This chain is absent in STI, but here the bulky Tyr-62(I) and Ile-64(I) (equivalent to Cys-14(I) and Ala-16(I) in PTI) shield His-57. Dissociation to I might be difficult because the interactions in the complex have to be broken cooperatively. The rigid partner molecules allow no conformational changes required for a stepwise (zipper) breakage of the interactions.

The inhibitor has a structure almost perfectly complementary to the enzyme with minimal adaptation required. This is in contrast to a flexible substrate chain with many degrees of freedom to be frozen to make interaction with the enzyme possible.[31-33]

Resonance Raman Labels: A Submolecular Probe for Interactions in Biochemical and Biological Systems

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Key aspects of many biochemical interactions occur when a relatively small molecule is in contact with a highly specialized, local environment. Elucidation in chemical terms of these ligand–active-site interactions represents some of the crucial problems of molecular biochemistry. Resonance Raman labels[2] provide a precise indicator or reporter technique to study the nature of events produced by biological active sites on the bound ligand. The basis of the method is the use of resonance Raman labels which, while yielding vibrational and electronic spectral data, are at the same time biologically active molecules. The technique will be introduced using relatively simple protein–ligand systems where time-dependent effects are not observed, such as drug–receptor[3] and antibody–hapten[4] interactions. In these the drug and hapten are simultaneously resonance Raman reporter groups and key biological components. Extension to the time domain is illustrated by enzyme–substrate reactions.[5-7] In these studies, through the use of substrates which are resonance Raman labels the conformation and electronic structure of the substrate in the enzyme's active site can be monitored as a function of time. Extension to other important areas such as membrane processes and nucleic acid–protein interactions will be presented in outline.

A resonance Raman label provides a detailed vibrational spectrum from a specially designed chromophore when it is bound to a biochemically active site. Specificity is achieved by synthesizing a chromophore which mimics as closely as possible a true biological component. Selectivity, the ability "to see" the label to the essentially complete exclusion of everything else present, is achieved by utilizing the resonance Raman effect. Resonance Raman spectra are obtained by illuminating a sample with laser light whose wavelength lies in an absorption band of a chromophore in the sample. Pronounced intensity enhancement of certain vibrations of the chromophore results thereby. The enhancement may be 10^3- to 10^6-fold compared to normal Raman spectra where the excitation wavelength is far from absorption bands. In practice, the resonance Raman effect allows spectra to be obtained from chromophores at concentrations of 10^{-4} M or less. At these concentrations in a complex biological system the resonance Raman spectrum from a given chromophore often dominates the recorded spectrum to the extent that it alone is observed. An example of such selectivity is shown by Figure 1a. The spectrum in Figure 1a was given by a chromophoric sulfonamide drug bound to the active site of the enzyme carbonic anhydrase in an aqueous buffer. Only the sulfonamide contributes to the absorption band near 450 nm (Figure 1, inset) as the other components absorb below 300 nm. By using either of the excitation wavelengths shown in the inset, the spectrum is that of the sulfonamide bound in the enzyme's active site (Figure 1a), unobscured by enzyme of solvent features.

The information content of resonance Raman spectra follows from two principles. The peak positions are a property of the ground electronic state, while peak intensities are strongly dependent on the excited electronic states as well as on the ground state. Thus, in a manner analogous to IR spectra, resonance Raman spectra contain features which represent vibrational modes of the ground electronic state. The region covered is approximately from 200 to 2000 cm^{-1}.

Paul Carey was educated at the University of Sussex in England. After receiving his Ph.D. in 1969 he joined the National Research Council of Canada as a postdoctoral fellow. His graduate work centered on the application of NMR to chemical problems. However, soon after arriving in Ottawa he became interested in using resonance Raman spectroscopy in biology. Although his interests in molecular biophysics are fairly catholic, he spends most of his time developing the kind of work described in this Account.

Henry Schneider was born in Montreal and received a B.Sc. from Sir George Williams University, a M.Sc. from the University of Western Ontario, and a Ph.D. from McGill. After 2 years of postdoctoral study at Cornell and some time in industry, he joined the National Research Council. His interests center about biochemical mechanisms, in particular those concerning membrane process. Both authors are Associate Research Officers.

