majority of the synthetic operations reported can be carried out in the presence of the unprotected indole nucleus, thus attesting to the mildness of the reaction conditions employed. There exists no need to reduce indole to indoline and then to regenerate the indole nucleus at a latter stage by oxidation as was deemed necessary, for example, in the Kornfeld–Woodward synthesis of lysergic acid.[12]

Further studies being carried out in our laboratories should greatly streamline the scheme presented. The application of this nitrile oxide cycloaddition chemistry to the construction of the rugulovasines and lysergic acid as well as other ergots is presently in progress and will be reported in separate accounts.[13]

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Binding of 5-Fluoro-L-tryptophan to Human Serum Albumin

Sir:

The rate of synthesis of 5-hydroxytryptamine (serotonin) in the brain appears to be determined by the concentration of tryptophan in the brain[1] which, in turn, is related to concentration levels of this amino acid in the blood plasma.[2,3] It has been demonstrated that tryptophan interacts strongly and stereospecifically with plasma albumin[4] and recently several ^{1}H NMR studies of the complexes formed between albumin and tryptophan have been reported.[5-7] These NMR experiments have not directly addressed the question of the number of binding sites for tryptophan on this protein and have generally been interpreted in terms of exhange rates between free and bound forms of the amino acid that are rapid. By using ^{19}F NMR spectroscopy to examine complexes formed between albumin and 5-fluorotryptophan, we have been able to establish that (1) there are at least two distinct binding loci for tryptophan on human albumin and (2) 5-fluorotryptophan at one of these sites is in slow exchange with the bulk amino acid.

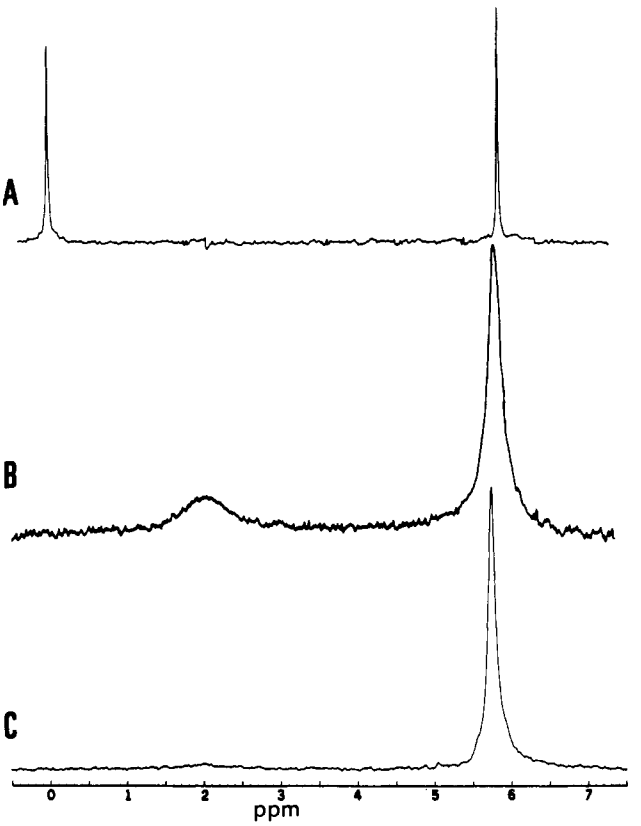

Figure 1. ^{19}F NMR spectra of 5-fluoro-L-tryptophan: trace A, 6 mM 5-fluorotryptophan (proton decoupled); trace B, 3.06 mM 5-fluoro-L-tryptophan and 1.03 mM human serum albumin (proton decoupling gated on during acquistion of the free induction decay); trace C, 3.06 mM 5-fluoro-L-tryptophan, 1.03 mM human serum albumin, and 4.00 mM L-tryptophan (gated proton decoupling). All spectra were recorded using a Varian Associates XL-100 spectrometer operating at 94.14 MHz. Sample temperatures were controlled at 25 ± 1 °C. The reference peak at 0 ppm was derived from a capillary containing a solution of *p*-fluorotoluene in toluene.

Commercial 5-fluorotryptophan (Aldrich) was resolved via its methyl ester by chymotryptic hydrolysis following the procedure of Tong et al.[8] The L isomer (mp 255–258 °C) showed a specific rotation $[\alpha]_D$ −19.5° at pH 5.9. Fatty acids were removed from crystallized human albumin (Schwartz-Mann) by Chen's procedure[9] and the protein had <0.15 mol of fatty acid/mol after this treatment. Chromatographic and electrophoretic experiments showed that the protein used for the NMR experiments was >80% monomeric. Samples for NMR spectroscopy were made up in a solvent containing 0.15 M NaCl, 0.05 M phosphate buffer, 1 mM EDTA, and 5% deuterium oxide and were adjusted to pH 7.4.

Some results are shown in Figure 1. Under conditions of complete proton decoupling the fluorine spectrum of 5-fluoro-L-tryptophan consists of a sharp singlet 5.843 ppm upfield from the reference signal provided by a capillary containing a 10% solution of 4-fluorotoluene in toluene (trace A). When human albumin is present at a concentration ratio of 1:3, the signal at 5.8 ppm is broadened substantially and a new resonance with a line width of ~60 Hz appears at 2.06 ppm (trace B). Experiments with glycerol solutions of the fluoroamino acid confirmed that the line widths were not due to sample viscosity. When L-tryptophan is added to a mixture of 5-fluoro-L-tryptophan and albumin two effects are noted: (1) the intensity of

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the low-field signal decreases in proportion to the amount of added L-tryptophan and (2) the upfield signal sharpens, approaching the line width of free 5-fluoro-L-tryptophan (trace C).

