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Mechanism of Adsorption of Hard and Soft Metal Ions to *Saccharomyces cerevisiae* and Influence of Hard and Soft Anions

SIMON V. AVERY†* AND JOHN M. TOBIN

School of Biological Sciences, Dublin City University, Dublin 9, Ireland

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The applicability of the hard-and-soft principle of acids and bases in predicting metal adsorption characteristics in a biological context was investigated for metabolism-independent uptake of the metal ions Sr^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, and Tl^{+} by *Saccharomyces cerevisiae*. Metal adsorption increased with external metal concentration (5 to 50 μM), although some saturation of uptake of the harder ions examined, Sr^{2+}, Mn^{2+}, and Zn^{2+}, was evident at the higher metal concentrations. Cation displacement experiments indicated that, with the exception of Tl^{+}, relative covalent bonding (H^{+} displacement) of the metals was greater at low metal concentrations, while weaker electrostatic interactions (Mg^{2+} plus Ca^{2+} displacement) became increasingly important at higher concentrations. These results were correlated with curved Scatchard and reciprocal Langmuir plots of metal uptake data. Saturation of covalent binding sites was most marked for the hard metals, and consequently, although no relationship between metal hardness and ionic/covalent bonding ratios was evident at 10 μM metal, at 50 μM the ratio was generally higher for harder metals. Increasing inhibition of metal uptake at increasing external anion concentrations was partially attributed to the formation of metal-anion complexes. Inhibitory effects of the hard anion SO_4^{2-} were most marked for uptake of the hard metals Sr^{2+} and Mn^{2+}, whereas greater relative effects on adsorption of the softer cations Cu^{2+} and Cd^{2+} were correlated with complexation by the soft anion $S_2O_3^{2-}$. Inhibition of uptake of the borderline metal Zn^{2+} by SO_4^{2-} and that by $S_2O_3^{2-}$ were approximately equal. The results showed good agreement with the hard-and-soft principle with respect to both the nature of bonding and preferred ligand binding of the metals examined, and the implications for biological systems are discussed.

The interaction of metals with biological cell surfaces is a prerequisite for intracellular accumulation, where metal ions may fulfill essential functions in cellular metabolism or, in certain cases, exert toxic effects towards cells (12). Thus, a detailed understanding of the mechanisms involved in metal adsorption can facilitate assessment of the impact of metals on biological systems. Considerable interest in such interactions has focused on microbial metal uptake (7). This is primarily because of the suitability of microorganisms for studying metal nutrition and toxicity at the cellular level and the important roles of these organisms in biogeochemical cycling and in biological food chains (12), although recent attention has also concentrated on their biotechnological potential in metal removal processes (14).

Despite the quite extensive literature available on metal-microbe interactions, few authors have attempted to relate differing mechanisms and/or relative levels of metal uptake or toxicity to the chemical characteristics of the metals under investigation. Some recent reviews and reports of metal-microbe interactions have considered the principle of hard and soft acids and bases as a means of predicting metal adsorption behavior (8, 9, 13, 19); however, to date no studies have examined the validity of this principle in a biological context. Pearson (18) originally proposed that the differential behavior of certain groups of bases could be attributed to their differing polarizability, and a survey of equilibrium values, based on thermodynamic and not kinetic considerations, yielded a further classification for metal cations (acids) according to whether they preferentially formed complexes with nonpolarizable (hard) or polarizable (soft) ligands. A refinement of this classification for biological systems was described by Nieboer and Richardson (17), who considered the electronegativity, charge, and ionic radius of metals in determining their relative class A (hard) or class B (soft) behavior. The principle predicts that hard metals, which are generally nontoxic and often essential macronutrients for microbial growth, bind preferentially to oxygen-containing (hard) ligands, whereas soft metals, which often display greater toxicity, form more stable bonds with nitrogen- or sulfur-containing (soft) ligands. Thus, no sequence of relative metal affinities for any particular ligand will be universal for all ligands.

