ChemComm
www.rsc.org/chemcomm

Nuclear inelastic scattering spectroscopy of iron–sulfur cubane compounds

Vasily S. Oganesyan,[ac] J. Elaine Barclay,[b] Sinead M. Hardy,[a] David J. Evans,[b] Christopher J. Pickett[b] and Upali A. Jayasooriya*[a]

[a] *School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich, UK NR4 7TJ. E-mail: u.jayasooriya@uea.ac.uk; Fax: 0044 (0)1603 592003; Tel: 0044 (0)1603 592711*
[b] *Department of Biological Chemistry, John Innes Centre, Norwich, UK NR4 7UH*
[c] *Centre for Metalloprotein Spectroscopy and Biology, University of East Anglia, Norwich, UK NR4 7TJ*

Received (in Cambridge, UK) 16th September 2003, Accepted 31st October 2003
First published as an Advance Article on the web 20th November 2003

The potential of NIS spectroscopy to study the iron–sulfur clusters in metalloproteins is illustrated using model compounds. The origin of the intense low energy transfer bands is discussed.

There are a large number of proteins containing iron–sulfur cubane clusters, and this number is continuing to grow rapidly. Interestingly, the $\{4Fe4S\}^+$ redox level has never been observed using resonance Raman spectroscopy in protein or model compounds, in marked contrast to the oxidised $\{4Fe4S\}^{2+}$ level, which is amongst the best characterised of metal–sulfur centres by the resonance Raman technique.[1] Destructive interference of scattering terms from different states is cited as the reason for the reduced cluster being such a bad resonance Raman scatterer. Therefore vibrational spectroscopic information on the reduced cluster in a protein environment is lacking. The novel spectroscopic technique of nuclear inelastic scattering, NIS (also known as, 'nuclear resonance vibrational spectroscopy, NRVS' and 'nuclear resonant inelastic X-ray scattering, NRIXS') has the potential to provide such information on iron atom centres in proteins irrespective of their electronic properties. This method involves the use of the Mössbauer absorption of a nucleus (^{57}Fe in the present study) to monitor the vibrational frequencies of the normal modes of that particular nucleus, an atom selective vibrational spectroscopy.[2] A synchrotron based technique that has now progressed to a level of sufficiently high resolution to be applicable for vibrational spectroscopy of condensed phases.[2,3]

Isotopically substituted tetraethylammonium salts of the anions $[^{57}Fe_4S_4(SCH_2Ph)_4]^{2-}$ **1** and $[^{57}Fe_4S_4(SCH_2Ph)_4]^{3-}$ **2** were synthesised following literature methods.[4]† Compounds were authenticated using ^{1}H-NMR, Mössbauer and IR spectroscopies and elemental analyses. Solid samples were anaerobically mounted into copper blocks (**1**, 250 mg; **2**, 100 mg mixed with 150 mg of **1** to provide sufficient sample) and covered with a 'captan' film and Be foil. NIS data were collected at the Nuclear Resonance beamline[5] at the European Synchrotron Radiation Facility in Grenoble, France.

NIS spectra of $[NEt_4]_2[^{57}Fe_4S_4(SCH_2Ph)_4]$ **1** both in the solid state and as a frozen 2 mM solution (a concentration relevant to biological samples) in acetonitrile are shown in Fig. 1. For ease of presentation, the solid-state spectrum is displaced by 0.015 units along the 'partial density of states', PDOS, axis. The results of a spectral simulation using an *ab initio* DFT calculation, of an *isolated* ion, is shown as a solid line. Amsterdam Density Functional (ADF) program version 2000.0.1 was employed. Density functional includes local exchange-correlation approximation with non-local gradient corrections of Becke for exchange and Perdew for correlation. The details of the DFT method used is given elsewhere.[6] The predicted frequencies have been scaled by a factor of 0.95, correcting for the overestimation of frequencies due to the systematic errors from approximations used for electron correlation.[6] Clearly there is very good agreement between the theoretical spectrum of the ion and that of the experimentally studied frozen 2 mM solution (25K), except for a large peak centred *ca.* 20 meV with a long background intensity tail to higher energy transfer. The solid-state spectrum shows relatively sharper peaks as expected, with again a significant background contribution. Details of this *ab initio* DFT calculation including the assignments and comparison with the data from other techniques reported in the literature, will be reported in a forthcoming paper.

