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Regular Article

Functional Transport Properties of Human Zinc Transporter 1: Kinetics and pH-Dependency

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Intracellular zinc (Zn^{2+}) homeostasis is essential for physiological and pathological processes and is strictly regulated by Zn^{2+} transporters. Zinc transporter 1 (ZnT1) is a ubiquitously expressed plasma membrane-localized Zn transporter that exports Zn^{2+} from the cytoplasm to the extracellular space. However, the functional transport properties regarding kinetics and driving forces of ZnT1 remain debatable. In this study, we established a cell-free proteoliposome assay system and demonstrated that ZnT1 transports Zn^{2+} with high affinity in pH-dependent and pH-independent manners. The K_m and V_{max} of pH-dependent Zn^{2+} transport were 0.40 μM and 15.13 nmol/min/mg protein, and those of pH-independent Zn^{2+} transport were 0.52 μM and 8.88 nmol/min/mg protein (low concentrations of Zn^{2+}), 3.02 μM and 17.59 nmol/min/mg protein (high concentrations of Zn^{2+}), respectively, suggesting biphasic kinetic components of Zn^{2+} transport. Even without pH gradient formation, ZnT1 exhibits potent Zn^{2+} transport activity. In pH dependency, Zn^{2+} transport activity was higher at an inside pH of 6.0 than at 6.5–7.5 for proteoliposomes, despite the same ΔpH of 0.5–1.5. The Zn^{2+} transport activity decreased at an outside pH of 8.0, despite an increase in ΔpH. Although previous studies have proposed that ZnT1-mediated Zn^{2+} transport activity is driven by a calcium (Ca^{2+}) gradient and not by a pH gradient, Ca^{2+} does not enhance Zn^{2+} transport activity in the presence or absence of a pH gradient. These results strongly suggest that ZnT1 protein transports Zn^{2+} optimally at a specific pH and exports excess intracellular Zn^{2+} even without ΔpH.

Key words zinc transporter 1, SLC30A1, zinc, pH, proteoliposome

INTRODUCTION

Zinc (Zn^{2+}) is an essential trace element that plays important roles in many physiological processes, such as normal growth and development, neurological function, metabolism, and immune response.[1,2] Zn^{2+} binds to approximately 10% of the structural and catalytic proteins to maintain and regulate their normal structure and function, while also acting as a signal messenger.[3] Intracellular Zn^{2+} deficiency is associated with growth retardation, cognitive dysfunction, and cancer, whereas excessive intracellular Zn^{2+} accumulation causes apoptosis, mainly due to an increase in reactive oxygen species caused by a functional decrease in superoxide dismutase.[4–7] As Zn^{2+} cannot be synthesized or degraded, the intracellular homeostasis of dietary Zn^{2+} is strictly regulated by two types of Zn transporters. Zrt- and Irt-like proteins (ZIP/SLC39) import Zn^{2+} from the extracellular space or intracellular organelles into the intracellular space, whereas Zn transporters (ZnT/SLC30) export Zn^{2+} from the cytoplasm into the extracellular space or intracellular organelles.[8]

Although most ZnTs are localized to intracellular organelles, only Zn transporter 1 (ZnT1/SLC30A1) is localized to the plasma membrane and exports Zn^{2+} to the extracellular space.[9–11] Experiments utilizing homozygous *Znt1* gene knockout mice indicated that *ZnT1* is essential for embryonic development.[12] Additionally, the expression and functional abnormalities of ZnT1 are involved in Alzheimer's disease, cancer, and primary aldosteronism.[13–17] A recent report indicated that ZnT1 is localized in the endosomes with a pH gradient by vacuolar ATPase as well as in the plasma membranes of human macrophages.[18] ZnT1-mediated Zn^{2+} filling into endosomes promotes endocytosis of toll-like receptor 4 and programmed cell death ligand 1 and reduces excessive inflammation and immunosuppression in hepatocellular carcinoma, thereby exerting an antitumor effect.[18] Thus, *ZnT1* regulates the intracellular Zn^{2+} concentration to prevent cytotoxicity at high Zn^{2+} concentrations.

