Roles of synthesis and degradation in the regulation of metallothionein accretion in a chicken macrophage-cell line

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Metallothionein (MT) is a metal-binding protein rapidly accreted in many tissues in response to trace elements or hormones. To gain an understanding of the regulation of MT accretion, rates of MT synthesis and degradation were determined by using a decay-kinetics technique. A chicken macrophage-cell line (HD11) that rapidly accretes incremental amounts of MT when stimulated with increasing concentrations of Zn^{2+} or Cd^{2+} was studied. The maximum rate of MT accretion occurred at $50\ \mu M$ - Zn^{2+} or $20\ \mu M$ - Cd^{2+} . The absolute rate of MT accretion was less in macrophages incubated with $25\ \mu M$ - as compared with $50\ \mu M$ - Zn^{2+} , owing to decreased and increased rates of MT synthesis and degradation respectively. The absolute rate of MT accretion was less in macrophages incubated with $10\ \mu M$ - as compared with $20\ \mu M$ - Cd^{2+} , owing to a decreased rate of MT synthesis with no change in degradation. Compared with macrophages continually incubated with $50\ \mu M$ - Zn^{2+} , removal of Zn^{2+} from medium previously containing $50\ \mu M$ - Zn^{2+} decreased the absolute rate of MT accretion, owing to decreased and increased rates of MT synthesis and degradation respectively. Removal of Cd^{2+} from medium previously containing $20\ \mu M$ - Cd^{2+} also decreased the absolute rate of MT accretion in macrophages. Unlike Zn^{2+} removal, the decrease in MT accretion was due to a decreased rate of MT synthesis with no change in degradation. When macrophages incubated with $50\ \mu M$ - Zn^{2+} were subsequently incubated with $20\ \mu M$ - Cd^{2+} , rates of MT synthesis and accretion were decreased as compared with cells continually incubated with $50\ \mu M$ - Zn^{2+} or $20\ \mu M$ - Cd^{2+} . When macrophages incubated with $20\ \mu M$ - Cd^{2+} were subsequently incubated with $50\ \mu M$ - Zn^{2+} , rates of MT synthesis and accretion were increased as compared with cells continually incubated with $50\ \mu M$ - Zn^{2+} or $20\ \mu M$ - Cd^{2+} . Switching the metal in the incubation medium did not influence the rate of MT degradation. Our results indicate that the rate of MT accretion is determined by variations in the rates of MT synthesis and degradation, depending upon the inducing metal and the concentration of the metal.

INTRODUCTION

Metallothioneins (MTs) are 6500 Da metal-binding proteins which contain 33 mol of cysteine/100 mol, but no phenylalanine or tyrosine [1–6]. An MT gene is expressed in response to either hormones or trace elements [7–9]. Upon MT-gene expression, the amount of MT mRNA and MT protein quickly increases several-fold from previously low levels [7–13]. Since MT accretion varies according to the magnitude and type of trace-element exposure [10,11,14,15], knowledge of the regulation of MT accretion would provide an insight into its ability to rapidly chelate trace elements.

The rate of MT accretion is dependent upon rates of the opposing processes of MT synthesis and MT degradation. Previously determined rates of MT synthesis and degradation [7,12,14,16–27] have not been determined in the same experiment or under the same experimental conditions so that relative contributions of these processes to MT accretion can be assessed. Previous studies indicate that the type of trace element chelated by MT [12,16–24] and trace-element status of the animal [16,20] affect the rate of MT degradation. Since the rate of isotope incorporation into MT is also affected by trace elements [7,12,14,18,22,24,25,27], it appears that MT accretion may be regulated by changes in its rates of both synthesis and degradation. An understanding of the relative contributions of protein synthesis and protein degradation is important for understanding the roles of MT in chelating and in releasing trace elements for nutritional, immunological or toxicological functions.

