Metallothionein Synthesis and Its Induction Mechanism: Correlation with Metal Ion Electronic Configurations and Softness Parameters

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(Received February 2, 1988)

Abstract

An examination of metallothionein induction by toxic metal ions reveals that induction is especially prominent by ions with the electronic configurations $(n-1)d^8$, $(n-1)d^9$, $(n-1)d^{10}$ and ns^2-$(n-1)d^{10}$. These electronic configurations are also those of both the 'softest' and many of the most toxic of the metal ions. The induction process for this protein appears to be one capable of sensing the electronic configurations of these species through the formation of a *trans* acting induction complex. The relative ability of toxic heavy metals to induce metallothionein is found to be correlated inversely with their softness parameters, σ_p. Examination of the acceptor properties of these inducing ions suggests that an –SH or –SeH group (soft base) is the critical reactant site for these ions, as these two species form stable bonds with ions that have such electronic configurations. The involvement of –SH or –SeH in the initial step of the induction process, *i.e.* as a component of the *trans* acting element, in a reaction with ions of such electronic configurations would provide the cell with an appropriate response to the presence of species capable of depleting its supply of glutathione, cysteine t-RNA, selenocysteine t-RNA and similar essential species containing –SH or –SeH. The enhancement of metal ion toxicity in states of selenium deficiency suggests that an –SeH containing molecule participates in this step. Two general mechanisms, based on the reaction of inducing metals with selenocysteine t-RNA, are suggested for the initial step in the induction process. The problem of species which are expected on the basis of their electronic configurations to induce MT, but which have not yet been shown to do so is apparently connected with the attempt to use non-labile complexes or extensively hydrolyzed or insoluble compounds as the inducing species.

Introduction

Soon after its discovery [1], metallothionein (MT) was linked with processes involving several of the toxic metals as well as the essential elements zinc and copper [2–8]. The synthesis of metallothionein is induced by a large number of toxic metals subsequent to their administration to animals, and this protein then binds the majority of these toxic metals, some quite firmly. Metallothionein induction has been recognized as a process by which cells protect themselves against the toxic effects of many metallic ions [3, 5, 9–15]. The relation of metallothionein induction to cadmium toxicity has been examined in considerable detail [8, 16–18]. The mechanism by which the metallothionein mediated reduction of cadmium toxicity occurs has been shown by Williams [19] to be related to the role metallothionein plays in regulating the cytosolic concentrations of such toxic ions. This utilizes a process related to metal ion buffering, in which the metallothionein maintains the concentrations of such toxic metal ions in a concentration range which is much lower than it would be otherwise. The nature of the key features of the metallothionein inducers, especially their softness and electronic configurations, and the relationship of this to metal ion toxicity is a matter of considerable importance. The purpose of the present study was to examine the hypothesis that the induction of the synthesis of this protein can be triggered by processes which sense those electronic configurations of the soft toxic metal ions and their immediate neighbors in the periodic table.

Metallothionein Induction

The induction of metallothionein is caused by a very large number of chemical species including many metal ions [2, 4, 10–12, 20], chelating agents [21, 22], dexamethasone [23], iodoacetate [12] and others [24]. Here we will be concerned only with the induction of metallothionein as a response to certain simple inorganic metallic ions and species with related electronic configurations. The metal ions reported to be inducers of metallothionein, together with the electronic configurations of some closely related species are collected in Table I. It is

0020-1693/88/$3.50

TABLE I. Species which Can Induce the Synthesis of Metallothionein and Species of Closely Related Electronic Configuration

