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Convenient Detection of Thiol Functional Group Using H/D Isotope Sensitive Raman Spectroscopy

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Raman spectra of several thiols (amino acids, peptides and organic) show that the C-S-H bending mode (β_{CSH}) shifts from ~850 cm^{-1} to ~620 cm^{-1} on deuteration of the thiol proton by simply dissolving them in D_2O/CD_3OD where detection by ^{1}H NMR is not possible. A nondestructive analytical tool for the detection of thiols in solid/neat and solution is developed.

Thiols form an important subclass of molecules in nature.[1] Naturally occurring thiols like glutathione (GSH), which is a tri-peptide containing a cysteine residue (Fig. 1),[2] is responsible for maintaining cellular redox balance.[3-5] GSH plays an important role in toxicology, drug metabolism, protein, DNA synthesis and transport[4, 6] and in the development/progression of cancer.[1, 7] It plays a protecting role against cellular damage by the reactive oxygen species (ROS)[5] generated during metabolism.[7, 8] Thiol containing pharmaceuticals such as penicillamine (Fig. 1), mercaptopurine, and captopril are effective in the treatment of many serious diseases like arthritis, hypertension, skin disease and cancer.[9] Sevral natural products containing thiol or disulfide such as coenzyme A,[10] thioterpineol, lipoic acid,[4, 10] and N-2-mercaptopropionyl glycine[11],[12] are known. Apart from these, thiol has widely used to prepare self assembled monolayer (SAM) on Au, Ag and other coinage metal surfaces.[13, 14] SAM modified surfaces are important for nanoscience, nanotechnology, molecular electronics and nonlinear optics.[15-20] Protein, cell and other biological species can be easily immobilized on SAMs,[21-23] which can be extensively used for biotechnological applications such as in tissue engineering and biosensing. Investigations of these areas of science require synthesis of thiols and their adequate characterization.[9]

Thiol group in a molecule is detected with the help of several colorimetric or fluorescence assays.[3, 24, 25] In such cases the detection is achieved at the cost of the material *i.e.* the thiol used can not be easily recovered. The most commonly used spectroscopic tools for the detection of thiols are FTIR and ^{1}H NMR. The S-H stretching frequency appears at ~2550 cm^{-1} in the FTIR spectrum of a thiol containing molecule. However, the intensity of this peak is very weak (S-H is a weak dipole) and is difficult to detect in dilute solutions of thiols. For alkyl thiols the thiol proton resonance is observed between 1.2-1.8 ppm in ^{1}H NMR.[26] This peak is often masked by alkyl protons in the case of long chain thiols[27, 28] and is not observed when measured in a protic solvent like CD_3OD or D_2O (Fig. S1-S7 in SI) as the thiol proton is readily exchanged with that of the solvent. This poses a practical problem in the detection of the thiol functional group, in particular, in water soluble molecules in spite of their importance in chemistry and biology. Hence a convenient non-destructive spectroscopic tool for the detection of a thiol functional group is extremely desirable.

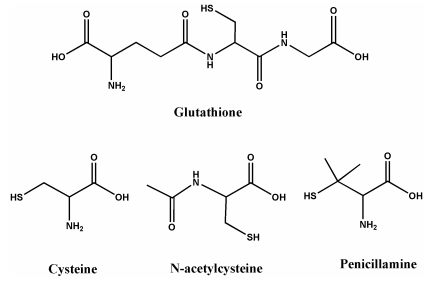

Fig. 1. Representation of biologically important thiols.

The Raman spectrum of thiol containing molecules show the C-S vibrations (ν_{CS}) in between 650-700 cm^{-1}, *i.e.* in a finger print region where most other functional groups do not interfere.[29, 30] However this fails to serve as a diagnostic feature as this vibration varies in position depending on the thiol and is also present in disulfides and thioethers.[31, 32] A while back it was reported that the C-S-H bending mode (β_{CSH}) in ethanethiol shifts from 870 cm^{-1} to 625 cm^{-1}, in the gas phase, on deuteration of the thiol proton.[33] In this manuscript we show that the β_{CSH}, observed around 850-900 cm^{-1} for different thiols (neat or in solution), shifts to 600-630 cm^{-1} on deuterating the thiol proton (resulting in RSD). Further, the deuteration can be performed by simply dissolving thiols in protic solvents like CD_3OD and D_2O. Using this technique the RSH functionality has been detected in a series of organic molecules, amino acids and peptides known to bear a thiol group. The ~250 cm^{-1} isotope shift observed in the β_{CSH} mode on deuterating the thiol proton is absent in disulfides.

