Contribution of Tyrosine Residues to the Optical Activity of Ribonuclease S

Sir:

We have undertaken the task of explaining the circular dichroism (CD) spectrum that arises from the tyrosine residues of the enzyme ribonuclease (RNase) S. An earlier investigation of the CD spectrum of the lowest energy tyrosine bands of RNase S has been reported by Strickland.[1] In the present study, we are primarily concerned with the higher energy ^1L_a bands. Our goal has been to ascertain the origin of the well-characterized anomaly in the CD spectrum of RNase S, which reveals itself as a region of positive ellipticity near 240 nm.[2-5] Since there is experimental evidence that this feature might arise from tyrosine residues,[2-5] it should be possible to determine its origin by the application of theoretical methods which we have successfully applied to a number of model compounds that contain phenolic chromophores.[6-8]

The theoretical formalism which we have utilized is similar to that developed by Bayley, Nielsen, and Schellman,[9] as extended by Hooker and Schellman[7] and Madison and Schellman.[10] It is a matrix-based theory which includes the configuration interaction of excited states. Thus, it is possible to express the rotatory strength of a given electronic transition in terms of interactions with all the other transitions of the molecule. The method inherently includes all of the common mechanisms by which optical rotatory power is generated by the interaction of molecular groups. However, the theory used for these calculations does differ from those utilized earlier in that certain modifications have been made to minimize origin dependence, which can arise when the Heisenberg transformation is applied to a limited basis set.[9] Nevertheless, the overall philosophy and structure of the theoretical formalism are unchanged.

In order to represent the dispersed nature of the π-electrons in the aromatic chromophores, all calculations were carried out in the distributed monopole approximation.[7] Furthermore, since the value which is chosen for the effective dielectric constant can affect the extent of interaction between electronic transitions, calculations were carried out for dielectric constants of both one and two.

The spectroscopic data which are required for the calculations are the same as those which have been used previously for model studies. Data for the peptide chromophores were taken from the paper by Bayley, Nielsen and Schellman.[9] Optical parameters for tyrosine were also the same as those used in previous investigations.[6-8] Even though RNase S contains amino acids such as phenylalanine, histidine, and cystine, which might be expected to make significant contributions to the CD spectrum, no attempt has been made to include side chain chromophores other than tyrosine in the calculations at this time.

Atomic coordinates for this investigation were based on the results of the X-ray diffraction study of RNase S by Richards, Wyckoff, and coworkers.[11] Our final calculations were based on the 7B set of coordinates which has been refined to fit X-ray intensities. These coordinates were kindly supplied to us by H. W. Wyckoff and T. Powers of Yale University. Hydrogen atoms were added to the α-carbon atoms, the peptide nitrogen atoms, and all of the atoms of the tyrosine side chains by assuming standard bond angles and bond distances.[12]

Atomic static charges for all atoms were determined from the data of Poland and Scheraga.[13] Hydrogen atoms were not added to the side chains of amino acid residues other than tyrosine, so the static charges of the affected atoms were adjusted to compensate for this fact.

Although the theory and associated programs are capable of carrying out the calculation of the optical properties of the entire RNase molecule, we have not attempted to do so at this time. Instead, we have included only the α-helical peptide residues, the six tyrosine residues, and the peptide residues which are associated with the carbonyl groups of the tyrosines. Thus, the residues other than tyrosine which fall in disordered regions and regions of β structure, have not been included. Only residues 2 through 12, 26 through 33, 50 through 58, and tyrosines 25, 73, 76, 92, 97, and 115 have been included in the calculation.

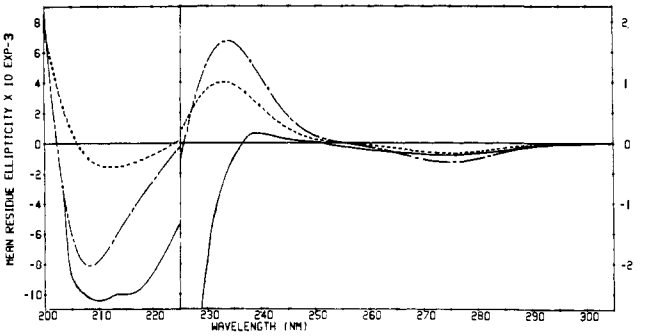

Figure 1. Theoretical and experimental circular dichroism curves for ribonuclease S. Line with alternating long and short dashes is theoretical curve for dielectric constant = 1. Line with short dashes is theoretical curve for dielectric constant = 2. Solid line is experimental curve based upon data from ref 2.

The results of our calculations are presented in Figure 1, where theoretical CD curves are shown for effective dielectric constants of one and two. The curves were calculated assuming Gaussian band shapes. All electronic transitions were assigned a half-width of 12 nm. The wavelength of each transition was set equal to the value determined in the calculation; i.e., calculated band splittings were used to calculate the theoretical CD curves. However, 7 nm was subtracted from the wavelength of each ^1L_b transition and 8 nm was added to the wavelength of each n-π^* transition, so as to bring the wavelengths of these bands into better agreement with experiment. This procedure is justified since the original wavelength of 282 nm[7] which was used in our calculations for the ^1L_b transition is artificially high. Furthermore, an energy of 212 nm was used for the n-π^* transition, because it occurs near that wavelength in aqueous solutions of simple amides.[9] However, it apparently occurs much higher in α-helical polypeptides,[14] so it was adjusted to 220 nm for purposes of calculating theoretical CD curves.

