ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Terbium Excitation Spectroscopy as a Detection Method for Chromatographic Separation of Lanthanide-Binding Biomolecules

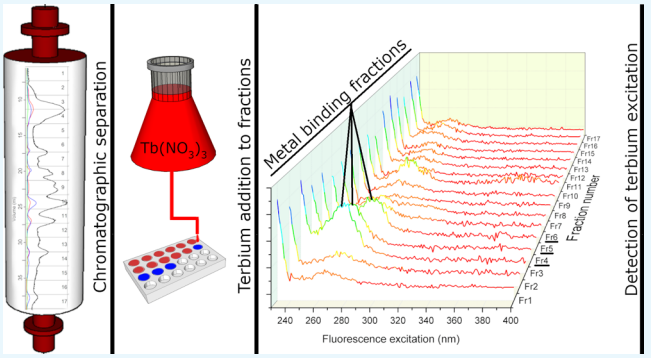
Wojciech Jurkowski, Marcus Heilmann, Anna M. Becker, Rainer Buchholz, and Thomas B. Brück*

Cite This: *ACS Omega* 2020, 5, 27050−27056

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Studies of biosorption and bioaccumulation of heavy metals deal mostly with challenging, inhomogeneous, and complex materials. Therefore, most reports describe only application studies, while fundamental research is limited to indirect methods and speculations on the binding mechanisms. In this study, we describe a method for detecting and isolating heavy metal-binding biomolecules directly from crude extracts. The underlying principle is terbium sensitization and fluorescence excitation spectroscopy used offline after a chromatographic run. Compounds interacting with metal ions inevitably change the coordination sphere of terbium, which is reflected in the excitation spectrum leading to metal-specific luminescence. Main advantages of our approach include simple, fast, and inexpensive experiment design, nondestructive measurements, and detection limits far below 1 mg. Here, we have applied our method for three promising biosorbents (green algae, moss, and cyanobacterium) and obtained first information on the character of active compounds isolated from each species.



INTRODUCTION

The phenomena of biosorption introduced many decades ago is a promising environmentally friendly approach for the removal of water contaminants. Until recently, many studies describing the application of this process have been published.[1] Parallel to screening and process development, much efforts have been put into fundamental research. In the 1960s, the ion-exchange properties of the brown algae-derived sugar polymer—alginate—have been elucidated by nuclear magnetic resonance (NMR) and X-ray diffraction for the first time.[2,3] Since then numerous reports have tried to shed light on the biosorption mechanisms using methods such as potentiometric titrations, scanning electron microscope with energy-dispersive X-ray spectroscopy,[4,5] Fourier transformed infrared spectroscopy,[6] X-ray photoelectron spectroscopy,[7] or atomic force microscopy.[8] Currently, cumulative data suggest that carboxylic, sulfonic, or hydroxyl functional groups are involved in the binding of metals on biomass components.[9] However, only few new metal-binding components have been actually isolated and characterized—those encompass fucoidan,[10] carrageenan,[11] pectines,[12] or mannans.[13] More recently, bacterial-derived extracellular polymeric substances[14] have been identified to enable metal binding. In general, research has focused more on the chemical mechanisms facilitating metal binding, than on isolating and identifying particular molecular components contained in a specific cellular matrix.[1] One main obstacle in these studies has been the lack of reliable tools for simultaneous detection and isolation of such components from biomass.

Much more has been achieved in the field of bioaccumulation, probably due to the smaller size of involved molecules. In contrast to biosorption, which is a rapid passive uptake of metals on the biomass surface, bioaccumulation represents a slower and active process, mediated by cross-membrane ion transporters.[15] A particular breakthrough discovery has been the phytochelatins,[16] which are required for the survival of organisms in heavy metal-contaminated environments by complexing and thus detoxifying them. In subsequent decades, further metal binding or transporting molecules have been identified through genomic and proteomic approaches including P-type ATPases,[17] CDF-proteins,[18] MFS-type importers,[19] and nicotinamine[20] to name just a few. These methods are very laborious and limited only to small molecules, rendering them useless for biosorption studies. More recently, liquid chromatographic methods suitable for separation of metal-binding molecules, including simple organic acids from hyperaccumulator plants[21,22] and

