Cysteine Conformation and Sulfhydryl Interactions in Proteins and Viruses. 3. Quantitative Measurement of the Raman S-H Band Intensity and Frequency

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ABSTRACT The bond stretching vibration of the cysteine sulfhydryl (SH) group in a typical protein generates a Raman band in the spectral interval 2500–2600 cm^{-1}, a region devoid of interference from any other fundamental mode of vibration of the protein. The relatively high Raman cross section associated with the S-H stretching vibration, the sensitivity of the vibrational frequency to hydrogen bonding interactions and side chain configurations, and the dependence of the Raman intensity on thiol-thiolate equilibria, combine to make the Raman SH band a potentially valuable marker of protein sulfhydryl interactions and a unique indicator of sulfhydryl participation in thiol-disulfide oxidoreductase activity. In order to exploit Raman spectroscopy for these purposes, accurate and precise measurements of Raman SH band profiles are required. We show here that the required precision and accuracy can be achieved by use of the Raman band corresponding to the stretching vibration of in situ nitrogen gas as a quantitative intensity and frequency standard. The Raman Q-branch center of the N_2 band occurs at 2330.7 cm^{-1}.

INTRODUCTION

The sulfhydryl group (SH) plays an essential and ubiquitous role in biology (Jocelyn, 1972). In addition to the sulfhydryl-containing amino acid cysteine, sulfhydryl groups occur abundantly in nonproteinaceous cellular factors, such as the glutathiones and coenzyme A. Although the sulfhydryl group is involved in a variety of enzymatic functions, including oxidoreductase, transferase, and lyase activities, mechanistic details and structural modes of substrate binding are not well understood. A typical case is the thioredoxin family of proteins, a large family of small proteins each containing a redox-active pair of cysteines capable of catalyzing enzyme-specific disulfide reduction (Laurent et al., 1964). Although the crystal structure of the oxidized disulfide form of *Escherichia coli* thioredoxin has been solved at 1.68-Å resolution (Katti et al., 1990), a high-resolution crystal structure is not available for any thioredoxin in the native dithiol form. Multidimensional NMR studies have provided information about the solution structure of *E. coli* thioredoxin, but do not probe directly the sulfhydryl groups at the redox-active center (Dyson et al., 1990).

Raman spectroscopy is well suited to investigating structural and chemical properties of protein sulfhydryl groups. The Raman S-H stretching band occurs in an interval of the vibrational spectrum (2500–2600 cm^{-1}) which is devoid of interference from any other fundamental mode of vibration of the macromolecule (Thomas, 1987). Additionally, the intrinsic intensity (Raman scattering cross section) associated with the S-H stretching vibration is relatively high, permitting Raman detection of a single sulfhydryl in a macromolecule containing thousands, or even tens of thousands, of chemical bonds (Prevelige et al., 1993; Li and Thomas, 1991). In recent work from our laboratory, we have developed empirical correlations between the Raman SH marker and hydrogen bonding interactions of the sulfhydryl S-H donor and S acceptor groups (Li and Thomas, 1991). Normal mode analysis has also permitted the correlation of Raman S-H and C-S stretching frequencies with specific configurations of the cysteinyl side chain (Cα-Cβ-S-H) (Li et al., 1992). Application of these results to *E. coli* thioredoxin has revealed interactions of the active-site sulfhydryls and has facilitated direct measurement of pK_a values governing thioredoxin thiol-thiolate equilibria (Li et al., 1993).

Raman spectrophotometric determination of sulfhydryl ionization equilibrium constants requires precise and accurately reproducible measurements of the SH vibrational band frequency and intensity. For proteins containing more than one sulfhydryl, multiple SH band components are expected. In the present work we show that the required spectral measurements can be accomplished by appropriate use of the Raman band of ambient molecular nitrogen, which is observed at 2331 cm^{-1} in spectra recorded in a conventional Raman sample illuminator at standard conditions (see Fig. 1).

The 2331 cm^{-1} band, which is assigned to the Q-branch of the fundamental vibration-rotation band of N_2 gas (Herzberg, 1950), is demonstrated to originate from air in the vicinity of the laser scattering focus. We show that the N_2 reference band serves as a convenient and reliable standard for frequency and intensity calibration of sulfhydryl marker bands. Specifically, we demonstrate that the quotient of intensities of a solute SH Raman band, and the N_2 reference band (I_{SH}/I_{2331}) is a reliable quantitative measure of sulfhydryl concentration over the range 0.006 to 1.5 M. For this purpose we employ the simple sulfhydryl reagent β-mercaptoethanol (βME). In a related paper (Li et al.,

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1993), the methodology described here is applied to thioredoxin for determination of the pK_a values of active-site cysteines.