The appearance of spectra such as trace B suggests the possibility of a simple two-site chemical exchange process in which free fluorotryptophan (5.84 ppm) undergoes exchange with protein-bound material (2.06 ppm) at an intermediate rate, leading to peaks which are somewhat broadened and shifted toward the weighted mean of the chemical shifts. Line-shape calculations show that to reproduce experimental spectra requires a rate constant for dissociation of the complex of $\sim 120\ s^{-1}$. However, saturation of the resonance at 2.06 ppm does not produce any significant change in the intensity of resonance at ~5.84 ppm, a result which demands a dissociation rate constant at least two orders of magnitude smaller than that predicted on the assumptions of simple two-site exchange, when the experimental values for T_1 (~0.5 s) of the upfield peak are considered. Thus, the upfield resonance observed does not represent solely free fluorinated amino acid but must be broadened by other interactions of 5-fluorotryptophan with the protein.

Increasing the sample temperature caused the signal at low field to move upfield and to broaden to the extent that it became lost in the noise level. Concommitantly, the upfield signal shifted to lower field and became quite broad, reaching a maximum width at ~45°C and then sharpening somewhat as the temperature was raised beyond this. Experiments above 60 °C were not possible owing to denaturation of the protein and, at this temperature, fast-exchange averaging of the fluorine signals had still not taken place. Attempts to understand the change in line shape observed at the various temperatures in terms of the two-site exchange model indicated above were not successful, again suggesting that the situation is more complex than this in the system under study.

Our interpretations of these and related data are the following. 5-Fluoro-L-tryptophan appears to bind to the protein at, at least, two chemically distinguishable protein sites. One of these leads to a substantial downfield chemical shift effect (3.8 ppm), causes the amino acid to become highly immobilized, as reflected by the transverse relaxation rate, and interacts strongly enough with the small molecule that exchange of this species with bulk 5-fluoro-L-tryptophan is slow. A second class of interaction sites is recognized by an appreciable line-broadening effect on the fluorine resonance of the bound amino acid. There is very little protein-induced chemical-shift effect at these sites and exchange with bulk fluoroamino acid from them appears to be rapid. The competition experiments indicate that both sets of fluorotryptophan binding sites are also binding sites for L-tryptophan, although the exchange rates for the unfluorinated amino acid are not revealed by our data.

These initial results suggest that earlier interpretations of ^1H NMR studies of the tryptophan–albumin system[5-7] are likely incomplete and should be reformulated to take into account the possibility of several tryptophan binding sites with some exchange rates in the slow exchange limit.

Additional work to characterize the fluorotryptophan–human albumin system, including determination of the exchange rates, is underway in our laboratory.

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Reactions of Superoxide with Iron Porphyrins in Aprotic Solvents. A High Spin Ferric Porphyrin Peroxo Complex

Sir:

Dioxygen complexes of iron porphyrins are of interest because of their proposed involvement in a variety of biological processes. Only three types of synthetic iron porphyrin dioxygen complexes have been reported, each of which is prepared by reaction of ferrous porphyrins with O_2: Fe(porph)L(O_2),[1,2] an analogue of oxymyoglobin; Fe(porph)O_2Fe(porph),[3] a μ-peroxo bis ferric porphyrin complex; and a partially characterized adduct formed from Fe^{II}(porph) and O_2 in the solid state.[4] Our interest in reactions of superoxide anion, O_2^-, with metalloporphyrins led us to investigate its reactions with iron porphyrins.[5] We report here the results of our studies, which include the preparation and characterization in solution of a new type of iron porphyrin dioxygen complex which we formulate as a mononuclear peroxo complex of a high spin ferric porphyrin, i.e., $[Fe^{III}(porph)(O_2)]^-$.

We initially investigated the reaction of KO_2 (solubilized by crown ethers[6]) with *ferric* porphyrins, Fe^{III}(porph)Cl. We found that three different reaction pathways occurred under different conditions. Specifically, the product obtained depended on the nature of the solvent, the presence or absence of traces of water, the concentration, and the temperature. The simplest reaction that we observed was one-electron reduction, similar to that observed for the reaction of O_2^- with $Mn^{III}(TPP)^+$ [7] or $Cu^{II}(phen)_2^{2+}$:[6]

$$Fe^{III}(porph)^+ + O_2^- \rightarrow Fe^{II}(porph) + O_2 \qquad (1)$$

Such a reaction was observed in Me_2SO or in Me_2SO/py, CH_3CN/py, or toluene/py (see Table I for exact conditions). In each of these cases, the Fe^{II}(porph) product is stabilized against reaction with O_2 by coordination of axial ligands, i.e., $Fe^{II}(TPP)(Me_2SO)_2$ or $Fe^{II}(TPP)(py)_2$. By contrast, in wet solvents or in relatively nonpolar solvents such as THF, toluene, or CH_2Cl_2, with no added base, μ-oxo dimer formation was observed (unless the solution was extremely dilute, see Table I):

$$2Fe^{III}(porph)^+ + O_2^- \rightarrow \rightarrow [Fe^{III}(porph)]_2O \qquad (2)$$

If, on the other hand, O_2^- was reacted with $Fe^{III}(TPP)Cl$ in DMF at −50 °C,[8] a third type of reaction was observed. The product visible spectrum (see Table I) most closely resembled that of Fe(TPP)(py)(O_2) as reported by Anderson et al.[9] with a small amount of μ-oxo dimer present as well. We conclude that reaction 3 occurred as previously reported by Hill et al.[10] for the reaction of O_2^- with Fe^{III}(PPIXDME)(ClO_4) under similar conditions.

$$Fe^{III}(porph)^+ + O_2^- \rightarrow Fe(porph)(O_2) \qquad (3)$$

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