. Consequently, even small changes in the value of the ϕ_{C-N} torsion angle of nucleotides, within the range typical of a *syn* or an *anti* conformation, can produce a significant effect on the amplitude and sign of the Cotton effect in the B_{2u} band.

The results so obtained are additional proof of the concept, which is now accepted by several authors,

Fig. 5. Downfield region of p.m.r. spectra of RNAase A, and of difference p.m.r. spectra of RNAase A in complex with nucleotides

Chemical shifts are expressed in p.p.m. relative to hexamethyldisiloxane (external standard). Numbers 12, 48, 105 and 119 stand for signals of C2-H protons of histidine residues in the complexes of RNAase A. I and II are signals of protons in the aromatic amino acid residues which shift as the complexes are being formed. Other conditions are described in the Materials and Methods section.

that there is no direct correlation between the c.d. spectrum of a nucleotide and its conformation.

This study has shown that non-additivity of the c.d. spectrum of the enzyme-nucleotide complex in the region of 250–320 nm is determined by the interaction between the asymmetric field of the protein matrix and the B_{2u} transition of the base, on the one hand, and by a change in the nucleotide conformation, on the other. The 'proper' c.d. spectra of 2'-CMP and 3'-CMP in complexes with RNAase A, and the contribution to the c.d. spectra of enzyme-nucleotide

complexes caused by the asymmetric field of the protein and/or by a change in the nucleotide conformation, were determined by studying the c.d. and p.m.r. spectra of complexes of RNAase A with nucleotides and their derivatives.

It would be safe to state that the effect of the protein on the c.d. spectrum of enzyme-nucleotide complexes, found in the present work for RNAase A, must be expected to be encountered in the c.d. spectra of any enzyme-ligand complex, and should therefore be taken into account when interpreting these spectra.

Fig. 6. Calculated c.d. spectra of 2'-CMP and 3'-CMP bound to RNAase A

(a) Contribution to the c.d. spectrum of the complex of 3'-CMP with RNAase A caused by a change in the nucleotide conformation. (b) Contribution to the c.d. spectrum of the complex of 2'-CMP with RNAase A caused by a change in nucleotide conformation. (c) (1) C.d. spectrum calculated for 3'-CMP in complex with RNAase A; (2) c.d. spectrum of 3'-CMP in solution. (d) (1) C.d. spectrum calculated for 2'-CMP in complex with RNAase A; (2) c.d. spectrum of 2'-CMP in solution.

References

- Beaven, G. H., Holiday, E. R. & Johnson, E. H. (1955) in *Nucleic Acids* (Chargaff, E. & Davidson, J. N., eds.), vol. 1, pp. 493–553, Academic Press, New York
- Cohn, W. E. (1950) *J. Am. Chem. Soc.* **72**, 2811–2812
- Crestfield, A. M., Stein, W. H. & Moore, S. (1963) *J. Biol. Chem.* **238**, 618–621
- Gorenstein, D. G. & Wyrwicz, A. (1974) *Biochemistry* **13**, 3828–3836
- Haar, W., Maurer, W. & Ruterjans, H. (1974) *Eur. J. Biochem.* **44**, 201–211
- Horwitz, J. & Strickland, E. H. (1971) *J. Biol. Chem.* **246**, 3749–3752
- Hummel, J. P., Ver Ploeg, D. A. & Nelson, C. A. (1961) *J. Biol. Chem.* **231**, 3168–3172
- Karabashyan, L. V., Dudkin, S. M., Shlyapnikov, S. V. & Karpeisky, M. Ya. (1973) *Izv. Akad. Nauk. SSSR Ser. Khim.* 1999–2003
- Karpeisky, M. Ya. & Yakovlev, G. I. (1975) *Bioorg. Khim.* **1**, 749–757
- Karpeisky, M. Ya. & Yakovlev, G. I. (1976) *Bioorg. Khim.* **2**, 1221–1228
- Kritzyn, A. M., Mikhailov, S. N., Kolobushkina, L. I., Padyukova, N. Sh. & Florentiev, V. L. (1975) *Izv. Akad. Nauk. SSSR Ser. Khim.* 1846–1850
- Meadows, D. H., Roberts, G. C. K. & Jardetzky, O. (1969) *J. Mol. Biol.* **45**, 491–511
- Milles, D. W., Robins, M. J., Robins, R. K., Winkley, M. W. & Eyring, H. (1969a) *J. Am. Chem. Soc.* **91**, 824–831
- Milles, D. W., Robins, M. J., Robins, R. K., Winkley, M. W. & Eyring, H. (1969b) *J. Am. Chem. Soc.* **91**, 831–838
- Nagyvary, J. & Provenzale, R. G. (1969) *Biochemistry* **8**, 4769–4775
- Oshima, T. & Imahori, K. (1971) *J. Biochem. (Tokyo)* **70**, 193–195
- Raj Bhandary, U. L. (1968) *J. Biol. Chem.* **243**, 556–564
- Richards, F. M. & Wyckoff, H. W. (1971) *Enzymes 3rd Ed.* **4**, 647–806
- Rogers, G. T. & Ulbricht, T. L. V. (1970) *Biochem. Biophys. Res. Commun.* **39**, 414–418
- Rogers, G. T. & Ulbricht, T. L. V. (1971) *Eur. J. Biochem.* **22**, 457–466

- Sakharovsky, V. G., Chervin, I. I., Yakovlev, G. I.,
Dudkin, S. M., Karpeisky, M. Ya., Shlyapnikov, S. V.
& Bystrov, V. F. (1973) *FEBS Lett.* **33**, 323-326
- Sela, M. & Anfinsen, C. B. (1957) *Biochim. Biophys. Acta*
24, 229-235
- Simons, E. R. (1971) *Biochim. Biophys. Acta* **251**, 126-131
- Strickland, E. H. (1972) *Biochemistry* **11**, 3465-3474
- Venkstern, T. V. & Bayev, A. A. (1967) *Absorption
Spectra of Minor Components and Oligonucleotides of
Ribonucleic Acids*, p. 35, Nauka, Moscow