EXPERIMENTAL METHODS

Spectrometer and sample configuration

Raman spectra were excited with the 514.5 nm line of an argon laser (Innova 70, Coherent, Inc., Santa Clara, CA) using approximately 200 mW of radiant power at the sample. The spectra were measured on a Spex Model 1401 scanning spectrophotometer, operating under the control of an IBM microcomputer. Fig. 1 shows the configuration of the sample cell (Kimax 34502) with respect to the incident laser beam and monochromator entrance slit. In this 90° scattering geometry, the image which fills the entrance slit comprises Raman scattering from both the liquid sample within the capillary cell and ambient air in the vicinity of the laser focus (*shaded rectangle* of Fig. 1 *b*). For spectral measurements the sealed capillary cells were thermostated at 6°C (Thomas and Barylski, 1970).

Survey spectra were collected at 1 cm^{-1} intervals with 2.0 s integration time and 8 cm^{-1} spectral slit width. Additional data were collected at 0.5 cm^{-1} intervals and with 4 cm^{-1} slit width in the region 2300–2700 cm^{-1}. Multiple spectra were signal averaged, as described (Li et al., 1993), to enhance signal-to-noise ratios. Liquid indene was employed as the primary standard to calibrate Raman frequencies to within 1 cm^{-1}.

All spectra were excited with the laser beam precisely aligned through the diameter of the sample cell, as verified by maximizing the signal intensity with respect to translation of the beam along the horizontal direction depicted in Fig. 1 *a*. This assured that the volume ratio of sample and air remained constant in successive experimental protocols.

Raman standards

In spectra obtained with the sample illumination geometry of Fig. 1, we consistently observed the distinctive and sharp Raman band at 2330.7 ± 0.5 cm^{-1}, assigned to the triple-bond stretching vibration of gaseous molecular nitrogen (Herzberg, 1950). In order to identify the location of the contributing N_2 molecules, we conducted numerous control experiments using different samples, capillaries of different dimensions and capillary mounts of different design. The most useful Raman standards were 0.01 M aqueous solutions of L-alanine-3,3,3-d_3 (MSD Isotopes, Montreal, Canada), 0.04 M CCl_4 solutions of deuteriochloroform ($CDCl_3$, Sigma Chemical Co., St. Louis, MO), and aqueous solutions of β-mercaptoethanol (Sigma) over a wide range of solute concentration.

The N_2 scattering volume was localized ultimately by determining the position and dimensions of its image on the monochromator entrance slit. The accuracy and reproducibility of this determination are discussed below. Subsequently, we employed the N_2 marker (2331 cm^{-1}) as the frequency standard for measurement of sulfhydryl Raman spectra in the region 2300–2700 cm^{-1}. The N_2 Raman band was also exploited as an intensity standard, as described in the Results and Discussion section.

In the case of βME and other sulfhydryl containing compounds, the spectral region 500–550 cm^{-1} was surveyed to ensure that no significant oxidation of thiols to disulfides occurred following sample preparation or data collection protocols. Data collection was generally completed within 3 h of sample preparation. Solvent and scattering backgrounds were subtracted from the signal-averaged spectra by difference methods described previously (Verduin et al., 1984). Curve fitting procedures were carried out using software developed in our laboratory (Thomas and Wang, 1988).