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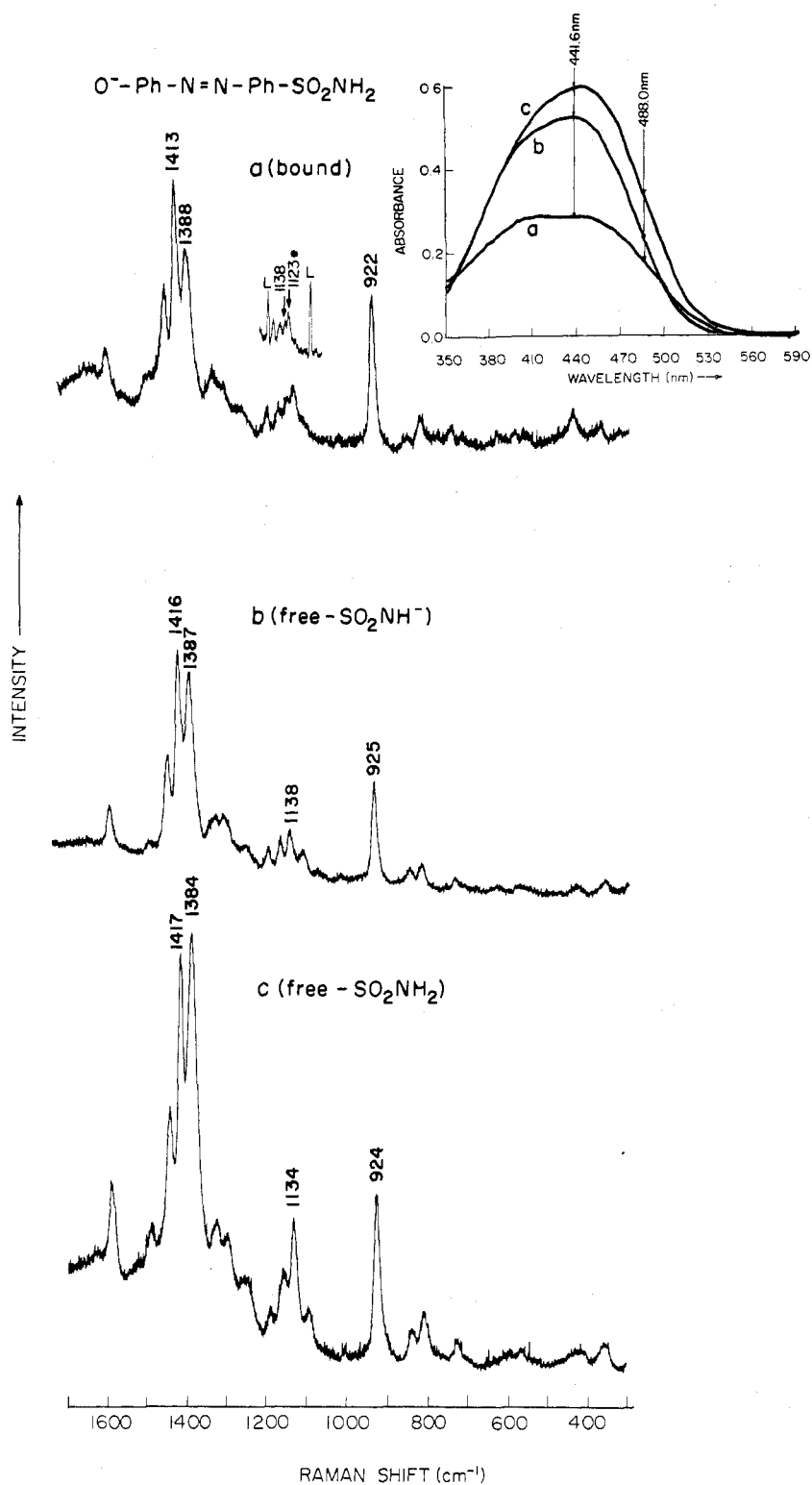**Figure 1.** Resonance Raman spectra of $^-OC_6H_4N{=}NC_6H_4SO_2NH_2$ (a) bound to carbonic anhydrase, pH 9.0, (b) the free $-SO_2NH^-$ form at pH 13.0 in aqueous solution, (c) the free $-SO_2NH_2$ form at pH 9.0. Concentrations of drug are $\sim5 \times 10^{-5}$ M. Inset: the corresponding absorption spectra. (From ref 3.)