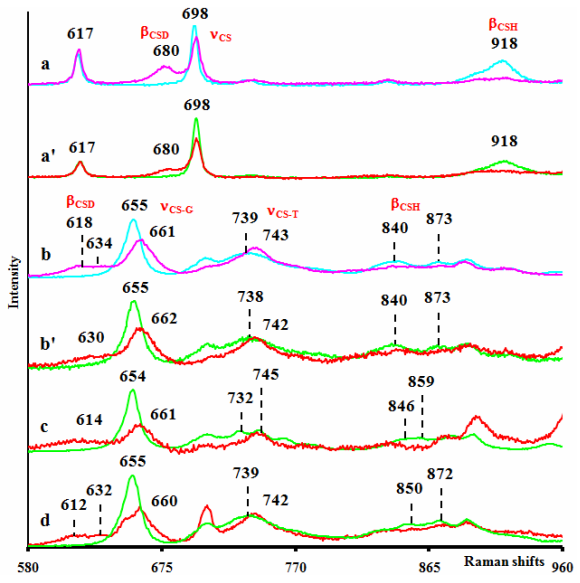

Fig. 2. The Raman spectra of (a) neat PhSH and PhSD; (a') PhSH in CH_3OH and CD_3OD; (b) neat $C_8H_{17}SH$ and $C_8H_{17}SD$ (b') $C_8H_{17}SH$ in CH_3OH and CD_3OD; (c) C_4H_9SH in CH_3OH and CD_3OD and (d) $C_{12}H_{25}SH$ in CH_3OH and CD_3OD. Sky blue and green spectra are for protonated species or solutions and pink and red spectra are for deuterated species or solutions. In solution spectra concentration was around 2-3 mM. Raman shifts are in cm^{-1}. ν is stretching mode, β is bending mode, G is gauche and T is trans conformation.

The β_{CSH} of neat PhSH occurs at 918 cm^{-1} and the ν_{CS} is observe at 698 cm^{-1} (Fig. 2a, sky blue).[34, 35] On deuterating the thiol proton (PhSD, Fig. 2a, pink), the peak at 918 cm^{-1} disappears and the β_{CSD} appears at 680 cm^{-1} i.e. 238 cm^{-1} shift is observed on replacing the thiol proton with deuterium. H/D isotope shifts of similar magnitude (i.e. 200-250 cm^{-1}) are also reported for the H-X-H bending modes of H_2O and H_2S. [36, 37] The H/D exchange can easily be performed by dissolving PhSH in CH_3OH (for H) or CD_3OD (for D). The data obtained in CH_3OH (Fig. 2a', green) and CD_3OD (Fig. 2a', red) solutions of PhSH indicate that the changes observed in the data (H/D isotope shifts) are identical to those obtained between neat PhSH and PhSD. In neat octanethiol ($C_8H_{17}SH$), two β_{CSH} modes are observed due to the presence of gauche (G) and trans (T) conformations across the C_1-C_2 bond.[38] These vibrations shift from 840 cm^{-1} and 873 cm^{-1} in neat $C_8H_{17}SH$ (Fig. 2b, sky blue) to 618 cm^{-1} and 634 cm^{-1} in neat $C_8H_{17}SD$ (Fig. 2b, pink). The same H/D isotope shifts are observed in the data obtained in CH_3OH (Fig. 2b', green) and CD_3OD (Fig. 2b', red) solutions of $C_8H_{17}SH$. Note that in the case of alkyl thiols the ν_{CS} for the G and T conformations, which are at 655 cm^{-1} and 739 cm^{-1} for $C_8H_{17}SH$ (both in neat and in CH_3OH solution), shifts to 661 cm^{-1} and 743 cm^{-1}, respectively, in $C_8H_{17}SD$ (both in neat and CD_3OD solutions). The > 200 cm^{-1} lowering on the β_{CSH} mode and ~6-7 cm^{-1} increase in the ν_{CS} mode is observed for a series of alkyl thiols (which includes butanethiol, dodecanethiol and ethanethiol, Fig. 2c, 2d, 3a and 3a'). In particular, the appearance of a new peak in the 600-630 cm^{-1} (at a lower energy than the C-S stretching vibration) on deuteration is clearly observed for all the thiols and provides a clear spectroscopic signature for the presence of thiol group in long chain alkyl thiols and in aromatic thiols in neat or in methanolic solution.[29, 35]