Species	Electronic configuration	References
Cr(III)	[Ar]$3d^3$	20
Mn(II)	[Ar]$3d^5$	20, but see 25
Fe(III)	[Ar]$3d^5$	20
Fe(II)	[Ar]$3d^6$	20
Co(II)	[Ar]$3d^7$	12, 20, 25–27
Ni(II)	[Ar]$3d^8$	12, 20, 25, 27–30
Cu(II)	[Ar]$3d^9$	12, 27, 31
Zn(II)	[Ar]$3d^{10}$	12, 25, 27, 32–34
Ga(III)	[Ar]$3d^{10}$	no evidence reported
Ge(IV)	[Ar]$3d^{10}$	no evidence reported
As(V)	[Ar]$3d^{10}$	no evidence reported
As(III)	[Ar]$3d^{10}4s^2$	no evidence reported
Se(IV)	[Ar]$3d^{10}4s^2$	20
Pd(II)	[Kr]$4d^8$	no evidence reported
Ag(I)	[Kr]$4d^{10}$	12, 34
Cd(II)	[Kr]$4d^{10}$	9, 12, 25, 27, 34–36
In(III)	[Kr]$4d^{10}$	30
Sn(II)	[Kr]$4d^{10}5s^2$	37
Sb(II)	[Kr]$4d^{10}5s^2$	no evidence reported
Te(IV)	[Kr]$4d^{10}5s^2$	no evidence reported
Pt(II)	[Xe]$5d^8$	38, but see 39
Au(I)	[Xe]$5d^{10}$	40–43
Hg(II)	[Xe]$5d^{10}$	11, 12, 25, 27, 34
Pb(II)	[Xe]$5d^{10}6s^2$	20, 29, 44
Bi(III)	[Xe]$5d^{10}6s^2$	20, 26, 45
Po(IV)	[Xe]$5d^{10}6s^2$	no evidence reported

immediately apparent that metallothionein induction is most typical of certain species in which the electronic configuration of the outermost electrons are $(n-1)d^8$, $(n-1)d^9$, $(n-1)d^{10}$ and $(n-1)d^{10}ns^2$. These configurations are noteworthy for being both polarizing and polarizable and thus include many of the soft acid metal ions. The correlation between the softness and the toxicity of metal ions is quite close [46–50] so it is not surprising to find that this group includes some of the most toxic metal ions: Hg^{2+}, Cd^{2+}, In(III) and Cu^{2+}.

Correlations of Toxicity, Metallothionein Induction and the Softness Parameter

As noted above, the toxicity of the majority of the heavy metal ions can be correlated with the softness parameter, σ_p which is inversely related to metal ion softness, as well as other physical parameters [46–51]. In our earlier examination of this correlation we found that the LD_{50} values of the softest of the heavy metals in mice correlated extremely well with this σ_p parameter [46], and subsequent studies by other investigators have confirmed these correlations [47–49, 52]. In connection with the present study, we have also examined some toxicity data on cell cultures of hepatocytes used in the study of metallothionein induction published by Durnam and Palmiter [11] and have found a similar correlation. For the ED_{50} values found for such cells and solutions of the heavy metal ions the correlation equation found was

$$ED_{50} = -231 + 3593\sigma_p \qquad (r = 0.833)$$

These ED_{50} values are the concentrations of the metal ion which kill one-half of the cells, and, as can be seen, the softest ions (with the lowest σ_p) are the most toxic.

This type of correlation suggested that there might be similar correlations between the softness parameter and some measure of the relative effectiveness of the toxic heavy metal ions in inducing the synthesis of metallothionein. Inasmuch as different investigators use very different systems to study the induction of metallothionein there is no generally accepted and readily available index of the relative ability of the different metal ions to induce metallothionein synthesis. Accordingly, we have examined the ability of the softness parameter to correlate metallothionein induction per mole of inducing metal ion in individual sets of experiments within which the data are strictly comparable and available in numerical form. Reports which compare the relative effectiveness of several different metal ions in the induction process for metallothionein are not common, and those that are available are based on several different biological systems for the examination of this induction process. We have found this kind of data in reports from five different laboratories and have examined the correlation of such data with the softness parameter, σ_p [53], a numerical measure of metal ion softness which decreases as metal softness increases.

