ELSEVIER

Biochimica et Biophysica Acta 1191 (1994) 291–298

BBA Biochimica et Biophysica Acta

Zinc transport and metallothionein induction in primary cultures of rabbit kidney proximal cells

Bertrand Gachot [1], Michel Tauc, Frida Wanstok, Luc Morat, Philippe Poujeol *

Service de Biologie Cellulaire, Département de Biologie Cellulaire et Moléculaire, Centre d'Etudes de Saclay, F-91191 Gif sur Yvette, France

(Received 1 October 1993)

Abstract

Primary cultures of isolated rabbit renal proximal cells were grown on collagen-coated permeable supports. The confluent epithelia were polarized, making possible the measurement of uptakes and effluxes across the apical and the basolateral membranes. Uptakes of ^{65}Zn were assessed under initial rate conditions, after 0.5 min incubation. The kinetic parameters of apical uptake were a J_{max} of 25.1 ± 5.3 pmol min^{-1} (μg DNA)$^{-1}$, a K_m of 43.3 ± 7.3 μM and an unsaturable constant of 0.105 ± 0.029 ($n = 7$) at 37°C. Cadmium competitively inhibited the zinc uptake, with a K_i value of 24.5 ± 7.3 μM. Basolateral uptake was characterized by a high capacity (J_{max} = 227.9 ± 46.6 pmol min^{-1} (μg DNA)$^{-1}$) and an affinity similar to that of the apical uptake (K_m = 35.4 ± 14.2 μM). Cadmium had no effect on the basolateral zinc uptake. Effluxes across the basolateral face of the epithelium always exceeded those across the apical face. Excess zinc in the culture medium induced the synthesis of metallothionein in the epithelia, as judged by the rate of [^{35}S]cysteine incorporation into a fraction of cytosolic proteins. Metallothionein induction did not appear to modify the kinetic parameters of the apical zinc uptake. These data suggest that separate saturable transport systems are responsible for the apical and basolateral zinc uptakes in proximal renal cells. Induction of metallothionein had no apparent effect on apical zinc uptake in this system.

Key words: Proximal tubule; Primary culture; Zinc; Apical; Basolateral; Uptake; Efflux; (Rabbit kidney cell)

1. Introduction

Zinc is the most abundant trace element in the body, and its importance as a nutrient is widely established [1]. Although the renal handling of zinc seems to differ markedly from that of other divalent cations [2], its transport mechanisms along the renal tubule are poorly characterized. Using isolated renal proximal cells, we previously showed that zinc was transported either as a free ion via a saturable carrier-mediated process and an unsaturable pathway, or complexed with cysteine or histidine, via a sodium-amino acid cotransport mechanism [3]. However, with this technique, all surfaces (brush-border and basolateral membranes) of the isolated cell were exposed to the substrate and it was therefore impossible to determine the site at which zinc uptake actually occurred. The simple geometry and free access to the apical and basolateral membranes of cultured monolayers are experimental conditions particularly suited to polarized membrane transport measurements. In this study, using a previously well-described model of primary culture of rabbit kidney proximal cells [4], we undertook to characterize apical and basolateral zinc transports. The induction of metallothionein and its relation to membrane transport was also studied.

2. Materials and methods

Animals. Young male New-Zealand white rabbits (body wt., 600–800 g) were used for the experiments. All animals were fed a standard diet and had free

* Corresponding author. Fax: +33 1 69083570.
[1] Present address: Clinique de Réanimation des Maladies Infectieuses, Hôpital Bichat-Claude Bernard, 46 rue Henri Huchard, 75018 Paris, France.

SSDI 0005-2736(94)00036-O

access to tap water. For cell preparations, kidneys were removed under sterile conditions from animals killed with 150 mg pentobarbital sodium and 1250 U heparin (Roussel, France) injected through the vein of the ear.

Primary cultures from sterile isolated proximal cells. Isolated proximal cells were prepared as previously described by Poujeol et al. [5], all steps being performed under sterile conditions. Briefly, kidneys were perfused with a medium composed of equal quantities of Dulbecco's modified Eagle medium (DMEM) and Ham F12 (Eurobio, Paris, France) containing 15 mM $NaHCO_3$, 20 mM Hepes (pH 7.5), 100 U/ml penicillin and 100 μg/ml streptomycin. Slices of the superficial cortex were then passed through a tissue press and successively filtered through 100-, 40- and 20-μm nylon meshes. Finally, cells were placed in a hormonally-defined culture medium composed of DMEM, Ham F12, 15 mM $NaHCO_3$, 20 mM Hepes, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, 5 μg/ml insulin, 50 nM dexamethasone, 10 ng/ml epidermal growth factor, 5 μg/ml transferrin, 30 nM sodium selenite and 10 nM triiodothyronine.

