depending upon the solar wind ram pressure and the local time, the field lines over Maitri can be mapped either to the outer boundary of the plasmasphere or to the inner boundary of the magnetosphere. This makes it a unique location. Further, the enhanced intensities during a geomagnetic storm are a clear indication of a certain acceleration mechanism becoming more active during such events. A detailed investigation of the origin of the particles and their possible acceleration mechanism is in progress.

The present conclusions have been arrived at from the data collected on 13 clear days from Antarctica during January–February 1994 as a part of the XIIIth Indian Scientific Expedition. More extensive campaigns are being planned during the ensuing southern summer months.

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ACKNOWLEDGEMENTS The logistic support for the above study was provided by the Department of Ocean Development (DOD), Government of India This work is supported by the Department of Space (DOS), Government of India Our thanks are due to Prof R K. Varma, Director, PRL, for his keen interest and constant encouragement We duly acknowledge the assistance given by Mrs Vijaya Mutagi in the fabrication of the instrument.

Received 5 December 1994, revised accepted 7 February 1995

Complexation and interaction modelling in a thorium(IV) – mycobacterial-siderophore system

H. J. MacCordick, O. Wendling and M.-S. Antony

Centre de Recherches Nucleaires et Université Louis Pasteur, Laboratoire de Chimie Nucléaire, F-67037 Strasbourg Cedex 02, France

Assessment of the role of bacterial cell components in the biosorption of actinide cations involves studies of the metal ion complexing properties of siderophores. In the present work, the interaction of thorium ions with mycobactin S from *Mycobacterium smegmatis* is examined *in vitro*, as a model system, using ethanol as the solvent medium. Formation of a labile addition compound is followed by UV spectrophotometry, which reveals characteristic absorption in the 330 nm region. Hydrolytic dissociation measurements with the ^{228}Th-labelled complex at pH 7 indicate lower stability than for the corresponding uranyl–mycobactin compound under analogous conditions. The properties of the thorium complex are rationalized by calculations of metal–ligand bond character and steric relationships at the molecular binding site.

APPLICATIONS of microorganisms and microbial constituents as complexing agents in the treatment of radwaste are finding widespread interest[1–3]. Previous work with a mycobacterial species has shown that this substrate is effective in the selective biosorption and retention of various actinide cations such as Th^{4+} and UO_2^{2+} or Am^{3+} on a Eu^{3+} carrier[3,4]. Measurements based on NMR and infrared spectroscopy applied to cellular extracts and model compounds have confirmed that the adsorption process involves cation interaction with specific cell components containing acidic functional groups such as those in phospholipids and non-esterified carboxylate[5].

The possible contribution of the mycobacterial siderophore in this respect has been tested with purified