In fungi, the cell wall is a multilaminate, microfibrillar structure consisting of up to 90% polysaccharide, with mannan–β-glucan being the main structural polymer in *Saccharomyces cerevisiae* (19). Other components include proteins, lipids, and pigments, and this diversity is reflected by the presence of a range of distinct potential metal complexation sites, e.g., carboxylate, phosphate, sulfhydryl, and amine groups (19, 20). As a consequence, the Irving-Williams series [Mn(II) < Fe(II) < Co(II) < Ni(II) < Cu(II) > Zn(II)], derived from the stability constants of transition metals with N donor ligands and often referred to in relation to metal adsorption to microorganisms, may not strictly apply to surfaces rich in oxygen-containing ligands, where more stable bonds with hard ions, e.g., Mn^{2+}, would be expected (13). The hard-and-soft principle also predicts that bonds formed between hard metals and hard ligands are predominantly ionic, whereas those of soft metal-ligand complexes are more covalent in character (18). Such inter-

* Corresponding author.
† Present address: School of Pure and Applied Biology, University of Wales, P.O. Box 915, Cardiff CF1 3TL, United Kingdom.

actions may be readily observable in simple inorganic chemical systems; however, Avery and Tobin (2) recently demonstrated that although bonding of the hard ion Sr^{2+} to denatured (dried and ground) yeast biomass could also be accounted for by electrostatic interactions alone, the structural complexity presented by the intact cell wall of live *S. cerevisiae* yielded complexes between Sr^{2+} and the cell wall functional groups of increased covalent character.

The purpose of the present investigation was to assess the extent to which the interactions of a range of metal ions with live, nonmetabolizing *S. cerevisiae* could be accounted for by the hard-and-soft principle. Cation displacement experiments characterized the degree of ionic versus covalent bonding of metals at the cell wall, while the influence of exogenously supplied representative hard and soft anions on uptake of the various metals determined preferred metal-ligand interactions.

MATERIALS AND METHODS

Organism, media, and growth conditions. *S. cerevisiae* X2180-1B was routinely maintained on solid medium comprising (in grams per liter) the following: malt extract, 3.0; yeast extract, 3.0; bacteriological peptone (Oxoid), 5.0; D-glucose, 10.0; and agar (Lab M no. 1), 16.0. For experimental purposes, cultures were grown in a liquid medium comprising (in grams per liter) the following: KH_2PO_4, 2.72; K_2HPO_4, 3.98; $(NH_4)_2SO_4$, 2.0; $MgSO_4 \cdot 7H_2O$, 0.12; $FeSO_4 \cdot 7H_2O$, 0.0022; $ZnSO_4 \cdot 7H_2O$, 0.004; $MnSO_4 \cdot 4H_2O$, 0.004; $CuSO_4 \cdot 5H_2O$, 0.004; D-glucose, 20.0; and yeast extract, 1.0. Cultures were grown at 25°C on an orbital shaker (150 rpm).

Metal uptake experiments. Cells from the late-exponential growth phase (72 h) were harvested by centrifugation (1,200 × *g*, 10 min) at room temperature and washed three times with distilled, deionized water in the absence of metabolizable substrates. The final cell pellet was resuspended in a few milliliters of distilled, deionized water. Cells were added to 100 ml of distilled, deionized water to a final concentration of 0.1 g (dry weight) 100 ml^{-1} in 250-ml Erlenmeyer flasks. The pH of the suspensions was adjusted to 5.5 by using 0.1 M NaOH or HNO_3. pH 5.5 was selected as a pH value high enough to be representative of environmentally relevant conditions but low enough to preclude extensive hydrolysis of the metals examined (3). After preincubation for 30 min (also in the absence of metabolizable substrates) at 25°C with rotary shaking and with the pH maintained at pH 5.5, either Sr^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, or Tl^+ (nitrate salts as NO_3^- are noncomplexing and do not influence the metabolism of *S. cerevisiae*) was added to the suspensions, to the desired final concentration, from appropriate stock solutions. The procedure for harvesting cells and the conditions of incubation described here yielded nonmetabolizing *S. cerevisiae* which did not accumulate metals by active processes (2). When desired, $NaNO_3$, Na_2SO_4, or $Na_2S_2O_3$ was added 5 min prior to the addition of metal salts. No precipitation of metals occurred over the range of ligand concentrations used. After 5 min of incubation in the presence of the metal, cells were separated by centrifugation (1,200 × *g*, 5 min), and the supernatant was removed for metal (including Mg^{2+} and Ca^{2+}) analysis. Uptake values were determined from the difference in final metal concentrations between control flasks without cells and test flasks. Mg^{2+} and Ca^{2+} displacement was determined from the difference in final external Mg^{2+} or Ca^{2+} concentrations between cell suspensions incubated in the presence and absence of metal.