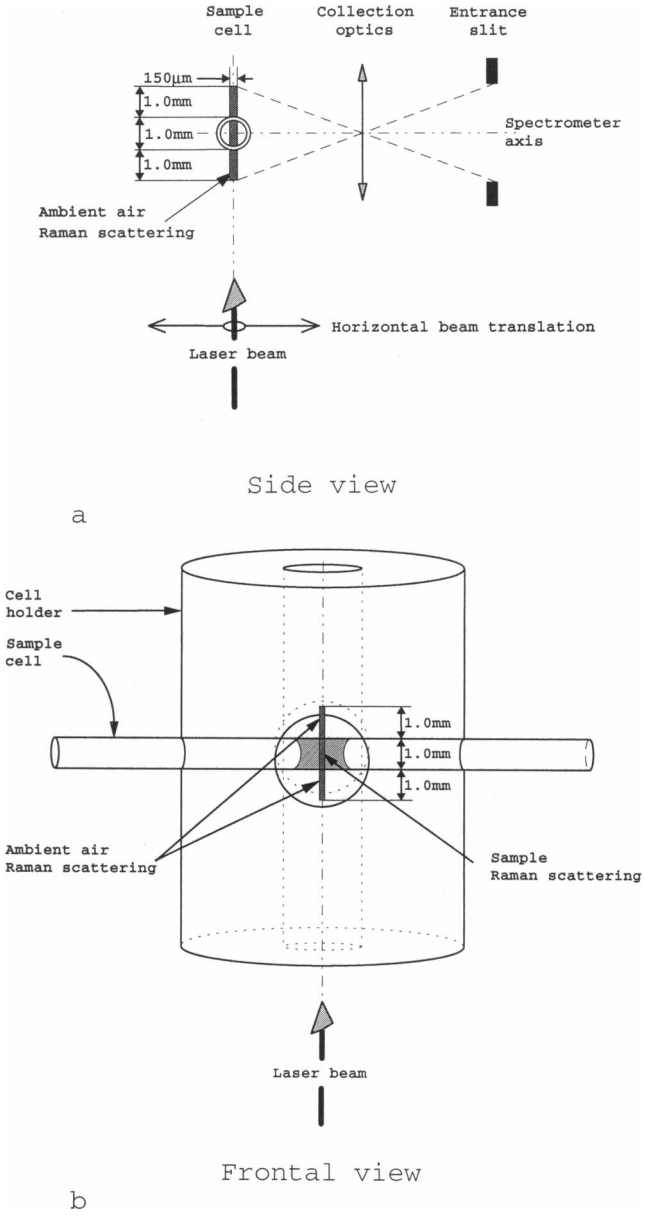

FIGURE 1 (*a*) Side view of the 90° scattering geometry in the sample illuminator of a Spex Model 1401 spectrophotometer. The laser beam (*lower left*) is manipulated in the horizontal plane to be impingent along a line intersecting the diameter of the capillary and perpendicular to its cylindrical axis. Raman scattering is collected from the shaded segment, which includes volumes of air both above and below the capillary sample cell as well as the volume of liquid within the capillary. (*b*) Frontal view of the sample cell holder (from top perspective) showing how the limits on Raman scattering from ambient air are determined by the upper and lower boundaries of the sample cell holder and the wall of the sample cell. The cross section of the shaded area was determined to be 3.0 mm by 67 μm, encompassing an air volume of 3.0×10^{-2} mm^3 and liquid volume of 1.50×10^{-2} mm^3 (see text).

RESULTS AND DISCUSSION

Characteristics of the sulfhydryl stretching band in proteins and viruses

Fig. 2 shows selected regions of the Raman spectrum of the bacterial virus P22. The P22 virion packages a double-stranded DNA chromosome in an icosahedral shell composed mainly of 420 copies of a 430-residue protein subunit (gp5) (Casjens, 1989). By analogy with model compounds, all of the Raman bands in this spectrum can be assigned in straightforward manner to specific residues of either the packaged dsDNA chromosome or the gp5

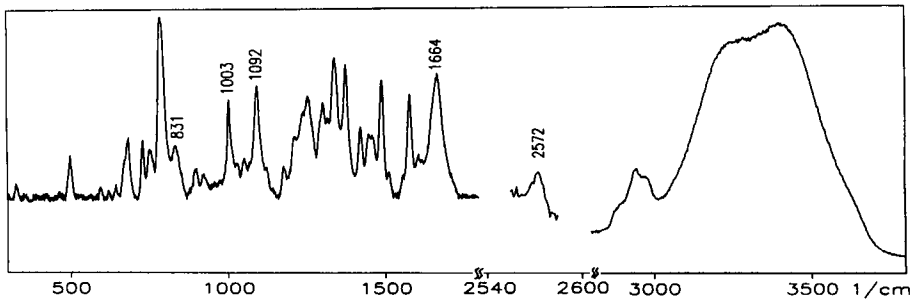

FIGURE 2 Raman spectrum in the intervals 300–1800, 2540–2600, and 2800–3800 cm^{-1} of the bacterial virus P22. The Raman SH stretching band of the single cysteine side chain (Cys^{405}) in the 430-residue coat protein subunit occurs at 2572 cm^{-1}. Also labeled are other representative Raman bands of coat protein subunits (1003 cm^{-1}, phenylalanine; 1664 cm^{-1}, amide I) and packaged dsDNA (831 and 1092 cm^{-1}, deoxyribose phosphate backbone). Detailed band assignments are given by Aubrey et al. (1992). The spectrum was obtained using 514.5-nm argon excitation from a sample of 5-μl volume at 12°C containing 80 μg of virus/μl in 10 mM Tris buffer (pH 7.5) + 200 mM NaCl + 10 mM $MgCl_2$. The intense Raman scattering of H_2O dominates the region 3100–3700 cm^{-1}.

subunit, as discussed in detail elsewhere (Aubrey, et al., 1992; Prevelige et al., 1993). For example, the bands at 1664 and 1003 cm^{-1} are due, respectively, to the peptide backbone (amide I) and phenylalanyl side chains (ring stretching) of gp5, while the bands at 831 and 1092 cm^{-1} are due to well characterized modes of the DNA deoxyribose-phosphate backbone. The intense Raman scattering in the 3100–3700 cm^{-1} region is due to H_2O solvent, and bands in the 2800–3100 cm^{-1} interval are due to CH stretching modes of both protein and DNA. The band at 2572 cm^{-1}, displayed in Fig. 2 using an amplified ordinate, is due to the single sulfhydryl group (Cys^{405}) in each gp5 subunit and illustrates the sensitivity of the method. This sulfhydryl represents one of 9000 chemical bonds in the protein subunit. Since the virus is composed by weight of about 44% protein, *the Cys^{405} sulfhydryl marker reflects 1 of roughly 20,000 chemically bonded groups in the P22 virion.*