However, the transport mechanism of ZnT1 remains unknown due to the lack of an appropriate quantitative transport assay for low concentrations of Zn^{2+} export. ZnT1 exhibited pH-dependent Zn^{2+} transport in a fluorescence assay using Zn^{2+} indicator in human embryonic kidney 293T (HEK293T) cells; however, the intracellular concentration of Zn^{2+} cannot be determined to calculate K_m and V_{max} values.[19] Moreover, ZnT1 exhibited pH-dependent Zn^{2+} transport with low Zn^{2+} affinity (K_m = 1.42 mM: H^+ indicator) in a fluorescence assay

using H^+ indicator in proteoliposomes containing purified ZnT1 variant.[20] As the proton gradient across the plasma membrane is negligible under normal physiological conditions in most cells, this pH-dependent mechanism alone cannot sufficiently explain ZnT1-dependent Zn^{2+} transport at plasma membranes. Therefore, previous studies have proposed that human ZnT1-mediated Zn^{2+} efflux activity is driven by a calcium (Ca^{2+}) gradient and not by a pH gradient.[21,22] In contrast, because ZnT1-localized endosomes are formed ΔpH by the vacuolar ATPase, the physiological significance of pH-dependent Zn^{2+} transport is controversial.

In this study, we observed that human ZnT1 transports Zn^{2+} in pH-dependent and independent manners at physiological concentrations using the highly sensitive radioactive $^{65}Zn^{2+}$, suggesting the pH-independent Zn^{2+} export at plasma membranes to the extracellular space and pH-dependent Zn^{2+} transport in the cytoplasm to acidic organelles.

MATERIALS AND METHODS

Expression and Purification of Human ZnT1 Protein A recombinant baculovirus encoding human *ZnT1* (accession No. NM_021194.3) cDNA was constructed using the Bac-to-Bac baculovirus expression system (Thermo Fisher Scientific, Waltham, MA, U.S.A.) according to the manufacturer's protocol. Human *ZnT1* cDNA was amplified by PCR using the primers (5′-CACCATGGGGTGTTGGG-GTCGGAACCGGGG-3′ and 5′-TCACAAAGATGATTCAG-GTTGTTTGTTTGG-3′). High Five cells (5×10^6 cells/10-cm dish) were infected with recombinant baculoviruses at a multiplicity of infection of 1 and cultured for an additional 48 h. The cells (1.6×10^8 cells) were suspended in a buffer consisting of 20 mM Tris–HCl (pH 8.0), 0.1 M sodium acetate, 10% glycerol, 0.5 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg/mL pepstatin A, and 10 μg/mL leupeptin and then disrupted by sonication using a TOMY UD-211 tip sonifier. The cell lysates were centrifuged at $800 \times g$ for 10 min to remove debris, and the resulting supernatant was further centrifuged at $160000 \times g$ for 1 h. The pellet (membrane fraction) was suspended in buffer consisting of 20 mM 3-(*N*-morpholino) propanesulfonic acid (MOPS)-Tris (pH 8.0), 10% glycerol, 10 μg/mL pepstatin A, and 10 μg/mL leupeptin at 8 mg protein/mL and was solubilized with 1% Foscholine-14. After centrifugation at $260000 \times g$ for 30 min, the supernatant was adjusted to pH 8.5 and applied to 1.25 mL of nickel-nitrilotriacetic acid (Ni-NTA) Superflow resin (Qiagen, Hilden, Germany). After incubation for 4 h at 4°C, the resin was washed with 10 mL of 20 mM MOPS-Tris (pH 8.0), 40 mM imidazole, 20% glycerol, and 0.1% *n*-decyl-β-D-thiomaltopyranoside. The human ZnT1 protein was eluted from the resin with 3 mL of the same buffer containing 300 mM imidazole.

Reconstitution Liposomes with a lipid composition corresponding to that of plasma membranes were prepared as previously described.[23] Briefly, 10 mg/mL liposomes consisting of 40% phosphatidylcholine, 30% phosphatidylethanolamine, 10% phosphatidylserine, and 20% cholesterol in weight ratio were prepared in a buffer consisting of 20 mM 2-(*N*-morpholino) ethanesulfonic acid (MES)–KOH (pH 6.0) and 1 mM DTT. The pH of the buffer was adjusted if necessary.