The total number of cells was estimated by microscopic observation of 20 μl of cell suspension diluted in 0.5% eosin solution. A final concentration of $20 \cdot 10^6$ cells/ml was usually obtained. Isolated cells were then seeded in collagen-coated culture wells containing 0.5 ml of culture medium (Millicell-HA 12 mm diameter, Millipore, Bedford, MA, USA) or 75 cm^2 culture flasks (Falcon, Becton Dickinson, UK) at a concentration of $1 \cdot 10^6$ cells/cm^2. The wells were placed in a 12-well culture dish (Costar, Cambridge, MA, USA) containing 1.5 ml of culture medium, thus representing the basolateral compartment. The medium was changed 3 days after seeding and then every other day. The antibiotics were definitively removed from the medium at the first change. Cultures were maintained at 37°C in a 5% CO_2-water-saturated atmosphere. Eight days after seeding, cultures reached confluency. Eight-day old cultures were used for experiments.

^{65}Zn uptake studies. In experiments on the temperature-dependence of the rate of uptake, cultures were preincubated for 15 min at the desired temperatures under air, in a shaking water bath at 60 oscillations/min. The incubation medium consisted of Hank's balanced salt solution (Eurobio, Paris, France) buffered with 20 mM Hepes (pH 7.4). The wells were rinsed three times with unlabelled medium, and apical uptakes were initiated by addition of 200 μl incubation medium inside the 12 mm diameter well and were terminated at the indicated times (or at 0.5 min for initial velocity measurements) by aspirating the medium then plunging and rinsing the well three times in ice-cold phosphate-buffered saline with 5 mM EGTA. The EGTA prevented further zinc uptake by chelating the free zinc in solution and reduced the amount of metal loosely or unspecifically bound to cell surfaces [6]. Basolateral uptakes were initiated by placing the well containing 200 μl apical unlabelled medium in a 12-well culture dish with 1.2 ml incubation medium per well and terminated as for apical uptake measurements.

All experiments were carried out in triplicate. The radioactivity was determined by scintillation counting (Minaxi, Packard Instrument Company, Downer Grove, IL, USA) after dissolving the cultured cells in 500 μl 2% SDS.

^{65}Zn efflux studies. Cultures were loaded with ^{65}Zn for 4 h at room temperature in Hank's balanced salt solution with 20 mM Hepes at pH 7.4, under a continuous air stream. After rinsing the wells in unlabelled medium to remove isotope from the extracellular space, apical and basolateral ^{65}Zn effluxes were measured simultaneously. Every 2 min, the totality of the medium (0.2 apical ml and 1.2 basolateral ml) was collected directly from the apical and basolateral sides of the culture well and replaced by fresh medium. The remaining radioactivity in the cultured cells at the end of the experiment was determined by scintillation counting after dissolving the epithelium in 500 ml 2% SDS.

DNA determination. DNA was measured by a modification of the fluorimetric micromethod of Switzer and Summer [7].

Transport data analyses. For uptake studies, kinetic parameters were determined by a simplex iterative method, using a PC/XT-like desk computer and a curve-fitting program (Enzfitter, Elsevier Biosoft, Cambridge, UK). For apical and basolateral zinc uptakes, the best adapted model was one involving a single carrier with a so-called 'diffusive' component according to the equation:

$$J = \frac{J_{max} \cdot [S]}{K_m + [S]} + K_d \cdot [S]$$

where J_{max} is the maximum rate of uptake expressed in pmol min^{-1} (μg DNA)$^{-1}$, [S] the concentration of zinc, K_m the apparent dissociation constant in μM and K_d a 'diffusional constant'. For metal competition experiments, the inhibition constant (K_i) was calculated by the method of Neame and Richard [8], according to the equation:

$$K_i = \frac{K_m \cdot [i]}{K_a - K_m}$$

where K_m is the value obtained in the absence of inhibitor, K_a the apparent K_m in the presence of inhibitor and [i] the concentration of inhibitor.

For efflux studies, the apical and basolateral efflux rate constants (i.e., the fraction of the total radioactivity lost per unit time) were calculated from the backaddition of the radioactivity in the efflux samples to the

radioactivity remaining in the cells, according to the Eqs. (1) and (2):

$$(k_a)_t = \frac{(C_a)_t}{C_{ep} + \sum_{i=t_f}^{t+1} [((C_a)_i + (C_b)_i] + 1/2[(C_a)_t + (C_b)_t]} \cdot (1/T) \quad (1)$$

$$(k_b)_t = \frac{(C_b)_t}{C_{ep} + \sum_{i=t_f}^{t+1} [((C_a)_i + (C_b)_i] + 1/2[(C_a)_t + (C_b)_t]} \cdot (1/T) \quad (2)$$

where $(k_a)_t$ and $(k_b)_t$ are the apical and basolateral efflux rate constants at time t, and $(C_a)_t$ and $(C_b)_t$ are the radioactivities lost from the apical and basolateral sides at time t and during the period T, respectively. C_{ep} is the radioactivity remaining in the epithelium at the end of the measurements, and t_f corresponds to the final time of the experiment.

Values reported in the text and figures are means ± S.E. Student's t-test was used for statistical analysis.