Rates of MT synthesis and degradation can be measured simultaneously using decay-kinetic techniques [28], by moni-

toring the decrease over time of an isotopically labelled amino acid in MT. We report the use of decay kinetics to study the regulation of MT accretion in a chicken macrophage-cell line induced to accrete different amounts of MT in response to various concentrations and sequences of Zn^{2+} or Cd^{2+} .

MATERIALS AND METHODS

Cell culture

Chicken HD11 macrophages transformed by a *myc*-containing MC29 retrovirus [30] were grown in canted-neck flasks (Corning Glass Works, Corning, NY, U.S.A.) containing RPMI-1640 medium + 25 mM-Hepes (Sigma Chemical Co., St. Louis, MO, U.S.A.) supplemented with 5% (v/v) fetal-bovine serum (Irvine Scientific, Santa Ana, CA, U.S.A.) and 50 mg of gentamicin sulphate (Gibco Laboratories, Grand Island, NY, U.S.A.)/l. These cells were incubated in a humidified air/ CO_2 (19:1) atmosphere at $41.5^\circ C$. Approx. 2 days before the start of each experiment macrophages were scraped from the flasks and 1.08×10^7 cells in 5 ml of medium were added to replicate 35 mm-diameter cell wells (Corning Glass Works).

Isolation and quantification of MT

MT was isolated by a reverse-phase (r.p.) h.p.l.c. procedure similar to that described by Richards & Steele [11], which purifies two MT isoforms from macrophage cytosol with 94% homogeneity [31]. Briefly, macrophage cytosol was heated at $60^\circ C$ for 10 min, centrifuged at 16000 g for 20 min and the supernatant injected into a Pecosphere-3C C_{18} cartridge column

Abbreviations used: MT, metallothionein; r.p., reversed-phase; Cd-MT and Zn-MT, MT induced by Cd^{2+} and Zn^{2+} respectively.

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(0.46 cm $\times$ 8.3 cm) with a 3 μ m particle size (Perkin-Elmer Corp., Norwalk, CT, U.S.A.). The column was maintained at ambient temperature and equilibrated with 10 mM- NaH_2PO_4 , pH 7.0. MT was eluted at 1 ml/min with a two-step linear gradient consisting of 0–10% of 10 mM- NaH_2PO_4 + 60% acetonitrile, pH 7.0, from 0 to 5 min, followed by a 10–25% gradient from 5 to 20 min. The eluent was monitored spectrophotometrically at 214 nm, and MT-containing peaks were integrated by a Maxima-820 chromatography workstation (Millipore Corp., Waters Chromatography Division). In order to quantify chicken macrophage MT induced by Cd^{2+} (Cd-MT) and Zn^{2+} (Zn-MT), Cd-MT and Zn-MT standards were purified from chicken liver [31]. These MT standards were found to have the same absorbance per unit weight at 214 nm.

Experiment 1. To assess the effect of the concentration of trace element on MT accretion, macrophages were incubated in medium supplemented with various concentrations of Zn^{2+} or Cd^{2+} . After 16 h incubation, three replicate wells of macrophages per treatment were lysed with a buffer containing 3 mM- NaH_2PO_4 , pH 8.0, 0.2% Nonidet P40, 0.5 μ M-phenylmethanesulphonyl fluoride, 10 μ g of phosphoramidon/l, 10 μ g of pepstatin/l and 250 ng of cystatin (Sigma)/l, and scraped from their wells. Complete cell lysis was ensured by freezing and thawing the homogenate three times. All samples were heat-treated, supernatants were injected into the r.p.h.p.l.c. column and MT isoforms were quantified as described above. Significant differences among treatment means were determined by the least-significant-difference test (SAS Institute Inc., Cary, NC, U.S.A.).

