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Speciation dynamics and bioavailability of metals. Exploration of the case of two uptake routes*

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Abstract: The steady-state biouptake of metals from complex media is outlined for the case of two different uptake routes. The analysis comprises the limiting situations of inert and labile complexes, and distinguishes between bioinactive and bioactive (lipophilic) complexes. Depending on the dynamic features, fluxes of the different species may be either coupled or uncoupled. The role of the lipophilic ligands may be important in the overall uptake process. Some specific cases are discussed.

INTRODUCTION

The bioavailability of metals in complex media is not only concerned with the equilibrium speciation of the system, but also with the dynamics of transformation of bioinactive metal species into active ones. Therefore, it is not generally sufficient to analyse the speciation and bioavailability of metals in complex media on the basis of their conversion kinetics at the biointerface [1] but we should take into account their transport and formation kinetics in the medium as well [2,3]. For the two kinetically limiting situations of inert and fully labile systems, the bioavailabilities of metal complexes were analyzed under conditions where the actual biouptake is described by a Michaelis–Menten type of steady-state flux, and the supply of the bioactive free metal is governed by diffusion of free metal or coupled diffusion of the different labile metal species. The resulting steady-state fluxes were given in terms of two basic quantities, i.e., the relative bioaffinity parameter (a) and the ratio between the limiting uptake flux and the limiting transport flux (b). The analysis precisely reveals under what conditions labile complex species contribute to the biouptake process.

Studies such as those on the uptake of cobalt(II) by carp [4] and zinc(II) by bacteria [5] indicate that indeed the simple "biotic ligand" and "free ion activity" models [1,6,7] are far from generally applicable, and that even for non-complex systems a (single) Michaelis–Menten flux equation is not sufficient to describe uptake in a wide range of metal concentrations. It seems that different types of adsorption sites and/or routes of actual uptake are simultaneously operational, and this may involve bioactive complexes next to the free metal [8,9].

Thus, it appears mandatory to analyze more involved biouptake schemes, with more than one type of biosurface site S, for example S1(adsorption + internalization) + S2(adsorption only) or S1(adsorption + internalization free metal) + S2(internalization lipophilic complex). For the limiting cases of inert and labile behavior of the different complexes the starting equations and the global ensuing flux behavior will be discussed. Coupling or uncoupling of the mass transfer of metal species with different labilities will be considered, and approximate biouptake rates will be formulated.

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ONE-ROUTE BIOUPTAKE

Starting points for the theory that relates metal speciation dynamics to biouptake fluxes can be found in ref. 3. The central element is the dimensionless Best equation which expresses the normalized steady state flux Q ($=J/J_u^*$) by the bioaffinity parameter a ($=K_M/c_M^*$) and the limiting flux ratio b ($=J_u^*/J_m^*$)

$$Q = \left[(1+a+b)/2b\right]\left\{1-\left[1-4b/(1+a+b)^2\right]^{1/2}\right\} \quad (1)$$

with J, J_u^* and J_m^* representing the flux, limiting biouptake flux, and the limiting transport flux in the medium, respectively, K_M the bioaffinity parameter and c_M^* the concentration of free metal in the bulk. Equation 1 applies to cases where the medium contains only free metal(M) and/or complexes(ML) with rates of dissociation and association so slow that they are inert on the effective timescale(δ^2/D), viz. $k_d\delta^2/D_{ML}$ and $k_a'\delta^2/D_M << 1$ [10,11]. If, however, the rates of association and dissociation of ML are so fast that on the effective timescale $k_d\delta^2/D_{ML}$ and $k_a'\delta^2/D_M >> 1$, the M ⇔ ML equilibrium is dynamic and contribution of ML to the biouptake has to be invoked [3].

