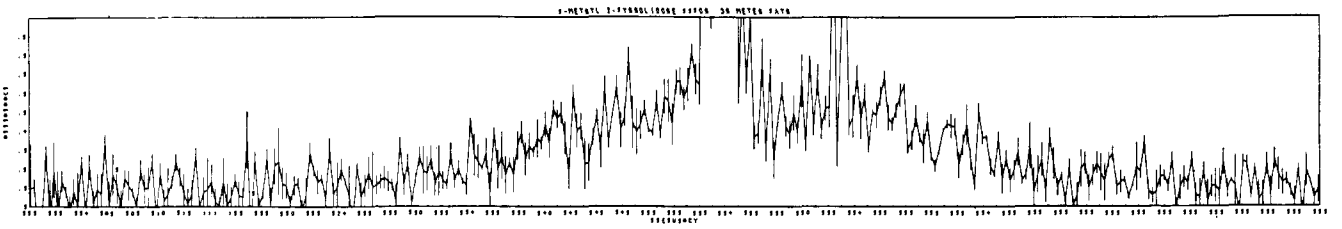

Figure 1. The infrared spectrum of *N*-methyl-2-pyrrolidone vapor from 100 to 200 cm^{-1}. The spectrum is of 39 m of gas at about 40°. The lines drawn on the spectrum are an indication of the noise level. The immense amount of structure seen in the spectrum is real.

spectrum also shows a complex series of bands with about a ten wave number spacing from 100 cm^{-1} to the lower limit of our spectrometer.

Under about 0.3-cm^{-1} resolution the spectrum of the gas phase splits into a vast series of lines. Part of this spectrum is shown in Figure 1. The number of bands and the spread of these bands over a few hundred wave number range in the gas-phase spectrum as well as the features of the liquid-phase spectrum can be explained by assigning the bands to transitions due to simultaneous changes of 0 or ±1 in three quantum numbers: n_1, the quantum number for the ring bending; n_2, the quantum number for the ring twisting; and m, the quantum number for the internal rotation of the methyl group. The spectrum is made complex by the complex nature of the motion of each low-frequency mode and by the large interactions among them. The out-of-plane bending has been predicted to have a double minimum potential.[4] The complexity of the spectrum makes a detailed assignment difficult, but it is the point of this note that the qualitative features of the spectrum cannot be explained without assuming a methyl rotation unhindered by a significant barrier. The spectrum we observe is qualitatively similar to the infrared spectra observed in molecules known to contain nearly free rotors.[5] (Some difficulties in interpreting the details of the spectra of even the much simpler molecules such as CH_3NO_2 remain unresolved![5])

It is difficult to put a lower limit on the barrier to internal rotation but nitromethane with a barrier of 6 cal and methylboron difluoride with a barrier of 14 cal show similar series of bands and so we postulate a barrier of this order of magnitude. All known molecules with barriers of this magnitude have been molecules in which there can be no threefold term in the barrier to internal rotation because of the symmetry of the molecule. This is not the case for our molecule and reasonable guesses for the barrier for the C^{α}–N bond of a polypeptide, for example, are on the order of 1000 cal/mol.[6] We cannot use the perturbation arguments of footnote 2 to explain the difference between our expectations and the observed result.

Instead we offer the following rationalization. Consider the methyl group rotated so that two of its hydrogens point at the two hydrogen atoms of methylene group 5. This would be expected to be a conformation of high energy, perhaps 1000 cal/mol above the minimum. However, in this configuration the remaining hydrogen of the methyl group points at the carbonyl oxygen. If we postulate an attraction between the hydrogen and the oxygen atoms,[7] we can explain the very low barrier as due to an "accidental" cancellation of the attractive and repulsive effects of the groups surrounding the methyl group.

Acknowledgment. It is a pleasure to acknowledge helpful conversations with Professors W. D. Gwinn, K. S. Pitzer, and A. Streitwieser, Jr.