Although we could not economically synthesise pure ^{57}Fe labelled **2** in a yield sufficiently high for the NIS experiment we have obtained the NIS spectrum of solid **2** diluted in **1** as shown in Fig. 2a, together with the *ab initio* DFT simulation. Also shown is the spectrum of the *ab initio* DFT simulation for a 50/50 mixture of the oxidised and reduced compounds for comparison. Again, reasonable agreement is found between the simulation and the experiment, except again for the intense low energy peak and the

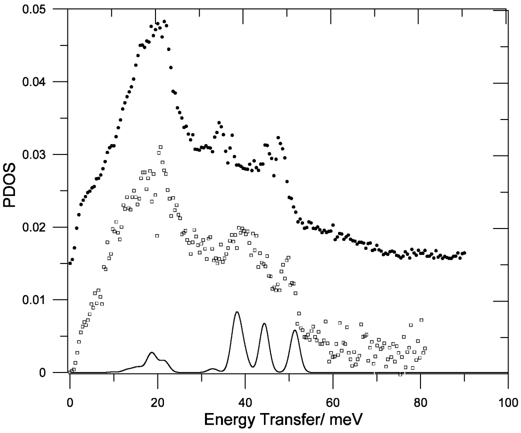

Fig. 1 NIS spectra of $[NEt_4]_2[^{57}Fe_4S_4(SCH_2Ph)_4]$ in frozen solution state (hollow squares) and solid state (filled circles, shifted 0.015 units along the PDOS axis for clarity), with the *ab initio* DFT prediction for an isolated ion (full line). All samples run at 25K.

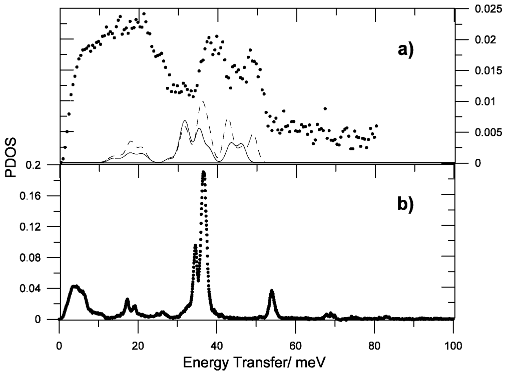

Fig. 2 (a) NIS spectrum of a mixture of $[NEt_4]_2[^{57}Fe_4S_4(SCH_2Ph)_4]$ and $[NEt_4]_3[^{57}Fe_4S_4(SCH_2Ph)_4]$ with the DFT prediction (full line) for $[NEt_4]_3[^{57}Fe_4S_4(SCH_2Ph)_4]$. Also shown is the DFT prediction for a 50/50 mixture of the above compounds (dashed line). (b) NIS spectrum of ^{57}Fe substituted tris(acetylacetonato)iron(III).

DOI: 10.1039/b311279a

background. This provides hitherto unavailable vibrational spectroscopic information on an $\{4Fe4S\}^+$ – centre.

