eq 3 indicates that the prolate conformation envelope becomes more elongated as C_n grows (Table II), i.e., that the relative weight of trans-rich conformers increases with C_n.

We have briefly described the application of surface resolution analysis to a basic problem in heterogeneous chemistry and demonstrated it by obtaining what seems to us a more realistic picture of the adsorption of monofunctional linear molecules. Other conformational aspects of adsorbates on inert and on catalytic surfaces are currently being explored by the new tool described here.

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Registry No. *tert*-Amyl alcohol, 75-85-4; naphthalene, 91-20-3; ethanol, 64-17-5; 1-butanol, 71-36-3; 1-hexanol, 111-27-3; 1-octanol, 111-87-5; 1-decanol, 112-30-1; 1-dodecanol, 112-53-8.

Ultraviolet Resonance Raman Spectra of Hemoglobin Excited at 200 and 218 nm: Tertiary and Quaternary Structure Differences

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We report resonance Raman (RR) spectra excited at 218 and 200 nm (Figures 1 and 2), using an H_2 Raman-shifted quadrupled YAG laser, for fluoromethemoglobin (metHb(F^-)), with and without the effector molecule inositol hexaphosphate (IHP). Selectively enhanced modes of tryptophan (218 nm) and tyrosine (200 nm) show clear intensity differences among these species, which are interpretable in terms of changes in the environment of aromatic residues at the subunit interfaces. Hemoglobin is a tetrameric protein which adopts alternative quaternary structures, T and R, associated respectively with the deoxy and ligated protein.[1] It is generally accepted that the switch between the two accounts for the main features of cooperativity in ligand binding by hemoglobin,[1,2] although the importance of intermediate structures remains a matter of controversy. Met forms of hemoglobin, which contain Fe^{III} and bound H_2O or added exogenous ligands, are in the R quaternary state; but in the case of metHb(F^-), addition of IHP switches the quaternary structure to T, as judged by a comparison of the crystal structure of IHP-bound metHb(F^-) from horse with that of human deoxyhemoglobin[3] and by NMR[4] and optical[5] studies.

Ultraviolet laser excitation has recently been shown to produce strong and characteristic enhancement patterns for the vibrational Raman modes of benzene[6] and its derivatives[7] and of protein aromatic side chains.[8-11] Asher[11] has shown myoglobin Raman

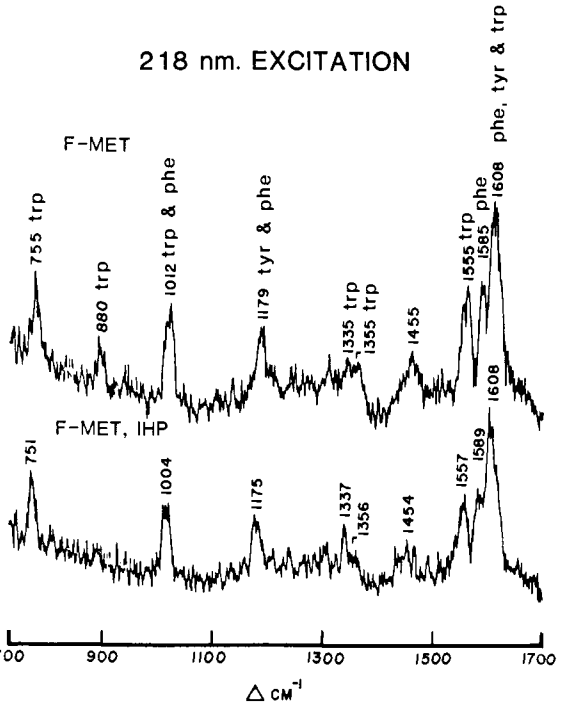

Figure 1. Ultraviolet resonance Raman spectra of 200 μM human hemoglobin in the metHb(F^-) form without (top) and with (bottom) IHP, with 218-nm excitation. Each spectrum is the sum of 10 scans collected with 0.05 Å/s accumulation time. All other conditions were as in ref 8. MetHb(F^-) was prepared using the method described by Asher et al.[17] IHP was added, in solid form, to 3 mol per mol of tetramer as described in ref 4. All solutions were buffered with 50 mM phosphate (pH 6.8); 50 mM tris buffer (pH 6.8) gave identical results.

