Differential selenium-dependent expression of type I 5'-deiodinase and glutathione peroxidase in the porcine epithelial kidney cell line LLC-PK₁

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The Se-dependent expression of two selenoproteins, cytosolic glutathione peroxidase (cGPx) and type I iodothyronine-5'-deiodinase (5'DI), was investigated in the porcine epithelial kidney cell line LLC-PK₁ in serum-free medium. The selenite-dependent expression of cGPx and 5'DI was revealed by enzyme-activity measurements, affinity labelling of 5'DI, metabolic labelling of proteins with ⁷⁵Se and steady-state mRNA analysis. The expression of the two enzymes strongly depended on selenite concentrations of the culture medium. cGPx required 2-fold higher selenite levels than 5'DI to reach half-maximal activity. The Se-dependent enzyme activities were approximately paralleled by the corresponding steady-state mRNA levels. The response of the two enzymes to Se supply was further characterized by kinetic Se-depletion and -repletion experiments. Upon

removal of medium selenite, cGPx activity decreased exponentially, whereas after an initial decrease over 1–2 days, 5'DI levels completely recovered during a further 2 days. These data indicate a differential Se-dependent regulation of the two selenoproteins, with 5'DI being preferentially supplied with the trace element Se, thus ensuring a continuous cellular capacity for thyroid-hormone activation, even under Se-deficient conditions. The abundant cGPx in cells with sufficient Se supply might serve as a cellular Se store which can be mobilized for the synthesis of more vital selenoproteins such as 5'DI under shortage conditions. Thus, a cellular hierarchy of selenoprotein expression, reflected by different individual regulation mechanisms at the transcriptional and post-transcriptional level, adds to the previously recognized tissue-specific hierarchy of Se retention.

INTRODUCTION

Se is an essential trace element for mammals [1]. Tissue Se is primarily found associated with proteins, but Se-containing transfer ribonucleic acids are also known [2–5]. Se is specifically incorporated into several distinct proteins as selenocysteine (Sec), utilizing a unique translation mechanism which recognizes an in-frame UGA *opal* stop codon as the Sec codon, together with specific downstream mRNA secondary structures called 'selenocysteine-inserting elements' [3,6–10]. Se also occurs randomly substituted for sulphur in the biosynthesis of selenomethionine-containing proteins [2].

The first identified mammalian selenoprotein is the cytosolic glutathione peroxidase (GPx; EC 1.11.1.9), belonging to an antioxidant enzyme family encompassing at least four members: the cytosolic (cGPx), the plasma (pGPx), the gastrointestinal and the phospholipid-hydroperoxide (PHGPx) glutathione peroxidases [11–17]. The cytosolic and plasma forms are homotetramers of 21 000–23 000-*M_r* subunits each with one Sec residue in the active site, whereas PHGPx is an 18 000-*M_r* monomer. As the second mammalian enzyme, type I iodothyronine deiodinase (5'DI) was identified as a selenoprotein [18–23]. 5'-DI is primarily found in liver, kidney and thyroid gland, catalysing the 5'-deiodination of L-thyroxine (T₄) to tri-iodo-L-thyronine (T₃), the thyromimetically active form of thyroid hormone [24]. 5'-DI, an integral membrane protein, is located in the cytosolic leaflet of the plasma membrane in kidney cells and on the cytosolic side of the endoplasmic reticulum membrane in liver [24]. The active 5'-DI enzyme is a homodimer [25] of 27 000-*M_r* subunits [26], which

contain one Sec residue each at the active site [18,21]. In all mammalian selenoenzymes so far known, the Sec residue plays a catalytically active role; like the bacterial selenoenzymes [4], all of these enzymes belong to the class of oxidoreductases.

Further mammalian selenoproteins with as-yet-unidentified functions have been partially characterized, such as the plasma selenoprotein P (SeP), which contains up to 10 Sec residues [8], selenoprotein W from rat muscle [27] and a mitochondrial capsule selenoprotein in spermatozoa [28]. ⁷⁵Se-labelling studies *in vivo* in rats indicate the existence of a total of more than 30 mammalian selenoproteins [29].