For the data of Maitani and Suzuki [20], the relationship between the number of moles of metallothionein induced per mole of metal ion (MT_{ind}) in the mouse liver by six metal species: Mn(II), Co(II), Ni(II), Zn(II), In(III) and Pb(II), and their σ_p values is given by the equation

$$MT_{ind} = 720 - 5530\sigma_p \qquad (r = -0.976)$$

The extremely high value of the correlation coefficient is noteworthy. The limiting value r could possibly have is -1.000 for a directly inverse relationship between the relative ability to induce metallothionein and the softness parameter. The relative induction of metallothionein (MT) in hepatocytes by the ions Cd^{2+}, Zn^{2+}, Hg^{2+}, Co^{2+} and Ni^{2+} which has been determined by Bracken and Klaassen [54], can be described by the equation

$$MT_{ind} = 4.322 - 34.44\sigma_p \qquad (r = -0.985)$$

TABLE II. Relative MT Induction (MT_{ind}) per Mole of Metal under Various Conditions

Metal ion	σ_p [53]	Mouse liver [20]	Mouse liver [11]	Mouse kidney [11]	HeLa cells [23]	Rat hepatocytes [54]	Mouse hepatocytes [12]	Mouse kidney [27]
Cd^{2+}	0.081		7595	6270	5	1.6	139	4.13
Zn^{2+}	0.115	67	5125	771	0.2	0.073	51	0.12
Cu^{2+}	0.104		5145	1334	0.09		9.4	0.070
Ag^{+}	0.073						101	
Hg^{2+}	0.064		10400	7791		2.13	59.4	28.5
Co^{2+}	0.130	10				0.024	31.3	0.094
Ni^{2+}	0.126	21				0.0073	2.58	0.110
Mn^{2+}	0.124	17						
In(III)	0.100	180						
Pb^{2+}	0.131	13						
Fe^{2+}	0.129	4						
r		−0.972	−0.968	−0.982	−0.942	−0.985	−0.678	−0.834
a		727.2	16715	17755	16.99	4.312	181.5	43.22
b		−5602	−106040	−150710	−152.2	−34.44	−1265	−360.5

Similar excellent correlations are found between the softness parameter and values for MT_{ind} for the induction of metallothionein in liver and kidney by the four metal species {Cd(II), Zn(II), Cu(II) and Hg(II)} reported by Durnam and Palmiter [11, 12]. The correlation equations found here are for mouse liver

$$MT_{ind} = 16\,710 - 106\,010\sigma_p \quad (r = -0.969)$$

and for mouse kidney

$$MT_{ind} = 17\,750 - 150\,700\sigma_p \quad (r = -0.982)$$

Once again the correlation is extraordinarily good, in fact it is of the sort that one would expect from some sort of a direct relationship between the parameters. Another set of data of this type on HeLa cells is from work of Karin and his collaborators [23], which involves only the three metal species Cd(II), Zn(II) and Cu(II). Here the correlation equation is

$$MT_{ind} = 16.99 - 152.2\sigma_p \quad (r = -0.942)$$

Data reported by Naganuma *et al.* [27] on metallothionein induction by six different soft metal ions in the mouse kidney is correlated with the equation

$$MT_{ind} = 42.1 - 354\sigma_p \quad (r = -0.812)$$

A somewhat poorer, but still significant, correlation involving seven soft metal ions as metallothionein inducers in cultured mouse hepatocytes is found in additional data of Durnam and Palmiter [12]

$$MT_{ind} = 181 - 1265\sigma_p \quad (r = -0.678)$$

The derived data on metallothionein induction per mole of metal ion which was utilized in obtaining these correlations is collected in Table II, along with the values of the correlation coefficients (r), the intercepts (a), and slopes (b) of the correlation equations obtained, all of the form

$$MT_{ind} = a + b\sigma_p$$

The values of MT_{ind} given in the Table are the relative values within each reported set of data.