Experiment 2. To investigate the regulation of MT accretion, decay kinetics were used to measure rates of MT synthesis and degradation. The technique employed had three experimental periods. During period 1 (labelling period), MT was induced by various treatments and newly synthesized MT and other cellular proteins were labelled with 3 μ Ci of [^{35}S]cysteine (1010 Ci/mmol; ICN Biomedicals, Costa Mesa, CA, U.S.A.)/ml for 16 h. During period 2 (wash-out period), cells were washed and incubated for an additional 3 h in fresh medium lacking the isotope. In period 3 (decay period), cells were washed and replenished with fresh medium. At 0, 6 and 12 h of the decay period, macrophages from three replicate wells per treatment were lysed, scraped and freeze-thawed three times to ensure lysis. Cytosols were heat-treated, supernatants were injected into the r.p.h.p.l.c. column and MT isoforms were quantified. MT isoforms were individually collected into liquid-scintillation vials and the ^{35}S radioactivity was quantified with a liquid-scintillation counter. Thus measurements of MT synthetic, degradative and accretion rates were made from 19 to 31 h after first subjection of macrophages to treatments.

Components added to the medium to induce MT accretion during the 16 h labelling period included 25 μ M- ZnSO_4 (low Zn^{2+}), 50 μ M- ZnSO_4 (high Zn^{2+}), 10 μ M- CdSO_4 (low Cd^{2+}) or 20 μ M- CdSO_4 (high Cd^{2+}). Macrophages incubated in low Zn^{2+} or low Cd^{2+} during the labelling period were incubated with the same components during the wash-out and decay periods (low Zn^{2+} /low Zn^{2+} ; low Cd^{2+} /low Cd^{2+}). Macrophages subjected to high Zn^{2+} or high Cd^{2+} during the labelling period were either maintained on the same treatment (high Zn^{2+} /high Zn^{2+} ; high Cd^{2+} /high Cd^{2+}), switched to medium with no supplemented trace elements (high Zn^{2+} /0; high Cd^{2+} /0) or to medium containing the high level of the other trace element (high Zn^{2+} /high Cd^{2+} ; high Cd^{2+} /high Zn^{2+}) during the wash-out and the decay periods.

The fractional rate of MT degradation was calculated as the slope of the regression of $\ln(\text{total } ^{35}\text{S} \text{ radioactivity in MT})$ versus time. The fractional rate of MT synthesis was calculated as the slope of the regression of $\ln(\text{specific radioactivity of } ^{35}\text{S} \text{ in MT})$

versus time. Specific radioactivity was calculated by dividing the total radioactivity by the quantity of MT. The quantity of MT accreted was regressed over time and the average quantity of MT accreted was calculated from this regression equation. The fractional rate of MT accretion was calculated by dividing the slope of the regression of MT quantity versus time by the calculated average quantity of MT present during the 12 h decay period. Since the difference between fractional rates of MT synthesis and degradation should be equal to the fractional rate of MT accretion, the validity of our measurements could be assessed. The absolute rates of MT synthesis and accretion were calculated by multiplying their respective fractional rates by the calculated average quantity of MT. Differences due to treatment for each of the fractional and absolute rates were determined by linear contrasts (SAS Institute Inc.).

RESULTS AND DISCUSSION

The rate of MT accretion in the macrophage-cell line is dependent on the concentration and type of trace-element inducer (Fig. 1). For instance, when cells were incubated 16 h in medium supplemented with 25 μ M- or 50 μ M- Zn^{2+} , 23 or 146 μ g of MT-1 was accreted per well. When cells were incubated 16 h in medium supplemented with 10 μ M- or 20 μ M- Cd^{2+} , 45 or 90 μ g of MT-1 was accreted per well. MT-1 accretion was maximally induced with 50 μ M- Zn^{2+} or 20 μ M- Cd^{2+} .

