Si-enterobactin from the endophytic *Streptomyces* sp. KT-S1-B5 – a potential silicon transporter in Nature?†

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Si-enterobactin (2a), a hexacoordinated complex of the siderophore enterobactin (2b) with silicon as the central atom, was isolated from an endophytic *Streptomyces* sp. occurring in *Piper guinensis* roots. The structure and absolute configuration were determined from NMR and MS data, and by X-ray diffraction. The orientation of the molecule along the pseudo-3-fold axis shows that the coordination environment of the silicon atom complexed with three bidentate ligands is $D_{3}$. We assume that 2a or related complexes may be involved in the transport of silicon in plants, diatoms, or other silicon-dependent organisms.

As part of our ongoing search for novel bioactive secondary metabolites from endophytes inhabiting Cameroonian medicinal plants, we investigated a *Streptomyces* sp., strain KT-S1-B5, isolated from the roots of *Piper guinensis*. Metabolic profiling by HPLC-UV-MS/MS, and subsequent dereplication by $^{1}H$ NMR using AntiBase, b delivered six known metabolites: $N$-acetylglucosamine, hopene B, aggreceride B, methyl $13$-methyltetradecanoate, and the siderophores nocardamine 3 and desoxynocardamine. In addition, evidence obtained via HPLC/ESIHRMS indicated the presence of protochelin5 and ferrioxamine E (see Fig. S7 in ESI†).

A further siderophore, enterobactin (2b), was also present, but was isolated as the unusual orthosilicate 2a instead of the expected iron complex. It was obtained in crystalline form after chromatographic separation, but was also found in the crude extract by ESIMS before further work-up, if the fermentation was performed in glass vials or better in the presence of traces of silica gel. In this communication, we report the isolation, structure elucidation and absolute configuration of 2a, referred to hereafter as Si-enterobactin.

The $^{1}H$ NMR spectrum (see Table S1 and Fig. S2, ESI†) of 2a showed three aromatic protons in the 1,2,3-positions, an exchangeable NH signal was observed at $\delta_{H}$ 10.17 ($d, J = 10.5$ Hz). The $^{13}C$ NMR data (Table S3 in ESI†) confirmed this interpretation. Of the ten $^{13}C$ NMR signals observed, the two at $\delta_{C}$ 169.5 and 167.2 had values indicative of acid derivatives. From the 2D NMR data (see Fig. S4 and S5 in ESI†), an $N$-(2,3-dihydroxybenzoyl)-serine subunit 1 was elucidated (Fig. 1). This unit is known to occur in the free state in *E. coli*,6 but is more important as a substructure of the iron-transporting salmochelins7 or in the cyclotrimer of 1, enterobactin (2b).8,9

The mass spectra displayed several pseudomolecular ions, but their masses ([m/z 345, 692, 714 at (–)-ESI; 391, 694, 716, 738, 760 at (+)-ESI]) did not match the monomeric or oligomeric catechol siderophores of type 1/2b or their iron complexes. However, the HRMS data did agree fairly well with the aluminium complex of enterobactin (2b), and very well with its silicon derivative (for assignments see the Experimental part).

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† Electronic supplementary information (ESI) available: $^{1}H$, $^{13}C$, and 2D NMR spectra (COSY, HSQC and HMBC), HRESI MS, and crystallographic data of Si-enterobactin (2a). CCDC 920703. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3cc44437f

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X-ray diffraction of single crystals grown from a methanolic solution confirmed the orthosilicate 2a (Fig. 2). The structure was solved by direct methods in the P2₁ space group and refined with the 2012 version of SHELXL. The presence of silicon was unambiguously established using the bond-valence method, where the charge of the central atom is calculated from the six bond distances observed. Further confirmation of silicon as the central atom was obtained from a ²⁹Si NMR spectrum, which showed a singlet at δ = -140.5 (see ESI†, Fig. S6). With 2b, we solved the first crystal structure of an organic silicon complex isolated from Nature.

Determination of the absolute structure of the molecule was also possible. The refinement of the Flack parameter gave a value of 0.018(8) and confirmed the absolute structure and the (S) configuration of all three serine residues. The orientation of the molecule along the pseudo-3-fold axis showed that the coordination environment of the silicon atom complexed with the three bidentate ligands is (partly disordered) methanol solvent. Si–O bond distances are 1.780(2), 1.786(2), 1.789(2), 1.793(2), 1.793(2), and 1.796(2) Å, indicating that the presence of hydrogen atoms can be excluded with confidence (Table S10, ESI†).