In the mammalian organism, a twofold hierarchy of Se utilization has been demonstrated *in vivo*. A comparison of the Se retention and accumulation capacities of various tissues reveals that Se is preferentially retained or accumulated in tissues such as brain, reproductive organs (testes, ovaries) and endocrine glands (thyroid, pituitary, adrenals, corpora lutea) [30,31]. The largest cellular pool of the protein-bound trace element under Se-adequate conditions is found in the cGPx fraction [31–35]. A preferential synthesis of selenoproteins other than GPx is observed under Se-deficient conditions, probably reflecting the biological importance of these proteins [7,29,31,33,36–38]. Previous experiments *in vivo* have shown that both cGPx and 5'DI expression is decreased in most organs of Se-deficient rats except thyroid, though the relative distribution of the two enzymes changed [18,24,31,39–44].

The present study focuses on the molecular hierarchy of Se distribution by examining cGPx and 5'DI expression in a homogeneous cell line. The porcine epithelial kidney cell line

Abbreviations used: ATG, aurothioglucose; BrAcT₄, N-bromoacetyl-L-thyroxine; cGPx, cytosolic glutathione peroxidase; 5'DI, type I 5'-deiodinase; pGPx, plasma glutathione peroxidase; PHGPx, phospholipid-hydroperoxide glutathione peroxidase; SeP, plasma selenoprotein P; Sec, selenocysteine; T₃, 3,3',5-tri-iodothyronine; T₄, L-thyroxine; rT₃, 3,3',5'-tri-iodothyronine.

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LLC-PK₁ growing in serum-free medium was chosen as it retains many characteristics of differentiated kidney proximal-tubule cells [45,46], such as hormonal responses, cell polarity and trans-epithelial transport, and exhibits abundant 5'DI activity [47,48]. In the present study the response of cellular 5'DI and cGPx enzyme activities, protein and mRNA levels towards various medium selenite concentrations, as well as the kinetics of Se depletion and repletion, were investigated. These data revealed differences in the Se-dependent regulation of the two selenoenzymes and provided insight on which level such a regulation might occur.

MATERIALS AND METHODS

Reagents

All reagents were of analytical or biochemical grade. Iodothyronines and *N*-bromoacetyl-L-[¹²⁵I]thyroxine (BrAc[¹²⁵I]T₄) (specific radioactivity ≥ 45 MBq/ μ g) were generously supplied by Dr. H. Rokos, Henning Berlin G.m.b.H., Berlin, Germany. L-3,3',5'-[¹²⁵I]Tri-iodothyronine (rT₃) (28–46 MBq/ μ g) was purchased from NEN—du Pont de Nemours, Bad Homburg, Germany. Sodium [⁷⁵Se]selenite of high specific radioactivity (31450 MBq/mg) was kindly provided by Dr. Zinn, Columbia, MO, U.S.A.

Cell culture

LLC-PK₁ cells were grown in 75 cm² plastic flasks or six-well plates (Nunc) at a density of 5×10^5 /cm² in a water-saturated atmosphere containing 5% CO₂ at 37 °C. As the standard medium, serum-free Dulbecco's modified Eagle medium-F12/Ham's (1:1, v/v; GIBCO—BRL) was used, supplemented with 1 mM sodium pyruvate, 250 munits/ml insulin, 10 μ units/ml vasopressin, 1 nM T₃, 200 nM hydrocortisol, 10 nM cholesterol, 25 ng/ml transferrin, and 47 nM sodium selenite [45]. Cells were split once a week at a ratio of 1:8, and were fed fresh medium on the fourth day of each passage. For biochemical assays, the cells were scraped and collected in 2–3 ml of ice-cold PBS/flask and homogenized by sonication.