The decay-kinetic technique used to measure rates of MT

Fig. 1. MT accretion in macrophages 16 h after incubation in medium supplemented with different concentrations of (a) Zn^{2+} or (b) Cd^{2+} (Expt. 1)

The cytosols were analysed by r.p.h.p.l.c. and the MT isoforms (■, MT-1; ▨, MT-2) quantified as described in the Materials and methods section. The second MT isoform eluted from the r.p.h.p.l.c. column was not quantified after Cd^{2+} induction. A different letter above each bar, within each isoform, denotes a significant ($P < 0.05$) mean difference. Abbreviation; ND, not detectable.

synthesis and degradation was validated to ensure that the amount of unlabelled cysteine in the medium was sufficient to minimize reincorporation of [^{35}S]cysteine into MT during the time MT fractional rates were measured without influencing fractional rates of MT synthesis and degradation [32]. The validity of MT fractional-rate measurements is supported by the observation that the difference between fractional rates of MT synthesis and degradation, which gives a calculated fractional rate of MT accretion ($k_s - k_d$), was very similar to the measured fractional rate of MT accretion over a broad range of conditions [see $(k_s - k_d)/k_a$; Table 1].

R.p.h.p.l.c. resolves two distinct MT isoforms from chicken macrophage cytosol [11,31]. The first isoform (MT-1) is predominant and is accreted in response to trace elements. Fractional rates of MT synthesis, degradation and accretion were similar for both isoforms; thus we limit further discussion to the first MT isoform eluted from the r.p.h.p.l.c. column.

Since rates of protein synthesis and degradation are zero- and first-order processes respectively [28], expression of these rates as such is useful for interpreting their contribution to the regulation of MT accretion. The MT synthetic rate is best expressed as an absolute rather than a fractional rate because it is most likely to be independent of the quantity of MT. The MT degradative rate is best expressed as a fractional rather than absolute rate because it is most likely to be dependent on the quantity of MT. It is useful to express the rate of MT accretion as either a zero- or first-order process, because it is a combination of synthetic and degradative processes.

The influence of metal type on the maximum rate of MT accretion varied with time. For the first 16 h after exposure, Zn^{2+} induced a 2-fold greater rate of MT accretion than did Cd^{2+} (Fig. 1; $20\text{ }\mu\text{M-Cd}^{2+}$ versus $60\text{ }\mu\text{M-Zn}^{2+}$), but from 19 to 31 h after exposure, rates of MT accretion were not significantly different (Fig. 2b). The similar fractional rates of MT accretion after induction by Zn^{2+} or Cd^{2+} from 19 to 31 h were accomplished with identical fractional rates of synthesis (k_s of 0.049 for both high Cd^{2+} /high Cd^{2+} and high Zn^{2+} /high Zn^{2+} ; Table 1). Accretion rates were also very similar during the decay period when macrophages were exposed to levels of Zn^{2+} or Cd^{2+} that were half that which resulted in maximal MT accretion rates ($10\text{ }\mu\text{M-Cd}^{2+}$ and $25\text{ }\mu\text{M-Zn}^{2+}$; Fig. 2a). At these lower trace-element concentrations, the similar accretion rates were accomplished with widely different fractional rates of synthesis and degradation. The fractional rate of degradation was more than

Fig. 2. Zn^{2+} - and Cd^{2+} -induced MT accretion in macrophages during the decay period (Expt. 2)

MT levels were quantified at 0, 6 and 12 h of the decay period. The decay period began 19 h after first exposure of macrophages to inducing levels of Zn^{2+} and Cd^{2+} . The fractional rate of MT accretion (k_a) is calculated as described in the Materials and methods section. (a) $\circ$, $10\text{ }\mu\text{M-Cd}^{2+}$; $\bullet$, $25\text{ }\mu\text{M-Zn}^{2+}$; (b) $\circ$, $20\text{ }\mu\text{M-Cd}^{2+}$; $\bullet$, $50\text{ }\mu\text{M-Zn}^{2+}$.

twice lower for macrophages exposed to Cd^{2+} (low Cd^{2+} /low Cd^{2+}) compared with Zn^{2+} (low Zn^{2+} /low Zn^{2+}), and the fractional rate of synthesis was sufficiently adjusted to result in similar rates of accretion (Table 1).