Enterobactin (2b), first isolated from Salmonella typhimurium, is known to form stable iron complexes, but the hexacoordinated complex 2a from a streptomycete is the first low-molecular weight organosilicate isolated from Nature. It is noteworthy that we have detected 2a and 2b using (−)ESI MS already after fermentation in Erlenmeyer flasks before a chromatographic work-up (Fig. S16, ESI†), although the yield of 2a was higher in the presence of traces of silica gel; in the presence of soil samples, however, 2a, 2b, or nocardamine were not formed. In separate experiments, enterobactin (2b) did not dissolve silica gel or diatomaceous earth at neutral pH during formation of 2a, and therefore a formation during work-up is unlikely. However, under slightly basic conditions, 2a was formed quantitatively from 2b in the presence of silica gel at ambient temperature, and a sandy soil sample formed the Si complex even without addition of a base (pH 7.01 in an aqueous suspension). This is not in contrast to our fermentation experiment in the presence of soil, as iron compounds present there are known to suppress the formation of siderophores. We conclude that enterobactin can incorporate silicate in the basic fermentation broth from glass or – in the natural habitat – soil. This reaction occurs nearly quantitatively, as the free 2b was detected as a trace component only in a few of our fermentations under Si limitation (Fig. S16 and also for further results, see ESI†).

Nevertheless, the formation of 2b is not completely unexpected. Recently, Süssmuth and co-workers synthesized a number of 2b complexes, among them also 2a. They found that the tris catechol derivative 2b is capable of binding silicic acid to form 2a at physiological pH, when cultivating E. coli in the presence of sodium silicate. Other synthetic catechol derivatives showed a similar behaviour.

In contrast to the occurrence of silicon in bacteria, its importance for plants, diatoms and certain other organisms like glass sponges is very well established. Generally, the silicon concentration is relatively high in plant tissues, and in some cases can exceed the concentration of nitrogen and potassium, reaching up to 10% of the dry mass. Horsetails belonging to the family Equisetaceae, as well as diatoms and glass sponges, require silicon as an essential nutrient. Silicon enhances growth in plants, and protects them from abiotic and biotic stresses such as infections by fungal pathogens and insects. In addition, it enhances the physical stability of plants, and is a cell wall substitute in diatoms.

The uptake of silicon occurs in plants via neutral silicic acid by means of transporter proteins of the aquaporin family (water channel proteins) that transport it to the shoot epidermis. Here it polymerizes to biogenic amorphous silica. Diatoms use functionally related proteins. The concentration of soluble silicic acid or silicate in the soil is moderate (0.1–0.6 mM) and often growth limiting. Therefore, it can be speculated that Si-enterobactin (2a) or related compounds of microbial origin are involved synergistically in the transport of biological silicon via mobilization of silicate, perhaps by means of associated endophytic microorganisms. The application of Si-catechol complexes as growth stimulators has already been tried, but was not very successful due to their low stability. In contrast, Si-enterobactin is remarkably stable, and survives in boiling water for more than 30 minutes. Removal of the silicon atom, whilst keeping the organic ligand intact, was achieved via a short treatment with hydrofluoric acid at room temperature; in this way, the 2b required for our previously discussed experiments was generated. Due to its stability, supplementation of the soil with Si-enterobactin or 2a-producing bacteria may have positive effects on plant growth. In particular, such supplementation may increase the harvest of silicon-demanding plants like rice. It may also be of interest to search for 2a or related low-molecular weight Si transporters in the tissue or the rhizosphere of Si-rich plants. Solutions of 2a may reach rather high silicon molarities of theoretically up to 2 M for silicic acid (as calculated for solid 2a, using molecular weight and density data; see ESI†, Table S1),
without losing solubility. In contrast, free silicic acid starts to polymerize into silica at concentrations >2 mM, although the intracellular concentration of silicic acid may reach values of up to 340 mM. Compounds related to 2a are therefore good candidates for the expected organic chelators maintaining supersaturated solutions in the Si pools of diatoms and other organisms.23

For general experimental procedures, see ref. 1.

Using our previously described methods,1 the endophytic Streptomyces sp. KT-S1-B5 was isolated from surface-sterilized roots of Piper guinensis (Piperaceae), a Cameroonian medicinal plant collected in Mbouda, Cameroon, in May 2008. The isolate sp. KT-S1-B5 was isolated from surface-sterilized Streptomyces roots of Piper guinensis (Piperaceae), a Cameroonian medicinal plant collected in Mbouda, Cameroon, in May 2008. The isolate 2a was found to produce Si-enterobactin (DH 921/3-2). We thank Mr R. Machnik and Dr M. John for the NMR spectra, Mrs F. Lissy and Mr A. Kohl for technical assistance, and Dr S. Hickford for language polishing. T.J.N.K., M.D.K.T., and F.T.M. thank the German Academic Exchange Service (DAAD) for fellowships.

Notes and references

13 CCDC 920703.