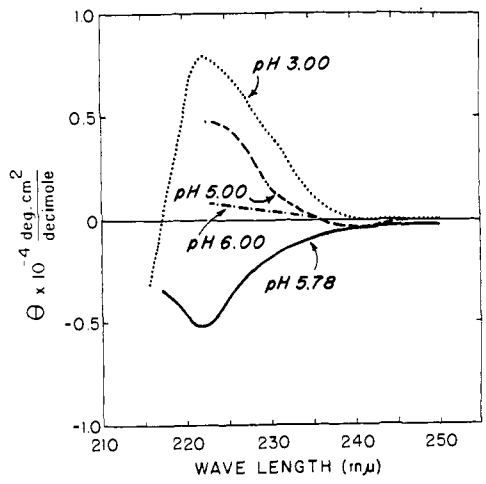

Figure 3. Circular dichroism spectra of poly-L-histidine in acetate buffer at ionic strength 0.10. Measurements were performed on a Jouan Dichrographe modified for 10-fold increased sensitivity. Formulas for calculating θ are given elsewhere[8]; 1-mm. and 0.5-mm. cell paths were employed. The same curve was obtained at pH 5.78 in unbuffered solution at ionic strength 0.10; the temperature was 25 ± 0.1°.

rather that it reflects the increasing dominance of a positive Cotton effect at shorter wave lengths.

In Figure 3 are shown the CD spectra at several pH values. As the pH is raised through the transition interval a negative band appears, the wave length maximum and sign of which are characteristic of the right-handed α-helix.[6] The magnitude of the maximum ellipticity is, however, only about $^1/_6$ of that observed for helical poly-α-L-glutamic acid.[12] There are at least two possible explanations for this. The first is that the molecule is only partially helical. The second is that the side chains contribute an oppositely signed band which effectively diminishes the ellipticity band arising from the peptide bonds in α-helical segments. At pH 3.0, the large positive band occurs at wave lengths corresponding to a known absorption band of the imidazolium ring in histidine.[13] The magnitude of the positive band would appear to be too large for the positive ellipticity band in this spectral interval which is associated with random coil polypeptides,[14,15] and the ORD at pH 4.0 shown in Figure 2 seems entirely incompatible with random coil optical activity uncomplicated by substantial side-chain contributions.

Urry and Eyring have recently reported the ORD spectra of the free amino acid.[16] They infer that the imidazolium ring contributes no Cotton effect in the 220-mμ region of the spectrum. Indeed, circular dichroism measurements of histidine performed in this laboratory reveal no band other than that due to the carboxyl, at wave lengths longer than 208 mμ. It does not necessarily follow, of course, that the 215-mμ imidazole absorption band is optically inactive in the random coil polymer at pH 3.0. We are currently examining the CD spectra of histidine-containing peptides. These results, along with the results of an investigation of the interaction of helical poly-L-histidine with hemin, will be reported in due course.

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(17) Research Career Development Awardee of the United States Public Health Service.

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The Cotton Effect Associated with Certain Tyrosine Residues in Ribonuclease[1]

Sir:

Conformation-dependent Cotton effects associated with the aromatic absorption bands of tryptophan and tyrosine have recently been reported in several proteins[2-4] and in poly-L-tyrosine.[5] Little information is