In order to compare accurately the intensity of a cysteinyl SH Raman band with other Raman bands in a typical protein, we show in Fig. 3 the complete Raman spectrum of the 108-residue protein, thioredoxin (Holmgren, 1989), using uniform ordinate and abscissa scales. The two sulfhydryl groups of thioredoxin (Cys^{32} and Cys^{35}) generate a complex band centered near 2569 cm^{-1}. Further details of this spectrum have been discussed (Li et al., 1993). Interestingly, the band at 2331 cm^{-1}, due to the gaseous N_2 molecules, exhibits about the same peak intensity as the sulfhydryl marker, although with a much narrower bandwidth. This is as expected for the Raman Q-branch of a fundamental vibration-rotation

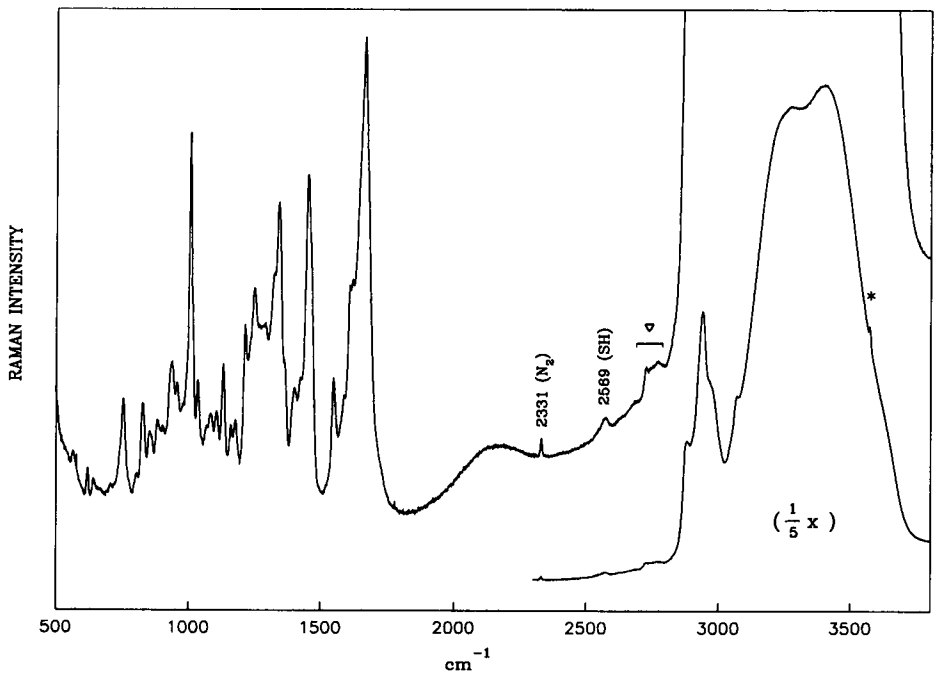

FIGURE 3 Raman spectrum in the region 300–3750 cm^{-1} of thioredoxin dissolved to 100 μg/μl in H_2O and adjusted to pH 4 with dilute HCl. The region 2300–3750 cm^{-1} is also shown at 1/5 amplification. No baseline corrections were employed. Other conditions are as given in Fig. 2. The asterisk (*) indicates a laser emission line; the arrow head (▽) indicates probable overtone and combination bands; and the Raman bands of the protein sulfhydryl groups (2569 cm^{-1}) and gaseous N_2 (2331 cm^{-1}) are labeled accordingly. Detailed assignments for other bands are given by Li et al. (1993).

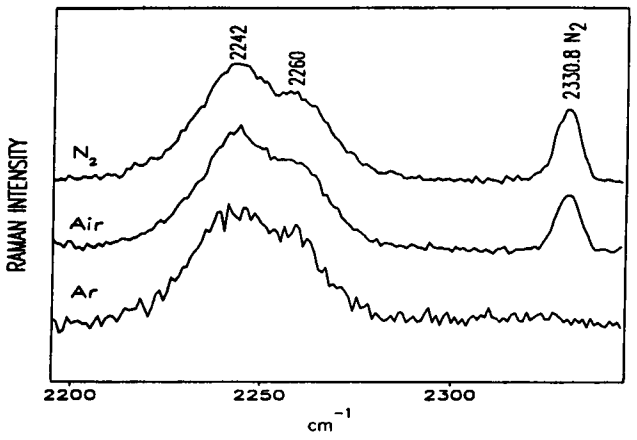

FIGURE 4 Raman spectrum in the region 2200–2350 cm^{-1} of L-alanine-d_3, 0.01 M in H_2O (pH 7.5). The sample was contained in a sealed glass capillary cell (Kimax 34502) mounted in the sample illuminator of the spectrometer (Spex Ramalog) with a controlled environment, as follows. Top spectrum, N_2 purge; middle spectrum, no purge (air at 101 kPa and 25°C); bottom spectrum, argon purge.