Received: May 8, 2020
Accepted: September 25, 2020
Published: October 14, 2020

https://dx.doi.org/10.1021/acsomega.0c02135
ACS Omega 2020, 5, 27050−27056

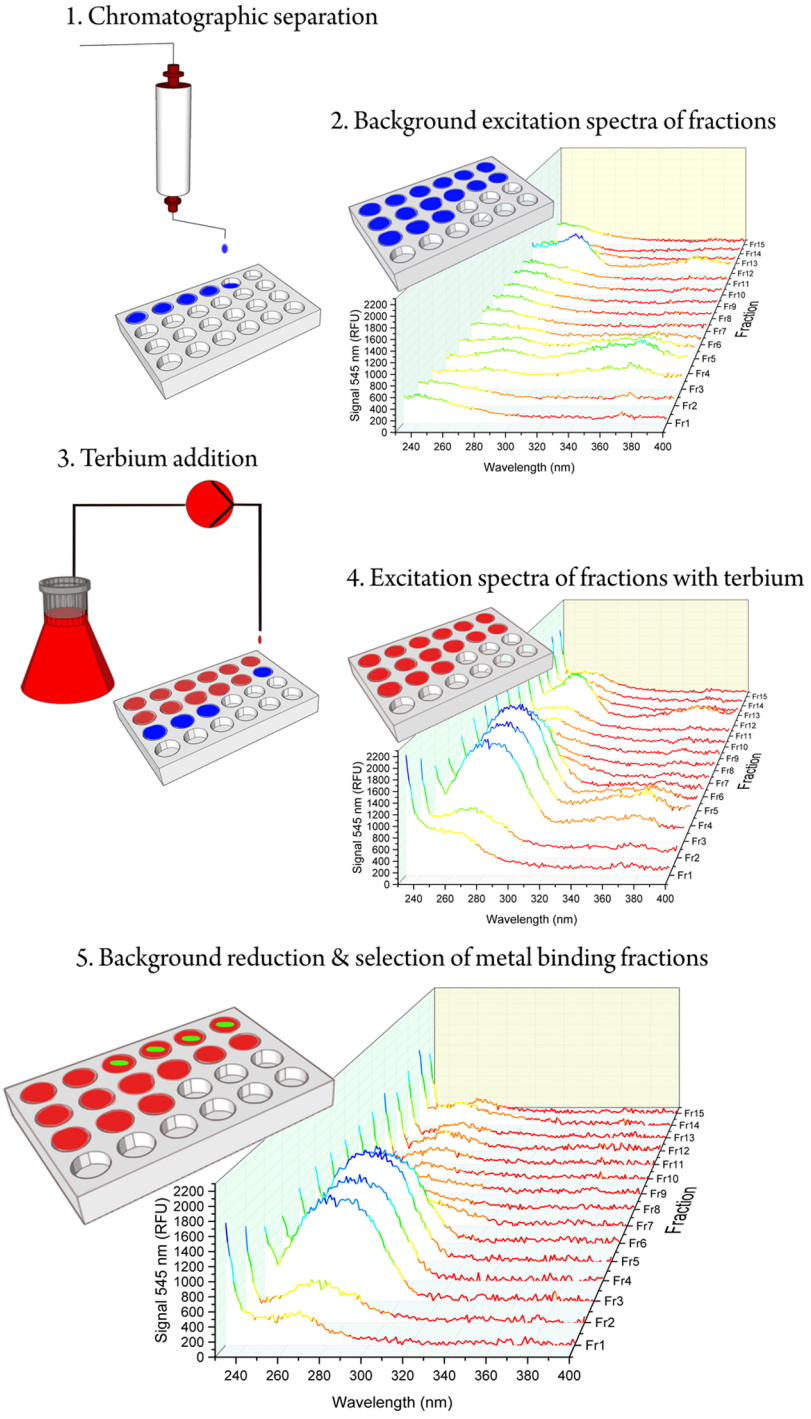


Figure 1. General workflow of the method.

algae,[23−25] have been reported. The detection of bound metals was performed with inductively coupled plasma−mass spectrometry (ICP−MS), being a state of the art method for elemental analysis.

General Method Description. In this study, we describe a new, preparative chromatographic method that allows rapid detection and fractionation of heavy metal-binding cellular components. This method is nondestructive and allows separation and isolation of metal-binding molecules in untreated extracts making it superior to the previously published separation methods employing ICP for detection. In contrast to approaches employing ICP, samples do not need to be spiked with the target metal prior to the chromatographic run. As our methodology relies on the capture of terbium-binding cell components in their native state, it facilitates further structural characterization, for example, via X-ray diffraction and NMR. Moreover, the method presented here is simple, inexpensive, and reliable.

The main feature of our method is the application of terbium(III) as a model ion. The extraordinary properties of lanthanide ions are rooted in their electron configuration. Throughout the series from La to Lu, the 4f valence shell is being filled prior to the 5s and 5p, which are responsible for the actual chemical properties of these elements due to their

https://dx.doi.org/10.1021/acsomega.0c02135
ACS Omega 2020, 5, 27050−27056

greater spatial spread. Hence, f-electrons are shielded and practically do not engage in chemical reactions but may on the other hand perform 4f−4f transitions between partially forbidden states. In effect, narrow and characteristic excitation and emission bands as well as extended luminescent radiation lifetimes can be observed.[26] Although luminescence can be readily achieved through direct excitation, the use of ligands increases the quantum yield dramatically by ligand-to-metal energy transfer (called sensitization), and provides shielding from solvent-induced nonradiative relaxation.[26]

While sensitization or solvent effects may easily alter the excitation wavelengths, the emission wavelengths are usually distinct and undergo only subtle changes. These properties have been recorded for interactions of Tb[27,28] and europium (Eu)[28] with ethylenediaminetetraacetic acid (EDTA) and calmodulin and even allowed to distinguish between metal-binding sites of the examined protein. Such behavior originates in the strict energy levels separating allowed electron states and is best depicted with a Jablonski diagram.[29] Energy necessary for excitation can be delivered via a variety of routes, thus yielding broad bands; conversely, an emission of photon is coupled to strict quantum states. In our case, the visible 545 nm band can be attributed to photons released during $^5D_4 \rightarrow {}^7F_5$ transition.