Labile complexes: Speciation and fluxes

For the case of labile complexes, i.e., complexes that are capable of maintaining equilibrium with M in a transport situation, the linear steady-state diffusion flux in the medium J_m can be written as [1]:

$$J_m = \bar{D}c_M^*\left(1/\bar{\delta}\right)(1+K')\left(1-c_M^0/c_M^*\right) = J_m^*(1+K')\left(1-c_M^0/c_M^*\right) \quad (2)$$

where $\bar{D}$ is the mean diffusion coefficient of M and ML, $\bar{\delta}$ the corresponding (mean) diffusion layer thickness, K' ($=K_{ML}c_{L,t}$) the stability constant of the complex ML times the total ligand concentration and the concentration of the free metal at the surface. Depending on the hydrodynamic conditions of the biouptake situation, $\bar{\delta}$ varies with $\bar{D}$ according to some power function $\bar{D}^\alpha$ with α usually between 1/3 and 1/2 [12].

TWO-ROUTE BIOUPTAKE

Two different routes for uptake of free metal

In general, this case corresponds to two Michaelis–Menten uptake pathways, i.e., two different routes each comprising a Langmuirian adsorption step and a first-order internalization rate. In the steady state the biouptake flux is:

$$J_u = J_{u,1}^* c_M^0/\left(K_{M1}+c_M^0\right) + J_{u,2}^* c_M^0/\left(K_{M2}+c_M^0\right) \quad (3)$$

which is to be combined with the diffusion flux of labile species in the medium, as given by eq. 2. J_m^* equals $(\bar{D}/\bar{\delta})c_M^*$ which reduces to $(D_M/\delta_M)c_M^*$ for the case of static systems or free metal alone. Using eqs. 2 and 3, the steady-state flux can be expressed in terms of c_M^* which leads to a cubic variant of the Best equation. This cubic equation gives the exact solution for this case. Limiting cases are found for (i) $c_M^0 \rightarrow c_M^*$, the case where mass transport is fast compared to the biouptake, for which J_u is given by eq. 3 with $c_M^0 = c_M^*$, and (ii) $c_M^0 \rightarrow 0$, the case of mass transport controlled biouptake with $J_u = J_m^*$. It is further interesting to analyze the limit where the flux is controlled by the biouptake, i.e. the case of low bioaffinity ($a >> 1$), to see under which conditions it is possible to consider just one of the sites. The most common situation is the combination $J_{u,1}^* < J_{u,2}^*$ with $K_{M1} < K_{M2}$, and Fig. 1 shows that for a difference of one order of magnitude between the maximum fluxes, it takes a difference of at least 10^3 between the bioaffinities to justify neglection of the second uptake route in the low concentration range.