The greater absolute rate of MT accretion in macrophages

Table 1. Rates of synthesis, degradation and accretion of the first isoform of MT eluted from the r.p.h.p.l.c. column (Expt. 2)

HD11 chicken macrophages were incubated in medium with $3\text{ }\mu\text{Ci}$ of [^{35}S]cysteine/well supplemented with either Cd^{2+} or Zn^{2+} . After 16 h, cells were washed and incubated for 3 h in chasing medium without added [^{35}S]cysteine but with the indicated treatments. Fresh chasing medium was added and macrophages in three replicate wells per treatment were harvested at 0, 6 and 12 h. MT was purified from cytosols by r.p.h.p.l.c. and ^{35}S in MT-containing fractions was determined. The fractional rates of MT synthesis (k_s), degradation (k_d) and accretion (k_a) and the absolute rates of MT synthesis and accretion were calculated as described in the Materials and methods section. Means within a column with different superscripts are significantly different ($P < 0.05$).

Treatment		MT fractional rates (h^{-1})				MT absolute rates ($\mu\text{g/h}$)	
Labelling medium	Chasing medium	k_s	k_d	k_a	$(k_s - k_d)/k_a$	Synthesis	Accretion
$10\text{ }\mu\text{M-Cd}^{2+}$	$10\text{ }\mu\text{M-Cd}^{2+}$	0.048 ± 0.006^b	0.014 ± 0.004^b	0.034 ± 0.003^{bc}	1.00	5.4 ± 0.7^c	3.8 ± 0.3^c
$20\text{ }\mu\text{M-Cd}^{2+}$	$0\text{ }\mu\text{M-Cd}^{2+}$	0.023 ± 0.008^c	0.004 ± 0.006^b	0.019 ± 0.003^c	1.00	4.6 ± 1.6^c	3.7 ± 0.7^c
$20\text{ }\mu\text{M-Cd}^{2+}$	$20\text{ }\mu\text{M-Cd}^{2+}$	0.049 ± 0.006^b	0.002 ± 0.006^b	0.049 ± 0.003^{ab}	0.96	12.7 ± 1.5^b	12.7 ± 0.8^b
$20\text{ }\mu\text{M-Cd}^{2+}$	$50\text{ }\mu\text{M-Zn}^{2+}$	0.077 ± 0.011^a	0.005 ± 0.004^b	0.064 ± 0.007^a	1.13	26.9 ± 4.0^a	22.3 ± 2.4^a
$25\text{ }\mu\text{M-Zn}^{2+}$	$25\text{ }\mu\text{M-Zn}^{2+}$	0.065 ± 0.013^{ab}	0.032 ± 0.008^a	0.033 ± 0.010^{bc}	1.00	2.9 ± 0.6^{cd}	1.5 ± 0.4^c
$50\text{ }\mu\text{M-Zn}^{2+}$	$0\text{ }\mu\text{M-Zn}^{2+}$	0.007 ± 0.008^c	0.047 ± 0.005^a	-0.039 ± 0.009^e	1.03	0.9 ± 1.1^d	-5.3 ± 1.2^d
$50\text{ }\mu\text{M-Zn}^{2+}$	$20\text{ }\mu\text{M-Cd}^{2+}$	0.012 ± 0.006^c	0.001 ± 0.003^b	0.011 ± 0.003^d	1.00	2.6 ± 1.3^{cd}	2.3 ± 0.7^c
$50\text{ }\mu\text{M-Zn}^{2+}$	$50\text{ }\mu\text{M-Zn}^{2+}$	0.049 ± 0.009^b	0.008 ± 0.006^b	0.040 ± 0.005^b	1.03	15.4 ± 2.9^b	12.4 ± 1.4^b

