Metals and microorganisms: A problem of definition

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Received 18 June 1992
Accepted 29 June 1992

Key words: Metals; Microorganisms

1. METALS AND MICROORGANISMS

Metals are directly and/or indirectly involved in all aspects of microbial growth, metabolism and differentiation. Many metals are essential, e.g. K, Na, Mg, Ca, Mn, Fe, Co, Ni, Cu, Zn, Mo, whereas others have no known essential biological function(s), e.g. Al, Ag, Cd, Sn, Au, Sr, Hg, Ti, Pb. All these elements can interact with microbial cells and be accumulated as a result of physico-chemical mechanisms and transport systems of varying specificity, independent of, or directly and indirectly dependent on, metabolism [1–4]. Some of these processes are of global importance being components of major biogeochemical cycles, including microfossil formation, iron and manganese deposition, silver and uranium mineralisation, as well as resulting in transfer to other organisms via food chains [2,5–8]. Some interactions are of biotechnological importance being relevant to metal removal and/or recovery from mineral deposits [9,10] and industrial effluents for industrial use or environmental bioremediation [1,11–15]. It follows therefore that their roles and functions within and exterior to microbial cells, and their provision in culture media [16], should be considered in all aspects of microbiology, including ecology, physiology, biochemistry, differentiation, molecular biology, genetics and biotechnology. Many works are now available on the multiplicity of microbial interactions with metals and their compounds [1,2,10–22] and referral to these is strongly recommended. The purpose of this article is to emphasise the importance of metals in microbiology and highlight some of the areas of potential confusion that exist, often resulting from terminology but also from inadequate cross-fertilization between complementary disciplines; the interface between microbiology and chemistry is especially important [5,18,23–26].

2. THE PROBLEM OF DEFINITION

Metal terminology is variable and often confusing, particularly in microbiological contexts. In
the Periodic Table, metals and metalloids can be considered to comprise all of the elements except the noble gases, and H, B, C, N, O, F, P, S, Cl, Br, I and At. Metalloids are Si, Ge, As, Se, Sb and Te. Groups Ia and IIA, the ‘s-block’ metals, form monovalent cations (alkali metals) and divalent cations (alkaline earth metals) respectively. Groups IIIb through VIIb contain the ‘p-block’ metals [26]. The transition elements are those whose ions have incompletely filled d orbitals [23]. The three transition series of the Periodic Table occupy rows 4, 5, and 6 and elements of the first and subsequent transition series can exhibit a wide variety of oxidation states, except for Group IIIb metals which have the III oxidation state only [26]. The lanthanides and actinides constitute a so-called inner transition series [26].

Table 1 shows metal ion classification as ‘Type-A’ and ‘Type-B’ metal cations, which is governed by the number of electrons in the outer shell, and transition metal cations [26] as well as the hard and soft acid classification [27]. Many biologically-related systems of metal classification are derived from such schemes which consider ligand preferences, a property which can underlie biological activity [16,18,25,26,28]. Oxygen-donor ligands are hard while sulphur donors are soft. In general, hard ligands bind to hard-metal ions and

