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Single-mode versus multimode calculations of Raman intensities of cytochrome *c*

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The simple single-mode model for Raman scattering is examined with regard to the relative Raman intensity ratios of cytochrome *c* at the 0–0 and 0–1 transition frequencies of S_1 and S_2. Interference between *A*- and *B*-term amplitudes as well as nonadiabatic effects are considered. The model is found to generate the experimentally observed intensity ratios only under the condition that the damping factors in S_2 (Soret band) and S_1 (Q band) are nearly equal. This condition is at variance with observation unless multimode effects are included to account for the large bandwidth of S_2 in cytochrome *c*. Use of the multimode model generates the observed Raman intensity ratios in S_2 without the need for *A*- and *B*-term interference effects.

The study of resonance Raman scattering intensity as a function of laser excitation wavelength (i.e., the Raman excitation profile, REP) can be a useful tool for the understanding of excited molecular states. A variety of phenomena can contribute to the overall resonance scattering amplitudes of a given Raman active vibrational mode. Within the adiabatic approximation two main sources of scattering amplitude are normally identified: the *A* term (Franck–Condon activity) and the *B* term (intermanifold vibronic activity).[1] Strictly speaking these sources must be considered in the full $3N$-6 vibrational subspace of the molecule. In fact, the shape of the REP of a given vibration may be dominated by other vibrations that are strongly coupled to the transition; and, furthermore, even when modes are weakly coupled to the electronic transition, if their number is sufficiently large, they will have a conspicuous influence on the shape of the REP.[2,3] Nonadiabatic corrections, though generally small, can also lead to detectable effects in the structure of the REP.[4]

If one ignores the multimode vibrational subspace and derives the REP from a single-mode *A*-term point of view, a very simple profile is obtained (particularly in the limit of weak Franck–Condon coupling, i.e., small potential well shifts). In this limit the only important Raman Franck–Condon factors involved in *A*-term scattering are $\langle 0|0\rangle\langle 0|1\rangle$ (at the zero–zero transition frequency) and $\langle 0|1\rangle\langle 1|1\rangle \simeq -\langle 0|0\rangle\langle 0|1\rangle$ (at the zero–one transition frequency). Thus, the single mode *A*-term amplitudes are equal in magnitude but opposite in phase. The REP then shows two peaks of equal intensity: one at the 0–0 frequency and the other at the 0–1 frequency of the electronic transition. However, if some *B*-term activity is also present, even within the single mode picture, interesting asymmetric interferences can occur due to the fact that the *B*-term scattering amplitudes have the same phase at both the 0–0 and 0–1 frequencies.

Recently a careful experimental study was made of the REP of the 1362 cm^{-1} (a_{1g}) mode in the Soret band (S_2) of ferrocytochrome *c*. The profile proves to be quite asymmetric.[2] Its shape is successfully rationalized in terms of an *A*-term model but within a multimode context.[2,3] On the other hand, calculations based on simple (single-mode) *A*- and *B*-term interferences have been applied to the REP's observed in metalloporphyrins and ferrocytochrome *c*.[5,6] In particular, Shelnutt[6] found that this model can generate a good qualitative fit to the *shape* of the asymmetric REP of the 1362 cm^{-1} vibration by using *B*-term interference from the vibronically coupled *Q* state (S_1) to alter the single mode *A* term of the Soret band (S_2). Large homogeneous bandwidths (900 cm^{-1} FWHM) were required to "fill out" the profile in the Soret band (this is inevitable for all single-mode models). Similarly, rather large homogeneous bandwidths (400 cm^{-1} FWHM) were used in the *Q* band region in order to minimize the differences between the observed and calculated Raman intensities for resonance with S_1 and S_2. Most significantly, it was found that this simple single-mode S_1/S_2 interference model failed on quantitative grounds and the predicted intensity ratio of the S_2 REP to the S_1 REP fell short of the observed ratio by a factor of 4, even when using the excessive 400 cm^{-1} bandwidth for S_1 (the maximum possible S_1 bandwidth is approximately 150 cm^{-1} and there is reason to believe that it is somewhat smaller, see Ref. 7).