Moreover, the resonance Raman features may be used to follow chemical events similar to those monitored in the IR, e.g., chemical rearrangement, conformation, bond distortion, and charge effects. The intensity dependence of resonance Raman features upon excited electronic states results, in practice, in the appearance of fewer features. The generalization "weak in the IR, strong in the Raman" often still holds. However, it cannot always be rigorously applied as a result of the large unsymmetrical molecules often studied. The occurrence of only a limited number of intense resonance Raman features brings conceptual simplicity and allows the development of realistic chemical models for biological phenomena. Furthermore, the dependence of resonance Raman scattering intensity upon excited states provides a direct or indirect monitor of these states. As a result, information on conformational changes in the excited state,[8-10] on the position of the

electronic dipole transition in a chromophore,[11] and on exciton coupling[12] can be elicited. A third spectral parameter in addition to line position and intensity is shape. Since line widths may reflect a distribution of closely related conformations, they are of value in probing heterogeneity[4] or conformational restrictions[13] in chromophore binding sites.

The normal Raman effect has yielded important new information on interactions and conformation in a host of biological molecules.[14a] In a sense the resonance Raman labeling technique and normal Raman studies are complementary. The former provides specific information from localized sites while a normal Raman spectrum can provide information on overall conformation, e.g., or the secondary structure of proteins or on phosphate–sugar backbone conformation in nucleic acids. For normal Raman studies, however, solutions have to be at least one or two orders of magnitude more concentrated, in terms of molarity, than those used for resonance Raman work. The utility of resonance Raman spectroscopy in studying *naturally* occurring biological chromophores has been the subject of a recent Account[15] and several reviews.[10,14] The labeling technique was developed[2] to overcome the limitation that most biological systems do not have a functionally important chromophore removed from the absorbance of the bulk of the system. Thus, the advantages inherent in the resonance Raman technique, low concentrations, selectivity, and specificity, can be extended to any system depending only on the ingenuity exercised in the design of the label. A general advantage of laser Raman spectroscopy is the ease with which it lends itself to experimental variations. Gas, liquid, and solid phases can be studied, and also flow systems and cryogenic equipment can be used with relative ease. The technology developed in other biophysical fields is therefore readily extendable to Raman and resonance Raman studies. The resonance Raman labeling technique requires a confluence of talents in spectroscopy, chemistry, and biology. Once these are brought to bear the method has considerable potential to follow events at the molecular or submolecular level in the most sophisticated biological environments.

Simple Theory and Units

Both Raman and resonance Raman processes are caused by a molecule inelastically scattering a photon of frequency ν (expressed in units of cm^{-1}) resulting in a photon of frequency ν' (cm^{-1}). Since total energy is conserved, $\nu-\nu'$ represents an energy level difference in the molecule, and in our case this is a vibrational quantum. Raman and resonance Raman spectra are normally reported (e.g., Figures 1, 3, 4) with $\nu-\nu'$ along

the abscissa in units of cm^{-1}. However the incoming photons are usually denominated in units not of cm^{-1} but of wavelength, i.e., ångströms or nanometers (10 Å = 1 nm and cm^{-1} = 10^8/Å). These relationships are best illustrated by reference to the inset in Figure 1. If the excitation wavelength is 4416 Å (in the blue region of the visible spectrum), the resonance Raman spectrum (Figure 1) extends approximately from the exciting line to 1800 cm^{-1} to low energy of 4416 Å. Thus, the resonance Raman spectrum is recorded by scanning the spectrometer from near 4416 to 4797 Å (the reciprocal of $((10^8/4416) - 1800)$ cm^{-1}).

Qualitatively, the origin of the resonance Raman effect has been understood for some time.[16] However, its quantitative application to molecular systems is still being actively pursued by theoreticians. The intensity of Raman scattering is proportional to the square of the polarizability tensor, whose elements are given by

$$(\alpha_{\sigma\rho})_{mn} = \frac{1}{h}\sum_e \frac{(M_\rho)_{en}(M_\sigma)_{me}}{\nu_{em} - \nu_0 + i\Gamma_e} + \frac{(M_\sigma)_{en}(M_\rho)_{me}}{\nu_{en} + \nu_0 + i\Gamma_e} \quad (1)$$

where the sum over the index e covers all of the eigenstates of the molecule; m and n are the initial and final states, h is Planck's constant, and Γ_e is a damping constant which takes into account the finite lifetime and, hence, linewidth of each molecular state e. The $(M_\rho)_{en}$, etc., are the electric dipole transition moments, e.g., along ρ from e to n. When ν_0, the frequency of the incident photons, approaches ν_{em}, the frequency of an allowed molecular transition, the denominator of the first term becomes very small and α consequently very large. This is the origin of the resonance Raman effect.