The excitation spectrum leading to the metal-specific emission at 545 nm may be altered in different ways. Changes can originate from sensitization, when the unknown molecule absorbs UV light, leading to a dramatic increase of luminescence quantum yield by ligand-to-metal energy transfer. However, also opaque compounds binding to lanthanides can increase quantum yields by shielding from solvent-induced nonradiative relaxation.[30] In such case, enhancements in the spectral region of direct metal excitation can be observed. Some molecules can also enhance such relaxation, which is leading to a reduced intensity of the excitation bands (luminescence quenching). All of these effects can be recognized in the sample-specific excitation spectrum.

As depicted in Figure 1, our method initially employs fractionation of a sample extract using a suitable size exclusion chromatography (SEC) column. Each fraction is then transferred onto a microplate to record the blank absorption and excitation spectra (for 545 nm emission) in the absence of terbium (background spectra). Subsequently, terbium is added to each fraction and the excitation spectra is recorded. The resulting spectral readings are then subtracted from the blank to yield metal-induced fluorescence data. The resulting spectra can be compared to the control spectrum of terbium in water (or buffer)—any differences suggest that the given fraction interacts with the metal.

RESULTS AND DISCUSSION

Metal-Binding Fractions Form *Calothrix brevissima* and *Physcomitrella patens*. A graphical overview of all collected data is presented for *Calothrix brevissima* in Figure 2. In this case, only fractions 3, 4, and 5 showed a significant change in luminescence after Tb addition. As these fractions were below or close to the exclusion limit of the column (30−70 kDa), the resolution is quite low as it represented a mixture of many cellular components. Due to relatively strong UV absorption at selected wavelengths, it can be presumed that these fractions contain proteins. At this point, it was unclear whether they also contained non-UV-absorbing molecules like polysaccharides, which are usually responsible for biosorption.

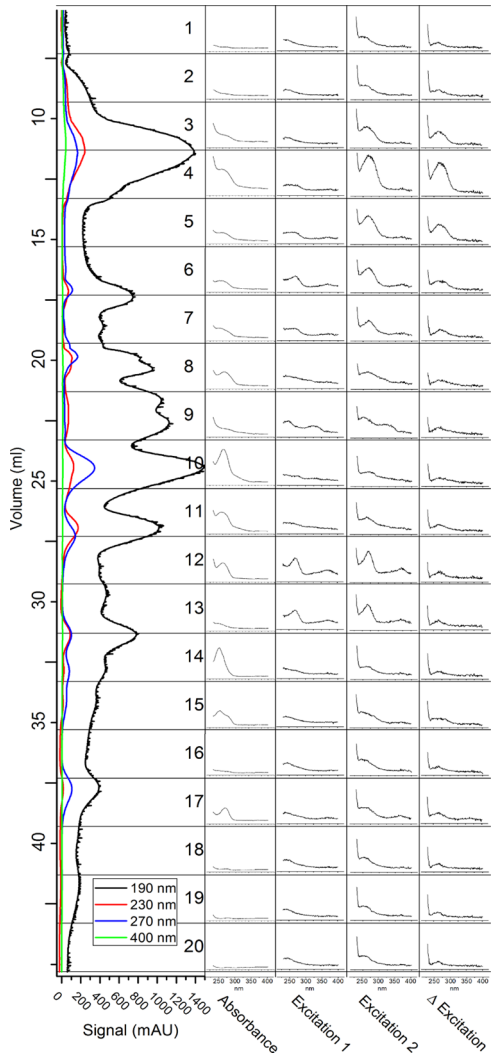


Figure 2. Chromatogram from raw extract of *C. brevissima* with fractional spectroscopy separated on the SEC column (Superdex-75 10/300 GL). Injection volume: 500 μL, F = 0.5 mL/min, 10 °C. UV detection—absorbance at 190, 230, 270, and 400 nm. Spectra from left: absorbance in the UV region, background excitation (1) without terbium, excitation (2) with added terbium, background subtracted excitation with terbium. All excitation spectra collected at 545 nm emission using the dual monochromator with xenon arc lamp source.

