Rates of Metallothionein Synthesis, Degradation and Accretion in a Chicken Macrophage Cell Line (43072)

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Abstract. **To understand the regulation of metallothionein (MT) accretion in a chicken-macrophage cell line, fractional rates of MT synthesis (FRS) and degradation (FRD) were measured by following decay kinetics of [^{35}S]cysteine in MT. To obtain valid measurements, we added various amounts of cysteine to medium to ensure that the isotope tracer was adequately diluted after MT was labeled in the presence of various levels of zinc. We also demonstrated that the measured fractional rate of MT accretion closely approximated the difference between FRS and FRD. All fractional rates were similar for the two MT isoforms isolated. FRD did not change during the 27-hr decay period, but FRS and fractional rate of MT accretion changed over time. FRS of MT was 0.097 and 0.012 hr^{-1} from 0 to 9 and 9 to 27 hr, respectively, after cells were incubated in medium supplemented with 50 μM zinc. FRD of MT was 0.020 hr^{-1}. Addition of 1100 μM unlabeled cysteine to medium supplemented with 50 μM zinc increased FRD and decreased FRS and fractional rate of MT accretion, as compared with not adding cysteine. Overall, these results indicate that rates of MT synthesis and degradation can both regulate MT accretion. Further experiments with various amounts of zinc and cysteine added to medium suggested that the effect of added cysteine on MT fractional rates was due to chelation of unbound zinc. Elimination of the cysteine effect on MT fractional rates was accomplished by adding more zinc to cysteine-supplemented medium. Thus, the concentration of unbound zinc affects the rates of MT synthesis and degradation.** [P.S.E.B.M. 1990, Vol 194]

Metallothioneins (MT) are small molecular weight metal-binding proteins which contain 33 mol% cysteine, but no phenylalanine or tyrosine (1–6). They are found in abundance in tissues of many species (1–9) and are easily measured using a variety of techniques (10–15).

A MT gene is expressed in response to certain trace elements or hormones (7–9). Upon MT gene expression, the amount of MT mRNA and MT protein quickly increases from low or nondetectable levels (7–11, 16, 17). MT rapidly chelates trace elements and slowly releases them over time. A clear understanding of the regulation of MT accretion would provide insight into the nutritional, toxicologic, and immunologic functions of this protein.

MT accretion is regulated by the opposing processes of synthesis and degradation. An understanding of MT accretion requires accurate and simultaneous measurements of fractional rates of MT synthesis (FRS) and MT degradation (FRD). Decay kinetic technique (18) is the method of choice for estimating FRS and FRD of proteins in the same experiment; however, limitations of the technique must be acknowledged and mitigated. A major source of error is the incorporation of labeled amino acids into MT during the decay period. FRS and FRD of MT are underestimated if, during their measurements, labeled amino acids from the degradation of labeled proteins are reutilized for MT synthesis. This source of error is reduced by adding enough unlabeled amino acid (18; chaser) so that, by dilution, amounts of isotopically labeled amino acid reincorporated into MT are minimal. The addition of a chaser amino acid is better suited for cell-culture systems than *in vivo* because greater than physiologic concentrations

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of unlabeled amino acids must be maintained to keep isotope reincorporation into MT at a minimum during the hours required to measure FRS and FRD of MT. Conditions used to minimize incorporation of label during the decay period must not influence rates of MT synthesis and degradation.

Decay kinetic techniques have been used to measure FRD of MT *in vivo* (19–26) and *in vitro* (16, 27–29). Interpretation of these studies was compromised by uncertainty regarding amounts of isotope reincorporated into MT during the time FRD was measured.

Isotope incorporation techniques have been used to measure MT synthetic rates *in vivo* (7, 23, 30–32) and *in vitro* (16, 33). The intracellular specific activity of isotopically labeled amino acid was not measured in these studies, thereby preventing calculation of the FRS of MT. Furthermore, FRD was not determined so that the balance of FRS and FRD can be used to assess their contribution to the regulation of MT accretion.

To more fully understand the regulation of MT accretion, we used decay kinetics to simultaneously measure its FRS and FRD in a chicken-macrophage cell line. These cells rapidly accrete two MT isoforms in response to zinc supplementation of medium. The validity of the technique used to measure FRS and FRD is based upon studies which examine various levels of isotope dilution during the decay period.