Equation 1 does not distinguish electronic and vibrational states. Examination of the interrelation between vibrational and electronic transitions needed for an understanding of the resonance Raman effect requires vibronic expansions based on eq 1. Several qualitative features have emerged from such work.[10,16] Franck–Condon factors are important in determining resonance Raman intensities, and this leads to the expectation that normal modes with a large shift in equilibrium geometry upon electronic excitation will produce intense resonance Raman features. Furthermore there are essentially two mechanisms for intensity enhancement,[16] one which depends on coupling to a single excited electronic state and a second which involves more than one excited state. These mechanisms can be distinguished by the wavelength dependence of Raman intensity in the preresonance region.[8]

Applications

Sulfonamides Inhibiting Carbonic Anhydrase. Carbonic anhydrase is a zinc metalloprotein which catalyzes the reversible hydration of CO_2. The discovery[17] that sulfonamides are strong inhibitors which bind to the active site with high affinity has proven to be of considerable therapeutic value.[18] However, in spite of extensive physicochemical studies,[19] including x-ray crystallographic analysis, the

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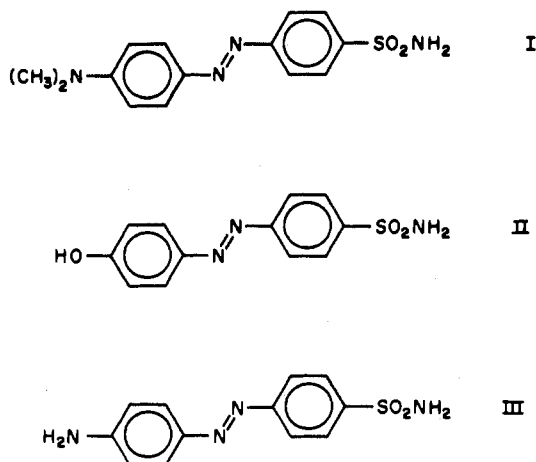
Figure 2. The structures of the chromophoric sulfonamides I, II, and III. (From ref 3.)

reasons for high affinity are not entirely clear. The resonance Raman labeling studies provide several new insights and possibilities. They show that the drug exists in the ionized form as $-SO_2NH^-$ when bound by the active site. In addition, they indicate that the geometry about the S atom in the bound ionized form differs from that in the unbound ionized form. Distortion from the geometry in aqueous solution also occurs for haptens upon binding to antibodies (see below and ref 4). These indications that ligand structure may change upon binding to protein receptors may have potential in assisting drug design. The view that ligand binding may influence protein structures is well established. However, direct experimental evidence for a change in ligand structure is relatively novel and suggests that the structure of the bound ligand should be considered in formulating structure–activity relations. Resonance Raman spectra may assist in establishing these relations.

Compounds I, II, and III (Figure 2), chosen for their chromophoric properties and high affinity for carbonic anhydrase, were each studied[3] when bound to several of the available forms of the enzyme. Figure 1 compares the resonance Raman spectrum of free aqueous II at pH 9.0 (Figure 1c) with that of the sulfonamide in the active site at the same pH (Figure 1a). Enzyme and solvent features are absent, as has already been noted. Three alterations are observed in Figure 1a: an increase in the relative intensity of the $1415/1388$-cm^{-1} bands, a shift in the 1134-cm^{-1} band to 1138 cm^{-1}, and the appearance of a feature at 1123 cm^{-1} not seen in Figure 1c. The spectral perturbations due to binding are all slight, but analogous changes are seen in each of the spectra of compounds I, II, and III when bound to four different forms of carbonic anhydrase.