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A Pronounced Solvent Effect on the Reversible Oxygenation of a Cobalt(II) Porphyrin System

Sir:

Numerous reports of 1:1 reversible oxygen complexes of cobalt(II) have appeared in recent years.[1] Although all of these complexes bind oxygen at low temperatures, only coboglobin[2,3] and a limited number of the cobalt Schiff-base complexes[4–6] reversibly bind oxygen 1:1 at room temperature. Many of the nonprotein cobalt complexes either lose oxygen or oxidize to a dimeric Co^{III}–O_2^{2-}–Co^{III} complex and/or other Co^{III} species. The ability of metal porphyrins to reversibly bind oxygen is of great biological significance since they are closely related to the oxygen carrying hemoproteins and coboglobin.

The reversible binding of oxygen to Co(II) porphyrins may be understood in terms of the equilibrium

$$\text{CoPpIXDME}\cdot\text{B} + \text{O}_2(\text{g}) \rightleftharpoons \text{CoPpIXDME}\cdot\text{B}\cdot\text{O}_2$$

$$K = [\text{CoPpIXDME}\cdot\text{B}\cdot\text{O}_2]/[\text{CoPpIXDME}\cdot\text{B}]p_{\text{O}_2}$$

where CoPpIXDME = cobalt(II) protoporphyrin IX dimethyl ester and B = base, *e.g.*, pyridine, 1-methylimidazole (CH_3-Im), dimethylformamide (DMF), etc.[7]

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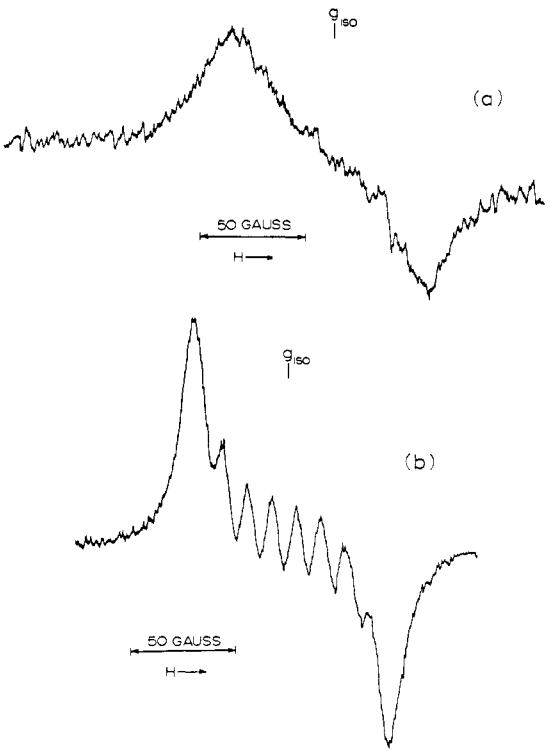

Figure 1. Esr spectra of CoPpIXDME·DMF·O_2: (a) dimethylformamide solution at 18°; (b) fluid dimethylformamide solution at −44°.

Previous studies,[8] using visible spectroscopy, of the effect of base, B, on the value of K in toluene solution indicated an increase in K with basicity in agreement with the results of Crumbliss and Basolo[5] on the cobalt Schiff-base complexes. The change in K with basicity is much too small to account for the difference in the oxygen carrying ability between CoPpIXDME and coboglobin, where B is an imidazole of a histidine residue. We wish to report preliminary results of the extension of our studies to other solvents. Polar solvents such as DMF (ϵ = 36.1) and acetonitrile (ϵ = 38.0) have a profound effect on the value of K. While CoPpIXDME·CH_3-Im in toluene (ϵ = 2.4) requires an oxygen pressure of 417 Torr for half oxygenation at −23°, CoPpIXDME·CH_3-Im in DMF requires only 12.6 Torr.[9] Thus there is a marked solvent effect, corresponding to a change in ΔF of 1.7 kcal/mol at −23°, favoring the oxygenation of CoPpIXDME·CH_3-Im in DMF over toluene.

The corresponding value for half oxygenation of CoPpIXDME·DMF in DMF is 40 Torr. This comparison of CoPpIXDME·CH_3-Im and CoPpIXDME·DMF both in DMF indicates that the degree of oxygenation at a given temperature and O_2 pressure is greater when CH_3-Im is the base. This result is consistent with our previous findings[8] that the greater the basicity of the axial ligand, the greater the affinity of the molecule for O_2 and with the findings of Crumbliss and Basolo[5] that at a given oxygen pressure the degree of oxygenation of Co(acacen) in solutions containing DMF is less than in solutions containing a substituted pyridine. But clearly the effect of the base on oxygenation is smaller than that of the solvent.