Materials and Methods

Cell Culture. Chicken HD11 macrophages transformed by a *myc*-containing MC29 retrovirus (34) were used in all experiments. The adherent macrophages were grown in canted neck flasks (Corning Glass Works, Corning, NY) and cultured in RPMI 1640 + 25 m*M* Hepes (Sigma Chemical Co., St. Louis, MO) supplemented with 5% fetal bovine serum (Irvine Scientific, Santa Ana, CA) and 50 mg/liter gentamicin-sulfate (Gibco Laboratories, Grand Island, NY). The cells were incubated in a humidified, 5% CO_2 atmosphere at 41.5°C and medium was changed every 48 hr. Approximately, 2 days prior to the start of each experiment, the macrophages were scraped from the flasks and 1.08×10^7 cells in 5 ml of the same medium were added to replicate 35-mm cell wells (Corning Glass Works).

Isolation and Quantitation of MT. MT was isolated by using a reverse phase (RP) high-performance liquid chromatography (HPLC) procedure developed by Richards and Steele (11). This procedure purifies two MT isoforms from macrophage cytosol to 94% purity (12). To isolate MT, macrophage cytosol was heated at 60°C for 10 min and centrifuged at 16,000*g* for 20 min. The supernatant was injected into a Pecosphere-3C C_{18} cartridge column (0.46 × 8.3 cm) with a 3-μm particle size (Perkin-Elmer Corp., Norwalk, CT). The column was maintained at ambient temperature and equilibrated with 10 m*M* NaH_2PO_4 (pH 7.0). MT was eluted at 1 ml/min with a two-step linear gradient consisting of 0–10% 10 m*M* NaH_2PO_4 + 60% CH_3CN (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 the MT-containing peaks were integrated by a Maxima-820 chromatography workstation (Millipore Corp., Waters Chromatography Division).

Purified chicken MT was used as the MT standard. It was purified from heat-treated hepatic cytosols using successive low-pressure molecular weight sizing and ion exchange chromatographic techniques, as described previously (12), followed by final purification by RP-HPLC.

Calculation of MT Fractional Rates. FRD of MT was calculated as the slope of the regression of natural logarithm of [^{35}S]cysteine dpm (total activity) in MT versus time. FRS of MT was calculated as the slope of the regression of natural logarithm of specific activity versus time. Specific activity was calculated by dividing total activity by the quantity of MT. To determine fractional rate of MT accretion (FRA), the quantity of MT was regressed as a function of time and the average quantity of MT present during the decay period was calculated from this regression equation. The FRA of MT was calculated by dividing the slope of the regression relationship by the calculated average quantity of MT. Since the difference between FRS and FRD should equal FRA, any error in our measurements would be evident. Differences due to treatment for each of the fractional rates were statistically determined by linear contrasts (SAS Institute Inc., Cary, NC).

Experiment 1. When using decay kinetics, underestimation of FRS and FRD of MT can be avoided if the isotopically labeled amino acid is made unavailable for MT synthesis during the time of measurement. This is often done by adding a sufficient quantity of unlabeled amino acid (18), as a chaser, to reduce the availability of labeled amino acid for MT synthesis. In Experiment 1, we simultaneously measured FRS, FRD and FRA of MT in the presence of various amounts of unlabeled cysteine added to the medium.

Macrophages were induced to accrete maximal amounts of MT by adding 50 μ*M* $ZnSO_4$ to the medium (12) in the presence of 5 μCi of [^{35}S]cysteine/well (1010 Ci/mmol; ICN Biomedicals Inc., Costa Mesa, CA). After 16-hr incubation, the cells in six wells were lysed with a buffer containing 3 m*M* NaH_2PO_4 (pH 8.0), 0.2% Nonidet P-40, 0.5 μ*M* phenylmethyl-sulfonyl fluoride, 10 μg/liter phosphoramidon, 10 μg/liter pepstatin, and 250 ng/liter cystatin (Sigma Chemical Co.) and scraped from their wells. Complete cell lysis was ensured by freezing and thawing the homogenate three times. The macrophages in other wells were washed and replenished with zinc-supplemented medium without [^{35}S]cysteine but with either 0, 1100, 2750, or 4400

μM unlabeled cysteine to provide isotope dilution during the decay period. After 3-, 9-, and 27-hr incubation, three replicate wells of macrophages per treatment were then lysed, harvested, and frozen and thawed. All samples were heat treated and supernatants injected into the RP-HPLC column and MT quantified. The MT isoforms were individually collected in liquid scintillation vials and the isotopes counted using a model 6880 liquid scintillation counter (Searle Analytic, Inc., Des Plaines, IL).