In the multimode *A*-term model[2,3] both the asymmetric shape of the Soret REP and the intensity were fit, since that model is not constrained by parameters derived from the *Q* band (i.e., the REP in S_2 is dominated by multimode *A*-term effects and can thus be scaled independently of the *B*-term coupling with S_1). Given the quantitative failure of the single-mode S_1/S_2 interference model, Shelnutt suggested that additional *B*-term sources from higher lying electronic states (e.g., S_3) may be active. The multimode model was criticized for requiring homogeneous bandwidths for S_2 which are smaller than those claimed for S_1 (see Ref. 7, however). The multimode model was therefore not considered as a serious alternative. As a matter of fact, in the multimode model, the homogeneous bandwidth is a "soft" parameter and can vary considerably without significantly altering the absorption bandwidth or the REP shape.[2,3]

In the present communication we show how additional *B*-term sources from higher lying electronic states are absent and that the modeling of the Soret REP must succeed within the S_1/S_2 system of electronic states. We see how the elaborate computer calculations for test-

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ing the single-mode S_1/S_2 interference model[6] can be mimicked by simple algebraic statements which reveal the limits of this model and how it must fail at the quantitative level. We conclude that the multimode A-term model appears to capture the essence of the Soret REP of the 1362 cm^{-1} mode. Generally speaking, as resonance with a strongly allowed electronic transition is approached, one expects the A-term to overcome the B-term sources of scattering.[1] It appears that the Soret band of cytochrome c is no exception.

A search for B-term sources of scattering at energies above the Soret state in the cytochrome c system was made at three separate wavelengths: at 337 nm,[2] and more recently at 317 and 257 nm.[8] At neither of the latter two wavelengths was Raman scattering from the heme group detected. In particular we note that the absorption spectrum of ferrocytochrome c shows a feature at about 315 nm [$\epsilon_{315}=36$ (mM–cm)$^{-1}$] which is comparable to the intensity of the Q band (S_1). In order to check more carefully for possible contributions from this "state," comparative studies were carried out on identical samples of ferrocytochrome c (23 μM concentration, 1.5 o.d. at Soret maximum in 5 mm curvette) using visible and near ultraviolet excitation. Raman scattering in the S_1 region is easily observed under these conditions and the 1362 cm^{-1} band can be compared directly to the 1 M $SO_4^=$ internal standard. At 514.5 nm excitation [$\epsilon_{515}=12.6$ (mM–cm)$^{-1}$] we find that the ratio of the peak height of the 1362 cm^{-1} band to the standard band is approximately 0.08. This agrees well with the experimental relative intensities presented previously by Shelnutt.[6] On the other hand, a sample of 23 μM ferrocytochrome excited at 317 [$\epsilon_{317}=32$ (mM–cm)$^{-1}$] shows no trace of Raman scattering from the heme chromophore. Only the internal standard band of sulfate is observed with a signal to (rms) noise level of approximately 70/1. We therefore conclude that no significant (A- or B-term) source of scattering from the state at 315 nm is present which is comparable to the scattering seen in S_1. Thus, since the feature at 315 nm and the Soret band are of comparable widths, the Soret band REP must be rationalized within the S_1/S_2 system of electronic states without recourse to coupling with electronic states to the blue of the Soret band.

Next we delineate the constraints of the single mode S_1/S_2 interference model[6] by presenting an algebraic analysis based on simple stick scattering amplitudes. Since a_{1g} modes do not couple the nearly degenerate x–y states associated with the heme group, it is sufficient to treat the x-polarized states of S_1 and S_2 independently of the y-polarized states (note that each set will lead to essentially the same REP). Thus, the two state model can describe the resonance behavior of a_{1g} modes as long as additional electronic states do not contribute significantly to the scattering amplitudes and the x, y splitting in S_1 and S_2 is small compared to the Raman active quantum (as observed in S_1).