These results from visible spectroscopy indicate that one might be able to obtain appreciable concentrations of the oxygen adduct of CoPpIXDME in DMF and CH_3CN at room temperature. Indeed this is borne out by esr measurements.

The esr spectra of cobalt protoporphyrin IX dimethyl ester in dimethylformamide solution at 18 and −44°, 760 Torr O_2, are shown in Figure 1. The spectra disappear in the absence of oxygen and are regenerated in the presence of oxygen. The intensity of the signal is enhanced by a factor of 50 on going from 18 to −44°, consistent with thermodynamic data[10] from visible spectroscopy measurements. The signal at 18° represents ~1% of the cobalt(II) present.

The fact that a spectrum is observed at room temperature is consistent with the formulation of Co(III)–$O_2\cdot^-$, since Co(II) shows no esr signal at room temperature in solution.[11] It is not consistent with the presence of a (Co(III)–$O_2\cdot^-$–Co(III)) species since room temperature spectra of superoxo-bridged complexes[12-14] show a 15-line spectrum from the splitting by two equivalent cobalt(III) atoms. The g values and Co splittings of previously reported isotropic spectra of Co(III)–$O_2\cdot^-$ complexes are given in Table I. The correlation between esr and visible spectra provides strong evidence that the reversible oxygen carrier in DMF and CH_3CN involves only Co(III)–$O_2\cdot^-$ and not peroxo or superoxo dimers. The substantial increase in K allows us to obtain thermodynamic data in fluid solution by both visible and esr spectroscopy.

The dramatic effect of the solvent on K appears to result from a stabilization of the polar Co(III)–$O_2\cdot^-$ species in the more polar aprotic solvents. Speculation

Table I. Isotropic Esr Parameters of Some Cobalt(III) Superoxo Complexes[a]

Compound	g_{iso}	a_{iso}^{Co}, G
CoPpIXDME·DMF·O_2	2.0197[b]	11.47[b]
CoPpIXDME·py·O_2	2.0207[c]	11.88[c]
CoPpIXDME·py·O_2	2.02[d]	11.8[d]
Co(acacen)py·O_2	2.0263[e]	13.70[e]
Co(acacen)DMF·O_2	2.0261[f]	13.33[f]
$[(NH_3)_5CoO_2Co(NH_3)_5](NO_3)_5$	2.0249[g]	11.4[g]

[a] Estimated errors, unless noted: g values ±0.0005; hyperfine splittings ±0.05. Values have not been corrected for the second-order hyperfine shift. [b] Measured from fluid dimethylformamide solutions at −44°. [c] Measured from fluid acetonitrile solutions at −43°. [d] Measured from fluid toluene solutions, ref 2. [e] Measured from fluid toluene solutions at −74°: B. M. Hoffman, D. L. Diemente, and F. Basolo, *J. Amer. Chem. Soc.*, **92**, 61 (1970). [f] Measured in toluene solution at −61°: see reference in footnote *e*. [g] From ref 12.

from deoxygenated solutions used in this study show only the five-coordinate species at 77°K. At higher temperatures, changes in the visible spectra of deoxygenated solutions with varying ligand concentrations are consistent only with five-coordinate species being formed under the conditions of the present experiments. Thus we have considered only five-coordinate species.

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as to whether this dipolar stabilization of the oxygen complex in DMF and CH_3CN may be provided by the protein in coboglobin awaits complete thermodynamic data on the effect of base, solvent, and porphyrin substituents for comparison with coboglobin. Work to this end is in progress.