Experiment 2. High levels of unlabeled cysteine added to medium to prevent incorporation of [^{35}S] cysteine during the decay period must not have an influence on the rate of MT synthesis. If the addition of unlabeled cysteine only reduces the incorporation of [^{35}S]cysteine into MT, values for FRS and FRD should increase equally. Since opposite effects on these fractional rates were observed upon cysteine supplementation of medium and FRA was markedly changed in Experiment 1, it was apparent that added cysteine had an effect other than intended. The observed decrease in FRS upon cysteine addition indicates either zinc chelation or cysteine toxicity.

To determine whether zinc chelation or cysteine toxicity was responsible for the observed effects of added cysteine in Experiment 1, a factorial experiment was designed with two and six levels of cysteine and zinc added to medium, respectively. There were three replicate wells of macrophages per treatment. After 16-hr incubation, macrophages in all wells were lysed, harvested, and MT isoforms isolated from heat-treated cytosols as described above. Data were analyzed by analysis of variance and differences among treatment means were determined by the least-significant differences test (SAS Institute Inc.). It was necessary to report the results per cell well rather than protein or DNA due to limitations in the volume of cytosol obtained. All of the cytosol was necessary for MT determination.

Experiment 3. The results of Experiment 2 indicated that cysteine supplementation of media resulted in zinc chelation. To compensate for zinc chelation, zinc concentrations were adjusted to ensure similar rates of MT accretion in macrophages regardless of amount of cysteine added to medium.

Macrophages were cultured in media supplemented with 2 μCi of [^{35}S]cysteine well and either 50 μM zinc and no added cysteine or 70 μM zinc and 550 μM added cysteine. After 16-hr incubation, the cells were washed and replenished with the previous medium with but without [^{35}S]cysteine. After 3-hr incubation to allow intracellular [^{35}S]cysteine to be diluted, medium in each well was renewed. Following 0, 3, and 9 hr of further incubation, macrophages from three replicate wells per treatment were lysed, harvested, and MT isoforms isolated, and activity was determined.

The medium containing 50 μM zinc was selected because Experiment 2 showed that it was the lowest concentration of zinc that maximized macrophage-MT accretion when no cysteine was added to medium. The medium supplemented with 70 μM zinc was selected because macrophages attained maximum MT accretion when this medium was further supplemented with 550 μM cysteine, as determined in Experiment 2. In the presence of 550 μM cysteine, concentrations of zinc greater than 70 μM either did not change or decrease the amount of MT accreted (unpublished data).

Experiment 4. An alternate way to minimize incorporation of [^{35}S]cysteine into MT during the decay period, when FRS and FRD of MT are measured, is to decrease the amount of [^{35}S]cysteine added to medium during the labeling period. This would decrease the amount of [^{35}S]cysteine incorporated into cell protein and therefore result in greater dilution of [^{35}S]cysteine arising from the degradation of labeled protein during the decay period in the presence of a constant concentration of unlabeled cysteine in chase medium.

Macrophages were cultured in medium supplemented with 80 μM zinc and 1100 μM cysteine and either 1 or 3 μCi of [^{35}S]cysteine/well. MT fractional rates were measured as described for Experiment 3.

Results

RP-HPLC resolved two MT isoforms from chicken-macrophage cytosol (11, 12). The first to elute from the column is the most prevalent isoform. The second MT isoform that elutes is present at approximately 15% of the amount of the first MT isoform. FRS, FRD, and FRA for these two isoforms were similar in all experiments, thus, only data for the first MT isoform eluted from RP-HPLC are presented.

Experiment 1. To minimize isotope reincorporation into MT during the time that fractional rates were measured, various amounts of unlabeled cysteine were added to medium. Cysteine addition significantly ($P < 0.01$) affected FRS, FRD, and FRA of MT when compared with cells cultured in medium without supplemental cysteine (Fig. 1). FRS and FRA of MT were decreased and FRD was increased by cysteine supplementation of medium. Opposite effects of cysteine supplementation on FRS and FRD of MT indicate that cysteine is affecting at least one of these rates by a mechanism different from minimizing [^{35}S]cysteine reincorporation into MT.