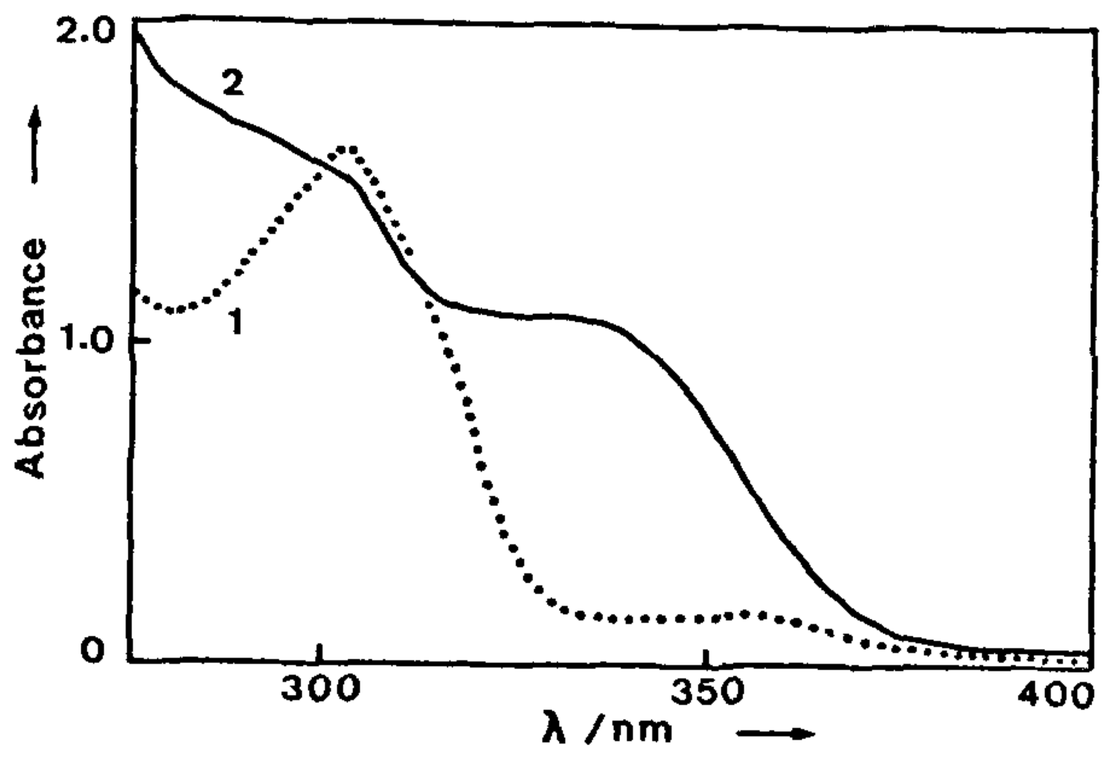
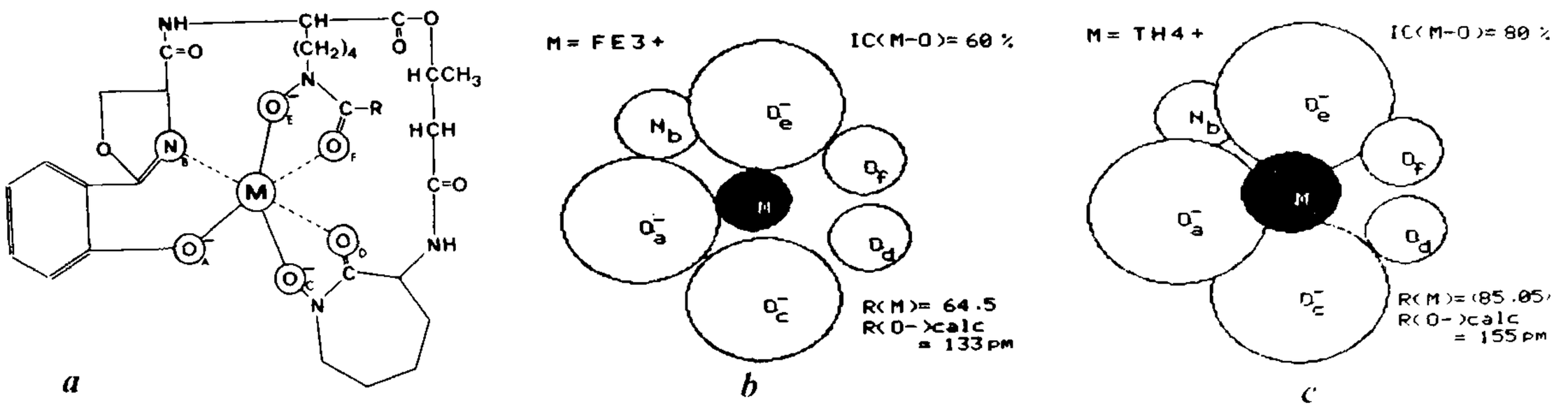