The change is attributed to an alteration in the structure of the sulfonamide on binding. The unbound form, at pH 9.0, is un-ionized. However the bound form possesses an ionized sulfonamide residue $-SO_2NH^-$, since the spectra of this form in solution at pH 13 and of sulfonamide bound at pH 9.0 are very similar. This finding is strengthened by the elimination of hydrophobic bonding or of changes in the azobenzene skeleton as sources of the spectral perturbations. Hydrophobic bonding effects were modeled by recording

the sulfonamide spectra in solvents of varying dielectric constant and in the solid phase. The spectra were found to be essentially insensitive to such changes. In particular, the intensity change and frequency shift observed upon binding could not be reproduced by varying the medium. This does not mean that hydrophobic bonding is not important as a source of binding energy, only that hydrophobic bonding does not produce the spectral changes. Additional insight is provided by vibrational analyses of azobenzene derivatives[20,21] which allow the prediction of the effect of change in conformation about the $-N=N-$ bonds on the resonance Raman spectra. These studies show that no evidence is found for conformational change in the azobenzene moiety upon binding.

In addition to providing direct information about the nature of the bound form of the ligand, the spectra also suggest why the binding constants are so large. The key lies in the conformation of the $-SO_2NH^-$ group which indicates that it may act as a transition state analogue.[22] A feature unique to the bound spectrum and not seen in the spectra of the free $-SO_2NH_2$ or $-SO_2NH^-$ forms is the new band near 1123 cm^{-1} (Figure 1a). It was proposed[3] that this new band results from a change in geometry about the S atom upon binding over and above that resulting from ionization. This finding, together with a comparison of bond lengths and angles, led to the proposal that the $-SO_2NH^-$ group is mimicking the CO_2–OH^- transition state of the natural reaction for carbonic anhydrase.

This example therefore shows that, using a very simple model, the free aqueous $-SO_2NH^-$ form, we have been able to present strong evidence for sulfonamide ionization in the active site. Moreover it was possible to rule out hydrophobic bonding and azobenzene structure changes as sources of the observed spectral changes. This contrasts sharply with other spectroscopic techniques, for example, fluorescence and absorption, where the observed data are more limited and cannot always definitively distinguish one cause to explain an observed effect.

Enzyme–inhibitor studies by other workers have followed the binding of zincon, a complex azo dye, to liver alcohol dehydrogenase and shown that binding probably occurs at the enzyme's zinc atom.[23] The competitive inhibition of trypsin by 4-amidino-4′-dimethylaminoazobenzene results in a change in the relative intensities of four of the inhibitor's resonance Raman features.[13]

Antibody–Hapten Complexes. An important step in the body's immune defense system is the recognition and binding of the invading species by antibody molecules.[24] These are proteins of mol wt 150 000, each molecule having two binding sites for the invading

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antigen. When proteins containing covalently linked artificial chromophores are injected into an animal, antibodies specific for the chromophore can be isolated from its blood. Then, using purified antibodies and free chromophores (called haptens) it is possible to study the initial antibody–hapten, hence the initial phase of the antibody–antigen, interaction. Resonance Raman labeling studies have been based on the chromophoric dinitrophenyl-hapten.[4,25] These have been studied bound to antibodies from rabbits[4] and bound to antibody molecules from tumors in mice[25] (MOPC antibodies). The binding sites in the former are heterogeneous, that is, they are not identical, whereas those of the MOPC antibodies from mice tumors are homogeneous.[24]

The resonance Raman spectra of haptens bound to antibodies revealed, for the first time, submolecular effects in the hapten caused by differences in antibody sites. Moreover the site effects of charge-transfer interactions and changes in the haptens' nitro groups could be separated and evaluated. In the resonance Raman spectra of bound dinitrophenyl-haptens containing a –N=N– linkage the difference in site structure of the rabbit antibodies was expressed in broadening of the –N=N– symmetric stretch.[4] Moreover large shifts in $\nu_{N=N}$ indicate that considerable distortion takes place about –N=N– single bonds in the binding site. Charge-transfer interactions between the dinitrophenyl group and an aromatic amino acid side chain were shown not to cause the observed shifts in the resonance Raman spectra of the bound haptens.

The changes in features coming from the nitro groups observed with rabbit antibodies[4] have been pursued in work on the MOPC antibodies.[25] For the latter simple haptens based on the 2,4-dinitroaniline skeleton were used. [15]N isotopic substitutions have been made to aid assignment and a spectroscopic study[9] revealed that *o*-nitroanilines, having at least one unsubstituted hydrogen, possess unusual properties due to the "ortho structure". Principally this is a band in the 1350–1400-cm^{-1} region (an example of a typical ortho structure spectrum is the 1275–1400 cm^{-1} region in Figure 3) which shows an unusually high degree of Raman intensity enhancement as λ_{max} is approached. This is probably due to large changes in the ortho structure occurring in the excited state. The sensitivity of intensity to such conformational differences suggests a novel resonance Raman application of possible general utility to probe restriction in ligand binding sites.[26a] This depends on the fact that resonance Raman peak positions are solely a property of the electronic ground state while intensities depend on excited states. Thus any restriction in a protein site which strongly favors the ground- over the excited-state equilibrium geometry may be expressed in little or no change in peak position but large changes in resonance Raman intensity compared to the free aqueous ligand.