An aliquot (30 μg protein) of purified human ZnT1 was mixed with liposomes (500 μg) and frozen at −80°C for at least 10 min. The mixture was diluted with reconstitution buffer consisting of 20 mM MES–KOH (pH 6.0 or 6.5), 20 mM MOPS–KOH (pH 7.0 or 7.5), 0.1 M potassium acetate, and 5 mM magnesium acetate. Reconstituted proteoliposomes were pelleted by centrifugation at $200000 \times g$ for 1 h at 4°C and then suspended in 200 μL of reconstitution buffer.

Transport Assay A reaction mixture (130 μL) consisting of 0.5 μg of protein incorporated into proteoliposomes, 20 mM MES–KOH (pH 6.0 or 6.5), 20 mM MOPS–KOH (pH 7.0 or 7.5), or 20 mM Tricine–KOH (pH 8.0), 0.1 M potassium acetate, 5 mM magnesium acetate, 10 mM KCl, and 100 nM $ZnCl_2$ ($^{65}ZnCl_2$: 70 MBq/μmol; RIKEN, Saitama, Japan) was incubated at 27°C. At the indicated time points, the proteoliposomes were separated from the external medium using centrifuge columns containing Sephadex G-50 (fine) to terminate transport. Radioactivity in the eluate was measured using a 3470 WIZARD auto-gamma counter (Revvity, Waltham, MA, U.S.A.).

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis The purification samples were denatured with sample buffer (final concentrations: 2% [w/v] sodium dodecyl sulfate (SDS), 2% [v/v] 2-mercaptoethanol, 10% [v/v] glycerol, 0.06% [w/v] EDTA, 1.2% [w/v] Tris–HCl, 0.2 mg/mL bromophenol blue), and separated by 10% SDS-polyacrylamide gel electrophoresis (PAGE) and visualized by Coomassie Brilliant Blue (CBB) staining and Western blotting against peroxidase-conjugated mouse monoclonal 6×His-tag antibody (Roche, Basel, Switzerland). Western blotting was performed according to the manufacturer's instructions (Cytiva, Tokyo, Japan).

Protease Treatment To determine the orientation of ZnT1 protein in proteoliposomes, protease treatment was performed as previously described.[24] Reconstituted proteoliposomes (30 μg) were incubated for 1 h at 37°C in a reaction mixture consisting of 20 mM MOPS–KOH (pH 6.0), 0.1 M potassium acetate, 5 mM magnesium acetate, and 0.1 μg trypsin (Promega, Madison, WI, U.S.A.). Digestion was terminated by the addition of PMSF to a final concentration of 1 mM. The reaction mixture was analyzed on a dot blot probed with rabbit polyclonal primary antibodies specific for the C-terminal region (E494-L507) of rat ZnT1 (Synaptic Systems, Göttingen, Germany) and peroxidase-conjugated goat polyclonal secondary antibodies to rabbit IgG (MP Biomedicals, Irvine, CA, U.S.A.). The background level was determined by adding 0.1 μg trypsin and 0.1% SDS. The luminescent images ($n=3$) were obtained using ImageQuant 800 (Cytiva), and quantified spot intensity using ImageQuant TL software (Cytiva).

Statistical Analysis The sample size was selected to allow for the statistical analysis of the results based on previous experiments.[25] All numerical values are presented as the mean±standard error of the mean (S.E.M.). A two-tailed paired Student's *t*-test or one-way ANOVA followed by Tukey's test for multiple comparisons was performed to determine the statistical significance of the results using GraphPad

Prism 10 software (GraphPad Software). The *p*-values with statistically significant results are presented in the figures (NS, not significant).

RESULTS

ZnT1 Transports Zn^{2+} in pH-Dependent and Independent Manners To exclude the influence of other proteins in the cells, we directly evaluated the transport activity of highly sensitive radioactive Zn^{2+} using a quantitative transport assay system with proteoliposomes containing only purified human ZnT1 protein. We confirmed the ZnT1 purification step *via* SDS-PAGE, followed by CBB staining and Western blotting with an anti-6×His antibody (Fig. 1A). Human ZnT1 was abundantly expressed in insect cells using a baculovirus expression system. The membrane fractions were largely solubilized with detergent, and the human ZnT1 protein was purified *via* Ni-NTA affinity chromatography. In the final fraction, a major band was detected at the expected apparent molecular mass of 55.3 kDa, and other bands were not detected. We reconstituted purified human ZnT1 protein into liposomes. The 54±6% of the C-terminal region of ZnT1 incorporated in proteoliposomes was accessible to trypsin digestion, indicating that approximately half of the ZnT1 was oriented with the cytoplasmic face directed to the outside of the proteoliposomes.