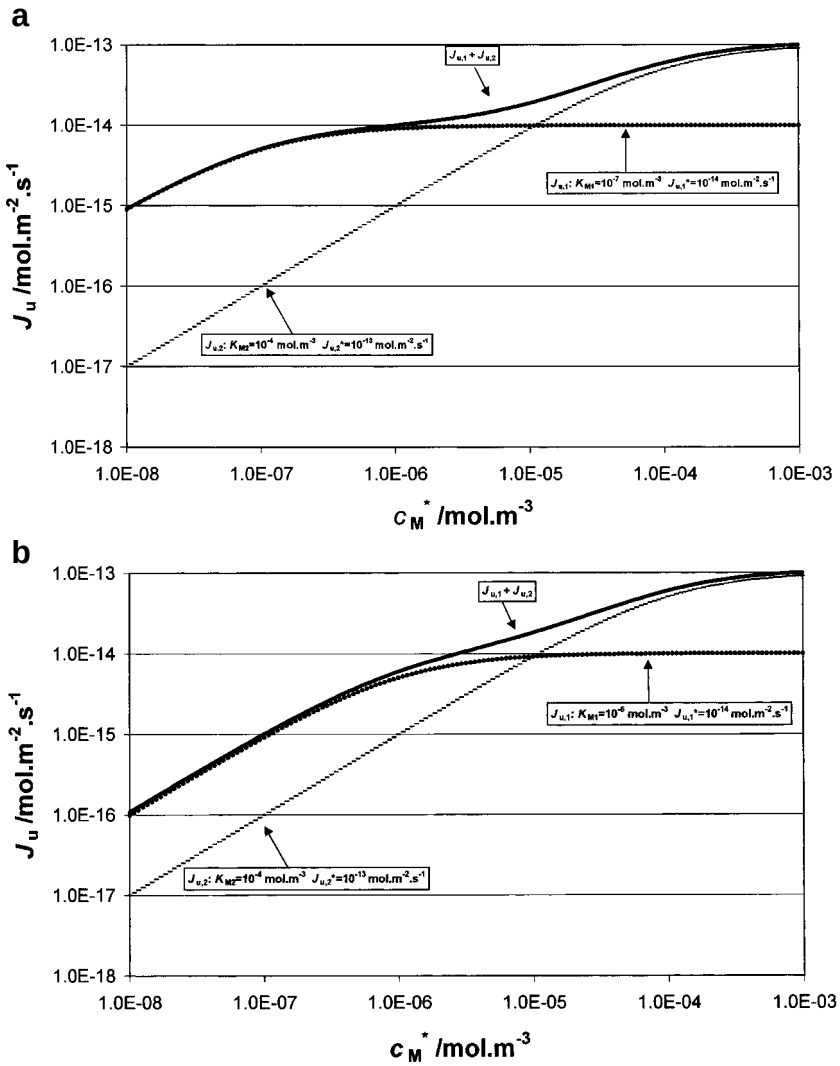

Fig. 1 Log J_u vs. log c_M^*. Two parallel Michaelis–Menten pathways: total uptake J_u ($J_{u,1}$+ $J_{u,2}$) (bold curve), $J_{u,1}$ with $J_{u,1}^* = 10^{-14}$ mol.m^{-2}.s^{-1} (dotted curve) $J_{u,2}$ with $K_{M2} = 10^{-4}$ mol.m^{-3} and $J_{u,2}^* = 10^{-13}$ mol.m^{-2}.s^{-1} (interrupted curve). Other parameters are: $c_M^* = 10^{-3}$ mol.m^{-3}, $D_M = 1 \times 10^{-9}$ m^{2}s^{-1}, $\delta = 5.0 \times 10^{-5}$ m. (a) $K_{M1} = 10^{-7}$ mol.m^{-3}; (b) $K_{M1} = 10^{-6}$ mol.m^{-3}.

S1(adsorption+internalization free metal) + S2(internalization lipophilic complex)

Figure 2 gives a sketch of the two-route biouptake considering free metal, complex ML, and lipophilic complexes MI, where I is the lipophilic ligand. The uptake of the lipophilic complexes is supposed not to proceed via discrete sites for the lipophilic ligand. Such a situation could be described by two parallel pathways, one of them of the Michaelis–Menten type, the other simply a first-order rate-limited internalization.

For the transport toward the biosurface there are two typical limiting situations:

- the lipophilic complexes are inert and the diffusion of MI is totally isolated,
- the lipophilic complexes are labile and the diffusion of MI, M and ML is coupled.

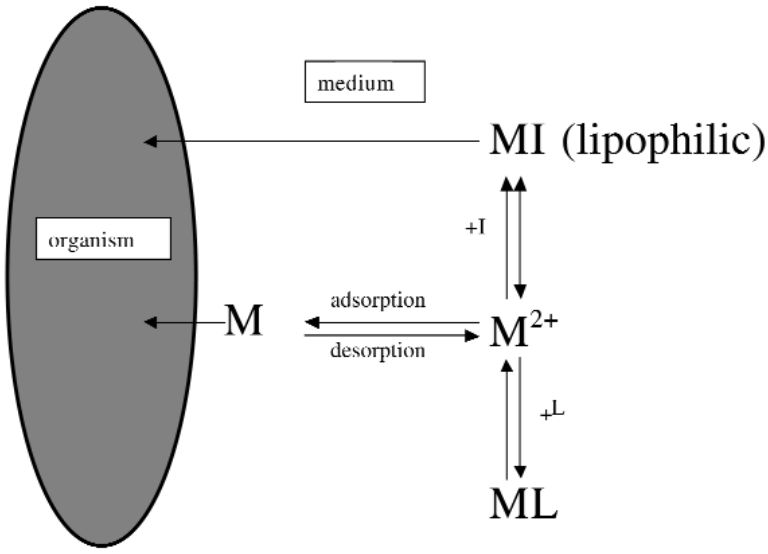

Fig. 2 Schematic representation of two-route biouptake with free metal and lipophilic complexes as the bioactive species.