Both FRS and FRA were calculated from separate linear-regression relationships for each treatment. This was necessary because FRS and FRA of MT did not remain constant during the time of measurement. Assuming 7 mol of zinc/mol of MT and a MT M_r of 6500 daltons, the net accretion of both MT isoforms from 0 to 9 hr would theoretically chelate over 95% of zinc that was present in the medium. This calculation indicates that FRS and FRA of MT decreased after 9 hr

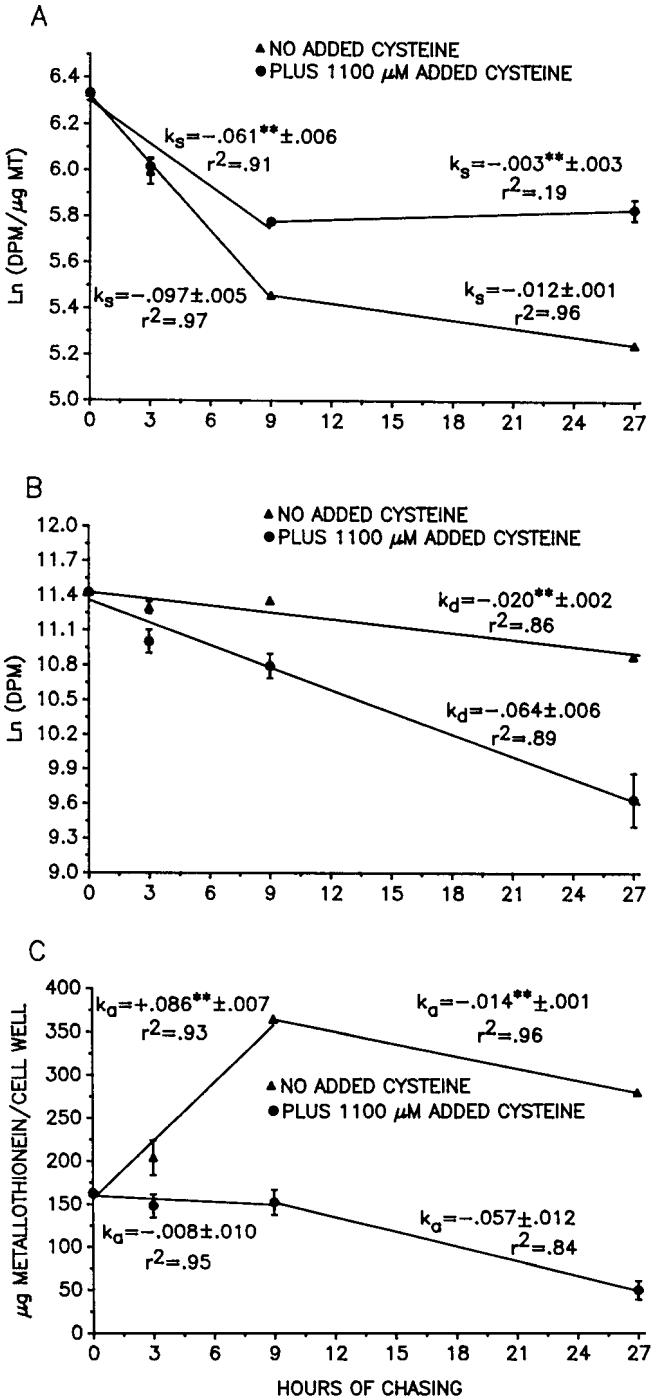

Figure 1. Macrophages per well (1.08×10^7) were incubated for 16 hr in medium supplemented with 50 μM zinc and 5 μCi of [^{35}S] cysteine/well. Macrophages from six replicate wells were then harvested. The cells in other wells were replenished with medium supplemented with 50 μM zinc and either 0 or 1100 μM unlabeled cysteine. At 3, 9, and 27 hr, three replicate wells per treatment were harvested. MT was isolated from heat-treated cytosol by RP-HPLC and fractional rates of synthesis (k_s) and degradation (k_d) were determined for each treatment from the slope of the decrease in specific (A) and total (B) activities of [^{35}S]cysteine in MT over time, respectively. The fractional rate of MT accretion (k_a) was determined for each treatment by dividing the slope of MT accretion over time (C) by its average accretion. ** Indicates significant ($P < 0.01$) treatment differences between fractional rates.

due to the removal of the zinc stimulus through chelation by apo-MT. FRD of MT, in contrast to FRS and FRA, remained constant over time. When 2750 or 4400 μM unlabeled cysteine was added to medium, the FRD, FRS, and FRA were not different from that obtained when 1100 μM was added (data not shown).

Experiment 2. Comparison of MT accretion in macrophages incubated 16 hr in medium supplemented with different amounts of zinc and cysteine enabled us to determine if cysteine supplementation affected MT fractional rates in Experiment 1 by chelating zinc or by being toxic to cells.