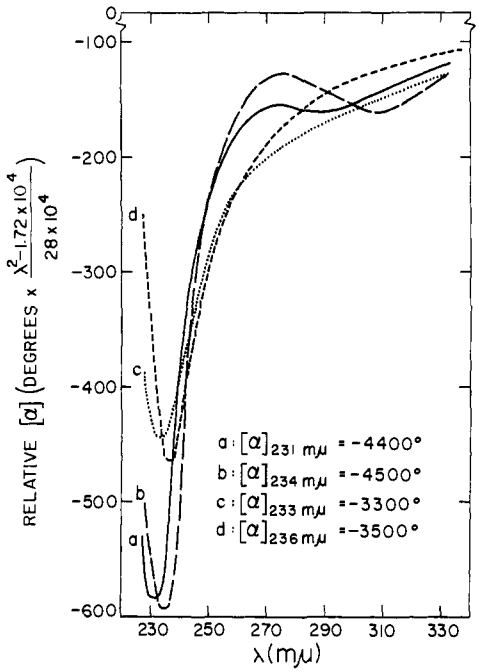

Figure 1. The ultraviolet optical rotatory dispersion of 0.48% pancreatic ribonuclease, in a 1-mm. cell, in (a) 0.15 *M* phosphate buffer at pH 6.2; (b) 0.15 *M* glycine–NaOH buffer at pH 11.5; (c) 0.1 *N* HCl; (d) 1.5% sodium dodecyl sulfate (recrystallized from isopropyl alcohol).

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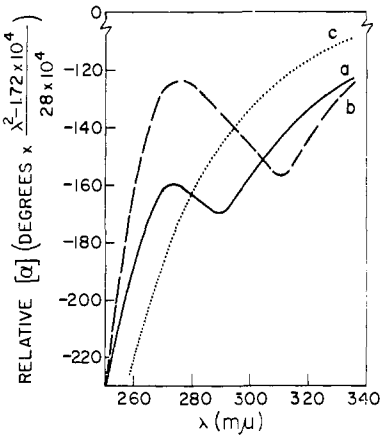

Figure 2. Rotatory dispersion of ribonuclease in the region of the tyrosine Cotton effect. Ribonuclease (0.48%, in a 0.5-cm. cell) (a) in 0.15 *M* phosphate buffer at pH 6.2; (b) in 0.15 *M* glycine–NaOH buffer at pH 11.5; (c) in 1.5% sodium dodecyl sulfate.

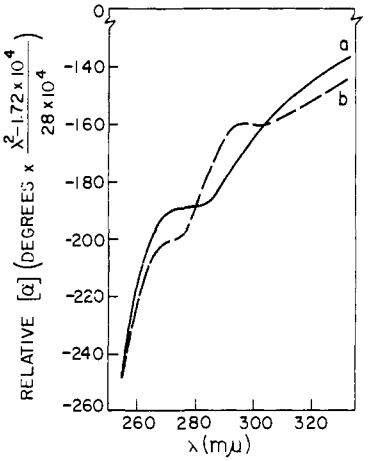

Figure 3. Rotatory dispersion of ribonuclease in the region of the tyrosine Cotton effect. Ribonuclease (0.48%, in a 0.5-cm. cell) in (a) 0.1 *N* HCl; (b) 0.1 *N* NaOH. The curves were obtained with freshly prepared solutions.

available, however, as to the nature of the interactions responsible for the production of these Cotton effects.

Since three of the six tyrosine residues in pancreatic ribonuclease ionize normally and reversibly, but the others are "buried" and ionize only at pH >12 in an irreversible manner,[6,7] it might be possible to distinguish between the contributions of the "normal" and "buried" residues to the rotatory behavior of this enzyme. Further, the low α-helix content of ribonuclease and the absence of tryptophan reduce the ambiguity in such a study of the contribution of tyrosine to the behavior of the optical rotatory dispersion (ORD) of this enzyme in the aromatic absorption region. In this communication, we wish to present evidence showing that a conformation-dependent tyrosine Cotton effect in pancreatic ribonuclease arises as a consequence of the asymmetric environment of the *normally ionizing* tyrosine residues in the native protein.

Crystalline, salt-free, beef pancreatic ribonuclease was obtained from Worthington Biochemical Corp., Lot No. R 609. ORD measurements were made with a modified Bendix Polarmatic recording spectropolarimeter. The ORD curves of ribonuclease, under various conditions, in the wave length range 220–350 mμ, are shown in Figure 1. The region of aromatic absorption (250–340 mμ) is shown in greater detail in Figures 2 and 3. The relative rotation given in these figures has been corrected in the usual manner for path length and concentration. To convert the relative rotation to a conventional [α], the values must be multiplied by the Verdet correction $28 \times 10^4/[\lambda^2 - (1.72 \times 10^4)]$. This has been done for the values of [α] at the minima of the curves shown in Figure 1.