For H^+ displacement experiments, the pHs of the suspensions were measured continually, by using a W.T.W.E56 precision glass pH electrode, and values at the time of addition of metal and later at equilibrium (30 s to 1 min after metal addition) were used to calculate H^+ displacement.

All glassware was washed with 1 M HNO_3 and rinsed thoroughly with distilled, deionized water prior to use. Dry weights of cells were determined by using tared foil cups dried to a constant weight at 55°C.

Metal analysis. Metals were analyzed by using a Perkin-Elmer 3100 atomic absorption spectrophotometer, fitted with a 10-cm single-slot burner head, and by using an air-acetylene flame. Metal concentrations were determined by reference to appropriate standard metal solutions. $NaNO_3$, Na_2SO_4, and $Na_2S_2O_3$ were found to interfere with absorbance values for Sr^{2+} only, and the method of standard additions of known amounts of Sr^{2+} was used to measure Sr^{2+} in ligand-containing solutions.

RESULTS

Metal adsorption to *S. cerevisiae* and displacement of Mg^{2+}, Ca^{2+}, and H^+. Sr^{2+} and Mn^{2+} (hard), Zn^{2+} and Cu^{2+} (borderline), and Cd^{2+} and Tl^+ (soft) were selected for experimental use as suitable representative metal ions of each of the groups of Pearson's classification (18). For all of these metals, uptake by live *S. cerevisiae* increased with increasing external metal concentration (5 to 50 μM), and maximum uptake levels after 5 min of incubation were approximately 14.9, 14.3, 14.2, 6.4, 26.7, and 23.4 μmol of metal g (dry weight)$^{-1}$ in the presence of 50 μM Sr^{2+}, Mn^{2+}, Zn^{2+}, Tl^+, Cu^{2+}, and Cd^{2+}, respectively (Fig. 1a to f). Uptake of each of the metals examined, under the conditions described, was attributed solely to metabolism-independent adsorption at the cell surface, as no change in levels of uptake occurred between 5 min and 1.5 h of incubation, while the subsequent addition of glucose resulted in a rapid and marked stimulation of metal accumulation which continued for >1 h (data not shown). A curvilinear relationship between adsorption values and the initial metal concentrations was evident for the hard ions Sr^{2+} and Mn^{2+} and the borderline ion Zn^{2+}, suggesting that some saturation of cell wall-binding sites occurred at the higher concentrations of these metals (Fig. 1a to c). In contrast, uptake of the softer metal ions Cu^{2+}, Cd^{2+}, and Tl^+ increased almost linearly over the same concentration range (Fig. 1d to f). When the metal uptake data were transformed and fitted to Scatchard and reciprocal Langmuir plots, the resultant graphs were nonlinear (Fig. 2 shows plots for Sr^{2+}), although in the case of Tl^+, the linearity of plots (not shown) was unclear because of the relatively large variability (i.e., standard error of the mean as a percentage of total uptake) in uptake values, resulting from low levels of Tl^+ uptake.

