

Analyst

Accepted Manuscript

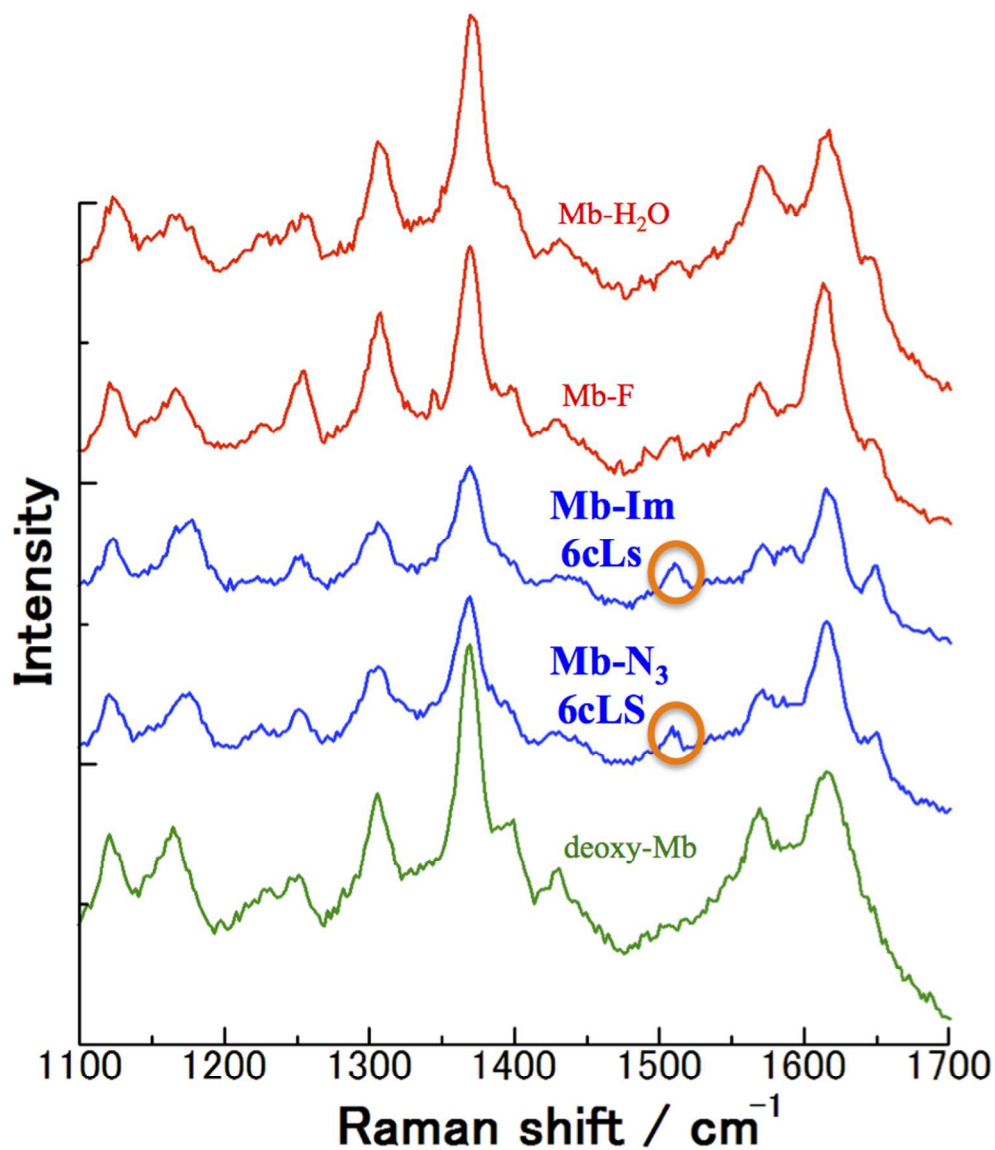


This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.



In our SERRS spectra, the peaks at 1510 cm⁻¹, which are assigned not to non-native 5-coordinated heme in the high spin state, but to native 6-coordinated heme in the low spin state (6cLS), were observed.

154x182mm (150 x 150 DPI)

COMMENT

Reply to the comment on “Sensitive marker bands for the detection of spin states of heme in surface-enhanced resonance Raman scattering spectra of metmyoglobin”

Cite this: DOI:
10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Yasutaka Kitahama,* Masatoshi Egashira, Toshiaki Suzuki, Ichiro Tanabe and Yukihiro Ozaki

In our SERRS spectra of metmyoglobin by excitation at 514 nm, the peak at 1510 cm⁻¹, which is assigned to the 6-coordinated heme in the low spin state, was observed by the addition of imidazole and NaN₃. Thus, the SERRS likely originates not from the non-native 5-coordinated heme, which is in the high spin state.