Determination of metallothionein in renal proximal cells. The cultured cells were trypsinized and resuspended in a minimal volume of buffer containing 10 mM Tris, 1 mM dithiothreitol and 0.1 mM phenylmethyl-sulfonyl fluoride (pH 7.4). They were disrupted by freezing and thawing in liquid nitrogen. Samples for metallothionein determination were prepared by centrifugation of the homogenate at 12000 × g for 3 min followed by heat denaturation of the supernatant at 95°C for 4 min. The heat denaturated proteins were removed by centrifugation at 12000 × g for 3 min. Protein content was determined in the heat stable fraction according to the Bradford method (Bio-Rad protein assay, Bio-Rad laboratories, Munich, Germany). After adjustment for protein concentration, the supernatant was analyzed by fast protein liquid chromatography (FPLC, Pharmacia, USA) equipped with a gel filtration column (Superose 12). After injecting samples (15 μg protein), the column was washed with 200 mM Tris-HCl (pH 7.4) at a flow rate of 0.4 ml/min, and 1-ml fractions were collected of which 500-μl aliquots were taken for scintillation counting. The absorbance at 280 nm was monitored. In another set of experiments, samples were analyzed by FPLC ion-exchange chromatography (Mono Q HR 5/5 column). The mobile phases were 10 mM Tris-HCl, 0.1% SDS (pH 7.4, buffer A), and 200 mM Tris-HCl, 0.1% SDS (pH 7.4, buffer B). After isocratic elution with 5 ml of buffer A, bound materials were eluted with a linear gradient of 20 ml of 10–200 mM Tris-HCl (0–100% buffer B). The flow rate was 1 ml/min and 1-ml fractions were collected. The absorbance at 280 nm was measured, and aliquots of 500 μl were taken for scintillation counting. The metallothionein-containing fractions were identified by elution of purified rabbit liver metallothionein (Sigma Chimie, France).

Two methods were employed to identify cellular metallothionein in the cultured monolayers. In the first the basal level of metallothionein was determined by incubating the heat stable fraction of the cytosolic proteins with ^{109}Cd. The labelled supernatant was analyzed by FPLC as described above. In a second set of experiments, the neosynthesis of metallothionein was assessed by the incorporation of [^{35}S]cysteine in the epithelia. At day 7 in culture, the medium in the flasks was replaced with complete medium containing [^{35}S]cysteine (2 μCi/ml). The following morning, after about 20 h of incubation, the heat stable fraction of the cytosolic proteins was eluted as described. In both sets of experiments, cellular metallothionein was determined under control conditions and after overnight incubation with 50 μM zinc in the culture medium. The concentration of zinc in the normal medium was 1.5 μM.

Chemicals. ^{65}Zn (as $ZnCl_2$), ^{109}Cd (as $CdCl_2$) and [^{35}S]cysteine were obtained from Amersham France (Les Ulis, France). All other chemicals were obtained from standard sources.

3. Results

3.1. Apical ^{65}Zn uptake

The apical uptakes of 15 μM zinc by cultured proximal cells incubated for different lengths of time at 4°C and 37°C are illustrated in Fig. 1. Zinc uptake showed a marked temperature dependence, particularly at incubation periods longer than 2 min. Subsequent experiments were performed at 37°C, and the uptake of zinc

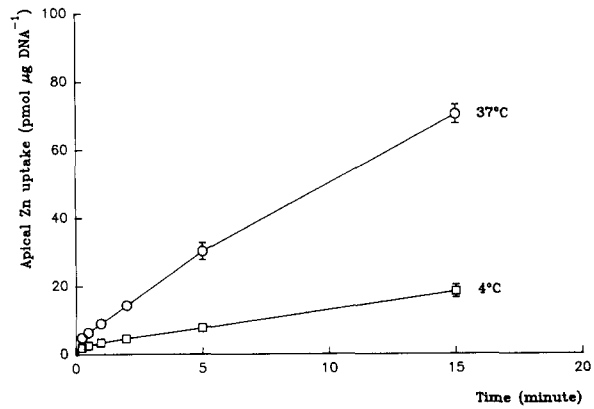

Fig. 1. Time and temperature dependences of apical ^{65}Zn uptake. Cell cultures were equilibrated for 15 min at the indicated temperatures. ^{65}Zn (15 μM) equilibrated at the corresponding temperature was added on the apical side of the monolayer, and uptakes were terminated at the indicated times. Each point is the mean (±S.E.) of three experiments.

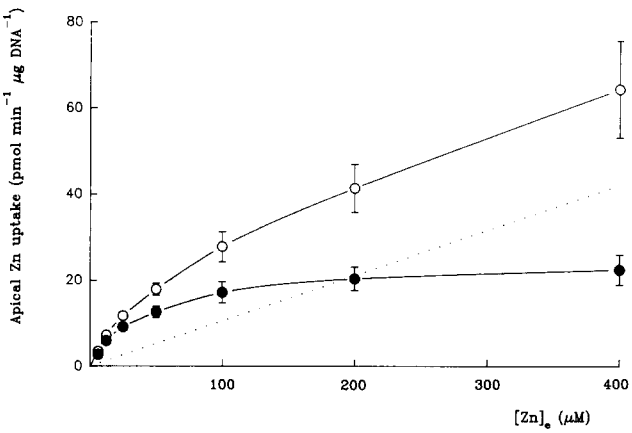

Fig. 2. Concentration-dependence of apical ^{65}Zn uptake. Cultured cells were incubated for 0.5 min at 37°C and increasing apical external Zn ([Zn]$_e$) concentrations. The unsaturable component (·····) was subtracted from the total uptake (○) to obtain the carrier-mediated transport (●). Each point represents the mean (±S.E.) of triplicate samples from seven different experiments.