The data in Figure 2 indicate that cysteine supplementation of medium results in zinc chelation. A greater concentration of zinc in cysteine-supplemented medium was required to attain the same maximal accretion of MT achieved in macrophages cultured in medium not supplemented with cysteine. Levels of zinc above 70 μM in cysteine-supplemented medium did not further increase accretion of either isoform of MT (data not shown), indicating maximal accretion at 70 μM zinc.

Experiment 3. The results of Experiment 2 showed that cysteine chelation of zinc was responsible for the effect of supplemental cysteine on MT fractional rates measured in Experiment 1. To avoid this effect of supplemental cysteine on MT fractional rates, we added more zinc to cysteine supplemented medium. Furthermore, by adding equal quantities of [^{35}S]cysteine to media differing in cysteine concentration, we varied the dilution of the isotope by unlabeled cysteine.

The FRS and FRD of MT, measured from 0 to 9 hr, were not significantly ($P > 0.05$) different whether macrophages were cultured in zinc-supplemented or zinc and cysteine-supplemented media (Fig. 3). The degradative rates obtained in Experiment 3 are similar

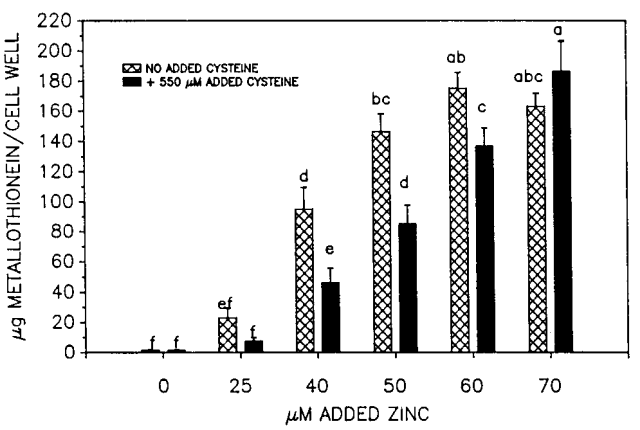

Figure 2. Macrophages per well (1.08×10^7) were incubated for 16 hr in medium supplemented with the indicated concentrations of zinc and cysteine. The cells were then harvested and MT was quantified by RP-HPLC of heat-treated cytosol. Each mean represents three replicates and a different letter above each bar denotes a significant ($P < 0.05$) mean difference.

to those measured in Experiment 1 with macrophages incubated in medium that was not supplemented with cysteine.

FRA of MT was significantly ($P < 0.05$) affected by treatment (Fig. 3). The difference in these measurements is surprising because no treatment differences were apparent for either FRS or FRD of MT. Since accretion is dependent on opposing synthetic and degradative processes, it is understandable that small and opposite changes in these two processes can greatly affect accretion.

Experiment 4. The FRS, FRD, and FRA of MT were not affected ($P > 0.05$) by amount of isotope added to each well (Fig. 4). The specific activity of the isotope when 3 μCi were added per well was similar to that in zinc and cysteine-supplemented medium used in Experiment 3.

Discussion

Valid measurements of FRS and FRD by decay kinetics require that the incorporation of labeled amino acids into MT during the decay period is minimal and that any attempts to block incorporation of label during the decay period do not effect rates of MT synthesis or degradation. Most investigators employ [^{35}S]cysteine as a label because this amino acid is highly enriched in MT compared with other proteins. Blocking incorporation of label during the decay period is usually accomplished by adding high levels of unlabeled cysteine to medium during the decay period. In Experiment 1, we found this approach to be inappropriate for minimizing [^{35}S]cysteine incorporation into MT during the time FRS and FRD of MT are measured. If isotope incorporation during the decay period occurs, measured values of FRS and FRD are lower than true values and preventing this artifact should result in estimates of FRS and FRD which increase equally. FRA should not be influenced by cysteine supplementation to media used during the decay period. Since measured values of FRS and FRD changed in opposite directions and FRA decreased with cysteine addition to medium, it is apparent that artifacts were introduced. Presumably, the increase in FRD of MT upon cysteine supplementation of medium in Experiment 1 was due to the ability of cysteine to chelate zinc (35). Cysteine chelation of zinc would quickly decrease the concentration of unbound zinc, resulting in disturbance of the equilibrium between MT-chelated zinc and unbound zinc. This disturbance may favor the release of zinc from MT and result in MT being more susceptible to degradation. Apo-MT has been found to be more susceptible to degradation in lysosomal extracts than metal-bound forms of this protein (36). Lower levels of unbound zinc would also decrease MT mRNA and MT protein synthesis. Using HD11 macrophages, we have found that removal of 50 μM zinc in medium significantly

