of excess 1-methylimidazole, expected to give ionic $[Cr(1\text{-}MeIm)_2(TPP)]^+[C_{60}^{\bullet}]^-$, and are of comparable magnitude to values reported for related 1:1 electrolytes in organic solvents.[15] In saturated frozen THF solution, the temperature-dependent EPR signals shown in Figure 3 are observed. Signals expected for Cr(III) are absent. This is because chromium(III) porphyrin signals extend over ca. 7000 G[15] and detection is limited by solubility. By comparison to previous EPR studies of $C_{60}^{\bullet-}$ which show signals centered at $g = 1.999$ (2),[2] 2.001,[4] and 1.997,[17] the observed signals centered at $g = 1.995$ are ascribed to $C_{60}^{\bullet-}$. Since coordination of $C_{60}^{\bullet-}$ to Cr(III) would be expected to lead to an EPR silent, integral-spin species via magnetic coupling, the observed signals indicate an ionic formulation. We are currently investigating the origin of the anisotropy of the observed signal.

The complex is very air sensitive in both solid and solution states. Visible spectral monitoring indicates that the oxidation products are C_{60} (λ_{max} 329 nm) and a mixture of $Cr^{IV}O(TPP)$ and $Cr^{III}_2(\mu\text{-}O)(TPP)_2$ (Soret 432 and 415 nm, respectively).[17] See the dashed line in Figure 1.

Spectral observations indicate that $Al^{III}(TPP^{\bullet-})$ (Soret 450 nm) is another metalloporphyrin complex which reacts with C_{60} to give a related $C_{60}^{\bullet-}$ redox product (Soret 420 nm).

In summary, with Cr(TPP) we have illustrated a productive route to isolable crystalline complexes having $C_{60}^{\bullet-}$ as the counterion. Unable to compete in solution with THF for ligation to $[Cr(TPP)]^+$, $C_{60}^{\bullet-}$ is a novel example of a very weakly coordinating anion. We are currently probing the details of the structural and magnetic interactions and exploring the generality of this synthetic route which opens up the possibility of isolating complexes with coordinated $C_{60}^{\bullet-}$.

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Unavoidable Time-Dependent Ellipticity Changes of Proteins in the Current CD Measurements

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What we report now is believed to be an unusually urgent topic for many investigators who measure circular dichroism (CD) spectra of proteins. We communicate here about unexpected results obtained with J-600 and J-720 of the Jasco J-series CD apparatus, which have been used by many investigators all over the world. A recent innovation of the CD apparatus has made it possible to magnify a slight change of ellipticity. The magnification itself seems not to be difficult. However, since the magnification was done over the full scale range of ellipticity in the measurements with a Jasco CD apparatus older than J-500, the magnification was limited by the capacity of the Y axis of the X-Y recorder. In other words, since it was impossible to magnify some particular part of ellipticity, for example, from 100 mdeg to 102 mdeg, we might have overlooked a slight, but significant, change.

It was found that the ellipticity of a protein decreases with an increase in measurement time. All of the present data were