A slightly different result was obtained in the case of the extract of the moss—*P. patens* (complete data in the Supporting Information). Interestingly, fraction 2 exhibits high UV absorption but no Tb interaction. In contrast, fractions 3, 4, and 5 interact with Tb but do not show intense UV absorption. This suggests that metal ions are selectively bound by cellular components, which do not exhibit a strong UV absorption, indicating that Tb does not interact with proteins. Furthermore, the direct comparison of the luminescence spectra of fractions 3 and 5 (see Figure 3) suggests the existence of at least two distinct compounds. Some more caution is required when analyzing fraction 10, which shows minor changes in the luminescence excitation spectra but is already luminescent prior to terbium addition. Nevertheless, the subtraction of endogenous luminescence excitation spectrum suggests that luminescence signal increased upon Tb addition. This could be verified by a luminescence emission scan with the excitation wavelength set

https://dx.doi.org/10.1021/acsomega.0c02135
ACS Omega 2020, 5, 27050−27056

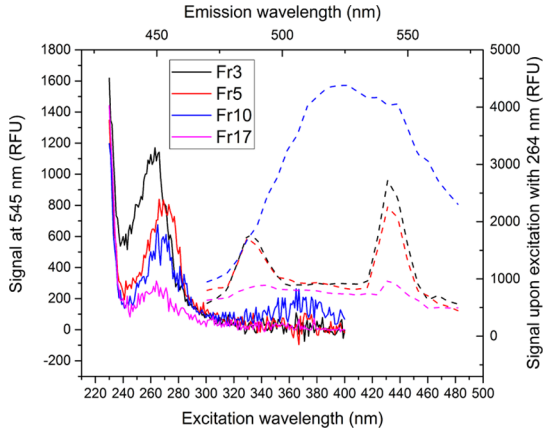


Figure 3. Luminescence excitation for 545 nm (solid line) and emission spectra for 264 nm (dashed line). Fraction 17 refers to deionized water used as a reference for the Tb signal.

to the excitation peak found in the sample (264 nm for fraction 10). This measurement (dashed line in Figure 3) revealed only a luminescence peak with very small metal influence around 545 nm suggesting that no metal-sample interaction occurred.

Comparison with Model Substances. Luminescence excitation spectra of Tb with some known metal chelators such as bovine serum albumin (BSA), EDTA, alginate, and fucoidan using the same procedure as for fractions of biomass extracts (Figure 3) have been recorded. In order to confirm that registered excitation maxima correspond to metal-specific emission, emission spectra at those excitation maxima have been recorded, showing no relevant luminescence interferences present (see the Supporting Information).

Depending on the complexed metal, it is reported that the corresponding metal−EDTA complexes absorb UV light at wavelengths between 220 and 240 nm.[31] However, when terbium is the central atom, the energy absorbed from UV light can be transferred to the metal and dissipated by luminescence at 545 nm. In our study, the endogenous Tb luminescence feature showed the highest emission upon excitation at 240 nm. Furthermore, due to the EDTA excess in our experiments, luminescence was observed only when the sample was excited between 230 and 250 nm, which indicated that no energy transfer, other than UV → EDTA → Tb, occurred. This UV-induced luminescence is equivalent to the luminescence measured for Tb−BSA complexes in our study.

In previous studies, only one absorption peak attributed to tyrosine in the range of 275−280 nm, and a low signal at 300 nm have been reported for BSA.[32,33] These data are in accordance with our absorption measurements. As expected, the recorded excitation spectrum of Tb−BSA contains the previously reported tyrosine signal, without the shift caused under the previously reported denaturating conditions.[33]

However unexpectedly, the highest signal was generated at 300 nm, where BSA itself exhibits only a low absorption. Although relatively low amounts of light are absorbed at this wavelength, far more of its energy induces the emissive state suggesting tighter coordination. Our findings indicate that the signal intensity correlates with increasing BSA concentrations and is nearly absent at very low protein levels, where excitation at 275 nm is dominant. This indicates that a different complex geometry is formed when BSA is supplied in excess. It is feasible that multiple proteins chelate the metal, thus forming sandwich complexes.

The respective Tb complexes with EDTA and BSA show a luminescence signal reduction upon excitation at 230 nm, with respect to signal intensity of Tb in water. It is feasible that both EDTA and BSA absorb the excitation energy at 230 nm before it is accessible for Tb. Therefore, no directly induced emission can occur.

Fucoidan and alginate solutions in the absence of Tb displayed very low or no absorption in the UV region, which corresponds well with their properties previously reported in the literature.[34,35] As sugar polymers do not absorb energy in this spectral region, they cannot transfer energy to the metal to induce luminescence. In fucoidan and alginate solutions, excitation spectra were similar to those recorded in water, which suggests that solvent-induced nonradiative relaxation is the most probable mechanism for the observed phenomena. In that context, the energy can be absorbed by the metal at a typical wavelength of 219 nm.[36]

In all three extracts of phototrophic species, active fractions showed excitation patterns, similar to water or polysaccharides. Two maxima were present: one at 230 nm or less (outside fluorimeter range) and one at about 264 nm. While protein complexes exhibit excitation according to their UV spectra but no Tb-specific excitation patterns, the reverse holds true for polysaccharide-containing samples. Our data indicate that carbohydrates are the active substances that can bind metals such as Tb.