We evaluated ZnT1-mediated transport activity into proteoliposomes (Fig. 1B). Human ZnT1 protein exhibited Zn^{2+} transport activity compared with that of control liposomes (negative control) without a pH gradient, and Zn^{2+} transport activity was enhanced twofold by ΔpH (Fig. 1C). pH-dependent Zn^{2+} transport was not observed in control liposomes lacking the human ZnT1 protein. In contrast, ZnT1 exhibited more potent Zn^{2+} transport activity, even without a pH gradient, at high Zn^{2+} concentrations than it did at low Zn^{2+} concentrations (Fig. 1D), strongly suggesting that the contribution of pH-independent Zn^{2+} transport activity increased at high concentrations of Zn^{2+}. The influence of Zn^{2+} binding to the ZnT1 protein and phospholipids was negligible compared with that of control liposomes and cold-incubated proteoliposomes (Fig. 1E).

ZnT1 Transports Zn^{2+} with High Affinity Kinetic analysis indicated that human ZnT1-mediated Zn^{2+} transport activity was accelerated in a dose-dependent manner and saturated at concentrations of 5–10 μM (Fig. 2A). ZnT1 yielded K_m and V_{max} values of 0.40±0.03 μM and 15.13±0.53 nmol/min/mg protein for pH-dependent Zn^{2+} transport, 0.52±0.13 μM and 8.88±1.37 nmol/min/mg protein (low concentrations of Zn^{2+}), and 3.02±0.45 μM and 17.59±1.00 nmol/min/mg protein (high concentrations of Zn^{2+}) for pH-independent Zn^{2+} transport, respectively (Fig. 2B). Interestingly, even without pH gradient formation, human ZnT1 was comparable to the pH-dependent Zn transport activity at high concentrations of Zn^{2+}. Moreover, the Eadie–Hofstee plot for the pH 6.0 condition indicates that biphasic kinetics of Zn^{2+} transport, supporting that the ZnT1 protein possesses pH-dependent high-affinity and pH-independent low-affinity Zn^{2+} transport components. Previous studies have reported intracellular Zn^{2+} concentrations at the 10^{-5}–10^{-12} M level,[26] strongly suggesting that the human ZnT1 protein exports Zn^{2+} to the extracellular space with or without a pH gradient at high Zn^{2+} concentrations in the physiological range.

Optimal Intracellular and Extracellular pH Is Important for Zn^{2+} Transport To further investigate pH-dependent Zn^{2+} transport activity, we examined the pH sensitivity inside and outside the proteoliposomes (Fig. 3). The ZnT1-mediated Zn^{2+} transport activity was significantly increased as the pH gradient increased among pH values outside and inside proteoliposomes that were 6.0–7.5 (Figs. 3A, 3B). However, the Zn^{2+} transport activity was higher at an inside pH of 6.0 than at 6.5, 7.0, or 7.5 for proteoliposomes, despite the same ΔpH of 0.5–1.5 (Fig. 3C). The Zn^{2+} transport activity decreased at an outside pH of 8.0 in proteoliposomes, despite the increased ΔpH (Fig. 3C). In our experiment conditions, inside pH 6.0 and outside pH 7.5, which is ΔpH = 1.5, were optimal for ZnT1-mediated Zn^{2+} transport. These results strongly suggest that ZnT1 is important in optimal intracellular and extracellular pH rather than the degree of pH gradient.

Ca^{2+} Gradient Did Not Enhance Zn^{2+} Transport As previous studies have proposed that ZnT1 exports Zn^{2+} *via* a Ca^{2+} gradient,[21,22] we investigated the effect of Ca^{2+} on Zn^{2+} transport activity. The inclusion of Ca^{2+} in liposomes (corresponding to extracellular Ca^{2+} addition) did not increase Zn^{2+} transport, either in the presence or absence of a pH gradient (Fig. 4). These results indicate that pH, but not Ca^{2+}, enhances ZnT1-mediated Zn^{2+} transport.

DISCUSSION

Previous attempts to determine the kinetics and pH dependence of ZnTs have been unsuccessful due to the lack of a method to examine the transport activity of low concentrations of minerals. In this study, we demonstrated that human ZnT1 transports Zn^{2+} with high affinity in pH-dependent and pH-independent manners, depending on the pH conditions inside and outside the cell. This is the first report describing the pH-dependent and pH-independent kinetic parameters and the optimal pH conditions inside and outside the membrane of ZnT1-mediated Zn^{2+} transport.