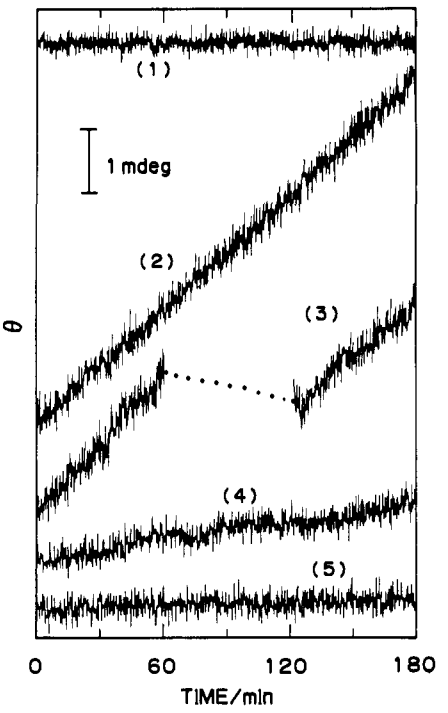

Figure 1. Time-dependent ellipticity changes of pantolactone and proteins at 25 °C: (1) ellipticity (θ) of 0.10% pantolactone at 219 nm, $\theta_{t=0} = -125.5$ mdeg; (2) and (3) θ of 1.2×10^{-5} M BSA at 222 nm, $\theta_{t=0} = -140.5$ mdeg. In the case of (3), the irradiation was cut off for 1 h; (4) θ of 1.0×10^{-5} M myoglobin at 222 nm, $\theta_{t=\theta} = -38.0$ mdeg and (5) θ of 6.8×10^{-6} M ribonuclease A at 210 nm, $\theta_{t=\theta} = -7.5$ mdeg. An upward change corresponds to a decrease of negative ellipticity. The light path length of the cell was 1 mm.

obtained by using CD cell thermostatted at 25 °C with a LAUDA RM6 and by running the apparatus in a room thermostatted at 23 ± 1 °C. Figure 1 shows a time-dependent ellipticity change of bovine serum albumin (BSA), myoglobin, and ribonuclease A together with the stability of the apparatus checked with pantolactone. The stability, checked with an ellipticity drift of pantolactone, was less than 0.2% of its total strength. However, the negative ellipticities of proteins decreased with time. The decrease continued for 11 h, the longest running time in the present work. The decrease at 222 nm was approximately 1.8 mdeg/h (1.3% of its initial strength/h) for BSA. A positive ellipticity of BSA at 192 nm also decreased with measurement time. Time dependence of ellipticity decrease was distinctly observed also for myoglobin (2.3% of its initial strength/h), while it was slight for ribonuclease A. These are results obtained with J-600 (band width, 1.0 nm). The same phenomena were observed with J-720. The ellipticity decrease of BSA at 222 nm was 1% of its initial strength/h with J-720 (band width, 0.5 nm). Figure 1 also shows the effect by the obstruction of the irradiation to the BSA sample. The irradiation was obstructed by leaving a metal of appropriate size in the light path. The ellipticity of BSA did not change as long as the light was cut off. The ellipticity began again to decrease substantially from the magnitude attained before the obstruction of light path. Since temperature of the sample part was confirmed to be unchanged by measuring it in the same situation as usual CD measurements (the CD cell was thermostatted, as mentioned above), there was no possibility that the proteins suffered thermal denaturation due to a rise in temperature (2-3 °C) of the sample room.

The dependence of ellipticity change on the measurement time might be due to some photoreaction in proteins, to which no attention has been paid so far. In order to examine the nature of the photoreaction, the reaction was prominently caused in a short time, as follows. The sample cell, the temperature of which was controlled at 25 °C by circulating water from the thermostat, was kept at the place which was 10 cm away from the lighting (arc discharge) point of a 450-W xenon lamp (the place where

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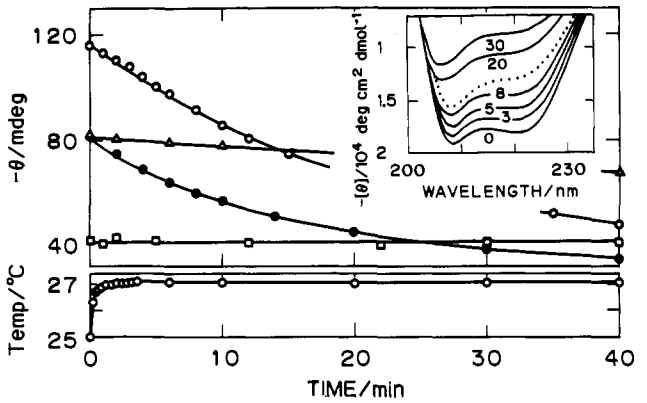

Figure 2. Ellipticity change of BSA (222 nm) and pantolactone (219 nm) as a function of irradiation time of the xenon lamp, dependence of CD spectra of BSA on the irradiation time (inserted figure), and the temperature (Temp) change with the irradiation time (below): (○) native BSA, (□) BSA in 6 M guanidine, and (△) pantolactone. These three were measured by circulating water at 25 °C into the cell: (●) BSA in the cell which was kept at 65 °C. In the inserted figure, numerical values indicate irradiaton time (min) of the xenon lamp (see text), and the dotted curve shows the CD spectrum of BSA denatured thermally at 65 °C. All data in this figure were measured with the cell with a light path length of 2 mm. Concentrations of BSA and pantolactone were 5.5×10^{-6} M and 0.032%, respectively.