incubated continually in $50 \mu\text{M-Zn}^{2+}$ (high Zn^{2+} /high Zn^{2+}) compared with $25 \mu\text{M-Zn}^{2+}$ (low Zn^{2+} /low Zn^{2+}) was due to an increased absolute rate of synthesis coupled with a decreased fractional rate of degradation (Table 1). The greater rate of MT synthesis with the highest level of Zn^{2+} is probably required to facilitate rapid Zn^{2+} chelation, and the lower rate of MT degradation may be the result of greater Zn^{2+} saturation of MT metal-binding sites, which would render this protein less susceptible to degradative enzymes. It has been shown that the metal-free form of MT is more susceptible to lysosomal proteinases *in vitro* than is either the Zn- or Cd-containing form of MT [33] and that the rate of MT degradation is inversely proportional to cellular Zn^{2+} content [29]. A greater absolute rate of MT accretion was measured in macrophages incubated continually in $20 \mu\text{M-Cd}^{2+}$ (high Cd^{2+} /high Cd^{2+}) as compared with $10 \mu\text{M-Cd}^{2+}$ (low Cd^{2+} /low Cd^{2+}). Unlike the case with increased Zn^{2+} concentrations, the greater absolute rate of MT accretion resulting from increased Cd^{2+} concentrations was due solely to a greater absolute rate of MT synthesis (Table 1).

Changes in the rates of both MT synthesis and degradation determine MT accretion after removal of Zn^{2+} from the medium. When macrophages accreted MT in response to $50 \mu\text{M-Zn}^{2+}$ and were then incubated in medium not supplemented with trace elements (high Zn^{2+} /0), the quantity of MT accreted decreased over time (Table 1). As compared with macrophages incubated with high Zn^{2+} /high Zn^{2+} , the decrease in MT accretion after Zn^{2+} removal was due to a decreased absolute rate of MT synthesis and an increased fractional rate of MT degradation. Karin *et al.* [12] have shown that the rate of isotope incorporation into MT decreased in HeLa cells after Zn^{2+} -supplemented medium was changed to one that was not supplemented with this trace element. Furthermore, they found that the MT degradative rate transiently increased 3-fold in HeLa cells for 8 h after Zn^{2+} -supplemented medium was changed to one that was not supplemented. Similar effects of Zn^{2+} removal on MT degradation have been reported in hepatocyte monolayers [29]. The fractional rate of MT degradation, we measured in macrophages incubated with high Zn^{2+} /0 remained constant ($r^2 = 0.92$) throughout the 12 h it was measured. The increased Zn-MT degradative rate we and others [12,29] have seen upon switching to medium not supplemented with Zn^{2+} may be due to a decrease in Zn^{2+} saturation of MT metal-binding sites.

When macrophages accreted MT in response to $50 \mu\text{M-Cd}^{2+}$ and then were incubated in medium not supplemented with any trace element (Cd^{2+} /0), absolute and fractional rates of MT accretion were less than in cells that were incubated with high Cd^{2+} /high Cd^{2+} (Table 1). This was due to a decrease in the absolute rate of MT synthesis upon removal of Cd^{2+} from incubation medium. The fractional rate of MT degradation was not changed by Cd^{2+} removal, in contrast with that seen upon Zn^{2+} removal.

When comparing results from macrophages incubated with high Zn^{2+} /0 or high Cd^{2+} /0, it is apparent that the MT degradative rate exceeded its synthetic rate after Zn^{2+} , but not after Cd^{2+} , removal (Table 1). This supports observations that MT degradation results in less cellular loss of Cd^{2+} as compared with Zn^{2+} [17,18].