We revealed that ZnT1 protein possesses biphasic kinetic components of pH-dependent and pH-independent Zn^{2+} transport in physiological Zn^{2+} concentration (Fig. 2). Because the intracellular concentration of Zn^{2+} cannot be determined in cell-based assays, the kinetic parameters were unknown in previous studies.[19] Moreover, previous calculations of the K_m value were limited to indirect evaluation of ZnT1 protein lacking C-terminal domain using an H^+ fluorescent indicator, which required a high Zn^{2+}concentration (1 mM) to detect transport.[20] Consequently, the reported K_m value (1.42 mM) was much higher than physiological Zn^{2+} concentration. In this study, ZnT1-mediated Zn^{2+} transport was directly evaluated using a highly sensitive ^{65}Zn radioisotope, yielding a K_m value (0.4–4 μM) within the physiological range. Previous reports using cell-based and cell-free assays also showed that ZnT1 protein transports Zn^{2+} in a pH-dependent manner, supporting our findings.[19,27] In contrast, it is unclear why Ca^{2+}

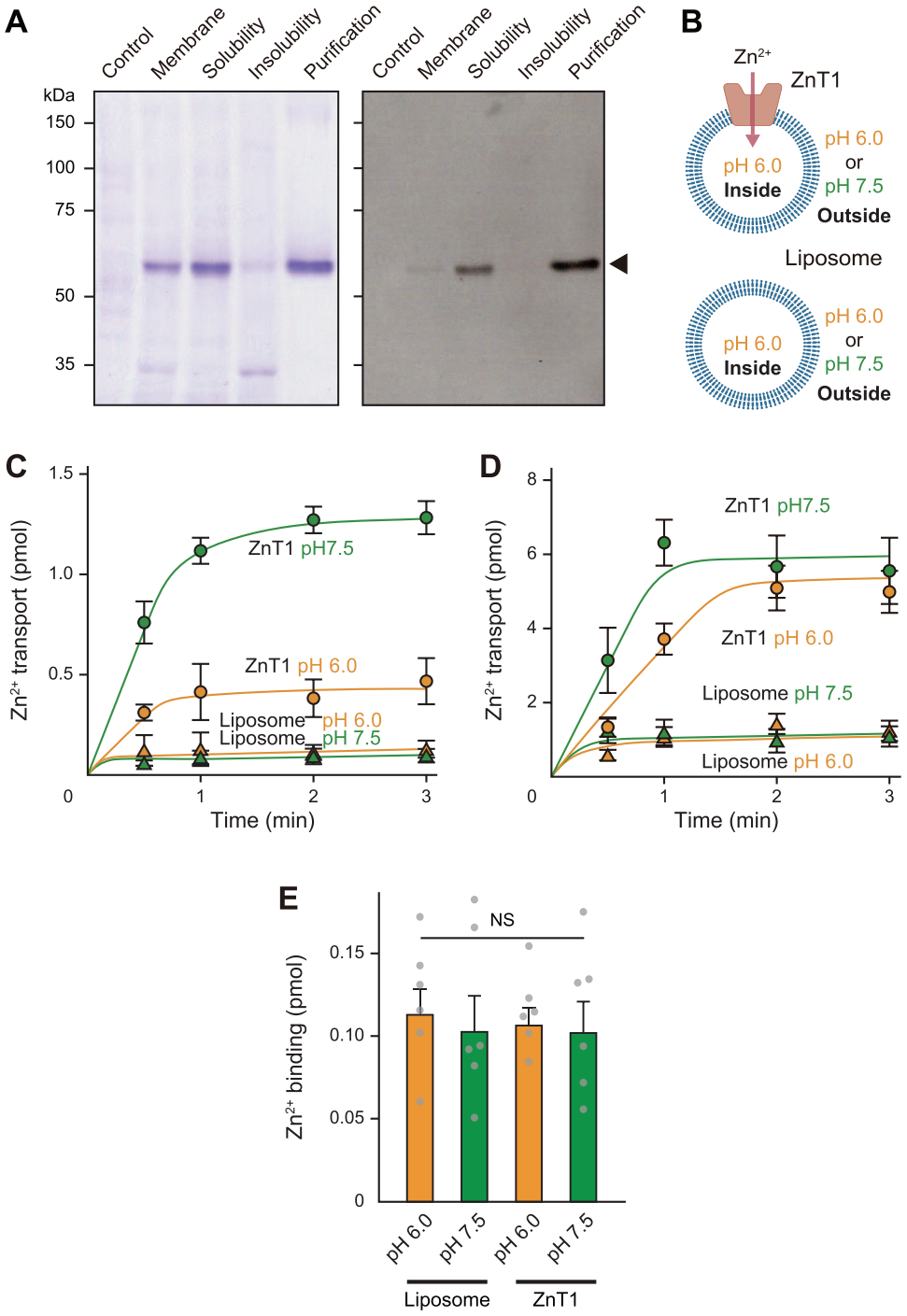

Fig. 1. ZnT1 Transports Zn^{2+} in pH-Dependent and Independent Manners

(A) Purification of human ZnT1 protein. The fractions were analyzed by SDS–PAGE and visualized by CBB staining (left: 2 μg) and by Western blotting with anti-6×His antibody (right, 2 μg). Arrowheads indicate the position of recombinant human ZnT1 protein. (B) ZnT1-mediated Zn^{2+} transport labeled with ^{65}Zn. The proteoliposomes containing ZnT1 protein (0.5 μg) or liposomes (negative control) at an