Enzyme–Substrate Reactions. A very promising extension of the resonance Raman labeling method is in systems where changes occur with time and several intermediates may be involved, as in enzyme catalysis.
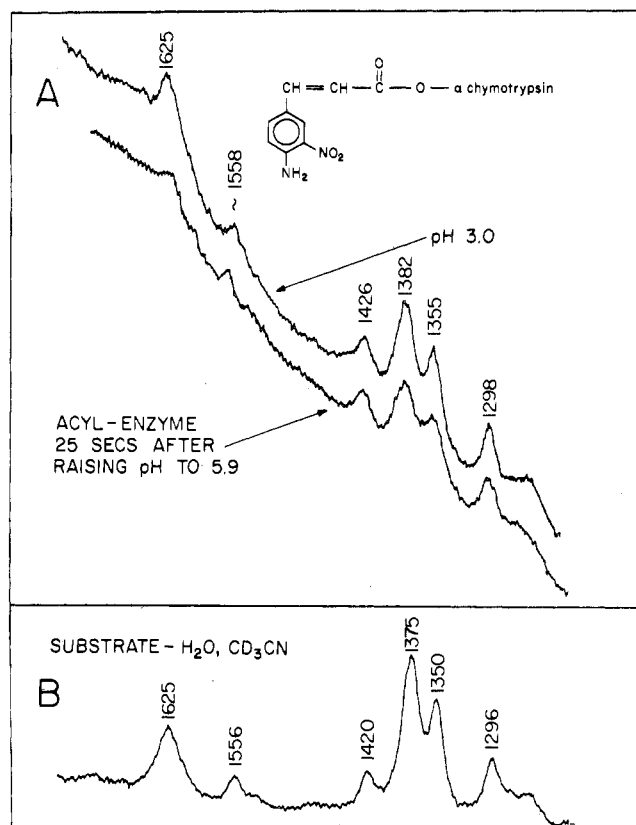
Figure 3. Resonance Raman spectra of: (A) 4-amino-3-nitro-*trans*-cinnamoyl-α-chymotrypsin at pH 3.0 (top) and pH 5.9 (bottom); (B) methyl 4-amino-3-nitro-*trans*-cinnamate. The spectrum of the unstable intermediate at pH 5.9 was obtained in a flow system. Essentially identical spectra were recorded in the range pH 5.9 to 7.0. (Adapted from ref 6.)

To study an enzyme–substrate reaction the substrate is chemically engineered to contain a group at or near the point of catalytic attack which gives an intense Raman spectrum.[5-7] Events which occur in the catalytic site which are mechanistically important are therefore expected to become accessible. This expectation is based on two facts. The first is the general demonstration described above with protein–ligand systems that submolecular events in ligands produced by active sites can be monitored. The second is that resonance Raman spectra are sensitive to the particular submolecular events postulated to be crucial steps in the mechanism of enzyme catalysis, for example, bond distortion and charge juxtaposition. At this point it should be emphasized that, although biochemists and in particular crystallographers have delineated the structure of several enzyme active sites, the development of experimental methods to permit distinctions between the various theories of enzyme catalysis remains one of the crucial problems in enzymology.[27]

The first enzyme–substrate complex studied by resonance Raman spectroscopy[5,6] is shown in Figure 3. It is the acyl-enzyme 4-amino-3-nitrocinnamoyl-chymotrypsin in which the cinnamoyl moiety is covalently bound at serine-195 of α-chymotrypsin. It is formed during the esterolysis of the methyl ester of 4-amino-3-nitrocinnamic acid. Apart from the luminescent background the spectra of the acyl-enzyme at