We thank Dr. Feis and Prof. Smulevich for their fruitful comment regarding our paper.¹ By changing ligand and pH, which affect a spin state of metmyoglobin (met-Mb), resonance Raman scattering (RRS) and surface-enhanced resonance Raman scattering (SERRS) spectra of met-Mb were measured.² In the RRS spectra, the peak that has been used for discrimination between the heme ion in the high or low spin state appeared at 1610 or 1640 cm⁻¹, respectively, although the corresponding SERRS peak was barely observed. We consider that the marker band for the spin state is not much enhanced by the surface selection rule.

Feis and Smulevich have commented that the heme in met-Mb on the citrate-reduced Ag becomes the non-native form through detachment from the heme pocket in the protein.¹ Indeed, the SERRS peak was observed at 1490 cm⁻¹, which is assigned to 5-coordinated heme b in the high spin state, by excitation at 406.7 and 413 nm (Soret band), while the corresponding RRS (ν_3) peak of native met-Mb appeared at 1480 and 1510 cm⁻¹, which is attributed to 6-coordinated heme b in the high and low spin state, respectively.³⁻⁵ By the interaction with Ag surface, also conformation of heme c in cytochrome *c* is changed and then is reflected in the spectra.⁶

In our paper,² however, the fig. 3 shows that the ν_3 peak in the SERRS spectra of met-Mb in the low spin state due to addition of imidazole and NaN₃, which was confirmed by the RRS spectra, appeared at 1510 cm⁻¹ with excitation at 514 nm (the α and β bands) despite the long incubation and exposure time, and no SERRS peak appeared at 1490 cm⁻¹. Even the peak at 1640 cm⁻¹, which is the marker band for the low spin state, can be seen in the SERRS spectra of met-Mb in the low spin state.² The peak at 1640 cm⁻¹ prefers to be enhanced by the resonance Raman effect with excitation at 514 nm,⁷ and then it has been sometimes clearly observed in the SERRS spectra.^{8,9}

Moreover, we compare our spectra with the SERRS spectra of Mb by excitation at the similar wavelength, namely, 532 and 514.5 nm (the α and β bands). In the SERRS spectra,^{9,10} the ν_3 peak was not observed at 1490 cm⁻¹, which is due to the non-native 5-coordinated heme in the high spin state, in a similar

way of our spectra. Thus, the SERRS spectra of Mb by excitation at 514 nm can act like they originate from the native 6-coordinated heme. On the other hand, the SERRS peak (ν_3) of protein-free β -hematin and Fe-protoporphyrin IX was well observed at 1490 cm⁻¹ even by excitation at 532 and 514.5 nm,^{11,12} although it appeared at 1510 cm⁻¹ in our spectra. Therefore, it has not been confirmed that our SERRS spectra are acquired from the protein-free hemin on the Ag as the ref. 11 and 12.

SERRS spectrum of deoxy-Mb on alkanethiol-protected Ag nanoparticles, on which Mb avoids contact with the surface,