For resonance with a single electronic state, $|e\rangle$, vibronically coupled to one additional state, $|s\rangle$, the A- and B-term amplitudes for the Raman transition can be written in the following explicit forms[1,4]:

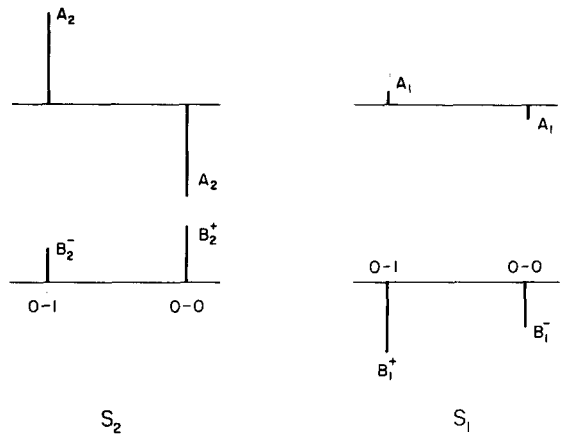

FIG. 1. The A- and B-term amplitudes for exact resonance with the 0–0 and 0–1 transitions of S_1 and S_2 are given. Explicit expressions for the various amplitudes can be found in Eq. (2) of the text. The phases of the amplitudes are given by the upward (+) or downward (–) direction of the stick amplitudes. The relative phasing between the A and B amplitudes is, *a priori*, unknown for a_{1g} modes. In this example we have chosen the phases so that the Raman intensity at the 0–1 transition of S_2 will be maximally enhanced. The zero-point energy separation between S_1 and S_2 is denoted by $|\Delta|$ in the text and corresponds to the separation between the two 0–0 transitions; the magnitude of Δ is 5800 cm^{-1} for ferrocytochrome c. The energy separation between the 0–0 and 0–1 transitions corresponds to the Raman active quantum $\hbar\omega$ and is 1362 cm^{-1} for this analysis.

$$A=\sum_v \frac{\langle g|R|e\rangle\langle e|R|g\rangle\ \langle 0|v\rangle\langle v|1\rangle}{E_v-h\nu_L-i\Gamma_e},$$

$$B=\sum_v \frac{\langle g|R|e\rangle\langle s|R|g\rangle\ \langle e|dV/dQ|s\rangle\ \langle 0|v\rangle\langle v|Q|1\rangle}{(E_v-h\nu_L-i\Gamma_e)(\Delta-\hbar\omega)} \qquad (1)$$

$$+\frac{\langle g|R|s\rangle\langle e|R|g\rangle\ \langle s|dV/dQ|e\rangle\ \langle 0|Q|v\rangle\langle v|1\rangle}{(E_v-h\nu_L-i\Gamma_e)(\Delta+\hbar\omega)},$$

where Q is the normal coordinate of the Raman active mode and v represents the occupation number of the excited vibrational state of $|e\rangle$. The transition dipole matrix elements are given by $\langle g|R|e\rangle$ and $\langle g|R|s\rangle$, while $\langle e|dV/dQ|s\rangle$ represents the vibronic coupling responsible for mixing the two crude adiabatic states $|e\rangle$ and $|s\rangle$. For simplicity, the damping factor Γ_e is taken to be independent of v (see Ref. 9). E_v is the electronic plus vibrational energy of the resonant state and $h\nu_L$ is the incident photon energy. The quantity Δ represents the zero-point energy separation of states $|e\rangle$ and $|s\rangle$ (i.e., $\Delta=E_e^0-E_s^0$) while $\hbar\omega$ is the energy of the Raman active vibration. This latter vibrational correction to the electronic energy gap between $|e\rangle$ and $|s\rangle$ represents the leading "nonadiabatic" correction to the REP.[4]