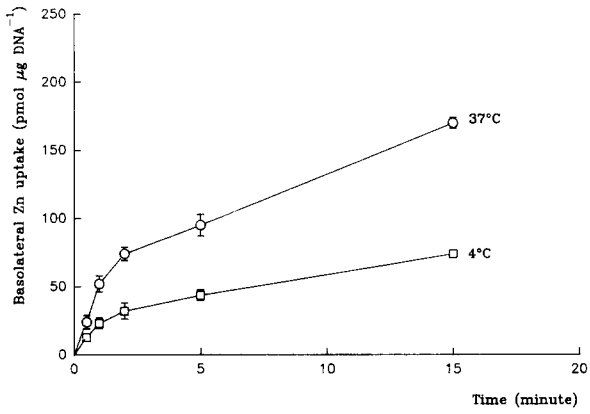

Fig. 4. Time and temperature dependences of basolateral ^{65}Zn uptake. Cell cultures were equilibrated for 15 min at the indicated temperatures. Wells were placed in dishes containing ^{65}Zn (15 μM) equilibrated at the corresponding temperature, representing the basolateral compartment. Basolateral uptakes were terminated at the indicated times. Each point is the mean (±S.E.) of at least three experiments.

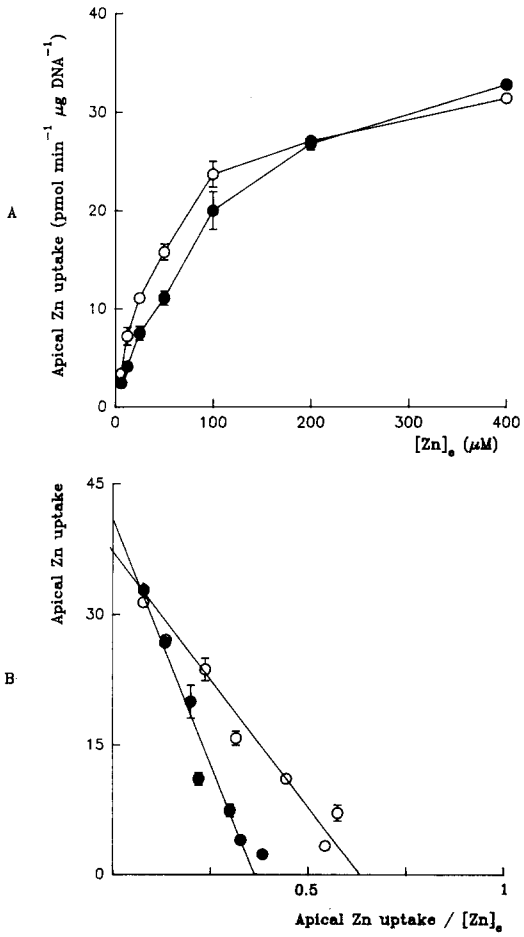

Fig. 3. Effect of cadmium on the kinetics of apical ^{65}Zn uptake. Cultured renal cells were incubated at 37°C and various apical external Zn ([Zn]$_e$) concentrations in the presence (●) or absence (○) of 30 μM $CdCl_2$, and initial (0.5 min) velocities of ^{65}Zn apical uptake determined. Each point is the mean (±S.E.) of three experiments. (A) Rate of apical uptake as a function of [Zn]$_e$. (B) Eadie-Hofstee linearization of data shown in (A), obtained by plotting apical Zn uptake against apical Zn uptake/[Zn]$_e$.

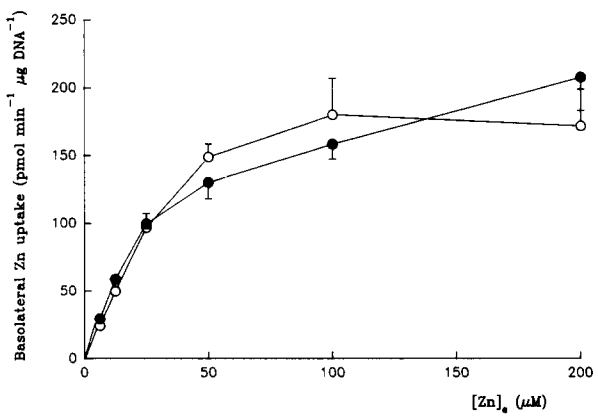

Fig. 5. Effect of cadmium on the kinetics of basolateral ^{65}Zn uptake. Cultured renal cells were incubated at 37°C and various basolateral external Zn ([Zn]$_e$) concentrations in the presence (●) or absence (○) of 30 μM $CdCl_2$, and initial (0.5 min) velocities of basolateral ^{65}Zn uptake determined. Each point is the mean (±S.E.) of three experiments.