The tyrosine Cotton effect observed in native ribonuclease at pH 6.2 is shown in Figure 2a. On exposure of ribonuclease to pH 11.5, the Cotton effect, centered at 278 mμ in neutral solution, shifted to 292 mμ (Figure 2b). This shift parallels that in the aromatic absorption band of tyrosine on going from the phenolic to the phenoxide form. Thus, the anomalous dispersion would seem to arise from the *accessible* residues, which are all fully ionized at pH 11.5. In contrast, ribonuclease in 0.1 *N* NaOH no longer showed the negative Cotton effect in the tyrosine absorption region but, instead, what appeared to be a very small positive Cotton effect (Figure 3b). This indicates that the ionization of the three "buried" tyrosine residues occurs concomitantly with the disruption of the interactions conferring optical activity on the accessible residues. This interpretation is consistent with the fact that the phenolic titration curve is fully reversible up to pH 11.5, while that obtained on exposure of the enzyme to pH values above 12 is irreversible.[6,7]

On acidification of ribonuclease to pH 1, the tyrosine Cotton effect may be seen to be greatly diminished (Figure 3a). Studies on ribonuclease by ultraviolet difference spectrophotometry[8] and by the "solvent perturbation" technique[9] indicate that only two of the inaccessible tyrosine residues become exposed on acidification to pH 1. Exposure of ribonuclease to sodium dodecyl sulfate (Figure 2c) results in a disappearance of the tyrosine Cotton effect. Bigelow and Sonenberg[10] have shown that in sodium dodecyl sulfate (0.01–0.1 *M*) only one of the "buried" tyrosine residues is exposed. These observations support the conclusion that interactions involving the "buried" tyrosine residues in ribonuclease do not make a major contribution to the tyrosine Cotton effect.

It should be noted that the tyrosine Cotton effect in ribonuclease is negative, in contrast to the positive Cotton effect obtained with the free amino acid.[5,11] Such a negative Cotton effect may be seen, however, in the ORD curve of an 8:2 copolymer of L-glutamic acid and L-tyrosine studied by Fasman, *et al.*[5] No comment on the sign of this Cotton effect was made by the authors.

From the availability of the tyrosine residues in native ribonuclease to iodination it has been concluded that two of the "buried" residues are tyrosines 25 and 97, while tyrosines 92 and 115 are readily available to

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iodination.[12,13] No definite information is available as to the remaining two residues. From our data it appears likely that residues 92 and 115 contribute to the aromatic Cotton effect.

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The Radical Anion of Dodecamethylcyclohexasilane[1]

Sir:

Radical anions are well known in which unpaired electrons occupy low-lying antibonding π molecular orbitals,[2] but only recently have radical anions of saturated systems (cyclopropane[3] and adamantane[4]) been reported. These latter discoveries prompted us to investigate the possibilities of radical-anion formation from alkylpolysilanes, wherein low-lying d orbitals might play an important role in accepting an electron. This communication reports the reduction of dodecamethylcyclohexasilane to give a paramagnetic species. The electron spin resonance spectrum of the species suggests that it is the anion radical of the parent cyclosilane, $[Si(CH_3)_2]_6^-$, in which the unpaired electron is delocalized over all six silicon atoms.

Reaction of a solution of dodecamethylcyclohexasilane in the mixed solvents tetrahydrofuran–1,2-dimethoxyethane (2:1) with sodium–potassium alloy at $-95°$ results in a blue-green solution which exhibits strong electron paramagnetic resonance. The spectrum becomes fully resolved upon warming to $-75°$. At low gain (Figure 1) it consists of about 19 equally spaced lines, with spacing 0.53 gauss and line width 0.11 gauss, centered at $g = 2.0032$. The relative intensities of the 13 central lines are 11:14:39:53:80:95:100:95:79:56:35:12:9, corresponding fairly well to the intensities expected for the central lines of a 37-line distribution, which would be expected if the unpaired electron interacted equally with all 36 protons.