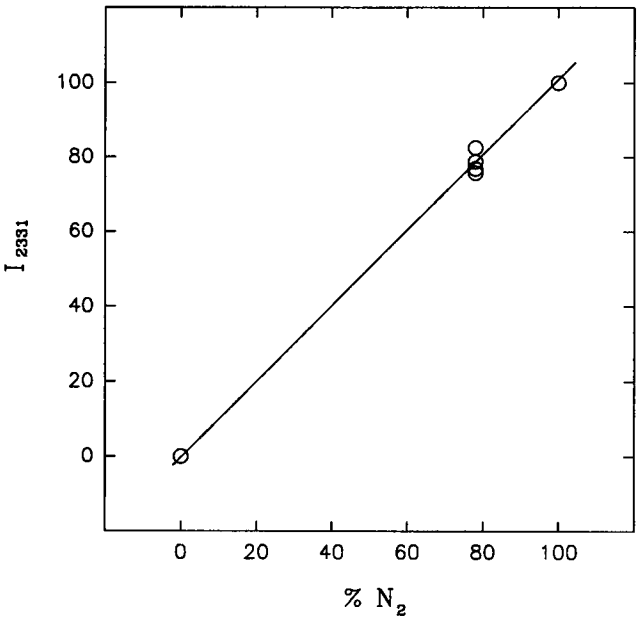

FIGURE 5 Plot of the dependence of the relative intensity of the 2331 cm^{-1} Raman band (N_2 Q-branch) on the nitrogen composition of the external cell atmosphere. The integrated intensity of the Raman bands (peaks at 2242, 2260 cm^{-1}) of L-alanine-d_3, measured above a baseline with tangents at 2200 and 2300 cm^{-1}, was used as the intensity standard.

band. The integrated intensities of the 2331 and 2569 cm^{-1} bands of Fig. 3 are in the approximate ratio 1:5.

Origin and characteristics of the nitrogen stretching band

Fig. 4 shows the Raman spectrum in the interval 2200–2350 cm^{-1} of an 0.01 M aqueous solution of L-alanine-3,3,3-d_3 (L-alanine-d_3). The data of Fig. 4 were collected for each of the following conditions in the sample compartment (Fig. 1) of the spectrometer: (i) ambient conditions (middle spectrum); (ii) during purge of N_2 gas (top spectrum); (iii) during purge of argon gas (bottom spectrum). The Raman bands of L-alanine-d_3 at 2242 and 2260 cm^{-1} in Fig. 4, which are assigned to C-D stretching vibrations (Aubrey and Thomas, 1991), provide a convenient basis for comparison of the intensity of the 2331 cm^{-1} band as a function of mole fraction of N_2 gas in the sample compartment of the Raman spectrometer. This is shown quantitatively in Fig. 5. The results of Figs. 4 and 5 confirm that the 2331 cm^{-1} band is due to gaseous N_2 in the sample compartment and that its intensity is linearly related to the molecular density of N_2. This was further confirmed in the 50–100 cm^{-1} region of the Raman spectrum by the detection of the pure rotational Raman spectrum of N_2 (data not shown).

The vertical dimension of the laser-illuminated volume of the sample cell holder (Fig. 1 *b*) is limited to 3.0 mm by the spectrometer design. Analysis of the image at the monochromator entrance slit at ambient conditions showed that 80% of the intensity of the 2331 cm^{-1} band originates from the