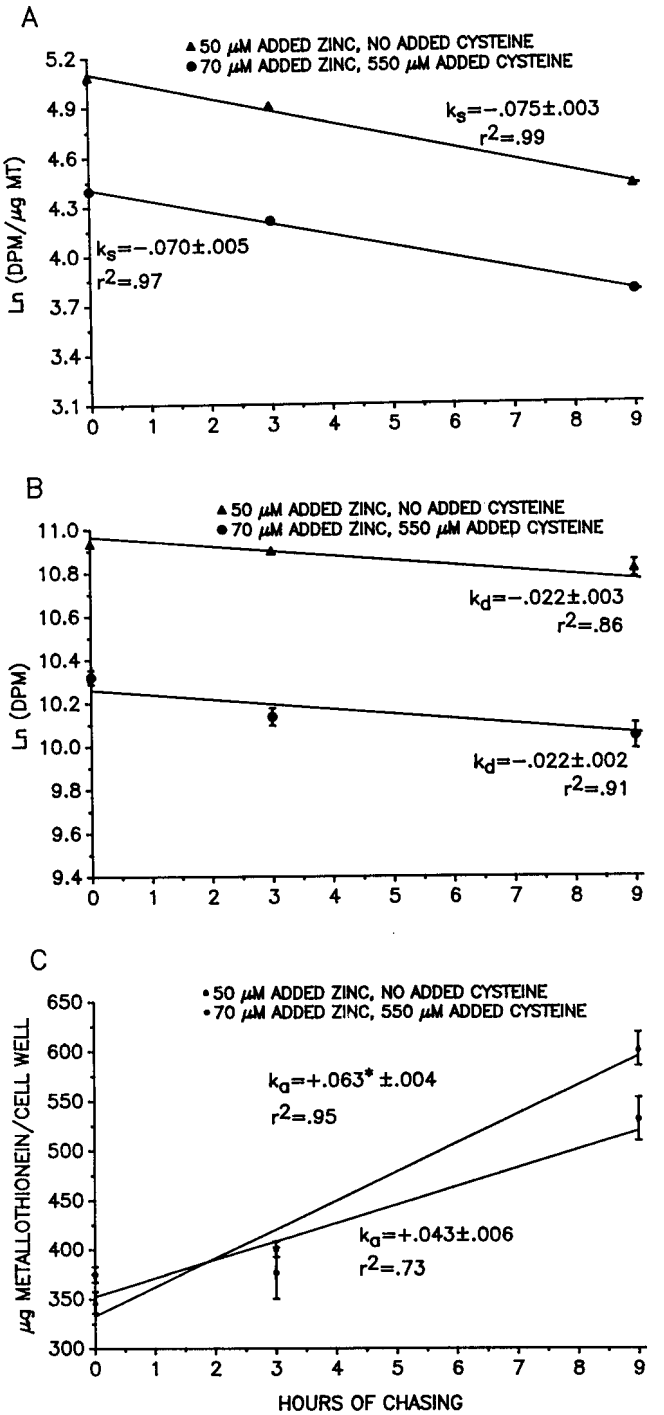

Figure 3. Macrophages per well (1.08×10^7) were incubated for 16 hr in medium supplemented with 2 μCi of [^{35}S]cysteine/well and the indicated concentrations of zinc and cysteine. To remove free label, cells were incubated an additional 3 hr in medium without [^{35}S] cysteine. Medium was again changed and after 0, 3, and 9 hr, macrophages from three replicate wells per treatment were harvested. The concentration of zinc and cysteine in medium was the same throughout the labeling and decay periods. MT was isolated from heat-treated cytosol by RP-HPLC and fractional rates of MT synthesis (k_s) and degradation (k_d) determined for each treatment from the slope of the decrease of specific (A) and total (B) activities in MT, respectively. The fractional rate of MT accretion (k_a) was determined for each treatment by dividing the slope of MT accretion (C) by its average accretion. * Indicates significant ($P < 0.05$) treatment differences between fractional rates.

