Environmental Science

Unravelling Cobalt Binding to Photosynthetic Bacterium by X-ray Absorption Spectroscopy

X-ray absorption spectroscopy | SUL-X beamline

Introduction

Anthropogenic release of heavy metals is recognised as dangerous for both environment and human health. Although some metals involved in metabolic functions are essential to living organisms, industrial activities may increase their level up to toxic concentrations.
Decontamination technologies are under study and bioremediation techniques that exploit bacteria, fungi, protists, and other microorganisms as pollution scavengers are gaining momentum due to their environmental sustainability. Photosynthetic microorganisms are extremely attractive, as they use cheap and widely available solar radiation as energy source. *Rhodobacter (R.) sphaeroides* (Figure 1) is a facultative photosynthetic bacterium able to grow under a wide range of environmental conditions and to tolerate and sequester heavy metals. It can be considered a valuable candidate for bioremediation of polluted sites, a sort of metal garbage collector of a not too far future. *Specifically, R. sphaeroides* shows a marked tolerance to cobalt ions [1].
In view of field applications, a full understanding of the interaction between *R. sphaeroides* and metal ions is crucial. The interaction of cobalt ions and phospholipids composing the photosynthetic membrane of the bacterium is investigated by using synchrotron radiation.

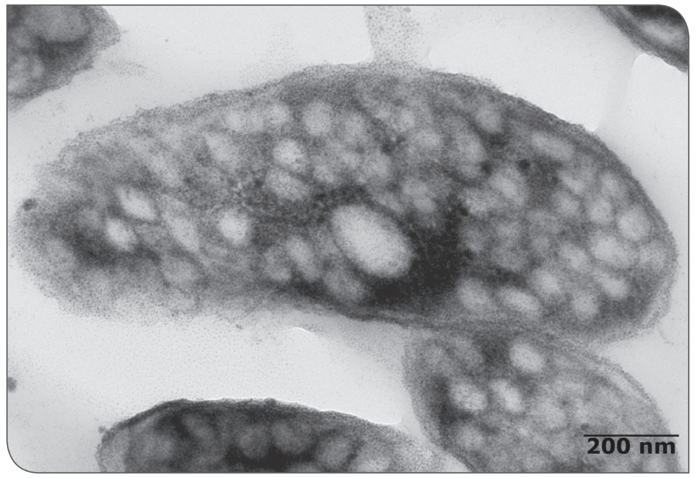

Figure 1: Transmission electron microscopy (TEM) image of a Rhodobacter sphaeroides cell.

Scientific Research

R. sphaeroides copes with very high concentration of cobalt ions by re-organising the expression of numerous enzymes, including the down expression of enzymes belonging to the chlorophylls and heme metabolic pathway, and the up-regulation of several proteins and enzymes involved in DNA degradation process. But the question of how the interaction of the bacterial cells and the ion take place still needs to be addressed.
Structural information on the fate of cobalt in *R. sphaeroides* cells exposed to high cobalt concentration has been obtained by X-ray absorption spectroscopy (XAS) measurements at the Co–K absorption edge taken at the SUL-X beamline at ANKA. The carotenoidless strain *R. sphaeroides* R26 has been used as it shows the most marked tolerance to cobalt ions among *R. sphaeroides* mutants [1]. Cobalt coordination in cells grown anaerobically under photosynthetic conditions and in presence of 5 mM $CoCl_2$ has been assessed by looking at three different samples: whole cells (WCCo), their cell envelope (CECo) and their soluble portion (SCo).
The X-ray absorption near edge structure (XANES) region can be conveniently used to infer the coordination symmetry of cobalt inner-shell: all recorded spectra show a small pre-edge peak at about 7.710 keV suggesting a slightly distorted octahedral first coordination shell, consisting of six light atoms (possibly oxygen and/or nitrogen). The radial distributions of WCCo, and SCo beyond 3 Å are comparable, indicating similar second coordination shells, whilst a small specific feature appears at 3.5 Å in CECo, suggesting a different second shell coordination (Figure 2, dotted spectrum).
The first coordination shell of the cell envelope (CECo) sample, obtained by fitting EXAFS signals, consists of six oxygen atoms having Co–O distance and Debye–Waller parameter of 2.08 Å and 0.006 $Å^2$, respectively. These values compare well with those found in cobalt chloride or cobalt acetate solutions, where a six-oxygen coordinated cobalt is expected. Interestingly, the region related to the second cobalt coordination shell of CECo samples can be fitted by using either two carbon atoms at 3.30 Å or two sulphur at 2.92 Å from metal without significant change of R-factor statistical parameter. This finding suggests that neither 6O–2C nor 6O–2S configurations can fully represent the second coordination shell, which is likely a mixture of the two. By implementing both model configurations in the fitting procedure and leaving their relative fraction as free parameter, a fifty-fifty contribution of the 6O–2C and 6O–2S fractions was found.
The soluble portion of the cells, SCo, was instead unambiguously fitted by using six-fixed oxygen atoms having a Co–O distance of 2.07 Å for the first shell. The fitting improved by adding a single carbon atom in the second shell with a distance of 2.8 Å, in agreement with the presence of a carbon belonging to carboxylate group binding to cobalt in a mono-dentate mode.
The data obtained by the whole cells sample, WCCo, shows a first shell containing six oxygen atoms and a second shell similar to that of SCo; however, using the same fitting procedure employed for CECo samples, a sulfur atom at a distance of 3.2 A has been found although at very low occupancy (5%), suggesting that in the whole cells the second shell contribution (6O–C) is the most populated one (95%).
These results clearly point out that six oxygen atoms in octahedral geometry are sufficient to explain the first shell of cobalt and that Co–C path is mandatory to fit the second shell in each sample. Interestingly, the cell envelope shows a mixed population of the cobalt second shell formed by equally populated fractions, one formed by two carbon atoms (as in carboxylic groups) and the other formed by two sulphur containing moieties. The distance of the sulphur atom from cobalt together with the six oxygen atoms found in the first shell indicates the presence of oxygen donor ligand having an oxygen atom bound to a sulphur atom, as in sulfoxide or sulfonate moiety.
The cell envelope of phototrophic bacteria comprises the peptoglycane, the outer cellular membrane, and the cytoplasmic membrane. Particularly, the outer membrane and cytoplasmic membranes contains sulfoquinovosyldiacyl glycerol, a class of lipids characterized by a fatty acid bound to the sulfoquinovosyl, a monosaccharide containing a negatively charged sulfonate group that makes this lipid the most probable candidate for cobalt binding on the cell envelop. This agrees with the sulfolipids overexpression in Co-exposed *R. sphaeroides* cells [2]. Differently, the second shell of SCo contains only one carbon atom (6O-C), evidencing that a carboxylate group is sufficient to bind cobalt in the soluble proteome of *R. sphaeroides*. Very small amounts of sulphur containing second coordination shell was detected for cobalt in the WCCo sample (sulfur fraction = 5%); this should not come as a surprise since the soluble fraction represents the largest portion in a cell, with the membrane representing only a very small portion.