spectra excited at ~230 nm with no discernable contributions from the heme, which dominates Raman scattering when visible laser excitation is used.[12]

The 218-nm spectra (Figure 1) of metHb(F^-) show several tryptophan (Trp) ring modes, as well as some contributions from tyrosine (Tyr) and phenylalanine (Phe).[9] The following changes in the Trp modes are seen upon IHP addition: (1) a 4-cm^{-1} downshift of the 755-cm^{-1} band; (2) dramatic intensity decreases for the bands at 880 and 1012 cm^{-1} (the latter produces an apparent downshift of the composite Trp and Phe band to 1004 cm^{-1}, close to the Phe frequency); (3) a shift in intensity from the 1355- to the 1335-cm^{-1} band. A 1355- → 1335-cm^{-1} intensity shift was previously observed[9] upon partial unfolding of the protein α-lactalbumin at low pH, but in that case the 876-cm^{-1} band moved up 4 cm^{-1}, without intensity lowering, while the 759-cm^{-1} band did not shift. The Hb A tetramer contains three pairs of symmetry-related tryptophans.[1,13] Two of these (α14 and β15) occur on the surface of the protein and are unaffected by changes in quaternary structure. The third pair (β37), however, occurs in the "flexible joint" region[13] near the interfaces between the βC and αFG helices. In the T structure, Trp β37 stacks against Tyr α 42, and this interaction is lost in the R structure; we attribute the RR alternations to this disruption. Perutz and co-workers[5] have shown that the T-R absorption difference spectrum for metHb(F^-) has a hyperchromic contribution at 294 and 302 nm attributable to Trp β37. We were unable to obtain satisfactory RR spectra near this wavelength (266 nm), but the 218-nm RR spectra show clear *hypo*chromism for the 880- and 1012-cm^{-1} Trp modes. We speculate that the Trp β37-Tyr α42 interaction produces a shift in Trp electronic oscillator strength from the 220-

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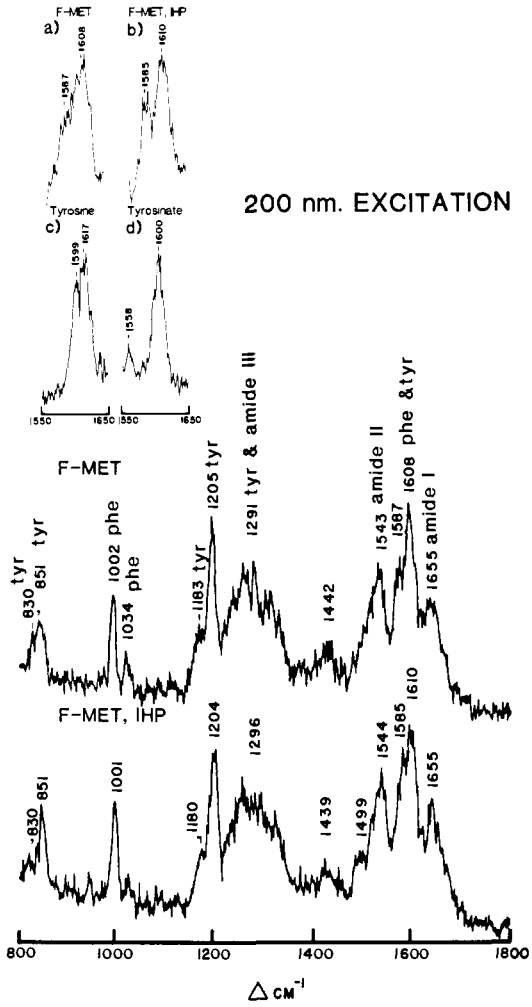