The compound $[NEt_4]_2[Fe_4S_4(SCH_2Ph)_4]$ crystallises in the monoclinic space group C_{2h}^5; $P2_1/c$ with 4 formula units per unit cell.[7] All ions are in general positions. All 'internal modes' are well accounted for by the simulation using the *ab initio* DFT calculation of an isolated ion, Fig. 1, because these modes do not show any significant dispersion across the Brillouin zone. However, the single ion DFT calculation does not take into account the external modes. The total of twelve ions (2 × 4 cations + 1 × 4 anions) within a unit cell gives the following external modes under the C_{2h} factor group, which is applicable at the zone centre – a total of 36 translational lattice modes and 36 rotational lattice modes. Each of these modes gives rise to a branch across the Brillouin zone normally with significant dispersion.[8]

Translational lattice modes;

$(6A_g + 6B_g + 6A_u + 6B_u)$ of the cations and $(3A_g + 3B_g + 3A_u + 3B_u)$ of the cubane anions, out of which the acoustic branches belong to $(A_u + 2B_u)$ irreducible representations.

Rotational lattice modes;

$(6A_g + 6B_g + 6A_u + 6B_u)$ of the cations and $(3A_g + 3B_g + 3A_u + 3B_u)$ of the cubane anions.

The literature reports[2] of all NIS investigations of molecular systems up to now are of compounds with one iron atom per molecule situated at or almost at the centre of mass of the molecule. In such a situation, to a very good first approximation, only the translational lattice modes will involve motion of the central iron atom and will show NIS activity. All the rotational lattice modes are NIS inactive because they involve restricted rotations of the molecular units about the centre of mass where the iron atom is situated. An illustrative example of such a system is tris(acetylacetonato)iron(III). It crystallises in the orthorhombic space group D_{2h}^{15}; *Pbca,* with eight molecules per primitive unit cell.[9] Therefore there are 45 external modes of which 21 are translational in character and 24 rotational in character. The observed NIS spectrum is shown in Fig. 2(b), and is characterised by relatively sharp features and a rather weak acoustic and lattice phonon region, because the rotational lattice modes do not contribute. Detailed analysis of this spectrum will be reported elsewhere.

The cubane clusters reported here are the only compounds, to the best of our knowledge, with iron atoms situated at a significant distance (*ca*. 2.1 Å) away from the centre of mass of the molecule, as a result, relatively large iron atom amplitudes of motion are to be associated with the rotational lattice modes. Therefore one would expect the rotational lattice modes of the cubane anions $(3A_g + 3B_g + 3A_u + 3B_u)$ to be also NIS active in this case in addition to the translational lattice modes that are expected in any event. We therefore assign the strong intensity *ca*. 20 meV mainly to the DOS of the rotational lattice modes.

Multiphonon activity is common to the vibrational spectroscopic techniques that accompany large momentum transfer. Inelastic neutron scattering, INS, is a good example, where the spectral data are reported without any corrections for multiphonon activity, but treated at the spectral simulation step by associating each fundamental internal mode with a set of external modes.[10] A relevant difference between NIS and INS in this context is that the hydrogen atoms, whose vibrational normal modes that dominate the latter, are almost never to be found at the centre of mass of the molecule, unlike the ^{57}Fe atoms in almost all of the molecules studied up to now by NIS. The multiphonon contributions are rigorously eliminated from the raw NIS data by a double Fourier transformation.[11] This method has been shown to work very well for the compounds studied up to now, where only translational lattice modes are involved. Therefore further experimentation is necessary to find the cause of the unusual background intensities shown by the cubane samples where, for the first time, rotational lattice modes are of importance.

In conclusion, it is found that the current state of nuclear inelastic scattering spectroscopy has sufficient resolution and sensitivity for real applications in bioinorganic chemistry. The observation of restricted rotations of the cubane molecules, with high intensity, in both the solid state and in frozen solution, shows the unique potential of NIS to access such vibrations for the first time in biological systems.

We acknowledge the assistance, discussions and encouragement provided by Drs Alexander I. Chumakov and Rudolf Rüffer during these experiments and the beamtime by ESRF, Grenoble, France. This work was supported by the Biotechnology and Biological Sciences Research Council and the EPSRC, UK.

Notes and references

† ^{57}Fe, 95.4% enriched metal powder (Isoflex, Russia) was converted to ^{57}iron(II) chloride and this was used to prepare cluster **1**. A portion of **1** was reduced to give sample **2**.