At higher gain about 25 of the expected 37 lines can be observed (Figure 2), but the pattern is complicated by the presence of two satellite spectra which appear to replicate the principal spectrum. The first has an intensity of about 5% of the main spectrum and corresponds to a doublet with a splitting of 15.8 gauss. Each half of the doublet contains at least 13 lines ($a = 0.53$ gauss, line width = 0.11 gauss). The second, also a doublet, has an intensity about 12% of that of the main spectrum. It contains at least 11 lines in each half, also with $a = 0.53$ gauss and line width 0.11 gauss. Because of the small hyperfine splitting of 5.25 gauss, the second satellite is almost superimposed on the main resonance lines. Within each satellite cluster, the relative line intensities correspond roughly to the binomial distribution for $n = 36$ mentioned above.

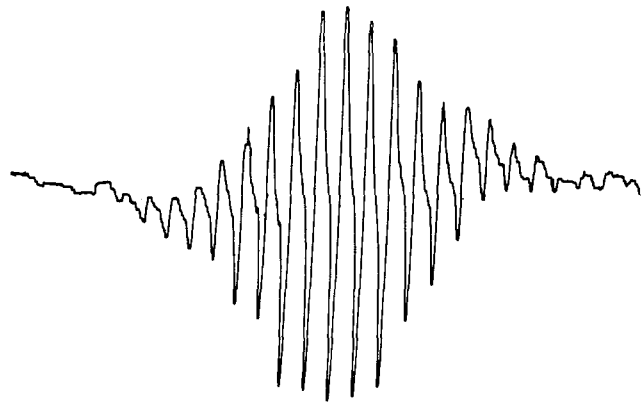

Figure 1. E.s.r. spectrum of radical anion at low gain, showing relative intensity of central lines.

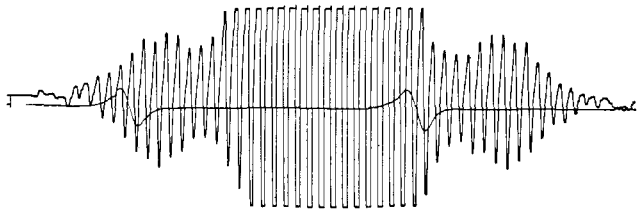

Figure 2. E.s.r. spectrum of radical anion at high gain, showing satellite spectrum with splitting 15.8 gauss attributed to ^{13}C. The spectrum of peroxylamine disulfonate anion in water, recorded simultaneously (double cavity), is superimposed for calibration.

The two doublets are not due to interaction with an alkali metal,[5] for the spectrum is identical when the anion radical is prepared using pure sodium or pure potassium instead of a mixture of the two metals. Tentatively, the satellites are assigned to splitting by carbon-13 and silicon-29. In the system under consideration these isotopes should produce doublet satellite patterns with relative intensities of 6.7 and 14.2% of the main spectrum, respectively, if delocalization is complete.

The observed e.s.r. spectrum suggests that the unpaired electron is delocalized equally over all six silicon atoms and so contacts equally all of the protons on the twelve methyl groups of the molecule. If this interpretation is correct, the unpaired electron must reside in a π- (or δ-) type orbital made up of 3d orbitals from the six silicon atoms.[6] In this model, because d-orbital combinations are considered, the six silicon atoms do not necessarily have to be coplanar for effective delocalization over the ring. However, ring interconversion is required to make all of the methyl groups equivalent on a time-average basis.

The e.s.r. signal was observed with undiminished intensity up to about $-50°$. Above this temperature the signal disappears rapidly and the solution becomes

(1) This research was supported by a contract from the Atomic Energy Commission and a grant from the Air Force Office of Scientific Research.
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