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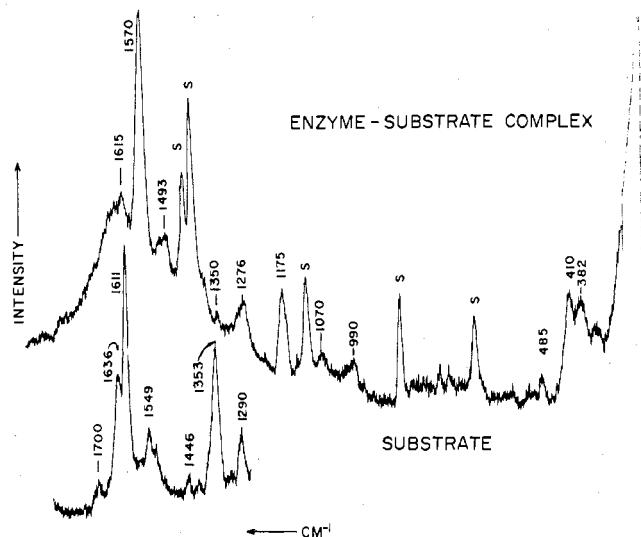
Figure 4. Resonance Raman spectra of the catalytic intermediate 4-dimethylamino-3-nitro(α-benzamido)cinnamoyl-papain (top) and the substrate methyl 4-dimethylamino-3-nitro(α-benz-amido)cinnamate (bottom). The substrate spectrum below 1290 cm⁻¹ contains only weak features and is obscured by solvent. S = solvent bands, resulting from dimethylformamide moving with intermediate during chromatography. (From ref 7.)

pH 3.0 (Figure 3A, top) and substrate (Figure 3B) are indistinguishable. Thus, at pH 3.0 the conformation of the acyl group in the active site is not noticeably perturbed from that in free solution. Crucially, the conformation about the ethylenic bonds remains essentially planar, trans and probably s-trans about the C=C—C=O single bond. At pH 3.0 chymotrypsin is inactive and the acyl-enzyme is stable for days. However, on raising the pH to 7.0 the enzyme becomes active (deprotonation of amino acid side chains necessary for catalysis occurs). The acyl-enzyme at active pH has a half-life of seconds and can be studied in a simple flow system[6] in which a steady state concentration of unstable intermediate is generated by mixing buffer and stable acyl-enzyme. The only spectral change observed following conversion to an active pH is a drop in intensity in the 1625-cm⁻¹ band (Figure 3A, bottom), a feature which has a high degree of C=C stretching character. The most likely explanation for this decrease is that, prior to deacylation, one of the most critical steps in the catalytic process, twisting, occurs about the C=C—C=O single bond. Thus, distortion takes place in the band next to the linkage undergoing cleavage. The generalization which emerges from this study is that the resonance Raman labeling technique can detect substrate distortion, one of the most discussed, yet one of the more difficult, possible sources of enzyme power to study experimentally.

A second study concerning an acyl-enzyme formed in reaction 2 has an even more dramatic appeal. The reason for this is clear from the spectra shown in Figure 4. The spectrum of the enzyme–substrate intermediate is totally distinct from that of the substrate or the product. The active site obviously produces drastic changes in the properties of the acyl residue. Again, it is tempting to consider these changes to be a direct expression of the catalytically important forces which are present in the active site. The spectra of substrate and product are dominated by ethylenic and ring modes in the 1600-cm⁻¹ region and a nitro feature near 1350 cm⁻¹. In contrast, the acyl-enzyme's spectrum shows
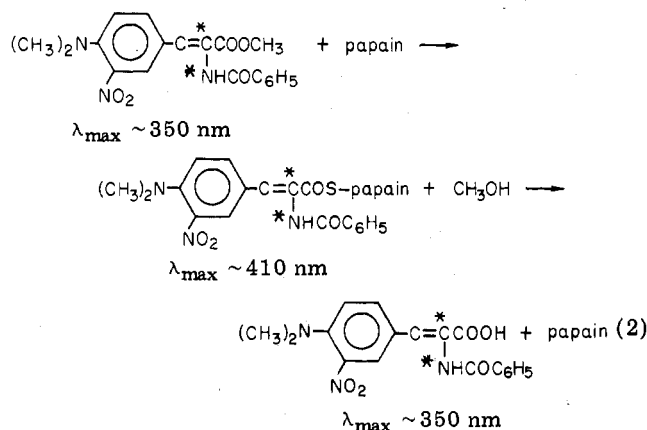
a very intense peak at 1570 cm⁻¹ and a peak of medium intensity at 1175 cm⁻¹. There is little evidence for the substrate's peaks in the spectrum of the intermediate, or vice versa.