It is unfortunate that a larger set of data is not available which includes all of the ions included in each of these smaller sets. Nevertheless, the induction process for metallothionein would appear to be directly linked to the softness parameter for the metal species involved. An approximate way to test this hypothesis using data from two laboratories is to reduce the data from two laboratories to a common basis on a scale relative to the results for Zn(II), a species common to both and then to test the combined set of data for correlation. When this is done for the hepatic data of Maitani and Suzuki [20] and that of Durnam and Palmiter [12], one obtains the relationship

$$MT/MT[\text{Zn(II)}] = 4.307 - 3.498\sigma_p/\sigma_p\text{Zn(II)}$$

$$(r = -0.787)$$

This is a level of correlation which is quite good considering that one set of data was obtained on whole animals and the other on hepatocytes. It must be kept in mind that it is not reasonable to expect data on different organs and/or different cell cultures to be correlated on any common basis as the accessibility of the inducing ions to the interior of the cells will not be comparable.

Mechanism of Metallothionein Induction

The triggering process which initiates the induction of MT synthesis can be assumed to be a reaction

between the inducing metal ions and some molecule whose concentration is monitored by the cell. It is very reasonable to suggest that the metals which induce MT are those which exert a large measure of their toxic action via their interactions with –SH and/or –SeH groups to varying extents depending upon the metal ion involved. It is expected that selenium will bond covalently to these heavier metal ions better than sulfur because of the somewhat larger covalent radius of Se (117 pm *versus* 102 pm for S), its more favorable situation for orbital overlap and its greater softness [55]. Possible candidates for this detector molecule are molecules which possess either –SH or –SeH groups whose presence is essential to their normal function. The involvement of selenium, rather than sulfur, in a critical site in the initial reaction site for metallothionein induction is consistent with the demonstrated enhancement of the toxicity of cadmium [56], mercury [57], silver [58, 59], *cis*-platinum [60] and copper [61] by states of selenium deficiency. The toxicity of all of these species is reduced by the induced metallothionein.

The induction of MT synthesis is now understood at the genetic level and regulatory elements in the MT gene have been identified. Two discrete heavy metal regulation sites have been identified in the 5′-flanking region and both are required for proper regulation of heavy metal response, with each providing different quantitative components of the total induction process. The proximal element confers high induction rates but low transcriptional efficiency while the distal element provides low induction rate with high transcriptional efficiency [6 and refs. therein].

There are two possible mechanisms for the initial step in the induction of MT synthesis by metal ions which are consistent with an initial reaction with an –SeH containing species.

Mechanism I

By analogy with the catabolite activator protein (CAP) of *E. coli* [28, 62], we propose that an –SeH containing molecule acts as the initial detector of metal ion presence. Following conformational change or other structural modification, which occurs on complexation to the metal, the metal ion detector protein can bind to the control sequences in the 5′-flanking region of the MT gene [6]. The binding event then allows transcription of the MT gene to be initiated. The relationship of the binding and the differential induction characteristics demonstrated for the proximal and distal regulatory regions [6] is not clear.

Mechanism II

An alternative mechanism which appears equally feasible at the present time is one based on analogy to the 'lac' operon [62, 63]. If a selenium containing species is bound to a regulatory region of the MT gene, introduction of an inducing metal that reacted with it could result in a complex that would be released and thus allow MT induction.

The model of reversible binding of inducer to cellular binding proteins (both CAP and lac) relies on the reversible nature of the interaction to introduce sensitivity to changes in intracellular inducer concentrations. Sensitivity to inducer concentration must also be a component of the model for the initial *trans* acting element-inducer complex. There is no *a priori* reason to discount models analogous to CAP or lac from the reversibility of complex formation point of view. It might be expected that due to the high affinity of either –SH or –SeH groups for the soft acid inducers, once the complex is formed, the duration of induction might be very long. This stable binding of inducing ions could explain the low levels of certain ions required for MT induction.