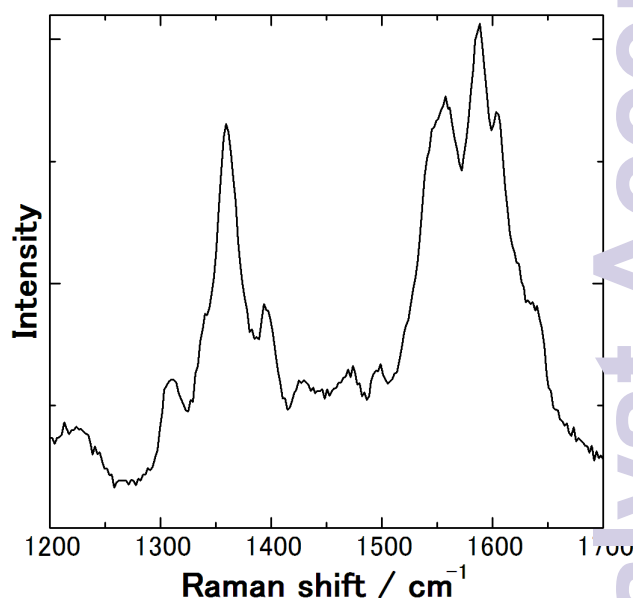


Fig. 1 SERRS spectrum of deoxy-Mb on alkanethiol-protected Ag

ARTICLE

1 was measured.¹³ Fig. 1 shows that the marker band for the high
2 spin state at 1610 cm⁻¹ barely appeared unlike the
3 corresponding RRS spectrum.² In this case, the difference
4 between the SERRS and RRS may be due not to the
5 detachment from the heme pocket by the interaction with the
6 Ag surface, but to the surface selection rule. The peak at 1560
7 cm⁻¹ (ν_2), which has been used for the marker band in the
8 SERRS spectra, appeared while another peak was observed not
9 at 1620 cm⁻¹ ($\nu(\text{C}_a=\text{C}_b)$), but at 1590 cm⁻¹ (ν_{37}).¹⁴ The ratio of
10 the two peaks in 1560–1620 cm⁻¹ was similar to the previous
11 results of deoxy-Mb on citrate-reduced Ag.²

12 In conclusion, Mb is affected by the Ag surface, whereas Mb
13 can still behave as the native protein, namely, the 6-coordinated
14 heme, whose ν_3 peak appears at 1510 cm⁻¹ in the low spin state.

Notes and references

15
16
17 ^a Department of Chemistry, School of Science and Technology, Kwansei
18 Gakuin University, Sanda, Hyogo 669-1337, Japan. E-mail:
19 kitahama@kwansei.ac.jp; Fax: +81-79-565-9077; Tel: +81-79-565-8349.

- 20 1 A. Feis and G. Smulevich, *Analyst*, 2015, .
- 21 2 Y. Kitahama, M. Egashira, T. Suzuki, I. Tanabe and Y. Ozaki,
22 *Analyst*, 2014, **139**, 6421.
- 23 3 G. Smulevich and T. G. Spiro, *J. Phys. Chem.*, 1985, **89**, 5168.
- 24 4 M. Feng and H. Tachikawa, *J. Am. Chem. Soc.*, 2008, **130**, 7443.
- 25 5 G. Kalaiivani, N. S. V. Narayanan, A. Sivanesan, A. Kannan, A.
26 Kaminska and R. Sevel, *RSC Adv.*, 2013, **3**, 6839.
- 27 6 D. H. Murgida and P. Hildebrandt, *J. Phys. Chem. B*, 2001, **105**, 1578.
- 28 7 B. N. Rospendowski, K. Kelly, C. R. Wolf and W. E. Smith, *J. Am.*
29 *Chem. Soc.*, 1991, **113**, 1217.
- 30 8 B. R. Wood, M. A. Khiavi, E. Bailo, D. McNaughton and V. Deckert,
31 *Nano Lett.*, 2012, **12**, 1555; In this case, the appearance of the peak
32 can be induced also by wavelength-dependent enhancement due to
33 localized surface plasmon resonance of single Ag nanoaggregate.¹⁵
- 34 9 S. Abdali, C. Johannessen, J. Nygaard and T. Nørbygaard, *J. Phys.*
35 *Condens. Matter*, 2007, **19**, 285205.
- 36 10 X. X. Han, H. Y. Jia, Y. F. Wang, Z. C. Lu, C. X. Wang, W. Q. Xu,
37 B. Zhao and Y. Ozaki, *Anal. Chem.*, 2008, **80**, 2799.
- 38 11 B. R. Wood, E. Bailo, M. A. Khiavi, L. Tilley, S. Deed, T. Deckert-
39 Gaudig, D. McNaughton and V. Deckert, *Nano Lett.*, 2011, **11**, 1868.
- 40 12 L. A. Sanchez and T. G. Spiro, *J. Phys. Chem.*, 1985, **89**, 763.
- 41 13 I. Tanabe, M. Egashira, T. Suzuki, T. Goto and Y. Ozaki, *J. Phys.*
42 *Chem. C*, 2014, **118**, 10329.
- 43 14 S. Hu, K. M. Smith and T. G. Spiro, *J. Am. Chem. Soc.*, 1996, **118**,
44 12638.
- 45 15 T. Itoh, K. Yoshida, V. Biju, Y. Kikkawa, M. Ishikawa and Y. Ozaki,
46 *Phys. Rev. B*, 2007, **76**, 085405.
- 47
48
49
50
51
52
53
54
55
56
57
58
59
60