To elucidate the model we display in Fig. 1, hypothetical relative magnitudes and phases of the A- and B-term contributions to the scattering amplitudes for resonance with both the S_2 (Soret) and S_1 (Q band) transitions. The amplitudes in exact resonance with the 0–0 and 0–1 transitions of each state are shown as sticks pointing upward or downward to represent, respectively, the positive or negative phase factors. In order to approximate the observed asymmetry of the REP in S_2,[2] the phase be-

tween the A- and B-term amplitudes has been chosen to maximally enhance the 0–1 scattering in the Soret region.[6] The nonadiabatic corrections to the B-term amplitudes have also been included. To be more specific, we use Eq. (1) to find single mode, single state resonance expressions ($h\nu_L = E_v$) for the A- and B-term amplitudes for S_1 scattering ($|e\rangle = S_1$, $|s\rangle = S_2$) and for S_2 scattering ($|e\rangle = S_2$, $|s\rangle = S_1$):

$$A_1 = M_{A1}[S_1]^2/\Gamma_1 , \tag{2a}$$

$$A_2 = M_{A2}[S_2]^2/\Gamma_2 , \tag{2b}$$

$$B_1^{\pm} = M^{\pm}_B[S_1][S_2]/\Gamma_1 , \tag{2c}$$

$$B_2^{\pm} = M^{\pm}_B[S_1][S_2]/\Gamma_2 , \tag{2d}$$

where the subscripts 1 and 2 label the first and second excited singlet states. The quantities $[S_1]$ and $[S_2]$ represent the electronic transition dipole matrix elements, while Γ_1 and Γ_2 are the damping factors associated with the two electronic states. The remaining terms labeled M represent Raman Franck–Condon factors for the A-terms and nonadiabatic vibronic coupling strengths for the B-terms [see Eq. (1)].

We now proceed to find a set of relationships between the A- and B-term amplitudes and the damping factors (Γ_1 and Γ_2) that must be satisfied in order for this single-mode model to be consistent with the experimentally observed resonance Raman intensities.[2,6] To begin with, we can use Fig. 1 as a guide to calculate the theoretical Raman intensity ratios. Squaring the summed amplitudes gives the ratio of the Raman intensities at the 0–1 and 0–0 transition frequencies of S_2:

$$R_{10}^{(2)} = I_{01}^{S2}/I_{00}^{S2} = (A_2 + B_2^-)^2/(B_2^+ - A_2)^2 . \tag{3}$$

Similarly, the ratio of the Raman intensities at the 0–1 and 0–0 transitions of S_1 is given by

$$R_{10}^{(1)} = I_{01}^{S1}/I_{00}^{S1} = (A_1 - B_1^+)^2/(A_1 + B_1^-)^2 \tag{4}$$

and, finally, the ratio of the Raman intensity at the 0–1 transition of S_2 to the intensity of the 0–0 transition of S_1 is given by

$$R_{10}^{(2,1)} = I_{01}^{S2}/I_{00}^{S1} = (A_2 + B_2^-)^2/(A_1 + B_1^-)^2 . \tag{5}$$

If we now define the parameters:

$$\Theta = A_2/B_2^- , \tag{6a}$$

$$\Phi = B_1^-/A_1 , \tag{6b}$$

$$\gamma = \Gamma_2/\Gamma_1 , \tag{6c}$$

we can use Eq. (2) to rewrite Eqs. (3)–(5) in the following manner:

$$R_{10}^{(2)} = (\Theta + 1)^2/(\mu - \Theta)^2 , \tag{7a}$$

$$R_{10}^{(1)} = (1 - \mu\Phi)^2/(1 + \Phi)^2 , \tag{7b}$$

$$R_{10}^{(2,1)} = (\Phi\Theta + \Phi)^2/(\gamma + \gamma\Phi)^2 , \tag{7c}$$

where μ is the ratio $M_B^+/M_B^- = (|\Delta| + \hbar\omega)/(|\Delta| - \hbar\omega)$. For the present application $|\Delta| = 5800$ cm^{-1} and $\hbar\omega = 1362$ cm^{-1}, so that $\mu = 1.6$. Using the experimentally determined values for the three ratios[2,6] we have $R_{10}^{(1)} \simeq 2$, $R_{10}^{(2)} \simeq 2$, and $R_{10}^{(2,1)} \simeq 48$ (see Ref. 10) which leads to a unique solution for the unknowns