Biochemical assays

5'DI activity was determined in the cell homogenates as described in [47,49,50], using ¹²⁵I-labelled rT₃ as substrate and measuring the release of radioactive iodide. Several inhibitors of 5'-deiodinases, including aurothioglucose (ATG), 6-n-propyl-2-thiouracil, iopanoic acid and the synthetic flavonoid EMD 21388 (6,4'-dihydroxy-3-methyl-3',5'-dibromoflavone) were employed to confirm the specific measurement of the type I 5'DI isoenzyme [24]. Affinity labelling of 5'DI with BrAc[¹²⁵I]T₄ was performed with the cell homogenates as described previously [26,50]. For the measurement of cGPx activity, the homogenates were centrifuged for 15 min at 13000 *g* (4 °C) to obtain crude cytosolic fractions [51]. Enzyme activity was determined spectrophotometrically in a coupled assay using 7 μ M t-butylhydroperoxide as substrate [52]. Protein concentrations were measured by the Bio-Rad dye-adsorption assay [53] with IgG as standard.

Northern-blot analyses

Total RNA was isolated from the cells by the Chirgwin method [54], electrophoresed on denaturing formaldehyde gels (1.1% agarose) and transferred on to Duralose membranes (Stratagene, Heidelberg, Germany) in a Bios (New Haven, CT, U.S.A.) diffusion blotting unit. DNA probes were labelled with

[α -³²P]dCTP (110 TBq/mmol, from Amersham, Braunschweig, Germany), using the Random Primed DNA Labeling Kit (Boehringer, Mannheim, Germany). Hybridization was performed at 42 °C by the protocol described by Maniatis and colleagues [55], and filters were washed with NaCl/sodium citrate (pH 7.0) buffers containing 0.1% SDS, using decreasing ionic strengths and increasing temperatures. The probe for 5'DI was a 774 bp PCR product amplified from rat liver cDNA with primers specific for the coding region [56]; for cGPx, an 850 bp insert from rat liver cDNA was used [57], the rat β -actin probe was a 1200 bp insert plus 150 bp from the pBS-vector (generously provided by Dr. J. Mazoub, Brigham and Women's Hospital, Boston, MA, U.S.A.), the 1378 bp probe for rat malic enzyme was provided by Dr. V. Nikodem (National Institutes of Health, Bethesda, MD, U.S.A.) [58], and the T₃ receptor probe was an endonuclease-*Xho*I-cut insert of the rat T₃R- α_1 pBS-KS plasmid provided by Dr. W. Chin (Brigham and Women's Hospital, Boston, MA, U.S.A.) [59]. The last probe specifically hybridized to the mRNA of the T₃R- α_2 isotype in LLC-PK1 cells, as indicated by the size of the labelled band. The cDNA probe clone 16C1 [60] for the rat plasma SeP was obtained from Dr. K. Hill (Vanderbilt University School of Medicine, Nashville, TN, U.S.A.) and the PIP349 plasmid containing a 349 bp cDNA insert of the rat PHGPx [10] was kindly provided by Dr. R. Brigelius-Flohé (Deutsches Institut für Ernährungs-forschung, Potsdam, Germany).

Dependence of cGPx and 5'DI expression on medium selenite concentration

To determine the Se-dependent expression of 5'DI and cGPx, LLC-PK₁ cells were seeded and cultured in serum-free medium containing graded selenite concentrations ranging from 0 to 1000 nM for 4 days. The cells were harvested for measurement of 5'DI and cGPx activities and for BrAc[¹²⁵I]T₄ affinity labelling of 5'DI. Total RNA was isolated from another aliquot of the cells and utilized for Northern-blot analysis.

Selenite repletion

The cells were seeded and cultured in selenite-free medium for 4 days, subsequently fed standard medium containing 47 nM selenite and harvested after defined time periods from 0 to 96 h. 5'DI and cGPx enzyme activities were measured, and 5'DI protein contents were analysed by affinity labelling.

Selenite depletion

The cells were grown in standard medium for 4 days before they were washed extensively and fed Se-free medium. The Se-depleted cells were harvested after time periods between 0 and 96 h.

Metabolic labelling with ⁷⁵Se

For the selective radioactive labelling of selenoproteins, the cells were precultured in medium containing approx. 50 nM sodium [⁷⁵Se]selenite for 4 days before they were washed and depleted of Se as described above for up to 4 days. Cells were lysed after defined time periods by addition of SDS/PAGE sample buffer, and equal amounts of protein were loaded on SDS/PAGE gels and processed as described above for autoradiography.