The first model[7] developed to account for the intermediate's spectrum was based on its similarity to the spectra of polyenes and azlactones and on its high Raman intensity. The intense band at 1570 cm⁻¹ was assigned to a mode from a conjugated double bond system and the band at 1175 cm⁻¹ to a single bond C–C or C–N mode, both modes coming from a group or groups in a conjugated chain. It was suggested that the α-benzamido group underwent rearrangement on the enzyme, –NH—C(=O)Ph becoming –N=C(OX)Ph. New results,[28] using an additional analogue of the original substrate and isotopic substitution (at the positions marked *), support the original assignments and show that the α-benzamido side chain makes a contribution to the deacylation kinetics and resonance Raman spectrum of the complex. However, an important new fact to emerge is that the appearance of the intense 1570-cm⁻¹ band is not dependent upon any possible α-benzamido side-chain rearrangement but probably results instead from charge effects. A series of model compounds,[28] based on the imidazole esters of cinnamic acid, mimics the absorption and resonance Raman properties of the acyl-papains studied. The crucial property of the imidazole esters is that they have a very strong electron-attracting group (imidazole) attached to the carbonyl and a strong electron-donating group (e.g., p-dimethylamino) at the other extremity of the cinnamoyl skeleton. Thus acting in concert through the chemical bonds these groups set up a highly polarized π-electron system. It is proposed that essentially the same sort of electron polarization occurs to the active-site bound acyl group. However, in the active site the polarization may occur through space by interaction of the acyl residue and protein. The electron-attracting power is considered to be provided by a positive charge in the active site, lying proximally to the carbonyl group. The imidazolium of histidine-159 is the prime candidate.

The generalization to be drawn from the papain studies in their present state is the suitability of the resonance Raman technique to monitor charge effects in chromophoric substrates. Taken with the work on chymotrypsin, two of the much discussed[27] possible sources of the catalytic activity of enzyme active sites

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have been shown to occur. These are substrate distortion and charge–substrate interactions. The ability of the resonance Raman technique to monitor such effects emphasizes its future potential in unraveling molecular aspects of enzymic catalysis.

Prospects

Two technological innovations now upon us will have immediate impact on the amount and nature of information available with the labeling technique. One is the development of lasers in the 330–415-nm region. Their availability expands greatly the number of compounds which can be used as resonance Raman labels by removing the restriction present hitherto that the chromophores absorb in the visible. Many more systems can thus be expected to become available for study. The other innovation increases the capability for studying rapid reactions, a factor of importance for biological processes in general. This development centers on the use of multichannel photoelectron detection which allow recording of spectra on the subsecond time scale. Pioneered in Raman spectroscopy by Delhaye and co-workers,[26b] multichannel detectors incorporate the advantages of the photographic plate and electronic detection and have obvious application for studying reactions in biology. Another approach, using existing technology, for the study of short-lived transients is to lower the temperature. Cryoenzymological techniques[29] could be fruitfully applied to following reactions between enzymes and specific chromophoric substrates, and it will be interesting to eventually compare the same enzyme–substrate reaction by both rapid Raman and Raman–cryobiological techniques.

New biological areas where the labeling technique is being applied are in studying membrane processes and nucleic acid–protein interactions. High quality spectra have been recorded for resonance Raman labels bound to the plasma membrane of bacteria.[30] These spectra are drastically altered by changes in the state of membrane energization, indicating the potential of the technique for studying membrane processes. The method lends itself to in situ studies of related systems, for example, nerve conduction in axons.

Using UV excitation, resonance Raman spectra of nucleic acids have been reported.[31] However, one disadvantage of UV excitation is that selectivity and specificity are lost, because of the many chromophores absorbing below 300 nm in complex biological milieu. To overcome this problem for nucleic acids it may be possible to use thio nucleic acids in which a ring $C{=}O$ is replaced by $C{=}S$. The sulfur analogues absorb in the 300–350-nm region. Spectra of some naturally occurring thio nucleic acids in tRNA have been reported,[32] but thio nucleic acids could equally well be used as labels, i.e., as synthetic analogues of natural substrates in ATP–myosin[33] reactions or in a host of other nucleic acid protein complexes, e.g., between RNA polymerase, DNA template, and RNA.[34]

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