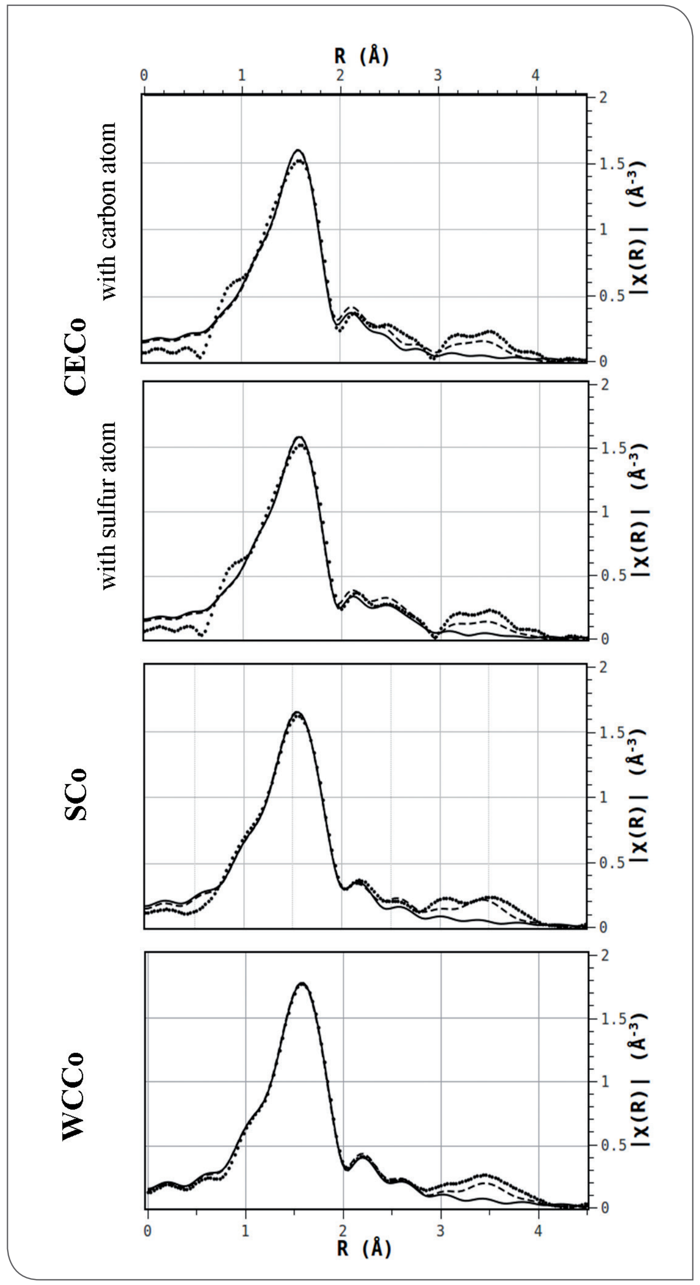

Figure 2: R. sphaeroides samples grown in 5 mM $CoCl_2$: experimental (dots), and calculated with (dotted lines) and without (full lines) multiple scattering paths. The data are shown in their respective Fourier transforms.

Conclusions

X-ray absorption spectroscopy was used to characterize the fate of cobalt ions present in the growth media of the photosynthetic bacterium *Rhodobacter sphaeroides*, a microorganism with high metal tolerance and considered a potential target for bioremediation processes.
Results show that, in the average, cobalt binds in whole cells mostly to carboxylate moieties, with a very small contribution of sulfoxide or sulfonate moiety. A detailed analysis shows that this contribution arises from the sole cell envelope, where the sulphur appear to be as important as carbon in the second coordination shell of cobalt, indicating that sulfolipids of the photosynthetic membrane can significantly contribute to cobalt binding in *Rhodobacter sphaeroides* [3].

Contributors

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