1 T. G. Spiro and R. S. Czernuszewicz, *Methods in Enzymology*, Ed. K. Sauer, 1995, **vol. 246**, 416.

2 See for example: H. Paulsen, H. Winkler, A. X. Trautwein, H. Grünsteudel, V. Rusanov and H. Toftlund, *Phys. Rev. B*, 1999, **59**, 975; U. A. Jayasooriya, S. A. Malone, A. I. Chumakov, R. Rüffer, A. R. Overweg and C. R. Nicklin, *Chem.Phys.Chem.*, 2001, **2**, 177; H. Paulsen, V. Rusanov, R. Benda, C. Herta, V. Schuremann, C. Janiak, T. Dorn, A. I. Chumakov, H. Winkler and A. X. Trautwein, *J. Am. Chem. Soc.*, 2002, **124**, 3007; A. I. Chemakov, R. Rüffer, O. Leupold and I. Sergueev, *Struct. Chem.*, 2003, **14**, 109.

3 See for example: A. I. Chumakov, R. Rüffer, O. Leupold and I. Sergueev, *Struct. Chem.*, 2003, **14**, 109; J. T. Sage, S. M. Durbin, W. Sturhahn, D. C. Wharton, P. M. Champion, P. Hession, J. Sutter and E. E. Alp, *Phys. Rev. Lett.*, 2001, **86**, 4966.

4 G. Christou and C. D. Garner, *J. Chem. Soc., Dalton Trans.*, 1979, 1093; J. Cambray, R. W. Lane, A. G. Wedd, R. W. Johnson and R. H. Holm, *Inorg. Chem.*, 1977, **16**, 2565.

5 R. Rüffer and A. I. Chumakov, *Hyperfine Interact.*, 1996, **97–98**, 589.

6 E. J. Baerends, D. E. Ellis and P. Ros, *Chem. Phys.*, 1973, **2**, 41; L. Versluis and T. Ziegler, *J. Chem. Phys.*, 1988, **88**, 322; G. te Velde and E. J. Baerends, *J Comput. Phys*, 1992, **99**, 84; C. Fonseca Guerra, J. G. Snijders, G. te Velde and E. J. Baerends, *Theor. Chem. Acc.*, 1998, **99**, 391; S. H. Vosko, L. Wilk and M. Nusair, *Can. J. Phys.*, 1980, **58**, 1200; A. D. Becke, *J. Chem. Phys.*, 1986, **84**, 4524; J. P. Perdew, J. A. Chevary, S. H. Vosko, K. A. Jackson, M. R. Pederson, D. J. Singh and C. Fiolhais, *Phys. Rev. A.*, 1992, **46**, 6671; L. Versluis and T. Ziegler, *J. Chem. Phys.*, 1988, **322**, 88.

7 B. A. Averill, T. Herskovitz, R. H. Holm and J. A. Ibers, *J. Am. Chem. Soc.*, 1973, **95**, 3523.

8 For an example of a pictorial description of external modes see: U. A. Jayasooriya and S. F. A. Kettle, *Phys. Rev. B*, 1984, **29**, 2227.

9 J. Iball and C. H. Morgan, *Acta Crystallogr.*, 1967, **23**, 239; I. Diaz-Acosta, J. Baker, W. Cordes and P. Pulay, *J. Phys. Chem. A*, 2001, **105**, 238.

10 G. J. Kearley, *J. Chem. Soc., Faraday Trans. 2*, 1984, **82**, 41; G. J. Kearley, *Nucl. Instrum. Methods Phys. Res., Sect. A*, 1995, **354**, 53.

11 V. G. Kohn, A. I. Chumakov and R. Rüffer, *Phys. Rev. B*, 1998, **58**, 8437; V. G. Kohn and A. I. Chumakov, *Hyperfine Interact.*, 2000, **125**, 205.