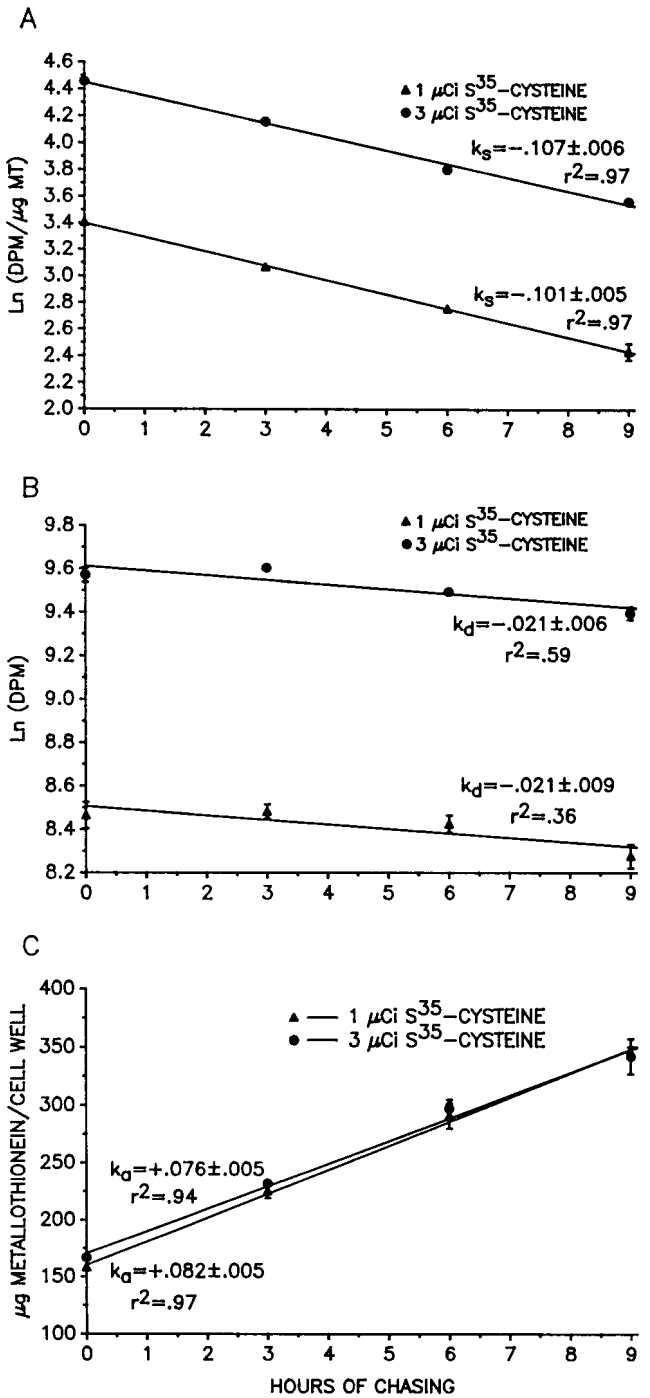

Figure 4. Macrophages per well (1.08×10^7) were incubated for 16 hr in medium supplemented with 80 μM zinc, 1100 μM cysteine, and either 1 or 3 μCi of [^{35}S]cysteine. To remove free label, cells were incubated 3 hr in medium without [^{35}S]cysteine. Medium was again changed and after 0, 3, 6, and 9 hr, macrophages from three replicate wells per treatment were harvested. The concentration of zinc and cysteine in medium was the same throughout the labeling and decay periods. MT was isolated from heat-treated cytosol by RP-HPLC and fractional rates of MT synthesis (k_s) and degradation (k_d) determined for each treatment from the slope of the decrease of specific (A) and total (B) activities in MT, respectively. The fractional rate of MT accretion (k_a) was determined for each treatment by dividing the slope of MT accretion (C) by its average accretion. No significant ($P > 0.05$) treatment differences between fractional rates were detected.

($P < 0.05$) decreases and increases FRS and FRD of MT, respectively, as compared with macrophages continually incubated with 50 μM zinc (37). Together, these changes explain the observed decline in FRA upon cysteine addition to the medium.

Results of Experiment 1 show the importance of measuring both total and specific activities as well as accretion rates when validating isotope-chasing techniques. For instance, had total activity only been considered (measuring FRD only), we would have been misled into assuming that cysteine supplementation of medium was necessary to minimize isotope reincorporation into MT to obtain an accurate FRD of MT. Karin *et al.* (16) supplemented medium containing 37 μM zinc with 10 mM cysteine in order to minimize reincorporation of [^{35}S]cysteine into MT when its degradative rate was measured in cultured HeLa cells by decay kinetics. Because they did not simultaneously measure MT synthesis using this technique or compare their degradative measurement with one obtained from cells cultured in less cysteine, it cannot be determined if cysteine chelation of zinc influenced the MT degradative rate.