a reflex mirror (M0) is set in a J-500 apparatus). Then, the cell was moved to another CD apparatus at appropriate intervals to measure the CD spectrum. Figure 2 shows the ellipticity change of BSA at 222 nm as a function of irradiation time of the xenon lamp and the dependence of its CD spectra on the irradiation time (inserted figure). In spite of circulating thermostatted water (25 °C) into the cell, the irradiation caused to ascend temperature of the sample part from 25 to 27 °C in the initial 1-min interval. However, it was unchanged thereafter (Figure 2). With an increase in the irradiation time, the ellipticity of BSA abruptly decreased at both 25 and 65 °C, although the ellipticity of pantolactone at 219 nm was almost unchanged. The change was not observed for BSA in 6 M guanidine. The ellipticity of the protein decreased at the same rate in sodium dodecyl sulfate (SDS), while it decreased more slowly in urea (not shown). The ellipticity change of BSA was smaller in urea than in SDS (not shown). The transformation of the CD spectrum induced by the present irradiation was apparently larger than that induced thermally at 65 °C (Figure 2). The CD spectra of BSA[1] and ribonuclease A[2] in various conditions can be well simulated by the curve-fitting method[3] with reference CD spectra determined by Chen et al.[4] The same reference spectra also gave a synthesized spectrum which excellently agreed with each experimental spectrum in Figure 2. The relative proportion of the α-helical structure was 18% for BSA irradiated for 45 min, as opposed to 66% in the native state.[1]

The present results indicate that the protein structure is altered by the irradiation of light used in the CD apparatus. The effect is less significant in the usual CD measurements, which require only several minutes for one sample, than those demonstrated in Figures 1 and 2. However, when the sample is left for a while in the light path, this effect becomes significant. This is the occasion when an experimental condition is changed as the cell is left in the light path. Indeed, many investigators are apt to leave the CD cell in the light path, when they measure CD changes with time or when they change temperature in the thermal denaturation studies. If the samples such as BSA and myoglobin are left for 10 h in the light path, 10–20% of total ellipticity would decrease due to this effect. The present phenomenon should be necessarily taken into consideration in the CD studies of proteins.

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Location of β-Sheet-Forming Sequences in Amyloid Proteins by FTIR

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The deposition of proteinaceous amyloid is characteristic of Alzheimer's disease (AD)[1] and type II diabetes.[2] Analysis of these amyloid deposits, or plaques, by X-ray diffraction[2,3] indicates that they are composed of fibrils which contain a structural motif that was first observed in the Bombyx mori silk fibril.[4] A low-resolution model for this structure, known as the cross-β fibril, was proposed by Pauling[4,5] but has not been significantly refined,[5] due to the fact that amyloid-forming proteins are extremely insoluble and do not readily crystallize.[6] Consequently, the intrastrand and interstrand interactions which direct formation of the constituent antiparallel β-sheet are not understood.[7,8] Krimm has suggested that isotope labeling of specific amides could be useful for the location of secondary structure within a protein sequence by Fourier transform infrared spectroscopy (FTIR).[9] Walters et al. have recently demonstrated that this approach is feasible for a complex, multiconformational peptide in solution.[10] We have extended this approach to allow the observation of specific transition dipole coupling interactions which are characteristic of β-sheet structure in the solid state. These studies reveal the existence of irregularities in cross-β fibril structure which cannot be otherwise observed and are incompatible with the Pauling model.

Antiparallel β-sheet structure is easily distinguished by FTIR due to a splitting of the amide I absorption which is caused by strong interstrand and, to a lesser extent, intrastrand transition dipole coupling (TDC) interactions.[9,11-13] This effect results in

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(13) The strength of TDC (ΔV) between two interacting dipoles can be estimated according to the following equation (α, β, and γ are angles which relate to the relative orientation of the dipoles, r = distance between the dipole centers).[10,11]

$$\Delta V \propto \frac{\cos \alpha - 3 \cos \beta \cos \gamma}{r^3}$$

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