When macrophages induced to accrete MT by Zn^{2+} were subsequently exposed to Cd^{2+} (high Zn^{2+} /high Cd^{2+}), absolute and fractional rates of MT accretion decreased as compared with cells incubated with high Zn^{2+} /high Zn^{2+} or high Cd^{2+} /high Cd^{2+} (Table 1). This was due to a decreased absolute rate of MT synthesis with no change in the fractional rate of MT degradation. In contrast, when macrophages induced with Cd^{2+} were subsequently exposed to Zn^{2+} (high Cd^{2+} /high Zn^{2+}), the absolute rate of MT accretion increased by almost 2-fold as compared

with cells incubated with high Zn^{2+} /high Zn^{2+} or high Cd^{2+} /high Cd^{2+} . This was due to an increased absolute rate of MT synthesis with no change in the fractional rate of MT degradation.

Previous studies indicate that Cd-MT has a substantially lower degradative rate than Zn-MT *in vivo* [16–18]. In general, our results show that Cd-MT is more resistant to degradative processes than is Zn-MT. Macrophages incubated with low Zn^{2+} /low Zn^{2+} had a greater fractional rate of MT degradation than those exposed to any level of Cd^{2+} . Macrophages incubated with high Zn^{2+} /0 had a greater fractional rate of MT degradation than those incubated with high Cd^{2+} /0. It is noteworthy that we did not obtain significantly ($P > 0.05$) different fractional rates of MT degradation between cells exposed to any level of Cd^{2+} and those incubated with high Zn^{2+} /high Zn^{2+} . We speculate that this may be due to greater saturation of MT by Zn^{2+} when cells were incubated with high Zn^{2+} /high Zn^{2+} as opposed to low Zn^{2+} /low Zn^{2+} . Thus the level of Zn^{2+} saturation of MT metal-binding sites at $50 \mu\text{M-Zn}^{2+}$ may be sufficient to make the Zn-MT as resistant to degradative processes as Cd-MT. Although we did not measure MT saturation, the percentage MT saturation can be calculated by dividing the amount of Zn^{2+} or Cd^{2+} available for binding (total quantity in medium) by the quantity of Zn^{2+} or Cd^{2+} that could be bound by the amount of MT accreted (μmol of MT $\times 7 \text{ mol}$ of Zn^{2+} /mol of MT). By calculation, there was a 4-fold greater Zn^{2+} saturation of accreted MT when macrophages were incubated with high Zn^{2+} /high Zn^{2+} as compared with low Zn^{2+} /low Zn^{2+} and a 1.6-greater Cd^{2+} saturation of accreted MT when macrophages were incubated with high Cd^{2+} /high Cd^{2+} as compared with low Cd^{2+} /low Cd^{2+} .

By converting fractional-rate-of-degradation (k_d) values into half-life ($t_{1/2}$) values by the equation:

$$t_{1/2} = \ln 2 / k_d \quad [28]$$

we measured a $t_{1/2}$ of 22 h when macrophages were incubated with low Zn^{2+} /low Zn^{2+} . This compares with 18 h for rat liver Zn-MT when measured *in vivo* [17]. When macrophages were incubated in medium with low Cd^{2+} /low Cd^{2+} , a $t_{1/2}$ of 50 h was measured. This compared with 86 h for rat liver Cd-MT [16,18]. When macrophages were incubated with high Zn^{2+} /0, a $t_{1/2}$ of 15 h was measured. Chen & Failla [29] reported a $t_{1/2}$ of 11 h for MT in rat hepatocyte cultures after the removal of Zn^{2+} from the medium. In cultured HeLa cells, the $t_{1/2}$ of Zn-MT was 36 and 11 h with Zn^{2+} -supplemented and -unsupplemented media respectively [12].

These experiments demonstrate that the rate of MT accretion is controlled by changes in both synthesis and degradation rates. In general, increases in MT accretion rates are a result of increased rates of synthesis and decreased rates of degradation. Under some circumstances, such as exposure to high levels of Cd^{2+} , changes in synthetic rates drive changes in rates of MT accretion. In other circumstances, such as removal of Zn^{2+} from the medium, changes in degradative rates appear to be important in determining changes in MT accretion rates.

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