Displacement of the cations Mg^{2+}, Ca^{2+}, and H^+ by metal adsorption was also examined over the same range of metal concentrations (5 to 50 μM); no release of Zn^{2+}, K^+, or Na^+ was detected following metal uptake. Increases in Mg^{2+} desorption were always proportional to those for Ca^{2+}, and data for these ions were collated. As observed for metal uptake, cation displacement increased at increasing metal concentrations; however, the pattern of increase was markedly different for Mg^{2+} plus Ca^{2+} and H^+ (Fig. 1g to l). At 50 μM Sr^{2+}, Mn^{2+}, and Zn^{2+}, displacement of H^+, indicating covalent bonding of the metal (2, 4–6), was not greater than double that observed at a 5 μM concentration of the same metal. Indeed, little increase in H^+ desorption occurred at

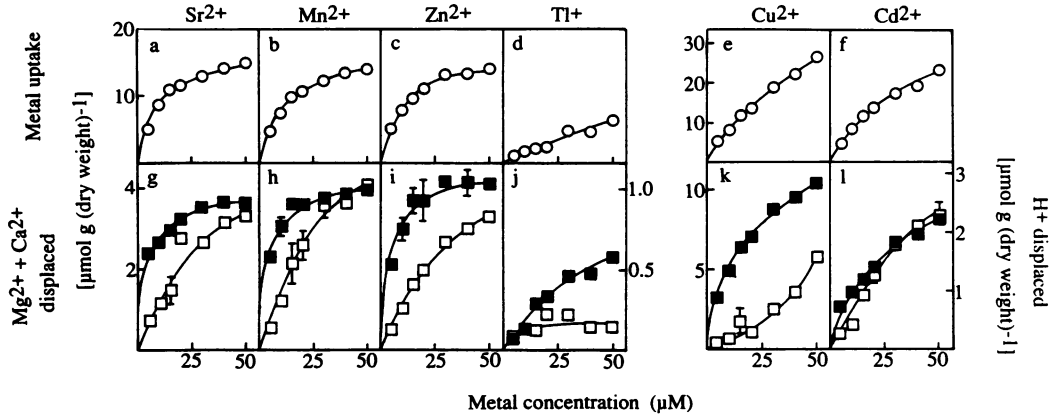

FIG. 1. Metal adsorption to *S. cerevisiae* and displacement of Mg^{2+}, Ca^{2+}, and H^+. Cells were incubated in distilled, deionized water (pH 5.5) in the presence of various concentrations of Sr^{2+} (a and g), Mn^{2+} (b and h), Zn^{2+} (c and i), Tl^+ (d and j), Cu^{2+} (e and k), and Cd^{2+} (f and l). (a to f) Metal uptake (○) after 5 min of incubation. (g to l) Mg^{2+} plus Ca^{2+} (□) and H^+ (■) displaced by the metal. Data are mean values from three replicate determinations, ± standard error of the mean when these values exceed the dimensions of the symbols.

>15 μM Sr^{2+}, Mn^{2+}, or Zn^{2+} (Fig. 1g to i). Some increases in H^+ release were apparent at up to 50 μM Tl^+, Cu^{2+}, and Cd^{2+} (Fig. 1j to l), and this was correlated with continued increases in the adsorption of these ions (Fig. 1e and f). In comparison with H^+, a more linear rise in Mg^{2+} plus Ca^{2+} displacement, corresponding to ionic bonding of metals (2, 4–6), resulted at increasing metal concentrations, although again this was not the case for Tl^+, with which no significant further increase in desorption of the divalent cations occurred at >10 μM metal (Fig. 1j).

As a consequence of the differential patterns of Mg^{2+} plus Ca^{2+} and H^+ displacement, ionic/covalent bonding ratios (calculated from relative Mg^{2+} plus Ca^{2+} and H^+ release) seemed to be markedly dependent on the metal concentration, and, with the exception of Tl^+, the ratio was increased at 50 μM in comparison with 10 μM metal (Table 1). The two- to threefold-greater values of ionic/covalent bonding ratios for Sr^{2+}, Mn^{2+}, and Zn^{2+} at the higher metal concentration were correlated with the saturation of covalent binding sites at lower concentrations of these metals (Fig. 1g to i). However, the largest proportional rise in the ionic/covalent bonding ratio resulted from increases in Cu^{2+} adsorption (Table 1), although this was more a consequence of the large displacement of Mg^{2+} and Ca^{2+} at elevated Cu^{2+} concentrations than of saturation of covalent binding sites (Fig. 1k). A decline in the ionic/covalent bonding ratio occurred at the higher concentration of Tl^+. Thus, although no relationship between hardness and degree of electrostatic bonding behavior was observable at 10 μM metal, at 50 μM, adsorption of metals of increasingly soft character to *S. cerevisiae*, with the exception of Cd^{2+}, was correlated with an overall decline in relative ionic bonding.

