

s8a.m7.p7 Structural determination of Bacterioferritin from *Desulfovibrio Desulfuricans* ATCC 27774. S. Macedo^{1,2}, P.M. Matias¹, E. Mitchell², A. Coelho^{1,3}, J. Le Gall^{1,4}, P. Lindley² & M.A. Carrondo¹. ¹Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República, EAN, Apartado 127, 2781-901 Oeiras, Portugal, ²European Synchrotron Radiation Facility, 6 rue Jules Horowitz, F-38042 Grenoble Cedex, France, ³Chemistry Department, Universidade de Évora, Apartado 94, 7001 Évora Codex, Portugal, ⁴Department of Biochemistry and Molecular Biology, University of Georgia, Athens, Georgia 30602.

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Iron is essential for the growth and development of most organisms. However, it is potentially toxic and, because of its high insolubility under physiological conditions, in short supply in biological systems¹. Ferritins fulfil the need of iron storage in a biologically accessible form in organisms as diverse as bacteria, fungi, plants and vertebrates.

In the specific case of bacteria, two types of iron storage proteins can be found: bacterioferritins and ferritins². Both have essentially the same architecture: they assemble in a 24-mer cluster and form a hollow roughly spherical construction. The iron storage cavity has a diameter of about 80 Å and can accommodate at least 2000 iron atoms² as an inorganic complex core. Each subunit (~20 kDa) folds as a 4-helix bundle capped by a final shorter helix at the C-terminal end. The 24 subunits are related by 4-fold, 3-fold and 2-fold symmetry axes (432 point symmetry). At the 3-fold axis there are channels that traverse the cavity.

Each monomer contains a dinuclear iron centre for the catalysis of Fe (II) oxidation, which is necessary for iron deposition in the sphere core. The oxidised iron is thought to move into the cavity through the 3-fold channels. Unlike ferritins, bacterioferritins also contain haems: 12 per 24-mer cluster. The haem is located at the 2-fold axis of the dimer, and its role is still not known.

We present the structure of Bacterioferritin from *Desulfovibrio Desulfuricans* ATCC 27774 (solved to a resolution of 1.95 Å with the MAD phasing method).

s8a.m7.p8 Crystal structures of photosynthetic reaction center and high-potential iron-sulfur protein from *Thermochromatium tepidum*. T. Nogi¹, I. Fathir^{1, 2}, M. Kobayashi^{2, 3}, T. Nozawa^{2, 3}, K. Miki^{1, 3, 4}. ¹Department of Chemistry, Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-8502, JAPAN. ²Department of Biomolecular Engineering, Graduate School of Engineering, Tohoku University, Aoba-ku, Sendai 980-8579, JAPAN. ³Center for Interdisciplinary Science, Tohoku University, Aoba-ku, Sendai 980-8579, JAPAN. ⁴RIKEN Harima Institute / SPring-8, Koto 1-1-1, Mikazukicho, Sayo-gun, Hyogo 679-5148, JAPAN.

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In photosynthetic purple bacteria, the electron transfer reactions of photosynthesis are performed by the following three components: the photosynthetic reaction center (RC), the cytochrome *bc*₁ complex, and the soluble electron carrier protein. The soluble electron carrier proteins are classified into two groups; the *c*-type cytochrome and the high-potential iron-sulfur protein (HiPIP). Compared to the case of the *c*-type cytochrome, little is known about the mechanism of the molecular recognition between the RC and HiPIP. We therefore determined the crystal structures of the RC and HiPIP from a purple bacterium, *Thermochromatium (Tch.) tepidum*, in order to elucidate the driving force of their interaction and their precise docking site.

The crystallization conditions of these two proteins have already been established^{1,2}. The structure of the RC was solved by the molecular replacement method, and refined at 2.2 Å resolution to a crystallographic *R*-factor of 23.1 % with a free *R*-factor of 28.7 %. The current model consists of four protein subunits, six kinds of prosthetic groups. In addition, seven detergents and one lipid molecules were assigned on the molecular surface of the trans-membrane region. On the other hand, the structure of the HiPIP was also solved by the molecular replacement method, and refined at 1.5 Å resolution to a crystallographic *R*-factor of 21.2 % with a free *R*-factor of 23.8 %. The HiPIP molecules were found to exist as monomers in the crystal, unlike the crystal structures of HiPIPs of other bacteria.

We will discuss the docking site of each protein in terms of the charge distribution of the molecular surface, and will compare the molecular recognition mechanism with that of the RC and *c*-type cytochrome.

In addition, *Tch. tepidum* is a thermophilic bacterium, which can grow up to 58 °C, the highest temperature of all known purple bacteria. Hence, the structure of the *Tch. tepidum* RC will be examined in terms of the stability of the membrane protein.

[1] Harrison P.M. & Arosio P. "The ferritins: molecular properties, iron storage function and cellular regulation", *Biochim Biophys Acta*, (1996), 1275: 161 - 203.

[2] Andrews S.C. "Iron Storage in Bacteria", *Advances in Microbial Physiology* (1998), 40: 281 - 351.

[1] Katayama N., Kobayashi M., Motojima F., Inaka K., Nozawa T., & Miki K. "Preliminary X-ray crystallographic studies of photosynthetic reaction center from a thermophilic sulfur bacterium, *Chromatium tepidum*", *FEBS Lett.*, (1994), 348: 158-160.

[2] Nogi T., Kobayashi M., Nozawa T. & Miki K. "Crystallization and preliminary crystallographic analysis of the high-potential iron-sulfur protein from *Thermochromatium tepidum*", *Acta Crystallogr.* (2000), D 56: 656-658.