

CoPf, a Co-porphyrin-containing protein of *D.gigas*.

Sergey A. Bursakov¹, Olga Yu. Gavel¹, Juan J. Calvete², Vanessa F. Cabral¹, Isabel Moura¹, and José J.G.Moura¹

¹REQUIMTE – CQFB, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal, and ²Instituto de Biomedicina de Valencia, C.S.I.C., Valencia, Spain

Co-porphyrin-containing proteins have been found earlier in sulphate reducing bacteria (J.J.G. Moura et al., Biochem. Biophys. Res. Commun. 1980, 92, 962-970). Monomeric violet coloured CoPf isolated from *Desulfovibrio (D.) gigas* was shown to contain a non-covalently bound Co (III) porphyrin-like cofactor. The molecular mass of the purified protein determined as 11838 Da by MALDI-TOF mass spectrometry (MS). CoPf shows spectra with maximum at 420, 580 and shoulder at 550 nm that are typical of a metalloporphyrin. Cofactor analysis was made on native protein, ClGu denaturated protein and acidified acetone-extracted cofactor. The positive ion mass spectrum of the unknown corrinoid indicated a molecular mass of 1355, identical to cyanocobalamin.

N-terminal and internal peptidic sequences were determined by combination of Edman degradation and nESI-MS/MS using a QTrap instrument. Total and free SH groups, and S-S bonds of CoPf were quantified by MALDI-TOF mass spectrometry. The two cysteinyl residues of the protein were involved in the formation of an intramolecular disulfide bond. Upon reduction, only one cysteine was accessible to alkylating reagents in 5 M ClGu-denatured protein.

BLAST alignment of the amino acid sequence of CoPf showed high similarity (72 to 82%) to the gamma subunit of the dissimilatory sulfite reductase from *D. vulgaris* (Hildenborough), *D. desulfuricans* G20, *Thermodesulforhabdus norvegica* and *Desulfotalea psychrophila*.