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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 ($\beta_{\text{CSH}}$) shifts from ~850 cm$^{-1}$ to ~620 cm$^{-1}$ on deuteration of the thiol proton by simply dissolving them in D$_2$O/CD$_3$OD 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. Naturally occurring thiols like glutathione (GSH), which is a tripeptide containing a cysteine residue (Fig. 1), is responsible for maintaining cellular redox balance.$^1$-$^5$ GSH plays an important role in toxicology, drug metabolism, protein, DNA synthesis and transport$^6$-$^8$ and in the development/progression of cancer.$^6$-$^7$ It plays a protecting role against cellular damage by the reactive oxygen species (ROS)$^8$ 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.$^9$Several natural products containing thiol or disulfide such as coenzyme A,$^{10}$ thioterpinol, lipoic acid,$^4$-$^10$ and N-2-mercaptopyrrolidine, are known. Apart from these, thiol has widely used to prepare self assembled monolayer (SAM) on Au, Ag and other coinage metal surfaces.$^{11}$-$^{14}$ 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 cannot 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$_3$OD or D$_2$O (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.

The Raman spectrum of thiol containing molecules show the C-S vibrations ($\nu_{\text{CS}}$) in between 650-700 cm$^{-1}$, i.e. in a finger print region where most other functional groups do not interfere. 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.$^{29}$-$^{32}$ A while back it was reported that the C-S-H bending mode ($\beta_{\text{CSH}}$) 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 $\beta_{\text{CSH}}$ observed around 850-900 cm$^{-1}$ for different thiols (neat or in solution), shifts to 600-630 cm$^{-1}$ on deutering the thiol proton (resulting in RSD). Further, the deuteration can be performed by simply dissolving thiols in protic solvents like CD$_3$OD and D$_2$O. 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 $\beta_{\text{CSH}}$ mode on deutering the thiol proton is absent in disulfides.
The presence of gauche (G) and trans (T) conformations across the neat octanethiol (C8H17SH), two are identical to those obtained between neat PhSH and PhSD. In the presence of H/D exchange can easily be performed by dissolving PhSH in CH3OH solution, shifts to 661 cm-1 and 876 cm-1 in H2O (Fig. 2b', blue) to 692 cm-1 and 640 cm-1 in D2O (Fig. 2b', pink), respectively. These shifts are consistent with the trend observed for alkyl and aromatic thiols so far. The VCS and βCSH 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 D2O. The corresponding disulfide shows no H/D isotope sensitive band in this region (Fig. 4a', green to brown). GSH show the VCS at 673 cm-1 and the βCSH mode at 820 cm-1 in H2O42 (Fig. 4b, green) which shift to 689 cm-1 and 620 cm-1, respectively, in D2O (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 C2H5SH in (a) CH3OH (green) and CD3OD (red) and in (a') H2O (sky blue) and D2O (pink), and of N-acetyl cysteine in (b) CH3OH and CD3OD and in (b') H2O and D2O (same colour code). Concentration was around 2 mM. Raman shifts are in cm-1, v is stretching mode, β is bending mode, G is gauche and T is trans conformation.

Fig. 2. The Raman spectra of (a) neat PhSH and PhSD; (a') PhSH in CH3OH and CD3OD; (b) neat C8H17SH and C12H25SD (b') C8H17SH in CH3OH and CD3OD; (c) C8H17SH in CH3OH and CD3OD and (d) C12H25SH in CH3OH and CD3OD. 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, v is stretching mode, β is bending mode, G is gauche and T is trans conformation.