Figure 2. As for Figure 1, but with 200-nm excitation. Each spectrum is the sum of 20 scans collected with 0.05 Å/s accumulation time. Inset: comparison of the high-frequency region of the 200-nm excited RR spectra of 200 μM metHb(F^-) without (a) and with (b) IHP, 600 μM tyrosine, pH 7.0 (c), and 600 μM tyrosinate, pH 12.0 (d). The protein spectra are each the sum of 30 scans, while the amino acid spectra are each the sum of 20 scans.

to the 270-nm region, analogous to the shift for nucleotide bases stacked in nucleic acid duplexes from the 260 nm to longer wavelength transitions.[14]

The 200-nm RR spectra (Figure 2) show strong enhancement of Tyr modes but also contributions from peptide vibrations (amide I, II, and III) as well as from Phe.[9] Hb A contains six pairs of symmetry-related tyrosines. One of these, α42, is H bonded to a negatively charged carboxylate group (Asp β99) in the T state, but an uncharged backbone carbonyl in the R state; the other tyrosines do not change their H-bonding significantly.[13,15] Pronounced alterations in the metHb(F^-) UV absorption at 279 and 287 nm upon IHP addition have been associated with tyrosine H-bond effects.[5] They can likewise account for the change in the 1600-cm^{-1} region of the 200-nm RR spectrum, which is highlighted in the inset of Figure 2, where it is compared with the 200-nm RR spectra of tyrosine and tyrosinate.[10] Ionization of tyrosine shifts the 1617- and 1599-cm^{-1} bands (ν_{8a} and ν_{8b}) to 1600 and 1558 cm^{-1}. Addition of IHP to metHb(F^-) produces a change in the 1608-cm^{-1} band shape and an apparent augmentation in the 1587-cm^{-1} shoulder. Both of these bands contain Phe as well as Tyr contributions,[9,10] but computer subtraction of the two spectra indicates a shift in the Tyr mode from 1617 and 1599 cm^{-1} to 1605 and 1582 cm^{-1}. While better data will be needed to confirm these values, they are suggestive of strong H bonding of Tyr protons, producing shifts in the direction of those seen upon deprotonation, as expected for a strong H bond to a carboxylate group. It may also be significant that the intensity ratio of the 830–851-cm^{-1} tyr doublet changes appreciably upon IHP addition (Figure 2). This doublet has been shown[15] to be sensitive to H bonding, but the relationships established for visible excitation are inapplicable to UV-RR spectra because of specific intensity changes at resonance;[10] the UV systematics have yet to be worked out.

These results demonstrate the feasibility of establishing conformational markers in the UV Raman spectra of hemoglobin, associated with the environment of tryptophan and tyrosine residues. These should be useful in studies of the structural dynamics associated with hemoglobin cooperativity.

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Functionalized Crown Ethers as an Approach to the Enzyme Model for the Synthesis of Peptides†,‡

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Formation of molecular complexes between organic hosts and guests has been achieved in several artificial systems[2] and successfully applied to enzyme models in the sense that host–guest complexes are formed prior to the reactions. However, only a limited number of such enzyme models have been designed for bimolecular synthetic reactions.[3] Here we wish to report a novel type of host as an approach to the enzyme model for the synthesis of peptides.

We tried to design novel hosts based on the general concept of enzyme catalysis,[4] in which the reactive covalent intermediate ($E–S_1$) is formed from the noncovalent complex ($E\cdots S_1$) and then reacts with the second substrate (S_2) to give the product ($S_1–S_2$) as shown in eq 1. Previous papers have reported that thio-bearing

$$E + S_1 \rightleftharpoons E\cdots S_1 \rightarrow E–S_1 \xrightarrow{S_2} E + S_1–S_2 \qquad (1)$$

† This paper is dedicated to Professor Shun-ichi Yamada on the occasion of his 70th birthday.

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