RESULTS

Selenite-concentration dependence of 5'DI and cGPx expression

The selenite concentration of the culture medium was found to regulate the steady-state levels of 5'DI and cGPx expression.

Figure 1 Se-dependent expression of 5'-DI and cGPx in LLC-PK₁ cells in serum-free medium

(a) Specific 5'-DI enzyme activity of cell homogenates (●) and specific activity of cGPx (○) after 4 days of culture at various selenite concentrations in the medium. Results are means $\pm$ S.D. for three independent experiments each with triplicate determination (where error bars are small the symbols obscure them). The values are expressed as percentages of the specific activities ('plateau' activities) at standard selenite concentration (47 nM); 100% corresponds to specific activities of $108 \text{ pmol} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$ (5'-DI) and $77 \text{ nmol} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$ (cGPx). (b) BrAc[¹²⁵I]T₄ affinity labelling of 5'-DI in cell homogenates after 4 days of culture at various selenite concentrations in the medium.

Figure 1(a) shows that, at zero selenite concentration, only marginal 5'-DI and cGPx enzyme activities ($\leq 5\%$ of maximum activity) could be detected. 5'-DI increased steeply above 0.5 nM selenite, whereas cGPx activity increased only beyond 1 nM selenite, requiring an about 2-fold higher selenite concentration for half-maximal activity (10 versus 5 nM for 5'-DI). Both enzymes reached plateau activities above 20 nM; however, at concentrations higher than 50 nM, 5'-DI activity decreased again, while cGPx showed a further slight increase above 500 nM. Simultaneous analysis of the amount of 5'-DI protein by BrAc[¹²⁵I]T₄ affinity labelling showed that the selenite-dependent 5'-DI enzyme activity was paralleled by the 5'-DI protein level (Figure 1b). A second BrAc[¹²⁵I]T₄-labelled protein with an M_r of approx. 18000 appeared to be expressed Se-dependently as well, but 5'-DI-specific inhibitors did not compete for BrAc[¹²⁵I]T₄ labelling (results not shown). The other bands (M_r 32000–70000) alkylated by BrAc[¹²⁵I]T₄ showed no selenite-dependent expression.

Northern-blot analysis revealed that the Se-dependent 5'-DI and cGPx enzyme activities were roughly paralleled by the corresponding steady-state mRNA levels as well (Figure 2). cGPx mRNA showed a continuous increase with increasing medium selenite concentrations, whereas 5'-DI mRNA was present at a very low level up to 20 nM selenite (hardly visible on the reproduction of the autoradiograms) and appeared to increase drastically at 47 nM. Like the corresponding enzyme activity and

Figure 2 Se-dependent mRNA levels

Total RNA was isolated from cells cultured for 4 days at various selenite concentrations and subjected to Northern-blot analysis. The blots were probed for the mRNA levels of the selenoproteins 5'-DI, cGPx, PHGPx, and SeP, as well as of the non-selenoproteins T₃R- α_2 and β -actin.

Figure 3 4-Day kinetics of Se repletion of LLC-PK₁ cells previously grown for 4 days in the absence of selenite

Specific 5'-DI enzyme activity (●) of cell homogenates and specific activity of cGPx (○) after repletion of the medium with 47 nM selenite. Results are means $\pm$ S.D. for three independent experiments, each carried out in triplicate, and are expressed as percentages of the final values measured after 4 days (where error bars are small they are obscured by the symbol). 100% corresponds to specific activities of $161 \text{ pmol} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$ (5'-DI) and $70 \text{ nmol} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$ (cGPx).

protein level, 5'-DI mRNA decreased again beyond 47 nM selenite. The mRNA levels of two further selenoproteins, PHGPx and SeP, were investigated; PHGPx mRNA showed no clear selenite-dependence, whereas the Se-dependent expression pattern of SeP mRNA was very similar to that observed for 5'-DI, showing a sharp peak level at the optimum selenite concentration of 47 nM. Notably, even at zero selenite concentration, all selenoprotein mRNAs were present at significant levels. The mRNA levels for the thyroid-hormone receptor isoform T₃R- α_2 and malic enzyme (not shown), the genes for which are known to be thyroid-hormone-responsive, exhibited no Se-dependent variations. Interestingly, β -actin mRNA levels, commonly used for normalization of Northern blots, were markedly influenced by selenite, tending to decrease with higher concentrations.