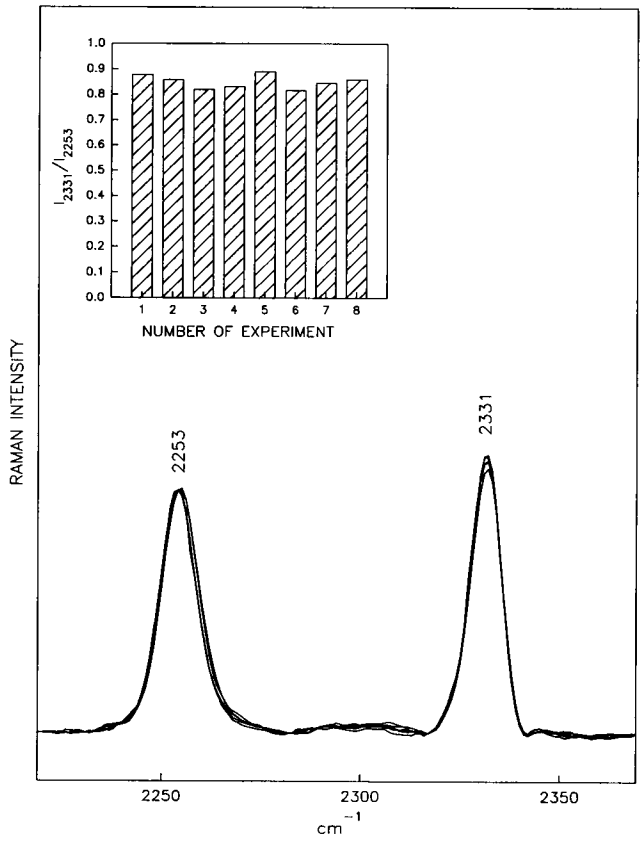

FIGURE 6 Raman spectrum in the interval 2220–2370 cm^{-1} of deuteriochloroform ($CDCl_3$) dissolved at 0.04 M in carbon tetrachloride. Spectral traces for eight independent experiments are superimposed. In each experiment the sample cell was aligned in the cell holder for precise horizontal alignment with respect to the incident laser beam (Fig. 1). Each trace is the average of 10 scans, and in each scan the data were collected at increments of 1 cm^{-1} with integration time of 5 s. The inset shows the integrated intensity ratio I_{2331}/I_{2253} measured in each independent experiment. The mean value of I_{2331}/I_{2253} (with standard deviation) is 0.850 (± 0.030).

volume of air (of which 78% is N_2) within 1 mm of the lower surface of the capillary cell, as depicted in Fig. 1 *a*. The remaining 20% of the band intensity originates from the volume of air within 1 mm of the upper surface of the cell. The predominating contribution of the former is ascribed to the much higher laser intensity measured at the proximal side of the capillary cell. Thus, in the vertical direction (Fig. 1), the limits on Raman scattering from N_2 are determined by the upper and lower boundaries of the sample holder and by the wall of the sample cell. In the horizontal direction, the boundaries of the N_2 scattering volume are determined by the diameter (150 μm) of the focused laser beam and the width (67 μm) of the image of scattered light which is condensed by the collection optics and projected through the monochromator entrance slit (400 μm) of Fig. 1 *a*. The total volume of air which contributes to the observed Raman scattering is calculated to be 3.0×10^{-2} mm^3.

Use of the 2331 cm^{-1} band as a quantitative intensity standard

The reproducibility of measurement of the intensity of the 2331 cm^{-1} band of N_2 was established through comparison with the intensity of the sharp and symmetrical C-D stretching band (2253 cm^{-1}) of liquid $CDCl_3$. Data obtained from repetitive experiments are shown in Fig. 6. Analysis of these data shows that the N_2 band intensity is accurately measurable, exhibiting a standard deviation of less than 4% in repeated experiments. Variations in the sample cell diameter, within the tolerances specified by the manufacturer (±3%), are believed to be the major source of these deviations.

Fig. 7 shows the interval 2300–2620 cm^{-1} from Raman spectra of aqueous solutions of βME containing different solute concentrations in the range 6–50 mM. The peak height of the sulfhydryl Raman band at 2586 cm^{-1} (I_{2586}) is normalized in each case to the peak height of the N_2 Raman band at 2331 cm^{-1} (I_{2331}). These data reveal a linear dependence of the peak intensity ratio (I_{2586}/I_{2331}) on βME concentration, expressed as moles per liter. The linear relationship is shown in Fig. 8 for βME concentrations extending from the millimolar range to values as high as 1.4 M. The linear fit of data points satisfies the equation

$$I_{2586}/I_{2331} = (230 \pm 5)\, C_{SH} - (0.7 \pm 0.1),$$

with a calculated correlation coefficient of 0.999. Similar results are obtained when integrated band intensities are used in place of the peak heights. These results show that the normalized intensity ratio I_{SH}/I_{2331} is a reliable indicator of sulfhydryl solute concentration in solution. Here, I_{SH} is the intensity of the sulfhydryl Raman band, computed either by peak height or band area. For proteins containing a single SH group and a well resolved SH Raman band, use of peak height simplifies the computation of I_{SH}. For proteins exhibiting multiple overlapping components of a complex SH Raman bandshape, either peak height or area may be used to compute each I_{SH}, after appropriate decomposition of the bandshape into its individual components (Li et al., 1993).

