Biol Metals (1991) 4:207–210

BIOLOGY OF METALS

Complexes of mycobactin from *Mycobacterium smegmatis* with scandium, yttrium and lanthanum

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Received April 15, 1991

Summary. The interaction of cations of group IIIb elements (Sc, Y, La) with mycobactin S in ethanol leads to the formation of 1:1 complexes which closely resemble the known aluminium compound with respect to ultraviolet absorption and fluorescence emission spectra. Determination of molar stoichiometry by spectrophotometry shows that this method can be conveniently applied to the estimation of purity in mycobactin samples. Hydrolytic dissociation measurements based on aqueous extraction of the labelled complexes in heterogeneous phase indicate a pronounced gradation in cation-binding stability, which increases from La (rapid and complete dissociation) to Sc (≈24% dissociation under similar conditions). The observed properties of the complexes are rationalized by semi-empirical model calculations, which suggest that ionic radius effects resulting from interaction of the IIIb cations with mycobactin S would not favour octahedral coordination of these elements as in the stable Fe(III) complex.

Key words: Mycobactin S – Group III cations – Optical spectra – Molecular modeling

Introduction

The interaction of metal cations with bacterial whole cells or their components is currently a subject of rapidly expanding interest in fundamental and environmental research fields. The role of microbial complexing agents in the cellular accumulation and toxicity of metals has recently been reviewed (Bauda and Block 1990; Birch and Bachofen 1990; Gadd 1990) and mechanisms relating to both intracellular and extracellular bonding have been described (Shumate and Strandberg 1985; Gadd 1990). In the former instance, knowledge of the reactivity of metal ions with bacterial siderophores is useful in assessing the specific complexing properties of these compounds.

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Our previous work on these lines has been directed towards the characterization of new metal complexes formed by a mycobacterial siderophore, mycobactin S; this choice appeared of particular interest in view of structural data available for one of these compounds (Hough and Rogers 1974). The use of a restrictive ionic model of mycobactin coordination (MacCordick 1985) has served as a guide to the experimental approach and has supported observations relating to the formation and stability of known complexes with Al^{3+}, Ga^{3+}, Cr^{3+} or Cu^{2+}, in place of Fe^{3+} (Snow 1970). By their nature, such considerations predicted general instability, or unfavourable reaction conditions, with most other metal ions. In agreement with the model, experimental evidence for the formation of complexes of mycobactin S with UO_2^{2+} and NpO_2^{+} (MacCordick 1988; MacCordick and Kadri 1988) revealed hydrolytic instability of these compounds and suggested that large or oxygen-containing cations of this type were more likely bound in an 'open' molecular configuration involving only part of the available chelating groups.

On the basis of similar predictions for trivalent ions of the lanthanide series and group IIIb (Sc, Y, La), we have examined the reactivity of cations of the three latter elements toward mycobactin S in non-aqueous medium. In its position common to both group and series, the lanthanum ion is of particular interest in this respect. In the present work, the characterization of novel siderophore complexes of these 'pre-lanthanide' elements is conducted with reference to the aluminium mycobactin compound since this represents a comparable artificial species; moreover, the optical properties and stoichiometry of this complex are well established.

Materials and methods

Biochemical preparations. Mycobactin S (MY) was obtained by growth of *Mycobacterium smegmatis,* strain 73.26 (Institut Pasteur, Paris) in iron-deficient broth cultures (MacCordick 1984). Solvent extraction of the Fe(III) complex was carried out accord-

ing to described procedures (Ratledge 1982 and references therein). The extract in ethanol was purified by chromatography on a column (70 × 2.5 cm) of Sephadex LH 20. Central fractions of the Fe-MY eluate were collected which had the highest absorbance ratio measured for $\lambda_{450}/\lambda_{375}$. In the isolated solid Fe-MY product, the iron content (corresponding to the effective 'complexing power' of the mycobactin) was determined by atomic absorption spectrometry. MY in the desferri form for cation coordination experiments was obtained from this material by treatment with 5 M HCl. The mean relative molecular mass of this mycobactin was taken as 813.

Volumetric solutions of M(III) cations (Sc, Y, La, Al) in absolute ethanol (10 ml, 5 mM) were prepared from the dried chlorides obtained by acid dissolution of the corresponding pure oxides (or metal, in the case of Al).