Selenite repletion and depletion kinetics

Upon feeding the cells standard medium (47 nM selenite) following a 4-day Se-free preculture, cGPx enzyme activity increased almost linearly for 3 days (Figure 3), apparently approaching a saturation value at day 4. 5'-DI activity, by contrast, exhibited a biphasic increase, with a transient plateau between days 1 and 2.

Figure 4 4-Day kinetics of Se depletion of LLC-PK₁ cells previously grown for 4 days in the presence of 47 nM selenite

Specific 5'-DI enzyme activity (●) of cell homogenates and specific activity of cGPx (○) after depletion of selenite from the medium. Results are means $\pm$ S.D. for three independent experiments, each performed in triplicate, and are expressed as percentages of the initial enzyme activities (where error bars are small they are obscured by the symbol). 100% corresponds to specific activities of 130 pmol $\cdot$ mg⁻¹ $\cdot$ min⁻¹ (5'-DI) and 102 nmol $\cdot$ mg⁻¹ $\cdot$ min⁻¹ (cGPx).

When the cells were depleted from selenite after a 4-day preculture period under standard conditions (47 nM selenite), cGPx activity decreased exponentially during 4 days ($t_{1/2} \approx 1$ day) (Figure 4), reaching 20% of the initial activity on day 4. 5'-DI activity decreased at a similar rate during the first 1–2 days of depletion, but then increased again steadily to reach levels equal to, or even higher than, those observed before Se depletion. 5'-DI activity on day 4 could be fully inhibited by either 100 μ M 6-n-propyl-2-thiouracil or 5 μ M ATG (results not shown), indicating that the restored deiodinase activity was not due to a compensatory induction of the Se-independent type II isoenzyme (containing a cysteine instead of a selenocysteine residue at the active site), which is only weakly inhibited by these compounds. Affinity labelling of 5'-DI in the cell homogenates from the repletion and depletion experiments yielded results analogous to the enzyme-activity results (not shown), confirming that the variations in enzyme activity were due to changes in the protein level.

Metabolic labelling of LLC-PK₁ cells with ⁷⁵Se

The time-dependent distribution of Se among the cellular selenoproteins during a selenite-depletion experiment was investigated by employing sodium [⁷⁵Se]selenite of high specific radioactivity in the predepletion culture medium. An autoradiogram of the metabolic labelling of Se-depleted LLC-PK₁ cells is shown in Figure 5 (to reveal the weak 5'-DI bands, the autoradiogram had to be overexposed with respect to the strongest bands.) After 4 days of depletion, at least 11 bands labelled by ⁷⁵Se could be distinguished (Figure 5; M. Gross, M. Oertel and J. Köhrle, unpublished work), with the majority of radioactivity incorporated into three proteins with M_r 18000, 23000 (cGPx), and 63000. During the 4-day depletion period, the intensity of most bands continuously decreased, except for 5'-DI and a new band with an apparent M_r of 32000, which was not visible before 48 h. The ⁷⁵Se-labelled M_r -27000 band of 5'-DI (co-migrating with the BrAc[¹²⁵I]T₄-labelled 5'-DI band; not shown) initially decreased during 24–48 h, but thereafter the signal increased again, corresponding to the results obtained by 5'-DI enzyme-activity measurements and BrAc[¹²⁵I]T₄ affinity labelling.

Figure 5 Se depletion kinetics of metabolically ⁷⁵Se-labelled LLC-PK₁ cells

Cells previously grown for 4 days in the presence of 50 nM sodium [⁷⁵Se]selenite were depleted of Se and harvested after the time periods indicated. Equal amounts of protein were loaded on to an SDS/PAGE gel, and the gel was subsequently autoradiographed for 2 weeks. The identity of the 5'-DI band was confirmed by running a BrAc[¹²⁵I]T₄-labelled cell homogenate in a neighbouring lane (not shown). The apparent M_r values for the bands were determined in several additional experiments using SDS/PAGE gels at various acrylamide concentrations and M_r marker proteins (not shown).

