Journal of
Synchrotron Radiation

ISSN 1600-5775

Received 22 April 2014
Accepted 5 May 2014

Response to *Comments on Structural studies of haemoglobin from pisces species shortfin mako shark (Isurus oxyrinchus) at 1.9 Å resolution by P. Ramesh et al. (2013). J. Synchrotron Rad. 20, 843–847*

Pandian Ramesh,[a,b]* S. S. Sundaresan,[b] Pon. Sathya Moorthy,[b] M. Balasubramanian[b] and M. N. Ponnuswamy[b]

[a]Laboratory of Structural Biology, Department of Molecular Cell Biology, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon 440-746, South Korea, and [b]Centre of Advanced Study in Crystallography and Biophysics, University of Madras, Maraimalai Campus (Guindy), Chennai 600 025, India. *E-mail: prms23@gmail.com

The following is a response to the points raised in the comment by Merlino *et al.* on our recent paper on haemoglobin (Hb) from the shortfin mako shark (SMS) (Ramesh *et al.*, 2013).

During the preparation of our paper, we planned to propose that SMS Hb belongs to a mixed-state conformation, but unfortunately we could not identify any related papers to support our statement. We referred to Naoi *et al.* (2001), whose structure had the same number of amino acids (AAs) and a similar kind of heme binding pocket. Naoi *et al.* solved two structures with different state conformation. With regard to those two states, our SMS Hb belongs to the deoxy T state conformation, and we confirmed that there is no ligand in the heme binding pocket, such as CO and O_2, in SMS Hb. We therefore concluded that SMS Hb belongs to the deoxy unliganded T state conformation (Ramesh *et al.*, 2013). We first purified native protein from shark blood and then performed the crystallization directly. Therefore, anticipating the aforementioned fact, we did not used a reducing agent during the purification and crystallization procedures.

We agree that there is a water molecule situated between Fe and the proximal histidine residue, and we created electron density maps of both the heme binding sites. The maps showed that the α chain does not contain a water molecule but there is a water molecule in the case of the β chain [see Figs. 4(*a*) and 4(*b*), respectively, of Ramesh *et al.* (2013)]. If a water molecule is present in both chains, we can say that Hb belongs to the aquomet form. In general, CO and O_2, considered as ligands, are found only in the relaxed R state; hence, we proposed the unliganded deoxy T state conformation.

In this regard, we compared our quaternary structure and heme binding site of SMS Hb with deoxy (1gcv) and carbonmonoxy (1gcw) forms of *Mustelus griseus* shark (MGS) Hb. From this comparison we found that our solved structure is most closest to the *Mustelus griseus* shark Hb deoxy T state conformation. On the basis of Vergara *et al.* (2007, 2008, 2009), and considering the comments of Merlino *et al.*, we are also very happy to propose a new state for our SMS Hb, belonging to the transition form from R to T with the heme binding pocket corresponding to a ferric Hb of the type α(hemichrome)β(aquomet) considered in the mixed-state form.

On the other hand, we cannot compare our overall structure with the one referred to by the commentators. The structure that they referred to contains 143 AAs in the α chain and 146 AAs in the β chain; but in our case there are 140 and 136 AAs in the α and β chains, respectively. The structure from Naoi *et al.* has the same number of AAs, and that is the reason why we made comparisons with the 1gcv and 1gcw structures. The structure referred to by the commentators and our SMS Hb structure have different numbers of AAs. The heme binding site of our structure has water in the β chain but in their case a water molecule is available only in the α chain, and so we cannot overlap and compare our structure heme binding site (SMS Hb heme α with α and SMS Hb heme β with β) with the structures referred to by the commentators. If we have to compare the structures then it is possible only when comparing the structure of the SMS Hb heme α with the β heme of their structure and SMS Hb heme β with their α heme, and it is not possible to compare the secondary structure of both (SMS Hb and the commentary structure) in the same way and it is also meaningless. A similar heme geometry is available only in the deoxy form of MGS Hb (Naoi *et al.*, 2001) and this kind of geometry was not proposed previously. That is the reason why we have not proposed that the heme binding pocket corresponds to a ferric Hb of type β(hemichrome)β(aquomet).

We referred to Naoi *et al.* (2001) for the following reason: SMS and MGS Hbs have the same number of AAs in both chains with a similar kind of heme geometry.

We have not referred to the commentary authors' papers (Vergara *et al.*, 2007, 2008, 2009; Vitagliano *et al.*, 2004, 2008) previously because: (i) SMS and the commentary-referred Hbs have a different number of sequences with different heme geometry; (ii) in our case there is a water molecule in the β chain and the commentary-referred structure has a water molecule in the α chain; and (iii) from this, we cannot compare the overall structure of our case with the commentary structure.

References

Merlino, A., Vergara, A. & Vitagliano, L. (2014). *J. Synchrotron Rad.* **21**, 832–833.
Naoi, Y., Chong, K. T., Yoshimatsu, K., Miyazaki, G., Tame, J. R. H., Park, S.-Y., Adachi, S. & Morimoto, H. (2001). *J. Mol. Biol.* **307**, 259–270.
Ramesh, P., Sundaresan, S. S., Sathya Moorthy, P., Balasubramanian, M. & Ponnuswamy, M. N. (2013). *J. Synchrotron Rad.* **20**, 843–847.
Vergara, A., Franzese, M., Merlino, A., Bonomi, G., Verde, C., Giordano, D., di Prisco, G., Lee, H. C., Peisach, J. & Mazzarella, L. (2009). *Biophys. J.* **97**, 866–874.
Vergara, A., Franzese, M., Merlino, A., Vitagliano, L., Verde, C., Prisco, G., Lee, H. C., Peisach, J. & Mazzarella, L. (2007). *Biophys. J.* **93**, 2822–2829.
Vergara, A., Vitagliano, L., di Prisco, G., Verde, C. & Mazzarella, L. (2008). *Methods Enzymol.* **436**, 425–444.
Vitagliano, L., Bonomi, G., Riccio, A., di Prisco, G., Smulevich, G. & Mazzarella, L. (2004). *Eur. J. Biochem.* **271**, 1651–1659.
Vitagliano, L., Vergara, A., Bonomi, G., Merlino, A., Verde, C., di Prisco, G., Howes, B. D., Smulevich, G. & Mazzarella, L. (2008). *J. Am. Chem. Soc.* **130**, 10527–10535.