Comparison with Other Methods. Most of the methods that have been described for separation and detection of metal-binding species apply ICP−atomic emission spectroscopy or ICP−MS,[37] which can be successfully coupled to a chromatographic system.[38−40] The advantages, including instant determination of multiple elements, very low detection limit, and independence of the sample matrix, render these methods a perfect choice for quantitative analysis of metal species. It is however necessary to add metals to the sample prior to chromatography. This in turn limits the selection of buffers to those not interacting with metals as well as the useable pH range. Furthermore, the sample fractions contain the metal already and cannot be used nor analyzed in their native form. It is also hard to purify the metal-binding species in a subsequent run without metal spiking because the retention times would vary and, as mentioned earlier, UV detection will not work for polysaccharides. Prior to the emergence of ICP-based detection, atomic absorption spectroscopy (AAS) was employed for the analysis of biomass fractions after a chromatographic run. Hence, the extracts of the cyanobacteria *Nostoc linckia* and *Nierembergia rivularis* loaded with Cd and Zn have been analyzed with AAS after fractioning by SEC.[41] Results obtained share similar advantages and limitations with previously mentioned studies using ICP; however, this methods suffers from low resolution and is associated with laborious sample handling.

Similarly, sensitized lanthanide luminescence has been used for detecting metal interactions with humic substances. Quenching of intrinsic fluorescence of those compounds as well as antenna effects were observed.[42] Using time-resolved luminescence spectroscopy, the same group managed to characterize competitive ion exchange by observing the decrease of sensitization upon addition of other metals.[43] While this is a remarkable solution for characterization of isolated compounds, it needs to be stressed out that without examination of the excitation spectra, some fractions of a chromatogram might be assigned as false negatives. The

https://dx.doi.org/10.1021/acsomega.0c02135
ACS Omega 2020, 5, 27050−27056

dependence of emission spectra on the chosen excitation wavelength is best depicted in Figure 4 (with corresponding emissions—see the Supporting Information). Using for example excitation of 300 nm, the analyst would miss the response from Tb−EDTA completely and almost all from Tb−alginate.

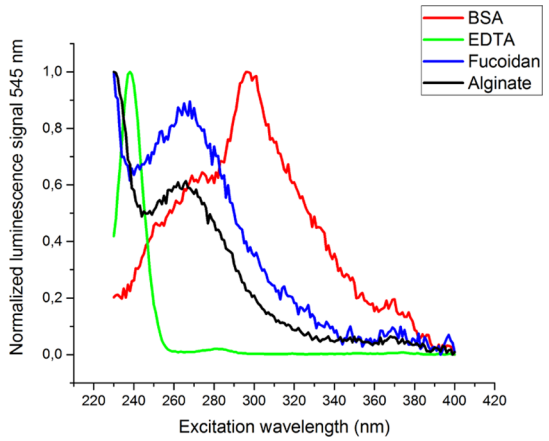


Figure 4. Luminescence excitation spectra for 545 nm for complexes of terbium with different metal chelators.

CONCLUSIONS

The herein presented method provides a chromatographic detection method for metal-binding molecules, which is compatible with previously published and very insightful chemical and enzymatically methods of biomass characterization. A cleavage at specific sites and subsequent sorption experiments, as shown for *Saccharomyces cerevisiae*,[13] would not be possible with other procedures because the fractions would contain the metal already.

One must be however aware of the limitations, which include a detection limit of approx. 0.1 mg (estimated gravimetrically by air drying of the active fractions), depending on the chelator. Although the method relies on terbium, lanthanides often substitute other metals in biological samples (e.g., Ca^{2+} or Mg^{2+} domains in proteins[44−46]) and occupy similar binding sites as other heavy metals.[47] This makes them suitable for probing many molecules; however, for absolute clarity, a direct complexation with the metal in question (and ICP−MS detection) should be applied as well.

EXPERIMENTAL SECTION

Extraction and Separation of Metal-Binding Components. In this particular experiment, a cyanobacterium (*C. brevissima*) and a moss (*P. patens*) were used. The extractions begun with grinding of 200 mg of dry and frozen biomass under liquid nitrogen. Next, ground biomass was suspended in 3 mL of deionized water and placed in a sonicator for 3 min (75% power, 50% time cycle). Disrupted cells were incubated for 1 h at room temperature and centrifuged for 15 min at 10,000 g at room temperature. The supernatant was filtered through a 0.45 μm syringe filter prior to the injection into the chromatography system.