The trend continues to hold for thiol molecules soluble in water. The β_{CSH} mode in C_2H_5SH shifts from ~870 cm^{-1} to ~620 cm^{-1} after deuteration[33, 39] in both CH_3OH/CD_3OD (Fig. 3a, green/red) and H_2O/D_2O (Fig. 3a', blue/pink) solvents. The increase in the ν_{CS} on deuteration of the thiol group, in this case, is ~16 cm^{-1} which is significantly more than those observed for the long chain thiols. Since the thiol protons are easily exchangeable in H_2O/D_2O, this analytical technique may be extended to several biologically relevant molecules bearing thiol groups.

N-acetyl cysteine (Fig. 1) is possibly the smallest analogue of a cysteine containing peptide. The C-S region in the Raman spectrum in methanolic solution shows two vibrations at 654 cm^{-1} and 685 cm^{-1} representing the C=O deformation (of amide group) and ν_{CS}, respectively[40] (Fig. 3b, green). In CD_3OD (Fig. 3b, red) the ν_{CS} appears at 688 cm^{-1} and the β_{CSH} appears at 638 cm^{-1}. Similarly the ν_{CS} and β_{CSH} shifts from 682 cm^{-1} and 876 cm^{-1} in H_2O (Fig. 3b' blue) to 692 cm^{-1} and 640 cm^{-1} in D_2O (Fig. 3b', pink), respectively. These shifts are consistent with the trend observed for alkyl and aromatic thiols so far. The ν_{CS} and β_{CSH} of cysteamine solubilized in water occurs at 664 cm^{-1} and 785 cm^{-1}, respectively.[41] These vibrations shifts to 676 cm^{-1} and 632 cm^{-1}, respectively, (Fig. 4a, blue to red) when dissolved in D_2O. The corresponding disulfide shows no H/D isotope sensitive band in this region (Fig. 4a', green to brown). GSH show the ν_{CS} at 673 cm^{-1} and the β_{CSH} mode at 820 cm^{-1} in H_2O[42] (Fig. 4b, green) which shift to 689 cm^{-1} and 620 cm^{-1}, respectively, in D_2O (Fig. 4b, red). GSSG the corresponding disulfide does not show any H/D isotope sensitive band in this region (Fig. 4b', green to brown). The difference spectra of these experimental spectra are shown in supporting information (Fig. S8-S10)

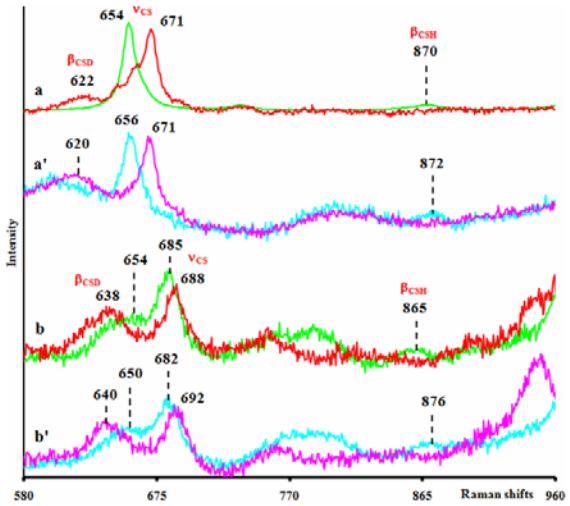

Fig. 3. The Raman spectra of C_2H_5SH in (a) CH_3OH (green) and CD_3OD (red) and in (a') H_2O (sky blue) and D_2O (pink), and of N-acetyl cysteine in (b) CH_3OH and CD_3OD and in (b') H_2O and D_2O (same colour code). Concentration was around 2 mM. Raman shifts are in cm^{-1}. ν is stretching mode and β is bending mode.