When the chelation of zinc by added cysteine was ameliorated by augmenting the level of zinc in the medium, measured rates of FRS and FRD were not changed. Experiment 3 demonstrates that adding additional levels of cysteine during the decay period was not needed to minimize isotope incorporation during the decay period. Experiment 4 demonstrates that isotope incorporation into MT during the decay period cannot be further decreased by reducing the ratio of [^{35}S]cysteine to unlabeled cysteine during the decay period. In all experiments, we obtained similar estimates of MT degradation except in Experiment 1 when cysteine was added to medium without further zinc supplementation. Based upon the constancy of MT degradative rates, within and among experiments, it appears that cysteine supplementation of medium is unnecessary to prevent incorporation of label during the decay period. The unsupplemented medium contained 416 μM cysteine, which is approximately twice the amount in chick plasma (38).

In Experiment 1, MT accretion increased from 0 to 9 hr and then decreased. This is due to the FRS of MT being greater and less than its FRD from 0 to 9 hr and 9 to 27 hr, respectively. The rate of isotope incorporation into MT has been shown to increase, then later decrease with time (7, 16, 30, 32). It appears that MT is rapidly accreted in response to added zinc, so that it can quickly chelate and lower the concentration of unbound zinc. Our calculations for Experiment 1 show that by 9 hr, sufficient MT had been accreted to bind 95% of zinc in the medium. Apparently, after 9 hr, the remaining unbound zinc was insufficient to induce high rates of MT synthesis. When the rate of

MT synthesis fell below its rate of degradation, the quantity of MT decreased. Perhaps a steady-state level of MT accretion can be achieved when enough zinc, released by MT degradation, stimulates MT resynthesis at the same rate that MT degradation occurs. The kinetics of MT accretion over time was different from that observed for tryptophan pyrollase (39) and arginase (40). These proteins, in contrast to MT, do not increase and then decrease in quantity before attaining a steady-state level of accretion. The kinetics for MT accretion are reasonable when one considers that high concentrations of zinc required to induce MT synthesis are potentially toxic to cells, thus, rapid chelation of zinc by MT is necessary.

FRD of MT, unlike FRS, did not change during the 27-hr decay period. Thus, the degradative rate of MT was not as sensitive as its synthetic rate to decreases in unbound zinc concentrations that occurred as a result of zinc chelation by newly synthesized apo-MT. In fact, changes in the degradative rate of MT only played a role in regulating MT accretion in Experiment 1 when an acute change in unbound zinc concentration was caused by cysteine addition to medium. Analogous to our results, the degradative rate of MT in cultured HeLa cells increases 3-fold upon zinc removal from medium (16). We have also seen, in our macrophage cell-line, an increase in the degradative rate of MT when 50 μM zinc was removed from medium (37). This lends support to the concept that rates of MT synthesis and degradation can both regulate MT accretion.

The half-life of MT ($t_{½}$) can be calculated from FRD values by the relationship $t_{½} = \ln 2/\text{FRD}$ (18). The $t_{½}$ that we have obtained for MT in Experiments 3 and 4 (32 hr) is similar to that reported for zinc-induced MT in HeLa cells (16). Our estimate of $t_{½}$, however, is approximately twice that measured *in vivo* in livers of zinc-injected rats (20). In Experiment 1, unbound zinc was effectively depleted by adding high levels of cysteine to medium, resulting in a marked depression in $t_{½}$ to 11 hr, which is identical to the $t_{½}$ in hepatocytes incubated in low zinc media *in vitro* (27).

By using RP-HPLC, we isolated two isoforms of MT from chicken-macrophage cytosol. We obtained similar fractional rates for each of these isoforms. Studies in the chicken have shown that there is only one MT gene (5). It is possible that two isoforms exist because of posttranslational modifications, although this has not been shown for MT in the past. Further work is required to reconcile these observations.

By simultaneously measuring FRS and FRD of MT, we have determined that MT accretion can be regulated through experimental treatment by both its rates of synthesis and degradation. FRS and FRA of MT did not remain constant, unlike FRD, when measured over 27 hr. When cysteine was added to medium supplemented with 50 μM zinc, all MT fractional rates were affected as compared with not adding cysteine to medium. These data suggest that both rates of MT synthesis and degradation are sensitive to unbound zinc concentrations and are possibly adjusted in order to maintain the concentration of unbound zinc in an appropriate range. Opposite changes in the FRS and FRD of MT permit rapid adjustments in MT accretion.

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