<table>
<thead>
<tr>
<th>Type-A metal cations</th>
<th>Transition metal cations</th>
<th>Type-B metal cations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron configuration of inert gas</td>
<td>One to nine outer shell electrons</td>
<td>Electron number corresponds to Ni°, Pd° and Pt° (10 or 12 outer shell electrons)</td>
</tr>
<tr>
<td>Low polarizability</td>
<td>Not spherically symmetric</td>
<td>Low electronegativity</td>
</tr>
<tr>
<td>‘Hard spheres’</td>
<td>V²⁺, Cr²⁺, Mn²⁺, Fe²⁺, Co²⁺</td>
<td>High polarizability</td>
</tr>
<tr>
<td>Mg²⁺, Ca²⁺, Sr²⁺, Al³⁺, Sc³⁺</td>
<td>Ni²⁺, Cu²⁺, Ti³⁺, V³⁺, Cr³⁺</td>
<td>‘Soft spheres’</td>
</tr>
<tr>
<td>La³⁺, Si⁴⁺, Ti⁴⁺, Zr⁴⁺, Th⁴⁺,</td>
<td>Mn³⁺, Fe³⁺, Co³⁺</td>
<td>Cu⁺, Ag⁺, Au⁺, Ti⁺, Ga⁺,</td>
</tr>
<tr>
<td>Hard acids</td>
<td>Borderline</td>
<td>Zn²⁺, Cd²⁺, Hg²⁺, Pb²⁺, Sn²⁺,</td>
</tr>
<tr>
<td>All Type-A metal cations plus Cr³⁺, Mn³⁺, Fe³⁺, Co³⁺, UO²⁺, VO²⁺</td>
<td>All divalent transition metal cations plus Zn²⁺, Pb²⁺, Bi³⁺</td>
<td>Ti³⁺, Au³⁺, In³⁺, Bi³⁺</td>
</tr>
<tr>
<td>In addition species such as BF₃, BCl₃, SO₃, RSO₂⁻, RPO₂⁻, CO₂⁻, RCO⁺, R₂C⁺</td>
<td>SO₂⁻, NO₃⁻, B(CH₃)₃</td>
<td>All Type-B metal cations minus</td>
</tr>
<tr>
<td>Preference for ligand atom:</td>
<td></td>
<td>Zn²⁺, Pb²⁺, Bi³⁺</td>
</tr>
<tr>
<td>N &gt; P</td>
<td></td>
<td>All metal atoms, bulk metals</td>
</tr>
<tr>
<td>O &gt; S</td>
<td></td>
<td>I₂, Br₂, ICN, 1⁺, Br⁺</td>
</tr>
<tr>
<td>F &gt; Cl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stability sequence:</td>
<td>Cations:</td>
<td>Ligands:</td>
</tr>
<tr>
<td>Stability α (charge/radius)</td>
<td>Irving-Williams series:</td>
<td>S &gt; I &gt; Br &gt; Cl = N &gt; O &gt; F</td>
</tr>
<tr>
<td>Mn²⁺ &lt; Fe²⁺ &lt; Co²⁺ &lt; Ni²⁺</td>
<td>Mn²⁺ &lt; Fe²⁺ &lt; Co²⁺</td>
<td></td>
</tr>
<tr>
<td>&lt; Cu²⁺ &gt; Zn²⁺</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
soft ligands bind to soft-metal ions. However, in a mixture of hard- and soft-metal ions, soft-metal ions may outcompete hard-metal ions for binding ligands [16]. Hard cations, e.g. Na⁺, K⁺, Mg²⁺, are generally small, exhibit high electronegativity and low polarizability and tend to participate in electrostatic bonding to ligands. Soft cations, e.g. Ag⁺, Cd²⁺, Hg²⁺, have low electronegativity and high polarizability and tend towards covalent bonding to ligands. Many essential metal ions, e.g. Fe³⁺, Ca²⁺, Mg²⁺, K⁺, are hard, whereas inessential toxic metals, e.g. Ag⁺, Cd²⁺, Hg²⁺, Sn²⁺, are soft. However, biologically inessential hard metals include Rb⁺, Sr²⁺ and Al³⁺, while a borderline category includes Cu²⁺, Ni²⁺, Zn²⁺, Co²⁺ [16]. The need for ‘borderline’ categories, which reflects gradually varying properties of metal ions rather than absolute distinctions [26], underlines the difficulty (or futility?) of rigorous definition. Other definitions are based on toxicity and environmental impact, although this can be highly variable and dependent on, e.g. the organism, element, speciation, concentration, physico-chemical parameters and anthropogenic activities [5,29,30]. Definitions may be particularly variable in the context of ‘heavy metals’. These are often defined as a group of approximately 65 metallic elements, of density greater than 5, with the general ability to exert toxic effects on microbial and other life forms [25,31,32]. However this imprecise definition includes elements with diverse physical, chemical and biological properties [25]. A further complication is that ‘heavy elements’ are defined as Pb, As, Cd, Hg, Sb, Se, Tl, In, Bi and Te [6]. This is again a diverse group, and, in fact As, Sb, Bi, Se and Te have elemental structures more typical of non-metals [6,26].

Organometallic compounds may be defined as compounds containing at least one metal-carbon bond [24]. Where such compounds contain metalloids, e.g. Ge, As, Se, Te, the term organometalloid may be used [24]. Organometal(loid)s arise in the environment from anthropogenic sources but also as a result of natural processes. Methylated derivatives of several elements occur naturally as a result of chemical and biological methylation, microorganisms playing highly significant roles in the latter process [24,33,34]. Such processes, as well as organometal(loid) degradation, are components of global biogeochemical cycles for elements including Hg, As, Sn, Pb, Se, Te, Ge and Sb [35,36].