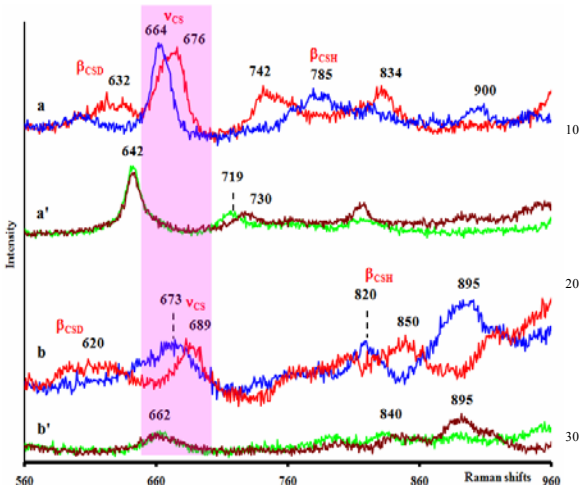

Fig. 4. The Raman spectra of (a) cysteamine, (a') cystamine and of (b) glutathione, (b') glutathione (S-S) solubilized in H_2O and D_2O. Blue and red spectra correspond to free thiols dissolved in H_2O and D_2O, respectively. The brown and green lines represent Raman data of the disulphides dissolved in H_2O and D_2O, respectively. The ν_{CS} stretching region is highlighted. Concentration was around 2 mM. Raman shifts are in cm^{-1}. ν is stretching mode and β is bending mode.

The experimentally observed H/D shifts in the Raman spectra of thiols can be corroborated to density functional theory (DFT, using BP86 functional, 6-311g* basis set in Gaussian 03 ver. C02)[43-45] calculated H/D shifts. DFT calculations on PhSH indicate that the β_{CSH} is at 908 cm^{-1} and it shifts to 672 cm^{-1} in PhSD (Fig. 5a', purple to blue). These values are in close agreement with the experimental data which shows the β_{CSH} shift from 918 cm^{-1} in PhSH to 680 cm^{-1} in PhSD (Fig. 5a, green to orange). Similarly, the β_{CSH} in C_2H_5SH is calculated to shift by 231 cm^{-1}, from 835 cm^{-1} to 604 cm^{-1}, on deuterating the thiol proton (Fig. 5b', purple to blue). The experimental data show a shift of 250 cm^{-1} in the β_{CSH} mode on deuteration (Fig. 5b, green to orange). Furthermore the DFT calculations successfully reproduce the shift of the C-S stretching vibration of C_2H_5SH to higher energies on deuterating the thiol proton.

DFT calculations are also used to calculate the effect of H/D isotopic substitution on the β_{CSH} and ν_{CS} modes of alkyl thiols having T or G conformations of the C_1-C_2 bond (Fig. 6). In the case of C_4H_9SH having a T conformation, the ν_{CS} and the β_{CSH} shifts from 715 cm^{-1} and 823 cm^{-1} to 728 cm^{-1} and 618 cm^{-1}, respectively, on deuterating the SH group (Fig. 6a, purple to blue). Alternatively, in G conformation, the ν_{CS} and the β_{CSH} shifts from 649 cm^{-1} and 852 cm^{-1}, 886 cm^{-1} to 673 cm^{-1} and 611 cm^{-1}, respectively, on deuterating the SH group (Fig. 6c). These calculations a) reproduce the relative energies of C-S vibration of thiols having G and T orientations and b) reproduce the shifts in β_{CSH} and ν_{CS} on deuteration of the thiol. These calculations also indicate the presence of the β_{CSD} modes of both G and T conformers energetically close to each other explaining in the broad β_{CSD} peak observed in the the experimental spectrum of C_4H_9SD at 614 cm^{-1} (Fig. 6b, orange). Similar broadening of the β_{CSD} band is also observed for both C_8H_9SD (Fig. 2b and 2b') and $C_{12}H_{25}SD$ (Fig. 2d).