The band at an apparent M_r of 63000 most likely represents SeP, although the calculated M_r of the unglycosylated protein is 41000 [8,61]. SeP, which might be translated in a ragged manner due to the presence of up to ten Sec residues, is also known to be heavily glycosylated [34,61], therefore, an aberrant migration behaviour on SDS/PAGE and a broadened band have been observed [34,61,62]. The protein with an apparent M_r of 18000 probably represents PHGPx [10,15,16]. The selenoprotein bands at M_r 55000 and 20000 have also been observed previously in other tissues and cells [17,29,33,51,63–65]. Except for 5'-DI, cGPx and the putative PH-GPx and SeP bands, the remaining selenoproteins found here still have to be identified.

It should be noted that, at M_r 18000 and 32000, both ⁷⁵Se- and BrAc[¹²⁵I]T₄-labelled bands were coincident (not shown); moreover, the BrAc[¹²⁵I]T₄-labelled band at M_r 18000 in Figure 1(b) showed a clear selenite-dependence very similar to that of cGPx, supporting the assumption that it in fact represents PHGPx. It is therefore tempting to conclude that selenoenzymes in general might have a strong tendency to be alkylated by BrAc[¹²⁵I]T₄ and other bromoacetyl compounds; this has also been found for proteins with 'hyperreactive' thiol groups [21,66,67]. The extremely nucleophilic character of the selenolate groups, especially if they are embedded in a hydrophobic environment (which should be expected in particular for PHGPx), renders this very likely. Thus, possibly some more of the bands labelled by BrAc[¹²⁵I]T₄ (Figure 1b) might in fact represent selenoproteins.

DISCUSSION

The data presented here show that, in the epithelial kidney cell line LLC-PK₁, the selenoproteins cGPx and 5'-DI are differentially regulated by the cellular Se status. The cGPx and 5'-DI enzyme activities in cells grown at varying selenite concentrations in the culture medium (Figure 1) indicate that there is an obvious preference for 5'-DI expression over cGPx at a low level of Se supply. The coherence of the BrAc[¹²⁵I]T₄-affinity labelling data with the corresponding 5'-DI enzyme activities confirms that the Se-dependent variations in enzyme activity are due to differences in the amount of enzyme protein. Furthermore, the Se-dependent steady-state mRNA levels of the two selenoenzymes (Figure 2)

showed variations that roughly corresponded to the measured activities, suggesting that Se regulates the expression of these proteins, at least in part, via transcriptional control and/or mRNA stabilization. Such a pretranslational regulation is an economic mechanism, since in case of Se shortage, it would avoid the production of protein fragments terminating at the Sec position due to the lack of Sec-tRNA^{Sec}. However, the maintenance of basal mRNA levels even during total Se deficiency may be important for the immediate synthesis of the most essential selenoproteins as soon as the Se supply is restored. A continuing transcription of selenoprotein genes under Se-deficient conditions has been shown by others as well and evidence for posttranscriptional regulation of cGPx has been presented [2,7,29,31,33,36–38,51,63,68,69].

The four selenoproteins, of which the Se-dependent mRNA levels have been determined here, can be tentatively grouped in three classes: (i) the selenoproteins whose mRNA levels appear to be continuously regulated by the Se supply (cGPx), (ii) the ones which exhibit a low basal mRNA level at low selenite concentrations and a steep up-regulation at higher selenite levels (5'DI and SeP), and (iii) a group whose mRNA levels are not affected at all by the Se supply and which therefore seem to be exclusively regulated via translational or post-translational control (PHGPx). For the last group of selenoproteins, the Se-dependent availability of Sec-tRNA^{Sec} might be an important factor regulating their biosynthesis [3,70], whereas expression of the first group appears to be mainly regulated on the mRNA level. The Se-dependent regulation of the intermediate group, on the other hand, probably takes place at both the mRNA and the translational level; in fact, such a combined regulation has been reported for SeP [36] and has been suggested to play a role in the preferential expression of certain selenoproteins at limiting Se supply.