Spectrophotometric measurements. Ultraviolet absorption spectra of the reacting components and M(III)-MY complexes were recorded in ethanol in the range 400–225 nm, using Beckman UV 5270 or Kontron spectrophotometers. This technique also served for determination of the molar combination ratio by spectrophotometric titration as described before (MacCordick 1988). In each case, the cation halide in ethanol was added stepwise to MY (0.16 mM) in the same solvent; absorbance values were measured at 340 nm.

Fluorescence spectra for MY and its complexes in similar solutions were obtained with a Fica 55 Mk II spectrofluorimeter, using an excitation wavelength of 340 nm and a bandwidth of 7.5 nm.

Hydrolytic dissociation measurements. Chloroform solutions of the complexes (1.76 mM), which were correspondingly labelled with radioactive indicators (^{46}Sc, ^{90}Y, ^{140}La; ≈11 kBq cm^{-3}), were shaken with equal volumes of water according to a procedure described elsewhere (MacCordick et al. 1985). Radioactivity in 1-cm^3 samples of the separated phases was counted with the aid of a Ge-Li detector (γ activity) or by liquid scintillation (β activity of ^{90}Y). The distribution of radioactivity between organic and aqueous phases was taken as an indication of dissociation of the complex.

Results and discussion

Absorption and fluorescence spectra

Electronic spectra for previously known metallo-mycobactin complexes have been described (Snow 1970). In the present case, results for the IIIb metal ions [M(III)] are compared with data reported for the aluminium mycobactin complex (Al-MY) in view of certain chemical similarities between these cations and Al^{3+}.

Addition of the M(III) ions in ethanol to a solution of MY in the same solvent leads to a general increase in absorption in the spectrum below 380 nm. The appearance of a peak between 378 nm and 330 nm with maximum at about 340 nm in the four cases (Fig. 1) is characteristic for complex formation, since neither the metal chloride nor MY alone show absorption in this region at the concentrations employed (0.2 mM). In the parent MY compound, the principal peak at 303 nm has been attributed to the presence of the 2-(*o*-hydroxyphenyl)oxazoline group, with additional overall absorption in the region below 300 nm arising from the Δ^2-acylhydroxamic acid moiety (Snow 1970). The characteristic shift of this former contribution to produce the peak of variable intensity at 340 nm indicates that bonding of the cation with MY must in all cases involve interaction with at least the phenolic oxygen of this hydroxyphenyl component, whose spectral contribution will be influenced by linkage with the complexed cation. The overall similarity in the electronic spectra of the four compounds is also evident in the fluorescence emission spectra (Fig. 2). In contrast, a correspondingly prepared europium(III) complex shows no fluorescence under these conditions, although its ultraviolet absorption spectrum is closely analogous to those of the other complexes. This difference is possibly due to a 4f-electron screening effect in the case of Eu(III).

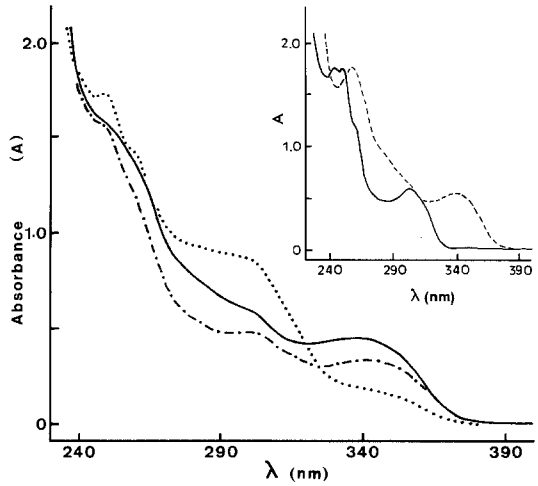

Fig. 1. Absorption spectra of M(III)-MY complexes (0.16 mM in ethanol). (·····) Sc-MY; (——) Y-MY; (-·-·-) La-MY. Insert: (- - -) Al-MY; (——) MY (S)