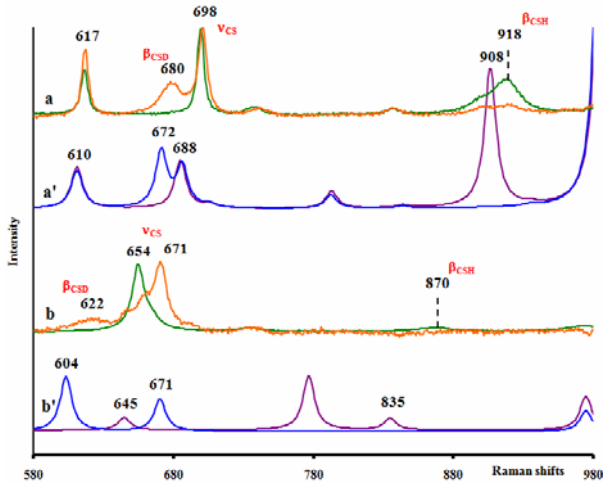

Fig. 5. The Raman spectra of (a) PhSH in CH_3OH (green) and CD_3OD (orange), of (a') PhSH and PhSD by theoretical predictions, of (b) C_2H_5SH in CH_3OH (green) and CD_3OD (orange) and of (b') C_2H_5SH and C_2H_5SD by theoretical predictions. In theoretical spectra violet and blue represents the protonated and deuterated results, respectively. Raman shifts are in cm^{-1}.

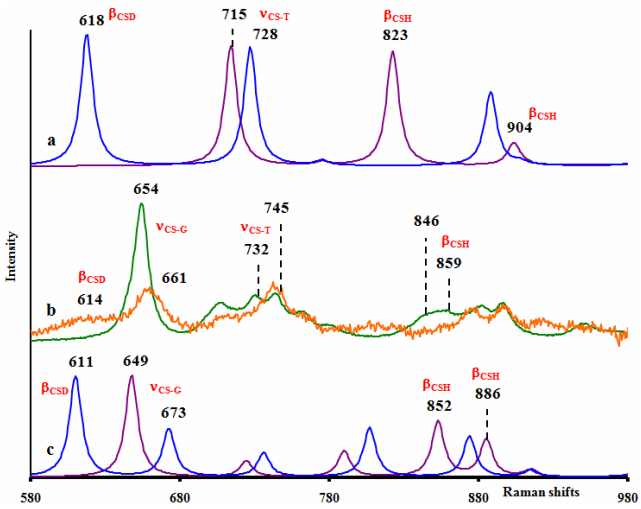

Fig. 6. The Raman spectra of (b) C_4H_9SH in CH_3OH (green) and CD_3OD (orange). Theoretically predicted results in (a) T conformation and in (c) G conformation. The purple and blue lines represent the calculated Raman spectrum for protonated and deuterated thiols, respectively. Raman shifts are in cm^{-1}. ν is stretching mode, β is bending mode, G is gauche and T is trans conformation.

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In summary, observation of a new peak below the C-S stretch and the shift of the C-S vibration to higher energies on deuteration of the thiol proton is found to be true for a broad range of thiol group containing molecules and may qualify a Raman signature of a thiol group. The protonated and deuterated thiols can be probed in neat or in solution resulting in similar H/D isotopic shifts. The deuteration can be performed in-situ by dissolving the thiol group containing molecule in CD_3OD or in

D_2O. The difference spectra of these theoretical results are shown in supporting information (Fig. S11-S12)

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Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental procedure, computational details, ^{1}H NMR spectra of thiols, the Raman spectra of above mentioned thiols along with their difference spectra and table of the spectroscopic data. See DOI: 10.1039/b000000x/

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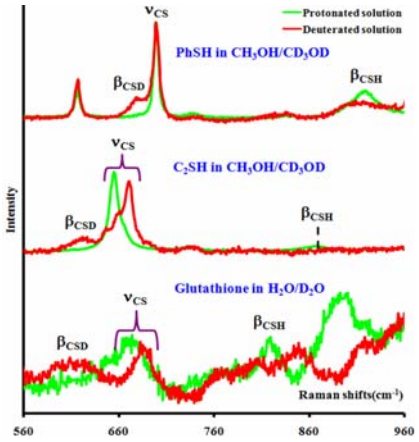
Graphics



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