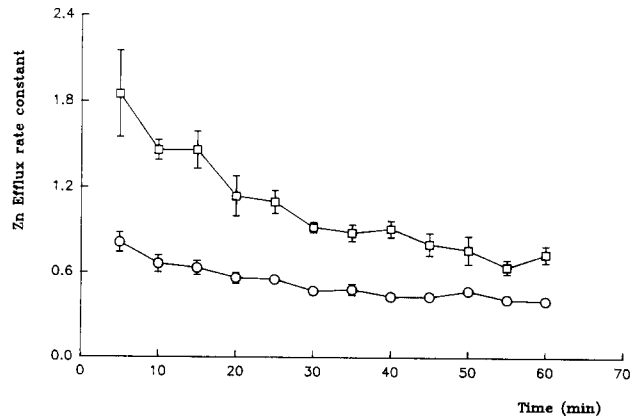

Fig. 6. ^{65}Zn effluxes from cultured renal cells. After loading with ^{65}Zn for 4 h at room temperature, apical (○) and basolateral (□) efflux rate constants were measured simultaneously at the indicated times. Each point is the mean (±S.E.) of three experiments.

after 0.5 min was chosen to represent the initial rate of entry.

The relationship between zinc uptake and increasing extracellular zinc concentration after 0.5-min incubation is shown in Fig. 2. The non-saturable component varied from one cell culture preparation to another. After subtracting these values from the total uptake values, initial rates of zinc accumulation can be seen to reach saturation within the 100–200 μM range. The kinetic parameters of apical zinc uptake obtained from these data are J_{max} 25.1 ± 5.3 pmol min^{-1} (μg $DNA)^{-1}$, K_m 43.3 ± 7.3 μM and K_d 0.105 ± 0.029 ($n = 7$) at 37°C.

The effect of cadmium on apical ^{65}Zn uptake is illustrated in Fig. 3. Analyses of the curves were made by plotting Zn uptake against the Zn uptake/external Zn ratio (Fig. 3B). Cadmium at a low concentration (30 μM) behaved as a competitive inhibitor of apical ^{65}Zn uptake, increasing the K_m from 54.3 ± 8.7 μM (control) to 125.8 ± 7.3 μM ($P < 0.01$). J_{max} was essentially unchanged, being 48.4 ± 1.9 pmol min^{-1} (μg $DNA)^{-1}$ under control conditions and 59.8 ± 3.5 pmol min^{-1} (μg $DNA)^{-1}$ in the presence of $CdCl_2$ (n.s.). The apparent K_i of cadmium was found to be 24.5 ± 7.3 mM ($n = 3$).

3.2. Basolateral ^{65}Zn uptake

Fig. 4 shows the basolateral uptakes of 15 μM zinc by 8-day old cultured proximal cells incubated for different lengths of time at 4 or 37°C. The temperature dependence was evident even at incubation periods as