$$\Theta = 7.9 , \tag{8a}$$

$$\Phi = 13.0 , \tag{8b}$$

$$\gamma = 1.2 . \tag{8c}$$

Equation (8c) is especially interesting since an upper limit of 50 cm^{-1} for Γ_1 (100 cm^{-1} FWHM) can be deduced from the width of the S_1 absorption band of ferrocytochrome c.[7] Thus, a maximum value for Γ_2 is found to be 60 cm^{-1} (120 cm^{-1} FWHM). The fundamental contradiction that arises is that the observed fullwidth of the Soret band (S_2) approaches 1300 cm^{-1}, an order of magnitude larger than the maximum FWHM allowed within this single-mode model!

How, then, do we account for the large absorption bandwidth of S_2 if we limit ourselves to a single-mode model? If we take the extreme view that the Soret band is predominantly homogeneously broadened with $\Gamma_2 \simeq 500$ cm^{-1} (1000 cm^{-1} FWHM), we find $\gamma \simeq 10$ and, using Eq. (7c), we have $R_{10}^{(2,1)} \simeq 0.75$ *not* the observed 48. In the previous analysis,[6] an attempt was made to resolve the problem by using an intermediate value for γ (900 cm^{-1}/400 cm^{-1} = 2.25). This choice of γ still predicts significantly larger relative scattering intensities in the Q-band region than are observed[10] [i.e., $R_{10}^{(2,1)} = I_{01}^{s2}/I_{00}^{s1} = 12$ (Ref. 6) vs $R_{10}^{(2,1)} = 48$ (experimental)]. This discrepancy can be deduced directly from Eq. (7c) where it can be seen that a change in γ by a factor of 2 (1.2 vs 2.25) results in a decrease of $R_{10}^{(2,1)}$ by a factor of 4. Clearly, the single-mode (A- and B-term interference) model fails to account simultaneously for the observed relative Raman intensities and the absorption bandwidths of ferrocytochrome c.

As we have remarked, a straightforward resolution of this dilemma results if we consider the effect of the full multimode subspace of a_{1g} vibrations which, in any case, is *observed* to be resonantly Raman active upon Soret band excitation.[2] If this subspace is incorporated into the calculation, the I_{01}^{S2}/I_{00}^{S2} ratio increases without the necessity of B-term interference. In addition to fitting the Soret band REP, this model explains the general shape of the Soret absorption band.[2,3] Inclusion of the multimode approach in the present analysis relaxes the restriction of Eq. (3) and allows $\Theta = A_2/B_2^-$ to take on values greater than 7.9 (i.e., the multimode A term alone generates the ratio $R_{10}^{(2)}$ and interference with the B term is no longer necessary to explain the data). This in turn [see Eq. (7c)] may lead to an increase of γ from the single-mode value of 1.2 (approximate upper limits on Γ_1 and Γ_2 are discussed in Refs. 2, 3, and 7). In any case, the entire single-mode analysis suffers a serious breakdown once the multimode effects are acknowledged, since the original choice of phasing in Fig. 1 (and Ref. 6) is no longer required.