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Laser Raman Spectra of Crystalline and Aqueous Glucagon

Sir:

Laser Raman spectroscopy is becoming an important technique in the investigation of the conformation of proteins and synthetic polypeptides both in the solid state and in solution.[1-7] Two spectral regions thus far are found useful in characterizing the conformation of the peptide backbone: one in the region 1630–1700 cm^{-1} (amide I) and the other between 1220 and 1300 cm^{-1} (amide III). Studies of the Raman spectra of synthetic polypeptides of known structure indicate that the amide I and III frequencies of α-helical, random-coiled, and β-pleated sheet forms are quite different. For example, the α-helical poly-L-alanine[8] has its amide I and III vibrational modes at 1660 and 1264 cm^{-1}, respectively, the random-coiled poly-L-glutamic acid[6] has the corresponding frequencies at 1665 and 1248 cm^{-1}, and the antiparallel β-polyglycine I[9] at 1674 and 1234 cm^{-1} (with a shoulder at 1220 cm^{-1}). However, there is some reluctance to accept these as characterizing frequencies for various conformations in proteins.[3] This is because peptide homopolymers have symmetry elements in the α helix and β structure. Selection rules can then restrict both the number and position of frequencies that may appear in the spectrum.[3] In the case of proteins, because of the variety of side groups, no symmetry elements exist and there should be no symmetry restriction.[3] In the Raman spectra of lysozyme, Lord and Yu[5] observed three resolved peaks at 1240, 1262, and 1274 cm^{-1} in the amide III region, but did not attempt to identify each of them with a certain conformation. Recently, Mendelsohn[10] has obtained the Raman spectra in the amide III region of eight globular proteins and concluded that α-helical and β-pleated sheet forms have amide III vibrations above 1250 cm^{-1} and the random-coiled structure below it. On the contrary, Yu, *et al.*,[1,2] have found one amide III line at 1227 cm^{-1} in the spectrum of thermally denatured insulin, which has been identified as a β structure.[2] In view of the present confusion in the assignment and the importance of the amide I and III regions in the assessment of protein conformation, it seems worthwhile to examine Raman spectra of a naturally occurring protein which is capable of undergoing the α helix–coil–β transition. We have now found that glucagon is an ideal model system.

Glucagon is a polypeptide hormone of 29 amino acid residues with a known sequence.[11] It has been reported that it exists in 75% α-helical structure in crystals[12-14] and that in freshly prepared acidic solutions glucagon is predominantly in the form of a random coil.[15] On standing at 26°, this acidic solution is gradually converted into a gel, which is found to consist of antiparallel β chains.[16] In this communication, we wish to present laser Raman spectra (in the region 1120–1700 cm^{-1}) of glucagon in these different conformational states and compare the amide I and III frequencies with those of synthetic polypeptides.

In Figure 1 are presented the Raman spectra of glucagon in crystals, freshly prepared aqueous solution (pH 2.25), and gels. In descending order, these spectra show a stepwise decrease in frequency of the amide III lines from 1266 (α helix) to 1248 (random coil) and then to 1232 cm^{-1} (antiparallel β). These frequencies are very similar to those of α-helical poly-L-alanine, random-coiled poly-L-glutamic acid, and antiparallel β polyglycine I, respectively. In the 1630–1700-cm^{-1} region, the amide I line of crystalline glucagon is seen at 1658 cm^{-1}. The shoulder near 1685 cm^{-1} may be due to the unsolvated random-coiled segments of glucagon (about 25%). It is of interest to note that in the amide III region there also exists a shoulder at 1235 cm^{-1}, which may be associated with the same structure (random coil). In Figure 1b the strong water line near 1640 cm^{-1} has obscured the amide I frequency of glucagon in freshly prepared aqueous solution. Upon gel formation, however, the amide I line has sharpened considerably and showed up in the spectrum (Figure 1c) as an intense sharp line at 1672 cm^{-1} (half-width about 15 cm^{-1}) on the sloping background of water. Again, the two frequencies, 1658 and 1672 cm^{-1}, are in agreement with the corresponding amide I frequencies of α-helical poly-L-alanine and polyglycine I. On the basis of present results, it appears that the substitution of homo side chains in synthetic polypeptides by a variety of side groups does not affect the frequencies of the strongly coupled amide I and III vibrations as much as might be expected.

We also report a Raman spectrum (Figure 2) obtained at an intermediate stage of gel formation. The structureless broad amide III line at 1248 cm^{-1} (Figure

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