An experimental CD curve for RNase S in phosphate buffer at pH 6.4 and 4° is also shown in Figure 1. The data for the experimental curve were taken from the work of Pflumm and Beychock.[2]

The results of our calculations for the ^1L_b transition appear to agree reasonably well with the experimental data, the experimental curve falling between the two theoretical curves. Examination of the ^1L_b band shapes on the lower wavelength side of the curves indicates that the value of 12 nm which was used for the bandwidth is too small. However, our results for the ^1L_b region are in reasonable agreement with the results of Strickland.[1] We do not calculate exactly the same rotatory strengths for several of the residues, but this is to be expected since we have not included the same chromophoric groups in our calculations.

As can be seen from Figure 1, our calculation does predict positive ellipticity in the region of 240 nm. This band arises entirely from the ^1L_a transitions of the tyrosine residues, which were centered at 226 nm in the calculation. The net rotatory strength was 0.019 DM for a dielectric constant of 1 and 0.006 DM for a dielectric constant of 2. Most

of this rotatory strength arises from tyrosine 92, which contributes 0.56 DM (ϵ = 2). However, a portion of this apparently arises from exciton interactions with tyrosines 25 and 97, which contribute rotatory strengths of −0.20 and −0.16 DM, respectively. All other ^1L_a transitions develop rotatory strengths at least an order of magnitude lower.

It is difficult to make definite assignments as to which residues are interacting with tyrosine 92, since all the states are mixed in the Hamiltonian matrix. However, one can obtain an idea as to the importance of certain interactions from the magnitude of the off-diagonal elements of the secular equation. On this basis, it appears that tyrosine 92 is interacting to a significant extent with the peptide transitions of residues 25 through 33, tyrosine 97, and its own peptide transition. However, it must be remembered that these off-diagonal terms of the secular equation are only interaction energies, so a large value for an off-diagonal term does not necessarily indicate a large contribution to the rotatory strength.

In any event, it is obvious that we can calculate sufficient rotatory strength from the ^1L_a bands of the tyrosine residues of RNase S to account for the experimentally observed region of positive ellipticity near 240 nm. In fact, as is apparent from Figure 1, the mean residue ellipticity in this region of the spectrum is grossly overestimated. There are several factors which could be responsible for this. The spectrum was calculated for a perfectly rigid conformation, whereas there certainly must be vibrational oscillations present in the real molecule which would be expected to diminish the intensity of the band. Furthermore, as was indicated above, the bandwidth of the ^1L_b transitions which was used to compute the theoretical curves was probably too small. Since the Cotton effects of the ^1L_b and ^1L_a bands are of opposite signs, a wider bandwidth for ^1L_b would reduce the magnitude of the ellipticity in the 240-nm region. In fact, if the bandwidths are regarded as adjustable parameters, the calculated curves can be brought into much better agreement with experiment. However, the most significant factor in the present case is simply that the rotatory strengths of the peptide n-π^* transitions have been seriously underestimated.

We have not really given much attention to the peptide bands. In fact, the peptide bands were originally included in the calculation simply to produce the required red shift of the ^1L_a band. However, the ellipticity calculated for the π-π^* region near 210 nm, with a dielectric constant of unity, appears to agree reasonably well with the experimental spectrum. Nevertheless, our calculation seriously underestimates the rotatory strength which should arise from the n-π^* transitions. Underestimation of the n-π^* rotatory strength is a problem which is common to other theories of the optical activity of polypeptides.[10] However, the discrepancy is even greater in the present case. This appears to be a property of the coordinate set which was used for these calculations. Preliminary calculations, which were carried out with RNase S coordinate set 2 (map 20) gave much larger rotatory strengths for the n-π^* transitions, in good quantitative agreement with the results reported by Madison and Schellman.[10] The origin of this difference is currently under investigation.

In conclusion, it appears that the region of positive ellipticity, which can be observed near 240 nm in the CD spectra of RNase S, definitely includes major contributions from the ^1L_a transitions of the tyrosine residues, especially from tyrosine 92. It is possible that other chromophoric groups might make contributions in this region. For example, the cystine residues could give rise to large rotatory strengths. However, the rotatory strengths which are calculated for the tyrosines are certainly sufficient to account for the effect.

The positive CD band near 240 nm might be somewhat weaker in RNase S than in RNase A.[2,4] This difference may be associated with a conformational difference in the vicinity of tyrosine 92. A more detailed investigation of the differences in the CD spectra of RNase A and RNase S is planned.

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Detection and Characterization of Radical Cations Produced by One-Electron Chemical and Electrochemical Oxidations of Organocobalt Compounds

Sir:

We wish to report the direct detection and characterization of radical cations produced by one-electron oxidation of organocobalt(III) compounds.

The study of the chemical and electrochemical *reduction* of organocobalt compounds has received considerable attention, particularly in the context of the chemistry of vitamin B_{12} derivatives and models.[1-4] In contrast, the corresponding *oxidation* processes have not thus far been extensively investigated and are poorly characterized. Studies on the oxidative cleavage of benzylaquobis(dimethylglyoximato)cobalt(III) provide indirect evidence for transient one-electron oxidation products (*formally* organocobalt(IV) species)[5-7] as do brief reports of electrochemical studies on organocobalt Schiff's base complexes.[4,8] We now report the direct detection and characterization of such species.

Spectral titrations at −78° of acidic aqueous methanol solutions (80 vol % methanol) of various organobis(dimethylglyoximato)cobalt(III) complexes ($[RCo(DH)_2(H_2O)]$, abbreviated [CoR] where R = alkyl or benzyl) with cerium(IV) nitrate demonstrated the occurrence of a stoichiometric 1:1 reaction, in accord with eq 1.

$$[RCo(DH)_2(H_2O)] + Ce(IV) \longrightarrow [RCo(DH)_2(H_2O)]^+ + Ce(III) \quad (1)$$