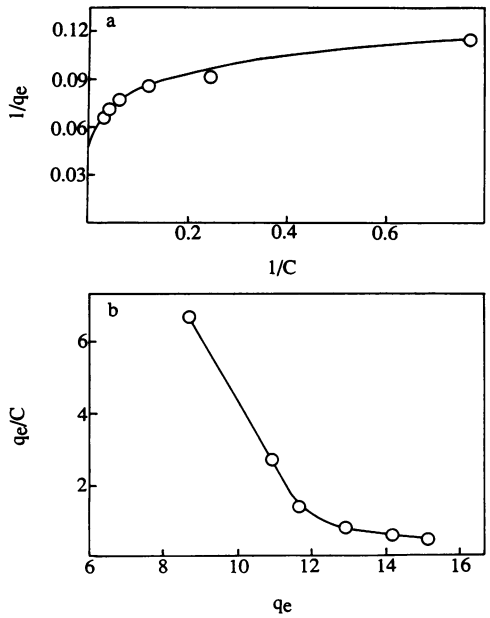

FIG. 2. Reciprocal Langmuir (a) and Scatchard (b) plots of Sr^{2+} adsorption by *S. cerevisiae*. The adsorption data (mean values) from Fig. 1a were transformed to give the plots. q_e, metal uptake by *S. cerevisiae* at equilibrium (micromoles gram [dry weight]$^{-1}$); C, final metal concentration in solution at equilibrium (millimolar).

Influence of exogenous hard and soft anions on metal adsorption to *S. cerevisiae*. The hard and soft anions (ligands), SO_4^{2-} and $S_2O_3^{2-}$, respectively, were supplied at various concentrations with the appropriate metal at 40 μM in order to assess their influence on metal adsorption to *S.*

TABLE 1. Ionic/covalent bonding ratios for bonding of metals to *S. cerevisiae*

Metal (concn, μM)	Mg^{2+} + Ca^{2+} displaced [μmol g (dry wt)$^{-1}$]	H^+ displaced [μmol g (dry wt)$^{-1}$]	Ionic/covalent bonding ratio[a]
Sr^{2+} (10)	1.10 ± 0.10	0.71 ± 0.02	1.55
Mn^{2+} (10)	1.21 ± 0.06	0.78 ± 0.06	1.55
Zn^{2+} (10)	1.04 ± 0.05	0.76 ± 0.07	1.37
Cu^{2+} (10)	0.66 ± 0.19	1.33 ± 0.08	0.49
Cd^{2+} (10)	1.55 ± 0.10	0.94 ± 0.08	1.65
Tl^+ (10)	0.45 ± 0.18	0.17 ± 0.03	2.65
Sr^{2+} (50)	3.31 ± 0.09	0.90 ± 0.03	3.68
Mn^{2+} (50)	4.13 ± 0.03	0.98 ± 0.05	4.21
Zn^{2+} (50)	3.33 ± 0.12	1.02 ± 0.01	3.27
Cu^{2+} (50)	5.65 ± 0.21	2.89 ± 0.06	1.96
Cd^{2+} (50)	8.28 ± 0.86	2.31 ± 0.05	3.58
Tl^+ (50)	0.57 ± 0.11	0.56 ± 0.04	1.02

[a] Calculated from relative Mg^{2+} + Ca^{2+}/H^+ displacement.