Figure 1. UV absorption spectra for MYS (•••, 1) and Th–MYS (——, 2) in ethanol



a *b* *c*

Figure 2. *a*, Site symmetry in ferrimycobatin S, M = Fe(III). For R, see ref 10 *b*, Computer graphic 'optimized' steric fit for cation-site interaction in Fe-MYS ($\gamma = 1.86$, $r_{Ocov} = 70.6$ pm [see text]) *c*, Computer graphic steric fit calculated for the Th/MYS interaction, using the computational parameters as for *b* IC = calculated ionic character (%) of M–O electrovalent bonds Radius overlap between the metal ion and O-atoms (Oa, Oc, Oe) signifies steric hindrance for molecular enclosure of the cation

mycobactin S (MYS) *in vitro* and metal cation complexes of variable degrees of stability have been obtained[6–8] with UO_2^{2+}, NpO_2^+, Sc^{3+}, Y^{3+} and La^{3+}. The experimentally observed trends in stability for these and other previously known complexes of mycobactin have been interpreted by calculations using a restrictive spatial model of cation–ligand site compatibility[9]. So far, studies of the affinity of the trivalent desferrimycobactin anion for cations of charge number higher than 3 do not appear to have been reported. In the present work, this question is investigated for the Th^{4+}–MYS system using optical spectroscopy and a refined version of steric modelling for the siderophore coordination site.

As in previous experiments[6,8], a solution of the metal chloride in absolute ethanol (10 mM) was added stepwise to an ethanolic solution of purified MYS (0.8 µmol, extracted from *Mycobacterium smegmatis*[10]) under conditions of spectrophotometric titration. The same method, using $FeCl_3$ and measurement of maximum optical density (OD) at $\lambda = 450$ nm as a function of molar ratios of the components, served as an analysis for the purity, that is, complexing power of the mycobactin sample. Results for the thorium system obtained in this way (Figure 1) showed a broad absorption shoulder centred at 330 nm (curve 2) that is not present in MYS itself, i.e. in its protonated, non-complexed form (curve 1).

This absorption, which in some MYS–cation systems may appear in the 340–350 nm region, is characteristic of metal ion complexation by MYS and normally corresponds to compounds of 1:1 stoichiometry. In the present instance, however, the presumed Th–MYS complex shows maximum absorption at $\lambda = 330$ nm for a Th:ligand ratio close to 2:1. Although the possibility of formation of a polynuclear complex of the type MYS–2Th cannot, a priori, be ruled out, preference is given to a formation–dissociation equilibrium of the type

$$Th^{4+} + MYS \rightleftharpoons Th^{IV}\text{–MYS},$$

in which a 1:1 complex is formed, but dissociates to the extent of ~50% in ethanol, formation becoming practically complete for an excess of thorium in molar proportion. This interpretation is based on the fact that (1) in alcoholic solutions of $ThCl_4$, thorium is present in dissociated form and not as a complex species[11], (2) the persistence of an absorption shoulder at ~303 nm throughout the addition of Th coincides with the peak typical of non-complexed MYS at this wavelength, indicating the presence of residual free ligand. Control experiments confirmed that $ThCl_4$ alone, at the concentrations employed, did not contribute significantly to absorption in this region.

The presumed lability of the Th–MYS complex was confirmed by measurements of its extent of hydrolytic dissociation at pH 7. In accordance with the foregoing experiments[6] with Fe–MYS and UO_2–MYS, a 1.76 mM chloroform solution of Th–MYS, labelled with ^{228}Th as an indicator nuclide, was shaken with an equal volume of demineralized water for 5 min. Gamma-ray counting of the ^{228}Th activity in the two phases then showed that 93% of the complexed thorium was released into the aqueous phase. This high extent of dissociation may be compared to the corresponding values of virtually 0% for Fe–MYS and ~20–32% dissociation for UO_2–MYS under analogous conditions[6].