The samples were run on a gel filtration column filled with Superdex-75, with deionized water as eluent, flowing at 0.5 mL/min. Temperature was set at 10 °C. Injection was performed manually with a sample volume of 500 μL. After passing through column, dead volume (5 mL) fractions of 2 mL were collected, regardless of the UV signal. Subsequently, 350 μL of each fraction was transferred into a 96-well microtiter plate. The absorption spectra as well as luminescence excitation spectra for 545 nm emission, that is characteristic for terbium, were recorded in order to assess background fluorescence of the sample (without Tb). Finally, the samples were mixed with 30 μL of 5 mM $Tb(NO_3)_3$ in deionized water, and the same luminescence excitation spectra were recorded again. The protocol consisting of three measurements and addition of terbium solution was executed automatically by the multiplate reader.

Additionally, spectra of Tb in the presence of various chelating agents: EDTA, BSA, fucoidan, and alginate were recorded, according to the procedure described above for crude extracts from the green algae *Picocystis* sp. For these measurements, the concentration of Tb was 100 μM, and ligands were in large excess—10 mM for BSA and EDTA and 500 mg/L for alginate and fucoidan.

Full list of chemicals and devices are given in the Supporting Information

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c02135.

Chromatograms for *P. patens* and *Picocystis* sp. extracts, excitation spectra of all fractions after and before Tb addition, excitation spectra of metal chelators before Tb addition, and emission spectra of Tb-chelates for selected wavelengths (PDF)

AUTHOR INFORMATION

Corresponding Author

Thomas B. Brück − *Werner Siemens Chair of Synthetic Biotechnology, Technical University of Munich (TUM), D-85748 Garching, Germany;* orcid.org/0000-0002-2113-6957; Phone: +49 89-28913253; Email: brueck@tum.de

Authors

Wojciech Jurkowski − *Werner Siemens Chair of Synthetic Biotechnology, Technical University of Munich (TUM), D-85748 Garching, Germany*

Marcus Heilmann − *Molecular Imaging and Radiochemistry, Clinic of Nuclear Medicine, Friedrich-Alexander Universität Erlangen-Nürnberg, D-91052 Erlangen, Germany*

Anna M. Becker − *Institute of Bioprocess Engineering, Friedrich-Alexander Universität Erlangen-Nürnberg, D-91052 Erlangen, Germany*

Rainer Buchholz − *Institute of Bioprocess Engineering, Friedrich-Alexander Universität Erlangen-Nürnberg, D-91052 Erlangen, Germany*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c02135

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We kindly thank the Bavarian State Ministry of the Environment and Consumer Protection for funding the project within the framework of ForCycle Project Group.