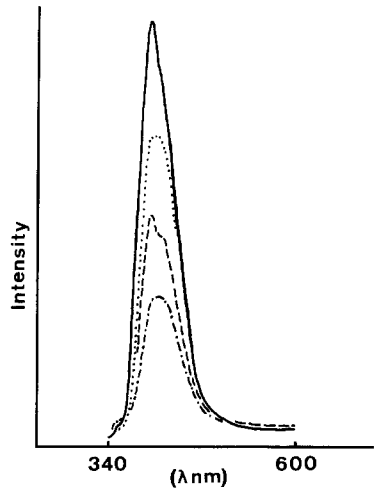

Fig. 2. Fluorescence emission spectra and λ_{max} values for M(III)-MY complexes (0.13 mM in ethanol). Excitation: 340 nm, band width 7.5 nm. Emission band width: 2.5 nm. (——) Al-MY (398 nm); (·····) Sc-MY (405 nm); (- - -) Y-MY (389, 412 nm); (-·-·-) La-MY (405 nm)

Stoichiometry

In the spectrophotometric titration measurements, the comparative reaction of Al^{3+} ions with MY in ethanol is a convenient standard of reference, since 1:1 stoichiometry in the Al-MY complex is implicit from results of mass-spectrographic analysis (White and Snow 1969).

Apart from its verification of stoichiometry, a characteristic feature of the Al/MY titration is relative slowness (several minutes) in attaining stable absorbance values following stepwise additions of the reacting component (Al^{3+}).

This time-dependent effect is not observed in the titrations with La(III) and Y(III), in which stable absorbance values are produced immediately and an absorbance plateau is reached for a molar ratio MY/M^{3+} = 1:1. With the exception of the scandium complex, which gives a relatively flat response and a less clearly defined stoichiometric plateau, these titrations are sufficiently sharp to permit use of this method for analyses of mycobactin preparations.

Hydrolytic dissociation

Measurements of MY complex stability in the presence of water are conveniently carried out by an extraction technique in which the complex containing the labelled cation is initially dissolved in an immiscible organic phase (chloroform).

For a complex concentration of 1.76 mM and a shaking time of 15 min, as employed previously for measurements with Fe(III)-MY (MacCordick et al. 1985), La-MY is completely dissociated at pH 7. The scandium complex undergoes only 24% dissociation under these conditions (Fig. 3), but hydrolysis is pronounced in the presence of acid; the action of 0.1 M HCl leads to 91% dissociation within the same reference time. This may be compared with a corresponding value of about 1% hydrolysis observed for Fe(III)-MY at this acidity.

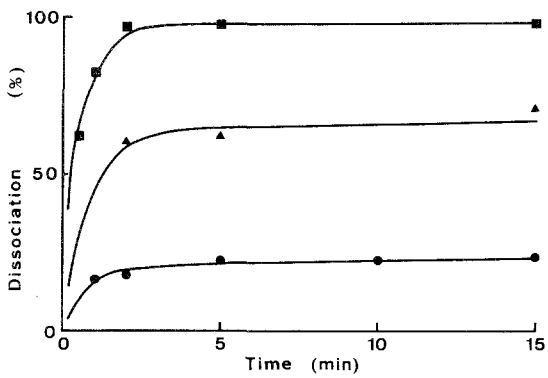

Fig. 3. Hydrolytic dissociation of M(III)-MY complexes at pH 7 (for conditions, see text). Estimated error ±6%. (●) Sc-MY; (▲) Y-MY; (■) La-MY

For the group IIIb-MY complexes, the relatively higher stability observed for Sc-MY is consistent with the generally stronger complex-forming tendency of Sc(III) in comparison to that of the lanthanide ions. The intermediate stability of Y-MY (≈70% hydrolysis) is compatible with a progressive variation in bonding strength for cations within the group. It is noteworthy that a similar stability sequence has been observed for complexes of these M(III) ions with enterochelin, on the basis of ion displacement measurements in the presence of exchangeable ferric ion (Rogers et al. 1980).

Modeling of MY complex formation

Model calculations of steric constraints in cation bonding have been performed previously for the complexing site in ferrimycobactin P, for which X-ray structural data are available (Hough and Rogers 1974). Close structural similarities in various ferrimycobactins have been pointed out (Snow and White 1969). In assessing the compatibility of the M(III) cations for bonding by the siderophore, it thus appeared reasonable to transpose computational data used for the mycobactin P model (MacCordick 1985) to the present context involving mycobactin S. This is further justified by the experimentally proven analogy in composition and observed stability of the respective metal complexes formed.

The calculated relative cation/site relationships for MY with the M(III) species are represented in Fig. 4 (b–e). For each of the group IIIb elements, the cations are apparently too large for six-coordinate complexation similar to that in Fe(III)-MY, but it is likely that they could undergo bonding interaction with at least one of the three negatively charged oxygen atoms of the acidic chelating groups (Fig. 4a). In this event, virtually no steric restrictions would be imposed.