These overall results are corroborated by predictions of molecular site modelling as described earlier[9] for metal complexes of mycobactin P. In the present case, this model was refined by using revised calculations of the effective ionic radius (r_{Oeff}) of the three negatively charged, electrovalent oxygen atoms (A, C, E; Figure 2 *a*) of the MYS cation-binding site, with the aid of the expression

$$r_{Oeff} = \left[\left(\gamma \cdot \frac{|\chi_O - \chi_M|}{(\chi_O + \chi_M)}\right) \cdot (r_O - r_{Ocov})\right] + r_{Ocov},$$

where the term containing the respective atomic electronegativity units (χ_O for oxygen and χ_M for the metal

cation) represents the M–O partial ionic bond character according to the Wilmshurst equation[12]; r_O is the purely ionic, single-bonded oxygen radius (176 pm)[13] and r_{Ocov} is the purely covalent oxygen radius, which has been assigned[14] values in the range 70.2 to 74 pm. Optimal values for the empirical constant γ and r_{Ocov} were obtained by iterative computation based on the best steric fit for cation and ligand site in the naturally occurring, most stable complex, Fe^{III}–MY. From the site-structural representations shown in Figure 2, it is seen that the Th cation (Figure 2 *c*) largely exceeds the calculated available volume of the site configuration as established from Fe–MYS (Figure 2 *b*), suggesting a resultant distortion of molecular symmetry that would expectedly lead to relative weakening of the metal–oxygen bonds. The higher degree of ionic character (~81%) calculated for the Th–O bond, together with the fact that only three of the four metal valencies can be engaged in MY complexation, may add further to this effect.

In spite of the lower stability of Th–MYS in comparison to UO_2–MYS under *in vitro* conditions, whole-cell biomass loaded with thorium cations appears to be more resistant to hydrolytic desorption than uranyl-loaded biomass. This apparently contradictory observation indicates that additional binding interactions, together with the protecting effect afforded by cellular incorporation, may be influential under complex natural conditions.

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Received 12 December 1994, revised accepted 27 January 1995

Partial unfolding of lactate dehydrogenase in the presence of low concentrations of guanidium chloride

Abhijit Chakrabarti†,*, Subrata Chattopadhyay and Chanchal K. DasGupta

Department of Biophysics, Molecular Biology and Genetics, University of Calcutta, 92, A P C Road, Calcutta 700 009, India
†Biophysics Division, Saha Institute of Nuclear Physics, 37 Belgachia Road, Calcutta 700 037, India

The presence of very low concentrations of guanidium chloride could partially unfold the tetrameric enzyme lactate dehydrogenase from pig muscle. The local partial unfolding in the tertiary structure of the protein was revealed from the study of quenching of the tryptophanyl fluorescence using the most widely used quenchers, acrylamide and iodide. Significant changes in the Stern–Volmer quenching constants as well as in the accessibility parameter of the tryptophan residues by the quenchers were observed in the presence of <100 mM guanidium chloride over the same in the absence of the denaturant. This indicated the presence of a partially unfolded state in the enzyme even at a low concentration of guanidium chloride which does not otherwise affect the enzymic activity or the secondary structure of the enzyme.

GUANIDIUM chloride (GdmCl) has been very frequently used, at a concentration >1 M, to denature proteins[1, 2]. However, by spectroscopic measurements, low concentrations of GdmCl have not been shown to impart any alterations in the tertiary structure of proteins. The presence of small amounts of GdmCl in the buffer during reactivation of denatured lactate dehydrogenase (LDH), as initiated by diluting highly concentrated solutions of the denaturant, has been shown to affect the rate of reactivation of the enzyme[3]. In many studies of protein folding, ~100 mM GdmCl is often present in the buffer during reconstitution from the completely unfolded state attained by the treatment with 4–6 M of GdmCl. The present experiments were aimed at analysing the effect of low concentrations of GdmCl on the tertiary structure of the tetrameric enzyme LDH from pig muscle, which has been the subject of extensive studies, and the folding pathways of the enzyme have been elucidated[4]. In the concentration range of 10–100 mM GdmCl, the activity of the enzyme remained unaltered and the secondary structure remained intact as indicated by circular dichroism (CD) measurements. The results from studies of quenching of tryptophanyl fluorescence indicated the presence of a partially unfolded state of the protein in the presence of a very low concentration of GdmCl.

*For correspondence.