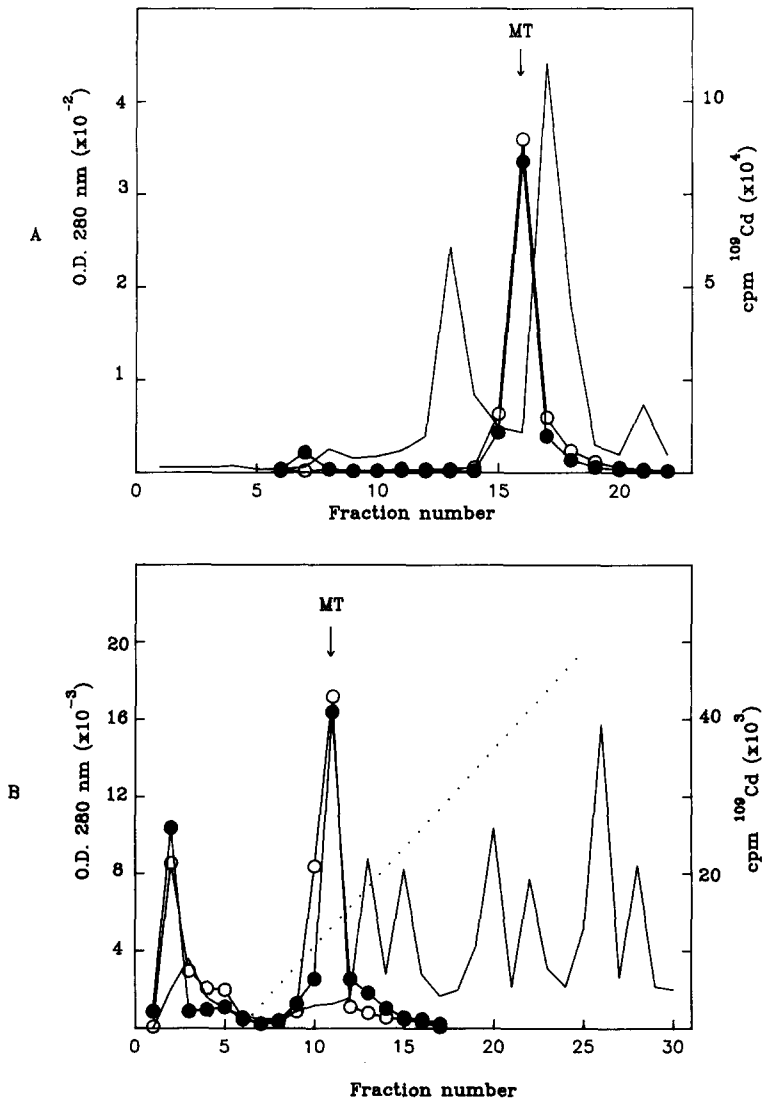

Fig. 7. Binding of ^{109}Cd by cytosolic proteins of proximal cells in culture. The heat stable fraction of the cytosolic proteins was incubated with ^{109}Cd and analyzed by fast liquid chromatography. Absorbance at 280 nm (solid line) and radioactivity (○, ●) in each fraction were determined. The experiments were performed under control conditions (●) or after overnight incubation with 50 μM zinc in the culture medium (○). MT indicates the site of elution of purified metallothionein. Representative experiments are shown. (A) Gel filtration chromatography. (B) Ion-exchange chromatography; the dotted line denotes the theoretical gradient.

short as 1 min. In subsequent experiments, the basolateral uptake of zinc after 0.5 min was chosen to represent initial rate conditions.

Fig. 5 illustrates the concentration dependence of basolateral zinc uptake and the effect of cadmium. After subtraction of the unsaturable component, initial rates of basolateral zinc uptake showed a nonlinear dependence on extracellular zinc concentration. Cadmium at a concentration of 30 μM had no significant effect on this uptake. The K_m was 35.4 ± 14.2 μM under control conditions and 43.4 ± 14.5 μM in the presence of cadmium (n.s.). The basolateral zinc transport system was shown to be of a high capacity, with a J_{max} of 227.9 ± 46.6 pmol min^{-1} $(\mu g\ DNA)^{-1}$ under control conditions, and unchanged in the presence of $CdCl_2$ (247.3 ± 44.6 pmol min^{-1} $(\mu g\ DNA)^{-1}$, n.s.).

3.3. ^{65}Zn efflux

Isolated cultured cells grown on permeable Millipore supports allowed for the separate measurement of zinc effluxes across the apical and basolateral membranes. Fig. 6 presents the ^{65}Zn efflux from the monolayer as a function of time. The basolateral efflux rate constant always exceeded the apical rate constant ($n = 3$).

3.4. Metallothionein synthesis by cultured proximal cells

As seen in Fig. 7, significant binding of ^{109}Cd into the metallothionein peak occurred under control conditions and when the cultured cells were incubated overnight with 50 μM $ZnCl_2$ in the medium. Excess zinc in the culture medium did not modify ^{109}Cd binding into the metallothionein peak.

When metallothionein synthesis was assessed by incorporation of [^{35}S]cysteine into the monolayer, metallothionein was detected only in the presence of excess zinc in the medium (Fig. 8). The incorporation of [^{35}S]cysteine into other proteins remained unaffected by the zinc concentration of the culture medium.

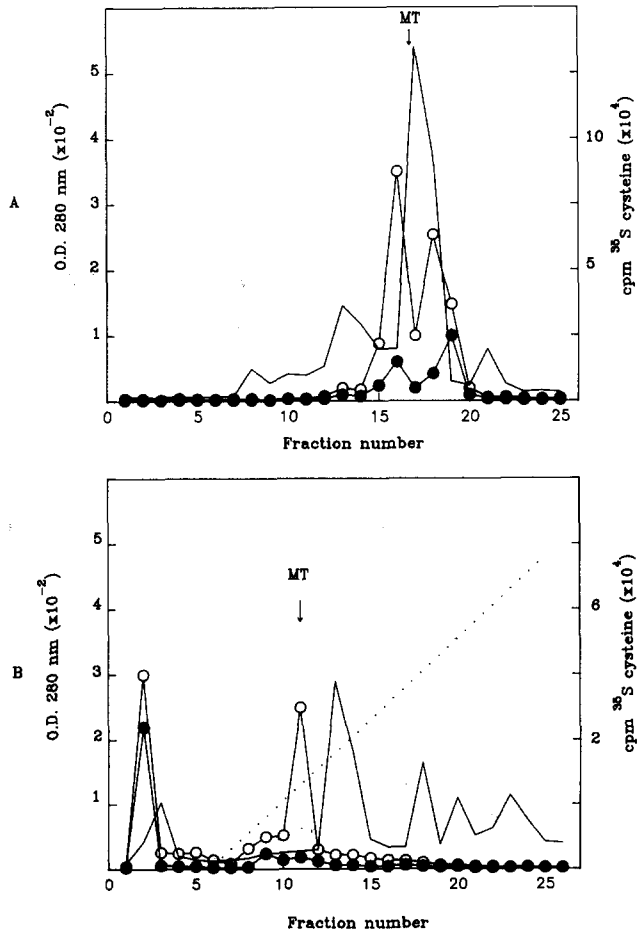

Fig. 8. Incorporation of [^{35}S]cysteine in cultured renal cells. After overnight incubation in medium containing [^{35}S]cysteine with (○) or without (●) 50 μM zinc, the heat stable fraction of the cytosolic proteins was analyzed by fast liquid chromatography. Absorbance at 280 nm (solid line) and radioactivity (○, ●) in each fraction were determined. MT indicates the site of elution of purified metallothionein. Representative experiments are shown. (A) Gel filtration chromatography. (B) Anion exchange chromatography; the dotted line denotes the theoretical gradient.