In conclusion, it is our view that in the Soret band of ferrocytochrome c the dominant scattering mechanism is, indeed, the multimode A term.[2,3,11] This view should be contrasted with the single-mode model which invokes significant A- and B-term interference effects to explain the REP but does not simultaneously account for the absorption bandwidths.[6] Some weak interference effects in S_2 from B-term coupling are possible, in our opinion,

and further experiments are needed in order to determine with accuracy the magnitude of this effect. In the *Q* band region, on the other hand, the theoretical analysis of resonance scattering is severely complicated by the significant interference between *A*- and *B*-term scattering mechanisms (with undetermined phase) as well as by the unusual polarization behavior.[10]

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[6]J. A. Shelnutt, J. Chem. Phys. **72**, 3948 (1980); see also J. Chem. Phys. **74**, 6644 (1981), where a multi mode point of view is included. However, the perturbation approach used there invokes ground state vibrational wave functions as a basis set and must diverge exponentially in coupling strength/number of CF active vibrations. It therefore is limited to cases in which there are only a small number of weakly coupled vibrations, and cannot incorporate the more extensive multimode model we regard as valid for the cytochrome *c* problem.

[7]The actual value for the FWHM of S_1 is probably somewhat less than 100 cm^{-1}; see P. M. Champion and R. Lange, J. Chem. Phys. 73, 5947 (1980); P. M. Champion and G. J. Perreault, J. Chem. Phys. 75, 490 (1981).

[8]B. Stallard (unpublished results).

[9]The assumption that the damping factor, Γ_e, is independent of the vibrational state is made in order to facilitate the comparison between the single-mode (Ref. 6) and multimode (Ref. 2) models, both of which employ this approximation. It is interesting to note, however, that if we assign different damping factors to the 0–0 and 0–1 transitions of S_2 (i.e., Γ_{20} and Γ_{21}) and consider only single-mode *A*-term scattering, we have [from Eqs. (2) and (3)] $R_{10}^{(2)} = \Gamma_{20}/\Gamma_{21} \simeq 2$ (experimental). This, however, would imply that the lifetime of the zero-point state of S_2 is two times shorter than the vibrationally excited state of S_2. This is quite an unusual result and so, for the present, we prefer the simplifying approximation $\Gamma_{ev} = \text{const}$.

[10]We have used the experimentally observed value for $R_{10}^{(2,1)} \simeq 48$ taken from Ref. 6 in which the single-mode model for explaining the REP of S_2 is put forth. In fact the 1362 cm^{-1} Raman line shows anomalous polarization when excited in the region *between* the 0–0 and 0–1 transitions of S_1 [see D. W. Collins, D. B. Fitchen, and A. Lewis, J. Chem. Phys. 59, 5714 (1973)]. This polarization behavior is not accounted for within the single-mode model as treated either here or in Ref. 6. In fact, the anomalous polarization behavior in S_1 may be due either to accidental degeneracy of Raman modes of different symmetry or to the lowering of the D_{4h} point group of the heme system. In the latter case the polarizability tensor for totally symmetric modes could contain an antisymmetric component. In the case of accidental degeneracy, the value of $R_{10}^{(2,1)}$ relevant to the single-mode model under discussion must have a *lower bound* of 48. If, on the other hand, the anomalous polarization has its origins in breakdown of symmetry, the REP in the S_1 region must be modeled to include antisymmetric scattering. In any case the extent of anomalous scattering at the 0–0 and 0–1 transitions of S_1 (the points of reference taken here) is greatly reduced from that seen midway between these bands (see Collins *et al.*). Furthermore, for S_2 excitation, no anomalous polarization is encountered. The dominant role of simple, symmetric, *A*-term scattering in the S_2 REP seems to successfully mask antisymmetric contributions to the polarizability tensor, whatever their origins. The argument presented here will show how the single-mode model is unable to rationalize, consistently, the bandwidth and the REP of S_2. Logic dictates that we employ parameters in our argument which favor the single-mode model. It is only for this reason that what may really be a lower limit of $R_{10}^{(2,1)}$ is accepted as its correct value, just as we have taken the phasing of the *A* and *B* amplitudes (Fig. 1) to be optimal for the single-mode model.

[11]We note that Soret band preresonance profiles of analogous a_{1g} modes in other heme systems are fit quite well using only the *A*-term contribution; see T. C. Strekas, J. A. Packer, and T. G. Spiro, J. Raman Spectrosc. 1, 197 (1973).