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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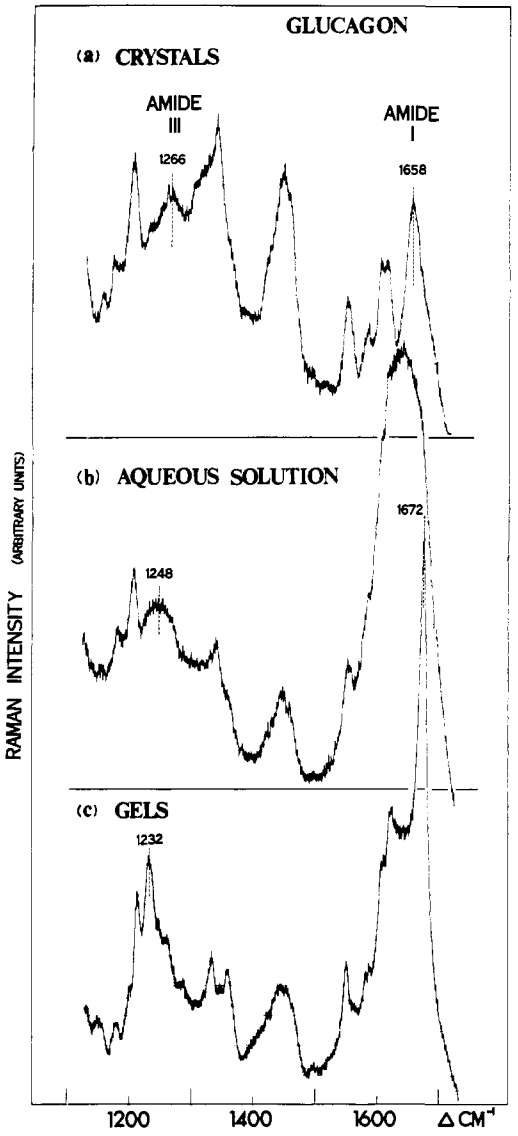

Figure 1. Raman spectra of glucagon in various states of aggregation. (a) Spectrum of crystalline glucagon (powder form): spectral slit width ($\Delta\sigma$), 4 cm^{-1}; sensitivity (s), 1000 cps full scale; rate of scan (γ), 10 cm^{-1}/min; standard deviation (sd), 1%; laser power (p) at the sample, 150 mW. (b) Spectrum of freshly prepared aqueous glucagon. The spectrum was obtained 1 hr after the solution was prepared: concentration (c), 20 mg/ml; pH 2.25; $\Delta\sigma$, 4 cm^{-1}; s, 2500 cps; γ, 10 cm^{-1}/min; sd, 1%; p, 250 mW. (c) Spectrum of gels formed from b on standing (~40 hr at 26°): $\Delta\sigma$, 5 cm^{-1}; s, 2500 cps; γ, 10 cm^{-1}/min; sd, 1%; p, 230 mW.

1b) has changed into a doublet: one at 1232 and the other at 1256 cm^{-1}. On further standing, we have observed that the intensity ratio of the 1232 cm^{-1} line to the 1256 cm^{-1} line increased and eventually approached the limit shown in Figure 1c. This indicates that an intermediate structure exists in the conversion of random-coiled to antiparallel β glucagon. A detailed discussion on the time-dependent Raman spectra of acidic glucagon solution will be given in a future publication.

It is certainly noteworthy to mention that infrared and Raman techniques do not detect the same amide I vibrations in both peptide homopolymers and proteins. In polyglycine I,[9] the infrared amide I bands appear at 1636 (strong) and 1685 cm^{-1} (medium), while the Raman amide I line shows up at 1674 cm^{-1}

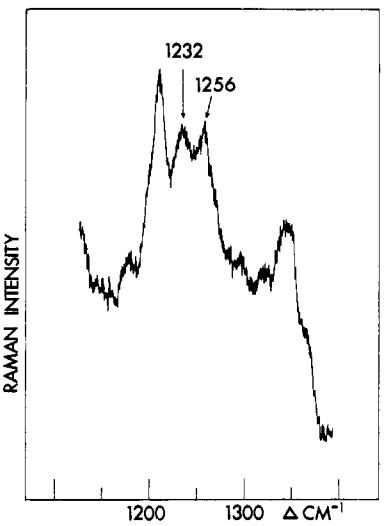

Figure 2. Raman spectra of incompletely formed gels of glucagon (from Figure 1b). This spectrum was obtained after 15 hr of standing at 26°. Conditions for the spectrum are the same as for Figure 1c.

(strong) as mentioned previously. In glucagon fibrils,[16] the infrared amide I bands are at 1630 (strong) and 1685 (weak), while the Raman amide I line is found at 1672 cm^{-1} (strong). In an earlier communication,[1] we have pointed out that the denatured fibrous insulin has the infrared amide I band at 1637 cm^{-1} and the Raman amide I line at 1673 cm^{-1}.

In order to determine the effect of water molecules on the conformation of fibrous glucagon, we obtained the spectrum of glucagon fibrils in the solid state. The amide I and III frequencies are found to be the same as those of Figure 1c, indicating that the antiparallel β structure of glucagon gels remains in the solid state.

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Intrinsic Basicities of Ammonia, Methylamines, Anilines, and Pyridine from Gas-Phase Proton-Exchange Equilibria[1]

Sir:

Measurements of the proton affinities of a variety of organic compounds by several different methods have been reported.[2] However, many important organic bases have not been investigated. The present work was stimulated by a recent determination of gas-phase

(1) Presented in part at the 3rd Conference of Structure Energy Relationships, Tallahassee, Fla., Feb 17–19, 1972.
(2) For a review article, see J. L. Beauchamp, *Annu. Rev. Phys. Chem.*, 22, 527 (1971).