■ REFERENCES

(1) Michalak, I.; Chojnacka, K.; Witek-Krowiak, A. State of the Art for the Biosorption Process—a Review. *Appl. Biochem. Biotechnol.* **2013**, *170*, 1389−1416.
(2) Haug, A. The affinity of some divalent metals to different types of alginates. *Acta Chem. Scand.* **1961**, *15*, 1794−1795.
(3) Steginsky, C. A.; Beale, J. M.; Floss, H. G.; Mayer, R. M. Structural determination of alginic acid and the effects of calcium binding as determined by high-field n.m.r. *Carbohydr. Res.* **1992**, *225*, 11−26.
(4) Wilke, A.; Buchholz, R.; Bunke, G. Selective biosorption of heavy metals by algae. *Environ. Biotechnol.* **2006**, *2*, 47−56.
(5) Tan, L.; Dong, H.; Liu, X.; He, J.; Xu, H.; Xie, J. Mechanism of palladium(ii) biosorption by Providencia vermicola. *RSC Adv.* **2017**, *7*, 7060−7072.
(6) Yan, G.; Viraraghavan, T. Mechanism of Biosorption of Heavy Metals by Mucor rouxii. *Eng. Life Sci.* **2008**, *8*, 363−371.
(7) Bertagnolli, C.; Uhart, A.; Dupin, J.-C.; da Silva, M. G. C.; Guibal, E.; Desbrieres, J. Biosorption of chromium by alginate extraction products from Sargassum filipendula: Investigation of adsorption mechanisms using X-ray photoelectron spectroscopy analysis. *Bioresour. Technol.* **2014**, *164*, 264−269.
(8) Pan, J.; Ge, X.; Liu, R.; Tang, H. Characteristic features of Bacillus cereus cell surfaces with biosorption of Pb(II) ions by AFM and FT-IR. *Colloids Surf., B* **2006**, *52*, 89−95.
(9) Davis, T. A.; Volesky, B.; Mucci, A. A review of the biochemistry of heavy metal biosorption by brown algae. *Water Res.* **2003**, *37*, 4311−4330.
(10) Paskins-Hurlburt, A. J.; Skoryna, S. C.; Tanaka, Y.; Moore, W.; Stara, J. F. Fucoidan: Its Binding of Lead and Other Metals. *Bot. Mar.* **1978**, *21*, 13.
(11) Veroy, R. L.; Montao, N.; de Guzman, M. L. B.; Laserna, E. C.; Cajipe, G. J. B. Studies on the Binding of Heavy Metals to Algal Polysaccharides from Philippine Seaweeds. I. Carrageenan and the Binding of Lead and Cadmium. *Bot. Mar.* **2009**, *23*, 59.
(12) Kohn, R. Binding of toxic cations to pectin, its oligomeric fragments and plant tissues. *Carbohydr. Polym.* **1982**, *2*, 273−275.
(13) Brady, D.; Stoll, A. D.; Starke, L.; Duncan, J. R. Chemical and enzymatic extraction of heavy metal binding polymers from isolated cell walls of Saccharomyces cerevisiae. *Biotechnol. Bioeng.* **1994**, *44*, 297−302.
(14) Li, W.-W.; Yu, H.-Q. Insight into the roles of microbial extracellular polymer substances in metal biosorption. *Bioresour. Technol.* **2014**, *160*, 15−23.
(15) Gadd, G. M. Heavy metal accumulation by bacteria and other microorganisms. *Experientia* **1990**, *46*, 834−840.
(16) Murasugi, A.; Wada Nakagawa, C.; HAYASHI, Y. Formation of Cadmium-Binding Peptide Allomorphs in Fission Yeast. *J. Biochem.* **1984**, *96*, 1375−1379.
(17) Odermatt, A.; Suter, H.; Krapf, R.; Solioz, M. Primary structure of two P-type ATPases involved in copper homeostasis in Enterococcus hirae. *J. Biol. Chem.* **1993**, *268*, 12775.
(18) Paulsen, I. T.; Saier, M. H. A Novel Family of Ubiquitous Heavy Metal Ion Transport Proteins. *J. Membr. Biol.* **1997**, *156*, 99−103.
(19) Khalfaoui-Hassani, B.; Verissimo, A. F.; Koch, H.-G.; Daldal, F. Uncovering the Transmembrane Metal Binding Site of the Novel Bacterial Major Facilitator Superfamily-Type Copper Importer CcoA. *mBio* **2016**, *7*, 1−11.
(20) Stephan, U. W.; Scholz, G. Nicotianamine: Mediator of transport of iron and heavy metals in the phloem? *Physiol. Plant.* **1993**, *88*, 522−529.
(21) Yen, T.-Y.; Villa, J. A.; DeWitt, J. G. Analysis of phytochelatin−cadmium complexes from plant tissue culture using nano-electrospray ionization tandem mass spectrometry and capillary liquid chromatography/electrospray ionization tandem mass spectrometry. *J. Mass Spectrom.* **1999**, *34*, 930−941.
(22) Callahan, D. L.; Roessner, U.; Dumontet, V.; Perrier, N.; Wedd, A. G.; O'Hair, R. A. J.; Baker, A. J. M.; Kolev, S. D. LC−MS and GC−MS metabolite profiling of nickel(II) complexes in the latex of the nickel-hyperaccumulating tree Sebertia acuminata and identification of methylated aldaric acid as a new nickel(II) ligand. *Phytochemistry* **2008**, *69*, 240−251.
(23) Wood, B. A.; Miyashita, S.; Kaise, T.; Raab, A.; Meharg, A. A.; Feldmann, J. Arsenic is not stored as arsenite−phytochelatin complexes in the seaweeds Fucus spiralis and Hizikia fusiforme. *Environ. Chem.* **2011**, *8*, 30−43.
(24) Zhang, W.; Tan, N. G. J.; Li, S. F. Y. NMR-based metabolomics and LC-MS/MS quantification reveal metal-specific tolerance and redox homeostasis in Chlorella vulgaris. *Mol. BioSyst.* **2014**, *10*, 149−160.
(25) Schaumlöffel, D.; Ouerdane, L.; Bouyssiere, B.; Łobiński, R. Speciation analysis of nickel in the latex of a hyperaccumulating tree Sebertia acuminata by HPLC and CZE with ICP MS and electrospray MS-MS detection. *J. Anal. At. Spectrom.* **2003**, *18*, 120−127.
(26) Bünzli, J.-C. G.; Piguet, C. Taking advantage of luminescent lanthanide ions. *Chem. Soc. Rev.* **2005**, *34*, 1048−1077.
(27) Wang, C.-L. A.; Aquaron, R. R.; LEAVIS, P. C.; Gergely, J. Metal-Binding Properties of Calmodulin. *Eur. J. Biochem.* **1982**, *124*, 7−12.
(28) Mulqueen, P.; Tingey, J. M.; Horrocks, W. D. Characterization of lanthanide (III) ion binding to calmodulin using luminescence spectroscopy. *Biochemistry* **1985**, *24*, 6639−6645.
(29) Dieke, G. H.; Crosswhite, H. M.; Crosswhite, H. *Spectra and energy levels of rare earth ions in crystals*; Interscience Publishers: New York, 1968.
(30) Bünzli, J.-C. G.; Eliseeva, S. V. Basics of Lanthanide Photophysics. In *Lanthanide Luminescence: Photophysical, Analytical and Biological Aspects*; Hänninen, P., Härmä, H., Eds.; Springer Berlin Heidelberg: Berlin, Heidelberg, 2011; pp 1−45.
(31) Dunstone, J. R.; Payne, E. A simple spectrophotometric method for determining magnesium, calcium, strontium, barium, cadmium and zinc with ethylenediaminetetra-acetic acid. *Analyst* **1959**, *84*, 110−113.
(32) Li, T.; Cheng, Z.; Cao, L.; Jiang, X.; Fan, L. Data of fluorescence, UV−vis absorption and FTIR spectra for the study of interaction between two food colourants and BSA. *Data Brief.* **2016**, *8*, 755−783.
(33) Sun, C.; Yang, J.; Wu, X.; Liu, S.; Su, B. Study on the fluorescent enhancement effect in terbium−gadolinium−protein−sodium dodecyl benzene sulfonate system and its application on sensitive detection of protein at nanogram level. *Biochimie* **2004**, *86*, 569−578.
(34) Salgado, L. T.; Tomazetto, R.; Cinelli, L. P.; Farina, M.; Amado Filho, G. M. The influence of brown algae alginates on phenolic compounds capability of ultraviolet radiation absorption in vitro. *Braz. J. Oceanogr.* **2007**, *55*, 145−154.
(35) Mmola, M.; Roes-Hill, M.; Durrell, K.; Bolton, J.; Sibuyi, N.; Meyer, M.; Beukes, D.; Antunes, E. Enhanced antimicrobial and anticancer activity of silver and gold nanoparticles synthesised using Sargassum incisifolium aqueous extracts. *Molecules* **2016**, *21*, 1633.
(36) Georges, J. Lanthanide-sensitized luminescence and applications to the determination of organic analytes. A review. *Analyst* **1993**, *118*, 1481−1486.
(37) Zawisza, B.; Pytlakowska, K.; Feist, B.; Polowniak, M.; Kita, A.; Sitko, R. Determination of rare earth elements by spectroscopic techniques: A review. *J. Anal. At. Spectrom.* **2011**, *26*, 2373−2390.
(38) Hann, S.; Koellensperger, G.; Obinger, C.; Furtmüller, P. G.; Stingeder, G. SEC-ICP-DRCMS and SEC-ICP-SFMS for determination of metal-sulfur ratios in metalloproteins. *J. Anal. At. Spectrom.* **2004**, *19*, 74−79.

