Analyst

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/analyst

Analyst

RSCPublishing

COMMENT

Comment on "Sensitive marker bands for the detection of spin states of heme in surface-enhanced resonance Raman scattering spectra of metmyoglobin" by Y. Kitahama, M. Egashira, T. Suzuki, I. Tanabe and Y. Ozaki

Cite this: DOI: 10.1039/xoxxoooooox

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

www.rsc.org/

A. Feis*[a] and G. Smulevich[a]

We contrast recently reported surface-enhanced resonance Raman spectra (SERRS) of myoglobin on silver nanoparticles with established knowledge about this complex. We conclude that the detected bands are not related to the spin states of the protein cofactor, being rather originated by a heme coordination change induced by the metal surface.

Surface enhanced Raman spectroscopy (SERS) is a promising spectroscopic tool for analytical purposes, as witnessed by numerous review articles in this journal.[1-5] In particular, possible applications of SERS to protein analysis are intensely investigated, owing to the relevant role of proteins in analytical methods in biology and medicine. A recent article in Analyst[6] presented a SERS study on a very well characterized heme protein, equine met-myoglobin, adsorbed on a popular SERS substrate, that is, an aqueous dispersion of citrate-reduced silver nanoparticles. The combination of plasmonic enhancement and electronic resonance of the heme chromophore (giving rise to surface enhanced resonance Raman spectroscopy, or SERRS) is expected to yield selective Raman spectra of the Fe-protoporphyrin IX cofactor modes with very high sensitivity. To demonstrate the connection between SERRS and resonance Raman (RR) spectra, the Authors compare RR and SERRS spectra upon addition of a variety of exogenous ligands, which can coordinate the heme iron. The RR spectra (Figure 2) display different band positions and intensities, depending on the ligand strength, as previously shown and rationalized in several articles about RR spectroscopy of heme models and heme proteins.[7-10] In contrast, the SERRS spectra (Figure 3) are scarcely influenced by heme ligation.

The Authors invoke the orientation of the protein on the Ag surface as the origin of the difference between RR and SERRS spectra. This interpretation does not take into account previous experimental work performed in distinct laboratories through several decades.[11-13] It has been shown in detail that the interaction between citrate-containing Ag colloids and met-myoglobin, which has a six-coordinate high-spin Fe-protoporphyrin IX under normal experimental conditions, gives rise to the SERRS spectrum of a pentacoordinated species. This has been attributed to the formation of a μ-oxo dimer, due to heme detachment from the protein matrix.[11] In particular, it has been reported that the pentacoordinated species is formed instantaneously, unless a flow system is used,[12] even under low laser illumination (e.g., 0.3 s at 6 mW for 406.7 nm excitation wavelength) and short protein/colloid incubation time (e.g., 2 min). Surface functionalization of the metal nanoparticles has been shown to be an alternative way to keep myoglobin in its native form.[13] For a correct interpretation of the spectra shown in Figure 3, a comparison should be made with the SERRS spectra of protein-free Fe-protoporphyrin IX[14] on a silver electrode at the same laser excitation wavelength (514.5 nm), or with the SERRS spectra of the related compound hematin[15] excited at 532 nm: the spectra are very similar, which suggests that the Authors have detected the SERRS spectrum of silver-adsorbed hemin. Moreover, the Authors should have displayed SERRS spectra with Soret band excitation of their met-myoglobin/Ag samples, as the marker bands enhanced at this excitation wavelength yield detailed information about heme coordination. The dissociation of heme from the protein can also explain the absence of an effect from exogenous ligands in Figure 3. For the above reasons, it is doubtful that the bands in the pH-dependent SERRS spectra (Figure 4) can be related to the alkaline spin-state transition of met-myoglobin.[10]

Finally, the reported spectra cannot be considered as reliable SERRS spectra of met-myoglobin as they possibly originate from a metal-induced artifact. Therefore these spectra are not suitable for bioanalytical chemistry applications, in contrast with the Authors' suggestion at the conclusion of their article.

Notes and references

[a] Department of Chemistry "Ugo Schiff", University of Florence, Italy
* Corresponding author

1 C. Lim, J. Hong, B. G. Chung, A. J. deMello and J. Choo, *Analyst*, 2010, **135**, 837.

2 H.-W. Cheng, S.-Y. Huan and R.-Q. Yu, *Analyst*, 2012, **137**, 3601.

3 J. A. Dougan and K. Faulds, *Analyst*, 2012, **137**, 545.

4 Y. Wang and S. Schlücker, *Analyst*, 2013, **138**, 2224.

5 M. Li, S. K. Cushing and N. Wu, *Analyst*, 2015, **140**, 386.

6 Y. Kitahama, M. Egashira, T. Suzuki, I. Tanabe and Y. Ozaki, *Analyst*, 2014, **139**, 6421.

7 S. A. Asher and T.M. Schuster, *Biochemistry*, 1979, **18**, 5377.

8 S. Choi, T. G. Spiro, K. C. Langry, K. M. Smith, D. L. Budd and G. N. La Mar, *J. Am. Chem. Soc.*, 1982, **104**, 4345.

9 S. Hu, K.M. Smith and T. G. Spiro, *J. Am. Chem. Soc.*, 1996, **118**, 12638.

10 A. Feis, M. P. Marzocchi, M. Paoli and G. Smulevich, *Biochemistry*, 1994, **33**, 4577

11 G. Smulevich and T. G. Spiro, *J. Phys. Chem.*, 1985, **89**, 5168.

12 M. Feng and H. Tachikawa, *J. Am. Chem. Soc.*, 2008, **130**, 7443.

13 G. Kalaivani, N. S. V. Narayanan, A. Sivanesan, A. Kannan, A. Kaminska, R. Sevvel, *RSC Adv.*, 2013, **3**, 6839.

14 J. L. Sanchez and T. G. Spiro, *J. Phys. Chem.*, 1985, **89**, 763.

15 B. R. Wood, E. Bailo, M. A. Khiavi, L. Tilley, S. Deed, T. Deckert-Gaudig, D. McNaughton, V. Deckert, *Nano Lett.*, 2011, **11**, 1868.