The βCSH of neat PhSH occurs at 918 cm-1 and the VCS is observe at 698 cm-1 (Fig. 2a, sky blue).34 On deuterating the thiol proton (PhSD, Fig. 2a, pink), the peak at 918 cm-1 disappears and the βCSH appears at 680 cm-1 (Fig. 2a, pink). These βCSH modes are observed due to the presence of gauche (G) and trans (T) conformations across the C-S bond.38 These vibrations shift from 840 cm-1 and 873 cm-1 in neat C8H17SH (Fig. 2b, sky blue) to 618 cm-1 and 634 cm-1 in neat C8H17SD (Fig. 2b, pink). The same H/D isotope shifts are observed in the data obtained in CH3OH (Fig. 2b', green) and CD3OD (Fig. 2b', red) solutions of C8H17SH. Note that in the case of alkyl thiols the VCS for the G and T conformations, which are at 655 cm-1 and 739 cm-1 for C8H17SH (both in neat and in CH3OH solution), shifts to 661 cm-1 and 745 cm-1, respectively, in C8H17SD (both in neat and CD3OD solutions). The > 200 cm-1 lowering on the βCSH mode and ~6-7 cm-1 increase in the VCS 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 βCSH mode in C2H5SH shifts from ~870 cm-1 to ~620 cm-1 after deuteration33,39 in both CH3OH/CD3OD (Fig. 3a, green/red) and H2O/D2O (Fig. 3a', blue/pink) solvents. The increase in the VCS 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 H2O/D2O, 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 VCS, respectively.40 In CD3OD (Fig. 3b, red) the VCS appears at 688 cm-1 and the βCSH appears at 638 cm-1. Similarly the VCS and βCSH shifts from 682 cm-1 and 876 cm-1 in H2O (Fig. 3b', blue) to 692 cm-1 and 640 cm-1 in D2O (Fig. 3b', pink), respectively. These shifts are consistent with the trend observed for alkyl and aromatic thiols so far. The VCS and βCSH 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 D2O. The corresponding disulfide shows no H/D isotope sensitive band in this region (Fig. 4a', green to brown). GSH show the VCS at 673 cm-1 and the βCSH mode at 820 cm-1 in H2O42 (Fig. 4b, green) which shift to 689 cm-1 and 620 cm-1, respectively, in D2O (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).
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) calculated H/D shifts. DFT calculations on PhSH indicate that the $\beta_{\text{CSH}}$ 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 $\beta_{\text{CSH}}$ shift from 918 cm$^{-1}$ in PhSH to 680 cm$^{-1}$ in PhSD (Fig. 5a, green to orange). Similarly, the $\beta_{\text{CSH}}$ in C$_2$H$_5$SH 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 $\beta_{\text{CSH}}$ mode on deuteration (Fig. 5b, green to orange). Furthermore, the DFT calculations successfully reproduce the shift of the C-S stretching vibration of C$_2$H$_5$SH to higher energies on deuterating the thiol proton.

DFT calculations are also used to calculate the effect of H/D isotopic substitution on the $\beta_{\text{CSH}}$ and $\nu_{\text{CS}}$ modes of alkyl thiols having T or G conformations of the C$_1$-C$_2$ bond (Fig. 6). In the case of C$_4$H$_9$SH having a T conformation, the $\nu_{\text{CS}}$ and the $\beta_{\text{CSH}}$ 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 $\nu_{\text{CS}}$ and the $\beta_{\text{CSH}}$ shifts from 649 cm$^{-1}$ and 852 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 $\beta_{\text{CSH}}$ and $\nu_{\text{CS}}$ on deuteration of the thiol. These calculations also indicate the presence of the $\beta_{\text{CSD}}$ modes of both G and T conformers energetically close to each other explaining in the broad $\beta_{\text{CSD}}$ peak observed in the experimental spectrum of C$_4$ H$_9$SD at 614 cm$^{-1}$ (Fig. 6b, orange). Similar broadening of the $\beta_{\text{CSD}}$ band is also observed for both C$_6$H$_{13}$SD (Fig. 2b and 2b’) and C$_{12}$H$_{25}$SD (Fig. 2d).

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$_3$OD or in...
D₂O. The difference spectra of these theoretical results are shown in supporting information (Fig. S1-S12).

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Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental procedure, computational details, ¹H 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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