The recent discovery of a unique codon for the incorporation of selenocysteine [64] provides an interesting possibility for an additional control level for MT induction. Metal binding to selenocysteine t-RNA thus provides a very reasonable first step in MT induction. By complexation of this material, which is present in only very limited amounts intracellularly, synthesis of selenoproteins would be repressed. This could manifest itself in several ways. If the MT *trans* acting element is a rapidly turning over selenoprotein, interruption of synthesis could cause induction to proceed due to exposure of formerly blocked control sequences. An additional possibility is that the MT gene could be induced by the product of aborted translation. Selenocysteinyl t-RNA is, in the classical interpretation, a suppressor t-RNA. In the absence of selenocysteinyl t-RNA, the selenocysteinyl codon, TGA, would be read as a stop codon. This would result in production of a truncated protein that could act directly as a regulatory agent. This regulatory scheme tightly couples the induction of MT to the selenocysteinyl t-RNA level. Of the mechanisms proposed, those in which reaction with a free selenocysteine t-RNA is the initiating step would seem to be most easily reconciled with the enhancement of metal toxocity in states of selenium deficiency. These mechanisms are consistent with the presence of carrier molecules for ions such as Cd^{2+} [25] to carry them across cellular membranes into intracellular space. Other inducing mechanisms may well be available to inducers of different sorts, such as dexamethasone.

Potential Metallothionein Inducers

An examination of Table I leads quickly to the realization that there are a number of species that

might be expected to have some ability to induce MT for which no evidence is presently available. These potential inducers include Ga(III), Ge(IV), As(V), As(III), Sb(III), Te(IV), Tl(I), Po(IV), Rh(II), Ir(II) and Pd(II). For some of these there are chemical reasons why the demonstration of MT induction might prove difficult. The σ_p value of Ga(III) is 0.99 (about the same as In(III)), which indicates that it should be an excellent inducer of MT. Its greater tendency to undergo hydrolysis, because its ionic radius is somewhat smaller than that of In(III), may make the demonstration of this more difficult. A similar situation may well account for the difference between Sb(III) and Bi(III). Rh(II) and Ir(II) undergo substitution reactions much more slowly than Co(II), but they would otherwise be expected to be species capable of inducing MT. Pd(II) is a species which should, like its closely related species, Ni(II) and Pt(II), be capable of inducing MT synthesis, though, quite unexpectedly, it appears not yet to have been examined. Te(IV) would seem to be a somewhat similar situation which may prove to be somewhat more effective than Se(IV). Softness generally increases as we examine analogous species going down a family. Ge(IV) should also be capable of causing some induction of MT synthesis, though it may be somewhat too hard an acid to be effective. As(III) should be about as effective as Se(IV), though As(V) may again be simply too hard to react prior to reduction. For two series of ions, Zn(II) to Ge(IV) and Sn(II) to Te(IV) with identical electronic configurations, the ability to induce the synthesis of MT is seen most clearly in those with the lowest oxidation state. From the data in Table II, one would predict that for σ_p values at or above about 0.136, there should be no MT induction via the mechanism operating to give the correlations reported here. Since, as noted above, the softness parameters usually drop in value as we go down a family in the periodic table, we would expect an enhancement of MT synthesis inducing ability as we move in this direction. For some species, such as Tl(III), their inherent oxidizing power will make them unstable in a biological milieu. For Tl(I), the ion is so large and its coordinating tendencies so weak, that it may be unable to form an effective bond with the appropriate group on the sensor molecule.

For an initial step of the induction process which involves reaction with an –SeH group one would expect that organometallic compounds such as CH_3HgCl, $(CH_3)_2SnCl_2$ and the like, that can react with such groups, might be also able to induce MT synthesis by this same type of mechanism. There are grounds to suspect that several of the species with appropriate electronic configurations, softness parameter values and chemical properties (ability to pass through cellular membranes and undergo a substitution reaction with the sensor molecule) will be found capable of inducing the synthesis of MT. If this model is correct, one might expect to find similar correlations for heavy metal induction of other cadmium binding proteins found in various organisms [65], though the evidence for a protective function of some of these against the toxicity of metals is rather limited [66].

Acknowledgements

We wish to acknowledge, with thanks, the support received for this work from the National Cancer Institute (Grant RO1-CA38997-02) and the National Institutes of Environmental Health Sciences (Grant RO1-ES02638-07).

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