As for Fe(III)-MY, model calculations indicate that the Al-MY complex should have a particularly favourable, fully chelated configuration (Fig. 4b) and indeed this compound is known to be comparatively stable to hydrolysis (Snow 1970). In the coordination of the group IIIb cations, Sc-MY represents the best combination in the sense that the smaller and more polarizing Sc^{3+} ion should form shorter and stronger bonds with chelating groups. This is reflected in the higher degree of covalent character estimated for the Sc-O linkage (≈30%). From these considerations, the ease of metal ion displacement under conditions of hydrolysis should increase with cation size throughout the group, and this is in fact observed.

Conclusion

The formation of artificially produced cationic complexes of mycobactin offers new and interesting possibilities in the study of the weaker bonding interactions of siderophores with metal ions in solution. Even transient interactions of this type may be of significance in

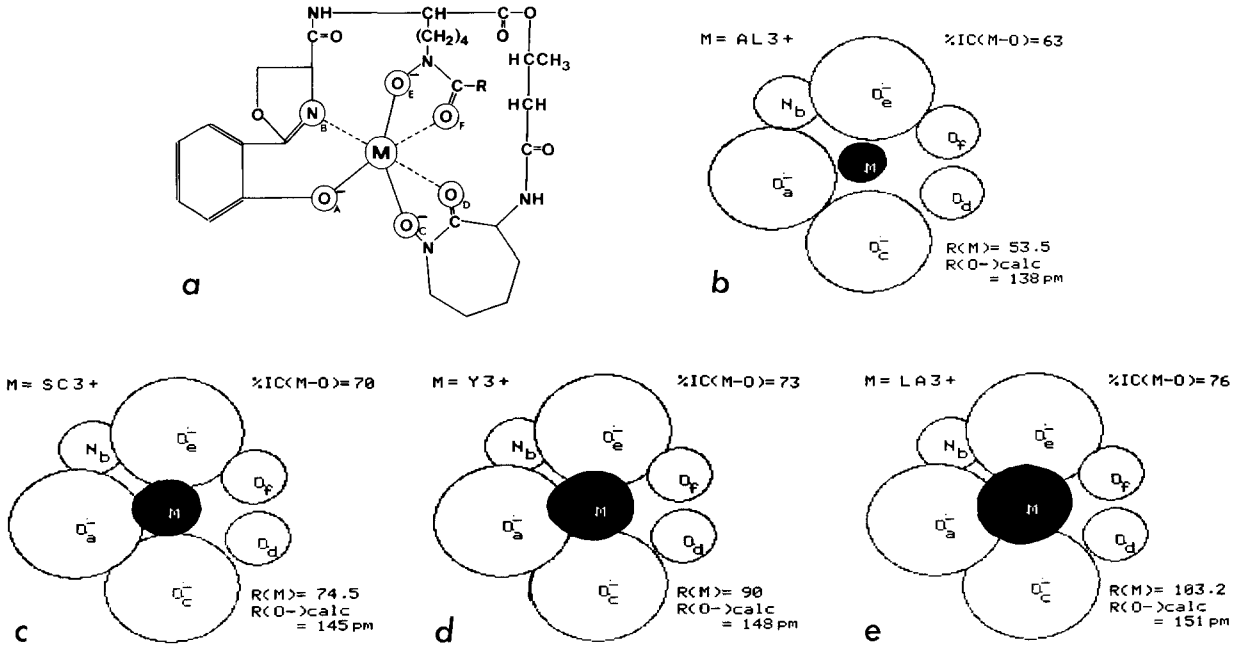

Fig. 4. (**a**) Site symmetry in mycobactin S. M = Fe(III) or other octahedrally coordinated cation. R = CH=CH—$(CH_2)_n$—CH_3 with $n = 14$ preponderant. (**b–e**) Computer graphics of calculated fits in M(III)-MY complexes. IC = calc. ionic character (%) of M-O electrovalent bonds. Radius overlaps between the metal ion and O^- atoms (O_a, O_c, O_e) represent steric hindrance for molecular enclosure of the cation

biosorption and migrational processes involving microorganisms under natural conditions. Model experiments dealing with these aspects are presently under consideration.

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