3. ESSENTIALITY

Essential metal ions have many functions in microbial cells, not all of which are fully characterized. Such functions may reflect their fundamental chemical properties, e.g. charge, size, redox properties and rates of ligand exchange [18]. Considerable attention has been focussed on metalloenzymes and it has been estimated that up to one-third of all known enzymes contain a metal ion as a functional participant [37]. This is likely to be an underestimate since most enzyme studies are carried out without consideration of metal requirements and in reaction buffer mixtures already containing, e.g. excess K⁺, Mg²⁺, Zn²⁺. Other important roles for metal ions include the formation of charge and concentration gradients across membranes which may be used in transport processes, intracellular compartmentation, osmotic responses and sensing (see, for example refs. 18, 22, 38, 39). Intracellular Ca²⁺ has an important and fundamental role as a second messenger in microbial eukaryotes [40]. Other major functions of metals include the stabilization of cellular structures, including cell walls, organelles and membranes, and biomolecules such as enzymes, proteins and nucleic acids [18]. Several also function as redox catalysts in cytochromes, iron-sulphur proteins, blue-copper electron-transfer proteins as well as in, e.g. oxidases, oxygenases and hydrogenases [18]. Deprivation of an essential metal ion will, by definition, ultimately result in death. However, broader definitions of essentiality may only refer to impairment of growth, reproduction or other function(s) in the absence of that essential metal and the fact that beneficial effects cannot be completely replaced by any other element [37]. This perhaps undermines the validity of using non-biological metal probes to investigate cellular functions of essential metals, e.g. Rb⁺, Cs⁺, or other monovalent cations as a probe for K⁺. However, under
some conditions some microorganisms may actually show greater affinities for certain non-biological inessential metal species, e.g. the replacement of cell K⁺ by Cs⁺ in cyanobacteria [41] and yeasts [38,39], where toxic symptoms may not be apparent in the short term.

4. TOXICITY

Virtually all metals, whether essential or inessential, can exhibit toxicity above certain threshold concentrations which for highly toxic metal species may be extremely low, e.g. Ag⁺ (Fig. 1). Major mechanisms of toxicity, particularly of ‘heavy’ metals, are generally a result of strong coordinating abilities [28]. Because of the multiplicity and variety of ligands found in microbial cells, almost every aspect of cell growth, metabolism and differentiation may be affected to varying degrees. Toxic effects include the blocking of functional groups of important molecules, e.g. enzymes, polynucleotides, transport systems for essential nutrients and ions, displacement and/or substitution of essential ions from cellular sites, denaturation and inactivation of enzymes, and disruption of cellular- and organelar-membrane integrity [28]. In an environmental context, virtually every index of microbial activity may be affected under conditions of metal pollution, e.g. primary productivity, nitrogen fixation, biogeochemical cycling of C, N, S, P and other elements (including metals), organic matter decomposition, enzyme synthesis and activity, etc. in aquatic and terrestrial ecosystems [30].

The toxicity of a given metal will depend on speciation and chemical properties as well as environmental factors, both in laboratory and field studies [16,31,42]. This may necessitate expression of a metal detoxification mechanism to ensure survival although, in some instances, it is debatable whether biologically-effective concentrations of potentially toxic metal species may occur. Where such factors as pH, $E_h$, other anions and cations, particulate and soluble organic matter (some microbially produced), clay minerals, salinity, etc. decrease biological availability by binding, complexation, precipitation or complexation, toxicity may be reduced or even eliminated [30,31]. It follows that analysis of microbial population responses to toxic metals in natural environments is highly complicated and subject to misinterpretation. In contrast to so-called ‘extreme’ environments (defined using human rather than microbial criteria) which may be characterised by a restricted microflora, a range of microorganisms from all the major groups may be found in metal-contaminated habitats; the ability to survive in the presence of apparently toxic concentrations is a common attribute [30]. Undoubtedly, such observations are influenced to a large extent by the physico-chemical characteristics of that environment.

In general terms, it is often stated that toxic metals affect microorganisms in natural environments by reducing numbers and diversity and enriching for and/or selecting a ‘resistant’ population. However, numerical estimates may provide little useful information in the metals context; there are well-known theoretical and practi-
cal difficulties in obtaining meaningful assessments of microbial numbers, diversity and activity in natural habitats in any case. It should also be noted that a metal-polluted environment may be detrimental to microorganisms for additional and/or alternative reasons to the presence of elevated total metal concentrations, e.g. extremes of pH, high salinity and nutrient limitation [29–32,42]. Some reasonable examples of metal-induced changes in natural microbial populations are derived from studies of the phylloplane (leaf surface) [43,44] although this may itself be considered to be an ‘extreme’ environment in some ways, prone to UV radiation and extremes of temperature and desiccation. Reductions in species diversity result in exposure to vehicular or industrially derived metal deposition and the melanin-pigmented polymorphic fungus *Aureobasidium pullulans* showed a positive correlation with lead, and other metals, whereas the ballistospore-producing yeast *Sporobolomyces roseus* was highly sensitive and declined in response to pollution; the use of such organisms as bioindicators of aerial metal pollution has been speculated upon [43,44].