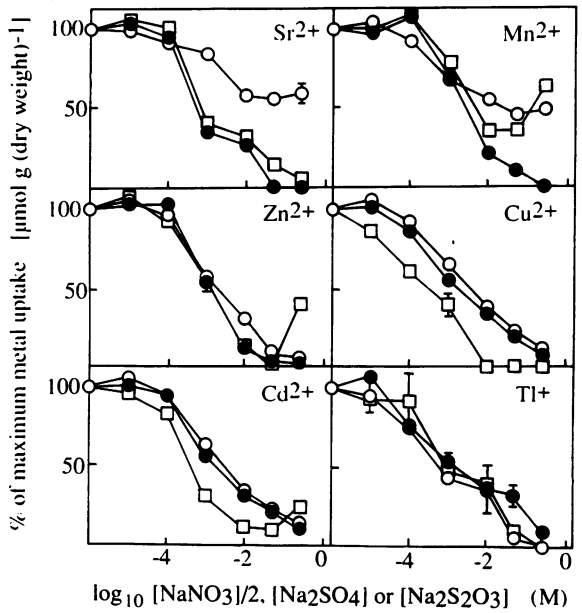

FIG. 3. Influence of exogenous hard and soft anions on metal adsorption to *S. cerevisiae*. Cells were harvested after 5 min of incubation in distilled, deionized water (pH 5.5) in the presence of various concentrations of $NaNO_3$ (○), Na_2SO_4 (●), or $Na_2S_2O_3$ (□) and 40 μM Sr^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, or Tl^+. Data are mean values from three replicate determinations, ± standard error of the mean when these values exceed the dimensions of the symbols.

cerevisiae. $NaNO_3$ was employed as a control so that the effects of cation competition by Na^+ on metal uptake could be distinguished from those of metal complexation. NO_3^- is considered a noncomplexing ligand because of its low stability constants with metal cations (23), and consequently, inhibitory effects on metal uptake may be attributed to the countercation (22). For all the metals examined, lower levels of uptake by *S. cerevisiae* were correlated with increasing external concentrations of anion (Fig. 3). Little inhibition of metal uptake was apparent at the lowest anion concentration examined (10 μM), although at 10 μM $Na_2S_2O_3$, Cu^{2+} uptake was only approximately 85% of that observed in the absence of added anions (Fig. 3). At higher anion concentrations, the availability of metals was apparently reduced, and in certain cases no metal uptake was evident. For example, Sr^{2+} uptake was not detectable at 0.05 or 0.25 M Na_2SO_4 (Fig. 3). At the highest anion concentration examined (0.25 M), the extent of ligand inhibition was reduced in certain cases. This was most evident for Mn^{2+}, Zn^{2+}, and Cd^{2+} uptake in the presence of $Na_2S_2O_3$ (Fig. 3). Na^+ ($NaNO_3$) also had a marked effect on metal uptake, although generally this was not greater than that observed in the presence of Na_2SO_4 or $Na_2S_2O_3$ when supplied at an equimolar final Na^+ concentration, and any additional effects of the latter were attributed to metal complexation.

The percent inhibition (calculated as 100 − percent maximum uptake) of metal uptake by $NaNO_3$ was subtracted from the percent inhibition observed in the presence of Na_2SO_4 or $Na_2S_2O_3$ at each anion concentration, thus giving values for inhibition that were attributable to anion complexation and excluded Na^+ effects. These values are presented in Table 2 as the ratio of percent inhibition by SO_4^{2-} to that by $S_2O_3^{2-}$ (calculated at 0.05 M anion). It is stressed that while the relative effects of SO_4^{2-} and $S_2O_3^{2-}$ were fairly consistent over the range of concentrations examined for each metal, those of Na^+ were more variable (Fig. 3), and the values presented consequently provide an indication of the observed trends in anion complexation rather than precise equilibrium data. Thus, complexation of the hard metals Sr^{2+} and Mn^{2+} by the hard anion SO_4^{2-} (as determined by inhibition of metal adsorption to *S. cerevisiae*) was approximately 1.4- and 4.7-fold higher, respectively, than that by the soft ligand $S_2O_3^{2-}$ (Table 2). Furthermore, softer metals displayed increased complexation by $S_2O_3^{2-}$, and the relative effects of this anion compared with SO_4^{2-} on metal uptake were most marked for Cu^{2+} and Cd^{2+}. In contrast to the other metals examined, neither Na_2SO_4 nor $Na_2S_2O_3$ consistently inhibited Tl^+ uptake more strongly than $NaNO_3$ (Table 2), although results for Tl^+ were more variable (Fig. 3).

