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High Protein Structural Flexibility Of A Truncated Hemoglobin From An Antarctic Cold-Adapted Bacterium.

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Although many cold-adapted marine species have been studied, we still have limited knowledge about molecular adaptations at low temperatures. The ability of cold-adapted organisms to survive at permanently low temperatures implies that to perform their physiological functions at adequate rates in freezing habitats, they have overcome constraints imposed by the cold environment through biochemical and physiological adaptations.

In the present study, we present the first detailed characterization of a cold-adapted bacterial Hb. The monomeric hemoglobin (Hb) from the cold-adapted bacterium *Pseudoalteromonas haloplanktis* have been cloned and over-expressed [1]. The fairly low redox potential suggests that the physiologically relevant form is Fe(III). Therefore, the protein in its ferric state (*Ph*-2/2HbO) has been characterized by spectroscopic (RR, UV-Vis, and EPR), kinetic measurements and different computer simulation approaches. The results indicate that this protein belongs to Group II (HbO) of the truncated Hbs family. In particular, sequence alignment of *Ph*-2/2HbO with other 2/2 Hbs, indicates that the conserved residues HisF8, TyrB10, TrpG8, TyrCD1 are in the typical positions for Group II Hbs [2,3]. On the proximal side, HisF8 is coordinated to the heme iron, as confirmed by the ν(Fe-Im) stretching mode at 223 cm^{-1} in the RR spectrum of the deoxy form. The heme distal pocket is characterized by the presence of a Trp (G8 position) and two tyrosyl residues (TyrCD1 and TyrB10).

The present study strongly indicates that the protein has unique features in the ferric state among Group II of 2/2 Hbs. In fact, unlike other bacterial Hbs, at neutral pH Fe(III) *Ph*-2/2HbO is characterized by one hexa-coordinated HS form (corresponding to that displaying a water molecule coordinated to the heme) and two (by RR) (Figure 1), or three (by EPR) hexa-coordinated LS forms. While one LS form should correspond to a deprotonated Tyr, the other two 6cLS forms are suggested to

correspond to either TyrCD1-O^- or TyrB10-O^-, coordinated to the heme Fe(III) atom and distinguished by different H-bonding properties for each heme bound Tyr. The MD simulations indicate that coordinated TyrCD1 is stabilized by TrpG8 and coordinated TyrB10 by both TrpG8 and TyrCD1. The lower redox potential of *Ph*-2/2HbO at pH 7.0 (i.e., - 80 mV *vs* SHE), compared to that of horse heart Mb and human Hb (+50 mV and +135 mV, respectively), indeed agrees with the presence of Tyr as axial ligand. Moreover, at least one Tyr coordinated to a heme iron has been identified by RR experiments upon excitation in the tyrosinate-Fe(III) CT band (near 500 nm). The spectra taken with the 514.5 nm excitation wavelength clearly show the enhancement of two polarized bands at 598 and 1509 cm^{-1}. These bands are assigned to the $\nu(Fe\text{-}O_{Tyr})$ and $\nu_{Tyr}(C{=}C)$ tyrosinate modes, respectively.

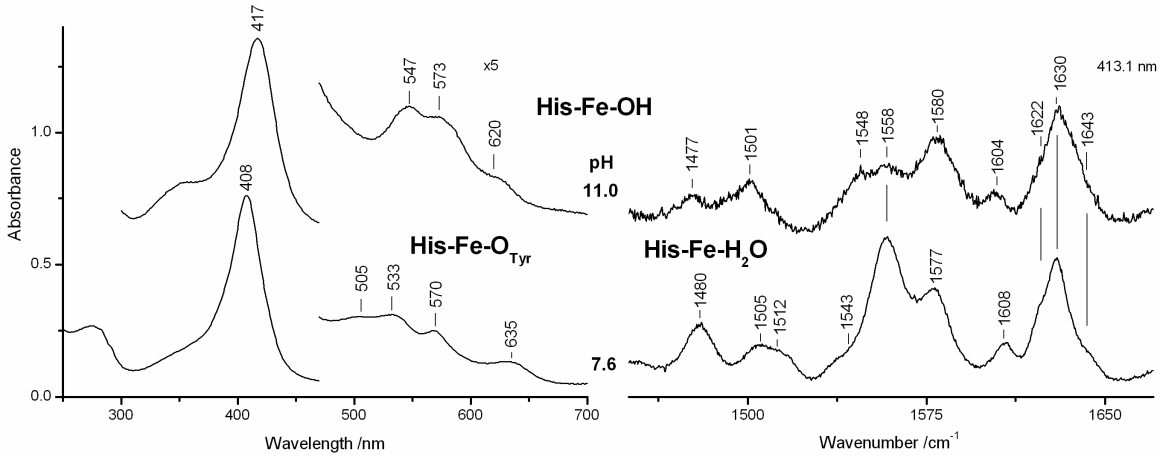

FIGURE 1. UV-Vis (left) and RR (right) spectra of ferric *Ph*-2/2HbO at different pH values. At alkaline pH a new form is observed which has been assigned to be a His-Fe-OH heme complex.

This is the first example in which both TyrCD1 and TyrB10 are proposed to be the residues alternatively involved in heme hexa-coordination by endogenous ligands. The ensemble of results indicates high protein structural flexibility, probably linked to the peculiarity of the cold environment which requires the maintenance of protein flexibility for supporting the cellular functioning.

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