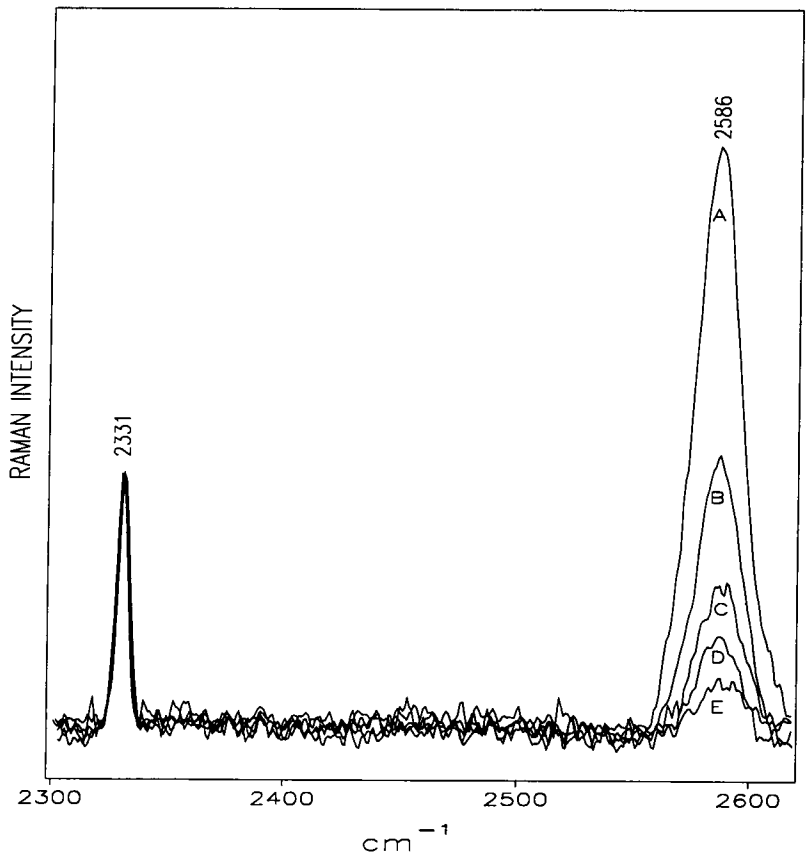

FIGURE 7 Raman spectra in the interval 2300–2620 cm^{-1} of aqueous solutions of βME. Concentrations of βME are as follows: (*A*) 50 mM; (*B*) 21.7 mM; (*C*) 12.5 mM; (*D*) 10.8 mM; (*E*) 6.25 mM. The intensity of the SH Raman band is normalized in each case to that of the 2331 cm^{-1} band of N_2. Data were recorded at 6°C.

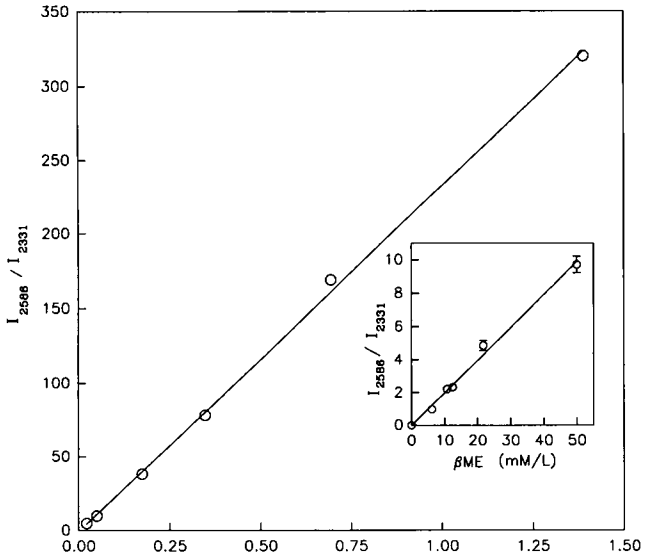

FIGURE 8 Plot of the intensity ratio I_{2586}/I_{2331} versus the molar concentration of βME in H_2O at 6°C, over a wide range of solute concentration. Data points for solutions with βME concentration in the millimolar range are shown in the inset at lower right.

CONCLUSIONS

The band observed at 2331 cm^{-1} in Raman spectra collected from solutions contained in capillary cells and maintained in a nitrogenous atmosphere is assigned to the Q-branch of the vibration-rotation band of gaseous N_2. The observed frequency, intensity, and bandwidth are as expected for the normal mode of molecular N_2. The linear dependence of the 2331 cm^{-1} band intensity upon nitrogen composition of the atmosphere in the sample compartment confirms the assignment and demonstrates that the band can serve as a reliable quantitative intensity standard. The intrinsic intensity of the N_2 Q-branch is relatively high, commensurate with that of the Raman SH band of a cysteine side chain. One indication of the high intrinsic intensity of the Raman SH marker is the detection in the P22 virus of a single cysteine sulfhydryl among the 430 amino acids per subunit. In a more typical globular protein-like thioredoxin, which contains 2 cysteine residues per 108 amino acids, the Raman SH bands and N_2 Q-branch exhibit comparable intensities.

The precise intensity of the N_2 Raman marker was found to be easily measured and accurately reproducible in independent experiments. This permits confident use of the N_2 Raman band as a reliable standard for monitoring cysteinyl SH Raman frequencies and intensities in protein solutions. Because the 2331 cm^{-1} band is not associated with any molecular species within the sample solution, its intensity is also invariant to changes in solute concentration, solution pH, ionic composition, and the like. The intensity of the N_2 marker is dependent only upon the molecular density of N_2 in the sample chamber and the geometry of laser-sample illumination. Both of these factors are easily controlled throughout a typical experimental protocol. Accordingly, the N_2 band is an appropriate standard for precise spectrophotometric determination of thiol ionization equilibria in proteins and their complexes (Li et al., 1993).