3.5. Effect of metallothionein induction on apical ^{65}Zn uptake

After overnight incubation with 50 μM $ZnCl_2$, apical zinc uptake assessed in initial velocity conditions was essentially unchanged: the K_m was 35.3 ± 21.9 μM under control conditions and 43.4 ± 25.2 μM after metallothionein induction (n.s.). The J_{max} was 21.0 ± 6.3 pmol min^{-1} (μg DNA)$^{-1}$ under control conditions and unchanged after metallothionein induction (25.5 ± 5.8 pmol min^{-1} (μg DNA)$^{-1}$, n.s., $n = 3$).

4. Discussion

In order to study apical and basolateral zinc transports in the rabbit proximal tubule, we chose primary cultures of freshly isolated proximal cells. Morphological, enzymatic and transport characteristics of these cells clearly showed that they were mainly of proximal origin [5]. Furthermore, Le Maout et al. [4] demonstrated that such isolated cells in primary culture preserved their proximal characteristics and recovered their membrane polarity when growing on collagen-coated permeable supports. Our results indicate that ionic zinc is transported into rabbit renal proximal cells through the apical membrane by a saturable, carrier-mediated process and an unsaturable pathway. The K_m value that we determined is in good agreement with data obtained from isolated renal proximal cells [3]. In addition, cadmium, the toxic homologue of zinc, is a potent competitive inhibitor of apical zinc uptake in cultured renal cells, suggesting that these two divalent cations share a common apical transport system. Few studies have attempted to characterize heavy metals transport systems in the kidney. Studying ^{109}Cd apical uptake by LLCPK1 cells, Templeton [9] did not find saturation kinetics in this system. However, uptakes in this study were measured at 4 h and thus did not represent initial velocities. The same applies to the data of Groten et al. [10] from primary cultures of rat renal cortical cells, since cadmium uptake was assessed after 22 h of incubation. Recently, Endo and Shaikh [11] found that cadmium uptake in primary cultures of rat cortical renal cells appeared to reach saturation with a K_m of 7 μM, and showed evidence of competitive inhibition by zinc and copper. However in the two latter studies, neither the isolated cells nor the resulting primary cultures were precisely characterized.

In the present study, we show that basolateral zinc uptake in cultured renal proximal cells is temperature-dependent and exhibits saturation within the 50–100 μM range. It would seem that distinctly different zinc transport systems exist at the two membrane surfaces of renal proximal cells since the basolateral J_{max} was about eight times that of the apical, although affinities of the two systems were similar. In addition, cadmium had no effect on basolateral zinc uptake. Our results are in agreement with data obtained with LLCPK1 cells, in which the basolateral uptake of cadmium was about twice the apical uptake [12]. Uptake by rat intestinal basolateral membrane vesicles had already been found to be saturable with a K_m of 24 μM [13], although these data were not subsequently confirmed in Caco-2 cells, an epithelial cell line derived from human large intestine [14]. The present study shows that zinc effluxes in cultured proximal kidney cells are also polarized, with evidence of an increased Zn permeability at the basolateral side of the epithelium. Taken together, these transport systems are adequate to explain both the proximal tubular reabsorption and the ionic zinc secretion, that have been found to occur in various experimental conditions [15,16].

In a second series of experiments, we investigated the induction of metallothionein by zinc in primary cultures of rabbit kidney proximal cells. A high level of metallothionein was found in renal proximal cells in culture, as revealed by ^{109}Cd binding of heat stable cytosolic proteins. This high basal level of metallothionein remained unaffected by previous incubation in a medium containing 50 μM $ZnCl_2$, suggesting that there was either no induction or that metallothionein was only synthesised in undetectable amounts. To be detectable with this technique, induction may have required higher concentrations of zinc or the use of cadmium, as suggested by data from LLCPK1 cells [17]. Evidence of cytotoxicity had been demonstrated in vitro at zinc concentrations as low as 125 μM [18]. Furthermore, using the highest non-toxic concentration of metal and RK-13 cells derived from rabbit kidney, Koizumi et al. [19] showed that induction of metallothionein synthesis by 100 μM zinc was 2-fold higher than by 15 μM cadmium. We therefore chose to employ zinc at a relatively low concentration. Incorporation of [^{35}S]cysteine in cultured renal cells confirmed that induction of metallothionein did in fact occur in the presence of 50 μM zinc in the medium, with only small amounts of protein being neosynthesized. Induction of metallothionein has already been shown to occur in the kidney, both in vivo [20] and in vitro, using established cell lines [17,19,21–24], or primary cultures of kidney cells of uncertain origin [25]. These studies clearly do not reflect the exact physiology of any specific nephron segment. Our results obtained from well-characterized primary cultures suggest that proximal tubular cells are able to synthesize metallothionein in the presence of excess zinc. The exact function of this metalloprotein in the kidney remains unclear. Chronic intoxication by cadmium is responsible for proximal tubular dysfunction [26], and intracellular metallothionein probably plays a role in protecting against the toxicity of cadmium [27]. Although a func-