TABLE 2. Relative influence of SO_4^{2-} and $S_2O_3^{2-}$ on metal uptake by *S. cerevisiae*

Metal	Ratio of % inhibition of uptake by SO_4^{2-} to that by $S_2O_3^{2-}$
Sr^{2+}	1.4
Mn^{2+}	4.7
Zn^{2+}	1.0
Cu^{2+}	0.2
Cd^{2+}	0.2
Tl^+	0

DISCUSSION

The results presented here indicate that the mechanism of adsorption of a range of metals to *S. cerevisiae* is related to their relative hardness or softness, although the clarity of such relationships may be obscured under certain experimental conditions because of their strong concentration dependence. The contribution of both ionic and covalent bonding to adsorption of all the metals examined here correlated with nonlinear reciprocal Langmuir isotherms of the metal adsorption data. Data which do not conform to the Langmuir model are generally taken to indicate multilayer adsorption; however, it has been suggested that nonconformity may also indicate adsorption behavior that cannot be accounted for by ionic or covalent bonding alone (6, 19). The importance of covalent bonding, albeit to a lesser extent than that of ionic bonding, to the overall adsorption of the hard metals Sr^{2+} and Mn^{2+} to *S. cerevisiae* is in agreement with previous studies using this organism (2) and with studies using marine algae (5), but not freshwater algae (4), and demonstrates that hardness does not necessarily preclude some degree of covalency. The large ionic radius and polarizability of Sr^{2+} and Mn^{2+} in comparison with the harder ion Ca^{2+}, which is considered to interact with biological ligands exclusively by electrostatic attraction (17), may account for some additional covalent bonding of the former ions. The relative degree of covalent interactions in bonding of the hard ions was particularly marked at lower metal concentrations, and consequently in that case no relationship between hardness or softness of the metals examined and ionic/covalent bonding ratios was apparent. However, this was not the case at higher concentrations, where, with the exception of Cd^{2+}, relative covalent bonding increased with metal softness. It should be stressed that although Cd^{2+} has been termed a soft metal in this paper in accordance with the classification of Pearson (18), the division between border-

line and soft metals is less clearly defined than that between borderline and hard metals (16). In fact, as a result of the lower product of its electronegativity and ionic radius, Cd^{2+} was regarded a borderline ion of slightly less class B (soft) character than Cu^{2+} by Nieboer and Richardson (17). This latter classification, intended to be more applicable to biological systems, correlates more closely with the present results.

The concentration dependence of the cation displacement behavior observed here suggests that a more accurate description of microbial metal adsorption in relation to the hard-and-soft principle is that the "capacity" of the cell surface for covalent bonding relative to ionic bonding increases with metal softness. Furthermore, examination of Fig. 1 suggests that the capacity of the cells for covalent bonding may be the principal limiting factor determining maximum levels of metal uptake. The comparatively small effect of concentration on relative ionic and covalent bonding of metals to algae previously described by Crist et al. (5) may have been because of the high (millimolar range) metal concentrations employed in their study. In the present work, relative covalent bonding was most marked at 5 to 10 μM metal.

The curved Scatchard plots for *S. cerevisiae* reported here are in agreement with numerous other studies of metal adsorption to microorganisms (10, 11, 25). The strong primary binding at low metal concentrations implied by these plots correlates with the pronounced concomitant covalent bonding observed here, while the weaker secondary interactions represented by the lower portion of the Scatchard plots may be accounted for by increased ionic bonding at these elevated metal concentrations. These results support observations from previous studies. From electron spin resonance studies of Cu^{2+} adsorption to the cell wall of the freshwater alga *Nitella flexilis*, Van Cutsem et al. (26) attributed increases in Cu^{2+} adsorption with Cu^{2+} concentration to the progressive increase in electrostatic interactions, relative to covalent interactions, with sites of progressively lower affinity for Cu^{2+}. Furthermore, Gardea-Torresdey et al. (9) reported that the activity of the carboxyl ligand in algal binding of copper(II) was more important at weak binding sites (high Cu concentrations) than at strong binding sites (low Cu concentrations).