The use of the 2331 cm^{-1} band as a reference intensity standard for sulfhydryls is not limited to applications involving thiol-thiolate equilibria (Li et al., 1993). Similar intensity standardizations are required for evaluating changes in the hydrogen bonding environments of cysteine sulfhydryls, including those associated with conformational switching in protein assemblies (Verduin et al., 1984). Additionally, quantitative determinations of Raman SH band intensities can be valuable in assessing exposure of protein sulfhydryl groups to chemical probes and monitoring stabilities of cysteine-metal complexes (zinc-finger domains). Finally, we note that the approach described here is not limited to protein solutions. Raman SH intensity changes can provide important insights into the identities of metal-binding sulfhydryls in crystalline proteins, including isomorphous derivatives employed in x-ray structure determinations.

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REFERENCES

Aubrey, K. L., and G. J. Thomas, Jr. 1991. Raman spectroscopy of filamentous bacteriophage Ff (fd, M13, f1) incorporating specifically deuterated alanine and tryptophan side chains: assignments and structural interpretation. *Biophys. J.* 60:1337–1349.

Aubrey, K. L., S. R. Casjens, and G. J. Thomas, Jr. 1992. Secondary structure and interactions of the packaged dsDNA genome of bacteriophage P22 investigated by Raman difference spectroscopy. *Biochemistry.* 31:11835–11842.

Casjens, S. 1989. Bacteriophage P22 DNA packaging. *In* Chromosomes, Eukaryotic, Prokaryotic and Viral. Vol. III. K. W. Adolph, editor. CRC Press, Boca Raton, FL. 241–261.

Dyson, J. H., G. P. Gippert, D. A. Case, A. Holmgren, and P. E. Wright. 1990. Three-dimensional solution structure of the reduced form of *Escherichia coli* thioredoxin determined by nuclear magnetic resonance spectroscopy. *Biochemistry.* 29:4129–4136.

Herzberg, G. 1950. Molecular Spectra and Molecular Structure I. Spectra of Diatomic Molecules. Second edition. Van Nostrand, Princeton, NJ. 658 pp.

Holmgren, A. 1989. Thioredoxin and glutaredoxin systems. *J. Biol. Chem.* 264:13963–13966.

Jocelyn, P. C. 1972. The Biochemistry of the SH Group. Academic Press, London. 404 pp.

Katti, S. K., D. M. LeMaster, and H. E. Eklund. 1990. Crystal structure of thioredoxin from *Escherichia coli* at 1.68 Å resolution. *J. Mol. Biol.* 212:167–184.

Laurent, T. C., E. C. Moore, and P. Reichard. 1964. Enzymatic synthesis of deoxyribonucleotides. IV. Isolation and characterization of thioredoxin, the hydrogen donor from *E. coli* B. *J. Biol. Chem.* 239:3436–3444.

Li, H., and G. J. Thomas, Jr. 1991. Cysteine conformation and sulfhydryl interactions in proteins and viruses. 1. Correlation of the Raman S-H band with hydrogen bonding and intramolecular geometry in model compounds. *J. Am. Chem. Soc.* 113:1157–1175.

Li, H., C. J. Wurrey, and G. J. Thomas, Jr. 1992. Cysteine conformation and sulfhydryl interactions in proteins and viruses. 2. Normal coordinate analysis of the cysteine side chain in model compounds. *J. Am. Chem. Soc.* 114:7463–7469.

Li, H., C. Hanson, J. A. Fuchs, C. Woodward, and G. J. Thomas, Jr. 1993. Determination of the pK_a values of active-center cysteines, Cys32 and

Cys35, in *E. coli* thioredoxin by Raman spectroscopy. *Biochemistry.* 32: 5800–5808.

Prevelige, P. E., D. Thomas, K. L. Aubrey, S. A. Towse, and G. J. Thomas, Jr. 1993. Subunit conformational changes accompanying bacteriophage P22 capsid maturation. *Biochemistry.* 32:537–543.

Thomas, G. J., Jr. 1987. Viruses and nucleoproteins. *In* Biological Applications of Raman Spectroscopy. Vol. 1, Raman Spectra and the Conformations of Biological Macromolecules. T. G. Spiro, editor. Wiley-Interscience, New York. 135–201.

Thomas, G. J., Jr., and J. Barylski. 1970. Thermostating capillary cells for a laser-Raman spectrophotometer. *Appl. Spectrosc.* 24:463–464.

Thomas, G. J., Jr., and A. H.-J. Wang. 1988. Laser Raman spectroscopy of nucleic acids. *Nucleic Acids Mol. Biol.* 2:1–30.

Verduin, B. J. M., B. Prescott, and G. J. Thomas, Jr. 1984. RNA-protein interactions and secondary structures of cowpea chlorotic mottle virus for in vitro assembly. *Biochemistry.* 23:4301–4308.