https://dx.doi.org/10.1021/acsomega.0c02135
ACS Omega 2020, 5, 27050−27056

(39) Winter, C.; Seubert, A. Usability of online-coupling ion exchange chromatography ICP-AES/-MS for the determination of trivalent metal complex species under acidic conditions. *J. Anal. At. Spectrom.* **2016**, *31*, 1262−1268.
(40) Zhang, B.; Li, F.; Houk, R. S.; Armstrong, D. W. Pore Exclusion Chromatography-Inductively Coupled Plasma-Mass Spectrometry for Monitoring Elements in Bacteria: A Study on Microbial Removal of Uranium from Aqueous Solution. *Anal. Chem.* **2003**, *75*, 6901−6905.
(41) El-Enany, A. E.; Issa, A. A. Cyanobacteria as a biosorbent of heavy metals in sewage water. *Environ. Toxicol. Pharmacol.* **2000**, *8*, 95−101.
(42) Kumke, M. U.; Eidner, S.; Krüger, T. Fluorescence Quenching and Luminescence Sensitization in Complexes of Tb3+ and Eu3+ with Humic Substances. *Environ. Sci. Technol.* **2005**, *39*, 9528−9533.
(43) Marang, L.; Reiller, P. E.; Eidner, S.; Kumke, M. U.; Benedetti, M. F. Combining Spectroscopic and Potentiometric Approaches to Characterize Competitive Binding to Humic Substances. *Environ. Sci. Technol.* **2008**, *42*, 5094−5098.
(44) Lars, D.; Ralph, G.; Renate, U.-H. Lanthanides as substitutes for calcium ions in the activation of plant α-type phospholipase D. *Biol. Chem.* **2014**, *395*, 791.
(45) Bakou, A.; Buser, C.; Dandulakis, G.; Brudvig, G.; Ghanotakis, D. F. Calcium binding site(s) of Photosystem II as probed by lanthanides. *Biochim. Biophys. Acta Bioenerg.* **1992**, *1099*, 131−136.
(46) Dudev, T.; Chang, L.-Y.; Lim, C. Factors Governing the Substitution of La3+ for Ca2+ and Mg2+ in Metalloproteins: A DFT/CDM Study. *J. Am. Chem. Soc.* **2005**, *127*, 4091−4103.
(47) Eriacho, R.; Carn, R. C.; Moore, J. L.; Rayson, G. D. Competitive Metal Ion Binding to Datura innoxia Root and Stem Tissue Materials. *J Anal. Mol. Tech.* **2016**, *2* (1), 1−6.

https://dx.doi.org/10.1021/acsomega.0c02135
ACS Omega 2020, 5, 27050−27056