Inert lipophilic complexes

Since the diffusion and the biouptake for pathway 1 are independent from those of pathway 2, we can look at this system as a sum of two Best equations, one for M and one for MI. The bioaffinity and the maximum biouptake flux of the lipophilic complex are generally high. Thus, it is a fair approximation to use $K_{MI} >> c_{MI}^0$, which means that the uptake of lipophilic complexes is essentially described by a first-order internalization rate. The total biouptake flux becomes:

$$J_u = J_{u,M}^* \, c_M^0 \Big/ \left(K_M + c_M^0\right) + J_{u,MI}^* \, c_{MI}^0 \Big/ K_{MI} \tag{4}$$

In the steady state, the transport and uptake fluxes of the lipophilic complexes are equal:

$$J = J_{u,MI}^* \, c_{MI}^0 \Big/ K_{MI} = J_{m,MI}^* \left(1 - c_{\mathrm{MI}}^0 \Big/ c_{\mathrm{MI}}^*\right) \tag{5}$$

which yields a simplified Best equation:

$$Q_{MI} = 1 \Big/ \left(a_{MI} + b_{MI}\right) \tag{6}$$

where Q_{MI} ($=J/J_{u,MI}^*$) is the steady-state flux normalized with relation to MI, a_{MI} ($=K_{\mathrm{MI}}/c_{MI}^*$) the bioaffinity parameter of the lipophilic complex and b_{MI} ($=J_{u,MI}^*/J_{m,MI}^*$) the bioconversion flux ratio for MI.

The total flux, normalized with respect to J_{u}^* for the free metal ($J_{u,M}^*$), can be written as:

$$Q = \left[(1+a+b)/2b\right]\left\{1 - \left[1 - 4b\Big/(1+a+b)^2\right]^{1/2}\right\} + \left(J_{u,MI}^* \Big/ J_{u,M}^*\right)\left[1\Big/\left(a_{MI} + b_{MI}\right)\right] \tag{7}$$

Analyzing eq. 6, there are two limiting situations: $a_{\mathrm{MI}} << b_{\mathrm{MI}}$ or $a_{\mathrm{MI}} >> b_{\mathrm{MI}}$. Since eq. 6 is only valid for large b_{MI} (high bioconversion capacity) and small a_{MI} (high bioaffinity), the normal limiting situation should be $a_{\mathrm{MI}} << b_{\mathrm{MI}}$. In this case, the limit to which Q_{MI} converges is $1/b_{\mathrm{MI}}$, or in terms of flux $J = J_{m,MI}^*$, meaning that the biouptake of inert lipophilic complexes will be controlled by diffusion of the complex MI from the bulk of the solution to the surface of the organism. Then the total concentration of the lipophilic ligand I is a key parameter of this route of biouptake.

Labile lipophilic complexes

Contrary to the inert case, the diffusion of MI is now coupled to that of M and ML , and consequently there is no certainty that $K_{MI} >> c_{MI}^0$, so the biouptake flux will be:

$$J_u = J_{u,M}^* c_M^0 \Big/ \left(K_M + c_M^0\right) + J_{u,MI}^* c_{MI}^0 \Big/ \left(K_{MI} + c_{MI}^0\right) \tag{8}$$