tion for metallothionein in the regulation of intestinal absorption of heavy metals has been proposed [28], similar evidence is lacking for renal transport [29]. In our model, metallothionein induction did not appear to modify the kinetic parameters of apical zinc uptake in renal proximal cells. Perhaps higher concentrations of zinc are necessary to cause a significant effect on apical transport. Furthermore, metallothionein may interfere with basolateral uptake or polarized zinc efflux, and these possibilities deserve further investigation.

In conclusion, the present study demonstrates that distinct saturable carrier-mediated systems associated with an unsaturable pathway are involved in zinc uptake by the apical and basolateral membranes of proximal tubular renal cells in primary culture. Low concentrations of zinc induce the synthesis of metallothionein in proximal cells. The exact function of kidney metallothionein remains to be determined, but there is no evidence that it is involved in the regulation of apical zinc uptake in proximal cells.

References

[1] Prasad, A.S. (1979) Annu. Rev. Pharmacol. Toxicol. 20, 393–426.
[2] Steele, T.H. (1973) J. Lab. Med. 81, 507–514.
[3] Gachot, B., Tauc, M., Morat, L. and Poujeol, P. (1991) Pflügers Arch. 419, 583–587.
[4] Le Maout, S., Tauc, M., Koechlin, N. and Poujeol, P. (1990) Biochim. Biophys. Acta 1026, 29–39.
[5] Poujeol, P. and Vandewalle, A. (1985) Am. J. Physiol. 249, F74-F83.
[6] Failla, M.L. and Cousins, R.J. (1978) Biochim. Biophys. Acta 538, 435–444.
[7] Switzer, B.R. and Summer, G.K. (1971) Clin. Chim. Acta 32, 203–206.
[8] Neame, K.D. and Richard, T.G. (1972) Elementary Kinetics of Membrane Carrier Transport, pp. 56–79, Blackwell, Oxford.
[9] Templeton, D.M. (1990) J. Biol. Chem. 265, 21764–21770.
[10] Groten, J.P., Luten, J.B., Bruggeman, I.M., Temmink, J.H.M. and Van Bladeren, P.J. (1992) Toxicol. in Vitro 6, 509–517.
[11] Endo, T. and Shaikh, Z.A. (1993) Toxicol. Appl. Pharmacol. 121, 203–209.
[12] Bruggeman, I.M., Temmink, J.H.M. and Van Bladeren, P.J. (1992) Toxicol. in Vitro 6, 195–200.
[13] Oestreicher, P. and Cousins, R.J. (1989) J. Nutr. 119, 639–646.
[14] Raffaniello, R.D., Lee, S.-Y., Teichberg, S. and Wapnir, R.A. (1992) J. Cell Physiol. 152, 356–361.
[15] Abu-Hamdan, D.K., Migdal, S.D., Whitehouse, R., Rabbani, P., Prasad, A.S. and McDonald, F.D. (1981) Am. J. Physiol. 241, F487–F494.
[16] Victery, W., Smith, J.M. and Vander, A.J. (1981) Am. J. Physiol. 241, F532–F539.
[17] Harford, C. and Sarkar, B. (1991) Biochem. Biophys. Res. Commun. 177, 224–228.
[18] Borovansky, J. and Riley, P.A. (1989) Chem.-Biol. Interact. 69, 279–291.
[19] Koizumi, S., Sone, T. and Kimura, M. (1985) J. Cell Physiol. 125, 223–228.
[20] Shaikh, Z.A. and Smith, J.C. (1976) Chem.-Biol. Interact. 15, 327–336.
[21] Webb, M. and Daniel, M. (1975) Chem.-Biol. Interact. 10, 269–276.
[22] Kimura, M., Otaki, N. and Kakefuda, T. (1979) Experientia Suppl. 34, 187–196.
[23] Felley-Bosco, E. and Diezi, J. (1991) Arch. Toxicol. 65, 160–163.
[24] Wan, M., Hunziker, P.E. and Kägi, J.H.R. (1993) Biochem. J. 292, 609–615.
[25] Cherian, M.G. (1980) Toxicol. 17, 225–231.
[26] Humes, H.D. and Weinberg, J.M. (1986) in The Kidney (Brenner, B.M. and Rector, F.C., eds.), Vol. 2, pp. 1491–1532, W.B. Saunders, Philadelphia.
[27] Nordberg, G.F. (1971) Environ. Physiol. Biochem. 1, 171–187.
[28] Cousins, R.J. (1985) Physiol. Rev. 65, 238–309.
[29] Foulkes, E.C. (1982) Dev. Toxicol. Environ. Sci. 9, 193–202.