In conclusion, although it is generally accepted that exposure to toxic metals does effect quantitative and qualitative changes in microbial populations in the environment, it may be difficult if not impossible to separate metal effects from those of environmental components, environmental influence on metal speciation and toxicity, and the nature of the microbial resistance/tolerance mechanisms involved.

Many of the above considerations apply equally in the context of laboratory studies. The specification and biological availability of a given metal should be considered, not only in metal-related investigations but also in the composition of microbial growth media to ensure adequate provision of essential metals [5,16,31]. The factors that determine metal bioavailability include those properties described previously with pH, organic and inorganic complexation, binding and precipitation being particularly important. Microbial growth may lead to changes in metal speciation as a result of pH alteration and the synthesis of extracellular products and metabolites for example, which may in turn affect the nature of both metabolism-dependent and -independent interactions of the metal species with the microbe, e.g. biosorption, intracellular accumulation [1,5]. In many cases however, the problem of metal speciation in laboratory media (and in the environment) cannot be adequately solved [16]. Measurement of individual metal species may be technically difficult in comparison to total metal concentrations, while, as mentioned, microbial growth and activity may also alter speciation. While the bioavailability of metals in laboratory media should be an important general consideration in any metal-related investigation, it has been concluded that it is unrealistic to carry out major speciation studies routinely [16].

5. SURVIVAL

It is commonly assumed that metal exposure leads to the establishment of a ‘resistant’ or ‘tolerant’ microbial population. Microbial survival largely depends on intrinsic biochemical and structural properties, physiological and/or genetic adaptation including morphological changes, and environmental modification of metal speciation, availability and toxicity, the relative importance of each being difficult to determine [14,30,31]. Terms such as ‘resistance’ and ‘tolerance’ are arbitrary, often used interchangeably without clear distinction in the literature, and may be based, often subjectively, on the ability to grow on a certain metal concentration in laboratory media [19,45]. Such determinations do not usually reveal whether adaptation has taken place in the environment and indeed may have little or no environmental relevance. It is perhaps more appropriate to define ‘resistance’ as the ability of a microorganism to survive toxic effects of metal exposure by means of a detoxification mechanism produced in direct response to the metal species concerned, e.g. bacterial reduction of Hg$^{2+}$ to Hg$^0$ [22,46–48] and the synthesis of metallothioneins and/or γ-glutamyl peptides (‘phytochelatins’) by yeasts [17,20,49,50]. ‘Metal tolerance’ may be defined therefore as the ability of a microorganism to survive metal toxicity by means
of intrinsic properties and/or environmental modification of toxicity [45]. Intrinsic biochemical and structural properties that determine survival may include possession of impermeable, pigmented cell walls, extracellular polysaccharide, and metabolite excretion, particularly where a given metal species is converted into an innocuous form by, e.g. binding or precipitation [30]. Such mechanisms have been termed ‘gratuitous mechanisms of resistance’ for obvious reasons [31]. However, distinctions are still difficult in many cases because of the frequent involvement of several direct and indirect mechanisms, both physico-chemical and biological, to microbial survival in the field or laboratory [45]. Microbial mechanisms implicated in survival in the presence of potentially toxic concentrations of metal species (as distinct from the environmental influences mentioned previously) include extracellular precipitation, complexation and crystallization, transformations including oxidation, reduction, methylation, and dealkylation, biosorption to cell walls, extracellular polysaccharide etc., impermeability, decreased transport, efflux, intracellular compartmentation and/or sequestration [1,2,17,19,21,30,31,33,38,47–50]. A given organism may directly and/or indirectly rely on several survival strategies though this is not always appreciated in the literature. For example, metallothionein synthesis is a mechanism of copper resistance in yeast [20,50] yet Cu²⁺ binding or precipitation around the cell wall and transport across the cell membrane must be components of the total cellular response [51].

6. CONCLUSIONS

The range of potential metal-microbe interactions is extensive [52] and it is clear that the relationship between a given organism, the metal species, and the environment (laboratory or ‘field’) are still incompletely understood. Several areas are now receiving intensive attention at the molecular and genetic level [46,53–55] while exploitation of certain interactions in environmental biotechnology is now widely accepted [9,11,12,15]. It is hoped that this article will draw attention to the complexity of metal interactions with microorganisms, their importance, and some of the factors, both microbiological and chemical, which should be taken into account in microbiological investigations.

REFERENCES