The surface heterogeneity of the *S. cerevisiae* cell wall that can be inferred from the curved Scatchard and reciprocal Langmuir plots of metal uptake, in addition to both ionic and covalent bonding of the metal ions, correlates with the known chemically multiphasic nature of the wall (19) and indicates the presence of a range of distinct potential binding sites. It should be noted that at the pH value examined here (pH 5.5), most of the potential cell surface ligands will already be in an unprotonated state (5), and consequently it is likely that at higher pH values, which would be more applicable to bacterial systems, the selective binding of hard and soft ligands would not be altered significantly, although the hydrolysis of certain metals in solution may affect overall uptake values (3). Carboxylate and phosphate moieties have been proposed as the major functional groups responsible for metal adsorption in denatured fungal biomass (25). However, these are hard (oxygen-containing) ligands, and it is unlikely that they alone could account for the large degree of covalent bonding reported here. The degree of covalency in metal-cell wall interactions is known to be much greater in live than in dead yeast cells (2), and it is probable that here, covalent bonding to intact cells was a consequence of metal complexation with the additional soft amine and sulfhydryl ligands that are active on the cell surfaces of live microorganisms (20, 21). The assumed preferential complexation of hard metals by hard ligands and of soft metals by soft ligands, upon which this argument is based, was confirmed in this investigation by uptake inhibition studies using exogenously supplied anions. As in other investigations of anion effects on metal uptake by microorganisms (22, 24), increasing anion concentrations resulted in overall increased inhibitory effects on metal uptake by *S. cerevisiae*, presumably through the formation of less cationic, neutral or anionic metal complexes. The small increase in Mn^{2+}, Zn^{2+}, and Cd^{2+} uptake that resulted at the highest $S_2O_3^{2-}$ concentration examined (0.25 M) suggested that, in contrast to the effect of coordination with Cl^- on cyanobacterial Cd^{2+} uptake (22), the formation of anionic metal complexes with $S_2O_3^{2-}$ may cause some facilitated adsorption to, or permeation through, the cell surface of *S. cerevisiae*. Considerable inhibition of metal uptake was also attributed to cation competition by the ligand counterion, Na^+. Inhibition by Na^+ was most marked for Tl^+, which correlated with the known strong competitive interactions of Tl^+ with other monovalent cations for microbial uptake (1). When ligand effects were apparent, inhibition of metal uptake by the hard anion SO_4^{2-} was most readily observable in the presence of hard (oxygen-seeking) metals, and that by the soft anion $S_2O_3^{2-}$ was most observable in the presence of soft (sulfur- or nitrogen-seeking) metals. Such relationships were also mostly evident in similar studies using other microorganisms (15, 24). In these investigations and elsewhere the tendency has been to evaluate results in comparison with stability constant values; however, the detailed examination of stability constant data can be time consuming, while an understanding of the chemistry underlying metal-ligand interactions would be preferential in many cases for immediately recognizing the potential impact of different ligands on metal bioavailability.

The present results indicate that the complex characteristics of microbial metal uptake correlate well with, and can be accounted for by, considerations based on the hard-and-soft principle. In addition to relating the nature of bonding and strength of interaction of individual metal ions to the various types of functional group present in cell walls, broader application of the hard-and-soft principle is relevant to the availability to microorganisms of both potentially toxic and biologically essential ions in complex ligand-containing media. Indeed, such considerations also apply to the specificity of metal-complexing ligands that may be released by microorganisms under conditions of metal stress (7). Furthermore, it should be emphasized that although this study was based on the yeast cell, it is likely that the results reflect processes that also occur in biochemical interactions in higher organisms, including those both at the cellular level and, as evidenced by crystallographic studies on metal complexes involving nucleotides and nucleic acid constituents (17), at the subcellular or molecular level.

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