The steady-state mRNA levels of the α_2 -isoform of the T₃ receptor (Figure 2) and malic enzyme (results not shown) were hardly affected by variations in Se supply, indicating that the thyroid hormone-dependent transcription of these genes was not influenced by the availability of the trace element. This appears reasonable, since the culture medium was constantly supplemented with 1 nM T₃, but not with the 5'DI substrate, T₄; therefore the Se-dependent alterations in local T₃ production capacity should not be expected to affect the expression of T₃-dependent genes. Surprisingly, β -actin mRNA, which is frequently used for the normalization of Northern-blot signals, tended to be slightly decreased at high selenite concentrations.

From the dependence of cGPx and 5'DI enzyme activity on the available amount of selenite, one might expect that the preferential expression of 5'DI should be also reflected by the kinetics of recovery upon selenite repletion (Figure 3). However, the Se-induced restoration of 5'DI activity after a prior Se depletion did not occur more quickly than that of cGPx. The biphasic increase of 5'DI activity during selenite repletion could be due to a delayed onset of an enhanced 5'DI gene transcription after restoration of the Se supply. A transient depletion of pre-existent mRNA could cause the levelling-off of the 5'DI increase after day 1; this first increase in activity, however, may be sufficient to meet the vital needs of the cell during the initial recovery period. Only after day 2, as newly transcribed mRNA becomes available, is a further increase of 5'DI activity accomplished. The approximately linear increase of cGPx activity, on the other hand, may reflect a faster Se-dependent response of cGPx gene transcription compared with 5'DI.

The preferential status of 5'DI in the cellular hierarchy of Se supply is revealed most impressively by the kinetics of Se depletion (Figure 4). Upon a sudden Se depletion, the synthesis

of cGPx obviously ceases and the release of Se resulting from the turnover of cGPx and other selenoproteins (Figure 5) can replenish the cellular pool for the production of more essential selenoproteins such as 5'DI. This selenite redistribution during depletion seems to occur to the selective benefit of the 5'DI and the selenoprotein with an apparent M_r of 32000 (Figure 5). The cellular content of 5'DI has been estimated as 2–5 pmol/mg of microsomal membrane protein in rat liver and kidney and in LLC-PK₁ cells [25,26,47], which is orders of magnitude lower than the amounts of cGPx. Since cGPx contains about 60–70% of the cellular Se under Se-adequate conditions in rats [34,64] and up to approx. 30% in LLC-PK₁ cells after 4 days of metabolic labelling [Figure 5 and results of further ⁷⁵Se-labelling experiments on LLC-PK₁ cells (M. Gross, M. Oertel and J. Köhrle, unpublished work)], cGPx might serve as an intracellular Se store. Similar data supporting a role of cGPx as a Se reservoir have recently been presented for hepatocytes and intact rats [32,36]. Since, apart from cGPx, the cell contains several additional antioxidant enzymes and compounds, cGPx is probably dispensable to a certain degree, such that under Se-shortage conditions, the Se liberated by cGPx turnover can be utilized for the synthesis of more vital selenoproteins such as 5'DI (the present study) or PHGPx [71]. Our observation that cGPx activities tended to increase further at very high medium selenite concentrations suggests that, under these conditions, the synthesis of even more cGPx protein may also serve to avert cytotoxic effects of Se by incorporating an excess of the element into protein. Thus, in addition to its function as a protective antioxidant enzyme, cGPx might play a dual role as a Se buffer in situations of both too low and too high (toxic) Se concentrations in the cell. Also, the observed rapid synthesis of cGPx upon selenite repletion (Figure 3) after Se deficiency could be important to immediately sequester the trace element in the cell in a non-toxic form.

To gain insight into the molecular mechanisms underlying the observed fine-tuned regulation of Se supply for the individual selenoproteins, further investigations will have to focus on the transcriptional activity of selenoprotein genes (to identify putative Se-dependent transcription factors and Se-responsive DNA sequences) and on the stability of selenoprotein mRNAs. Moreover, several unidentified selenoproteins remain to be functionally characterized, and the roles of putative Se transport and storage proteins, such as SeP, will have to be clarified.

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