We consider the situation of coupled transport of labile complexes ML and labile lipophilic complexes MI. Assuming that L is in excess over M, so that $c_L \approx c_{L,t}$, we will have the following equilibria and equilibria constants in solution:

$$M + I \Leftrightarrow MI \qquad K_{MI} = c_{MI}/(c_M c_I)$$

$$M + L \Leftrightarrow ML \qquad K' = K_{ML} c_{L,t} = c_{ML}/c_M$$

The average diffusion coefficient $\bar{D}$ for the system is:

$$\bar{D} = \left(D_M c_M + D_{ML} c_{ML} + D_{MI} c_{MI}\right) \Big/ c_{M,t} \tag{9}$$

with $c_{M,t} = c_M + c_{ML} + c_{MI}$. The diffusion flux can be represented by:

$$J_m = J_m^* \left(1 - \frac{c_{M,t}^0}{c_{M,t}^*}\right) = J_m^* \left(1 - \frac{c_M^0}{c_M^*}\left(\frac{1 + K' + K_{MI} c_I^0}{1 + K' + K_{MI} c_I^*}\right)\right) \tag{10}$$

The dimensionless parameters *a*, *b* are different when the labile complexes ML are present [3]. In the steady state the flux becomes:

$$J = J_{u,M}^* \left(\frac{c_M^0}{K_M + c_M^0}\right) + J_{u,MI}^* \left(\frac{c_{MI}^0}{K_{MI} + c_{MI}^0}\right) = J_m^* \left(1 - \frac{c_M^0}{c_M^*}\left(\frac{1 + K' + K_{MI} c_I^0}{1 + K' + K_{MI} c_I^*}\right)\right) \tag{11}$$

This expression can be rearranged in terms of the dimensionless parameters *a*, *b*, a_{MI}, b_{MI} and χ ($=c_M^0/c_M^*$):

$$b\left(\frac{\chi}{a + \chi}\right) + b_{MI}\left(\frac{\chi c_I^0/c_I^*}{a_{MI} + \chi c_I^0/c_I^*}\right) = \left(1 - \chi\left(\frac{1 + K' + K_{MI} c_I^0}{1 + K' + K_{MI} c_I^*}\right)\right) \tag{12}$$

The interesting question is whether the free lipophilic ligand is taken up by the organism or not. This is a key issue in the discussion of the biouptake in presence of labile lipophilic ligands.

a) The lipophilic ligand I is consumed by the organism:
The concentration of lipophilic ligand at the surface of the organism is then likely to be much smaller than the concentration in the bulk solution: $c_I^0 << c_I^*$. Then, if the term c_I^0/c_I^* tends to zero and $K_{MI} c_I^0 << 1$, eq. 12 is simplified to:

$$b\left(\frac{\chi}{a + \chi}\right) = \left(1 - \chi\left(\frac{1 + K'}{1 + K' + K_{MI} c_I^*}\right)\right) \tag{13}$$

which results into a modified Best equation. If it is further assumed that $K_{MI} c_I^* << 1$, we obtain the Best equation, with the only difference that now $\bar{D}$ is given by eq. 9. This is not a trivial case in the biouptake situation since it implies that there is a small concentration of lipophilic complex compared to the free metal, but it is still relevant because uptake of MI may be much larger than

uptake of free metal. Note that the condition $c_I^0/c_I^* \rightarrow 0$ is a severe one since it is likely that $b/a << b_{MI}/a_{MI}$.

b) The lipophilic ligand I is not consumed by the organism:
Only if the concentration of lipophilic ligand at the surface of the organism is approximately equal to the concentration in the bulk solution ($c_I^0 \approx c_I^*$) then the term $(1+ K'+ K_{MI}c_I^0)/(1+ K'+ K_{MI}c_I^*)$ is practically 1, and eq. 12 is simplified to:

$$b\left(\frac{\chi}{a+\chi}\right) + b_{MI}\left(\frac{\chi}{a_{MI}+\chi}\right) = (1-\chi) \tag{14}$$

This is formally identical to the case of two parallel Michaelis–Menten pathways where the flux expression for MI might be simplified if $K_{MI} >> c_{MI}^0$ (see above).

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