inside pH 6.0 were incubated in a buffer solution containing Zn^{2+} at either pH 6.0 (yellow) or pH 7.5 (green) at 27°C. (C, D) Time-course of ZnT1-mediated Zn^{2+} transport at 100 nM (C) and 5 μM (D). (E) The influence of Zn^{2+} binding to the ZnT1 protein in pH-dependent and independent manners at 100 nM and 4°C compared with that of liposomes at 100 nM and 27°C. Data are presented as mean ± S.E.M. (n=6-8). NS: not significant (one-way ANOVA followed by Tukey's test).

dependence was observed in the previous reports; however, the ZnT1 protein structures with bound H^+ or Ca^{2+} have yet to be elucidated. Future investigations of the ZnT1 structures with bound H^+ or Ca^{2+} are expected to clarify the debatable issue regarding Zn^{2+} transport mechanisms.

We demonstrated that ZnT1 protein transports Zn^{2+} in an optimal pH-dependent manner (Fig. 3). Although plasma membranes, such as the brush border membrane of the small intestine and the proximal renal tubule, exhibit a pH gradient, previous reports suggest that ZnT1 is localized at the basolateral membrane, where no pH gradient exists in these tissues.[28,29] In contrast, Yang *et al.* recently reported that ZnT1 was abundantly localized to the early endosomes of macrophages, and exhibited antitumor effects in mouse

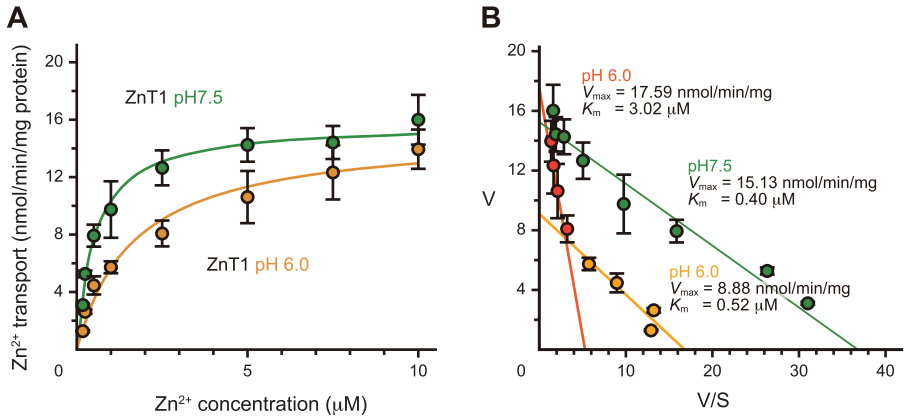

Fig. 2. ZnT1 Transports Zn^{2+} in the Physiological Concentration Range

(A, B) Dose-dependence of human ZnT1-mediated Zn^{2+} transport (A) and an Eadie–Hofstee plot (B). The proteoliposomes containing ZnT1 at an inside pH 6.0 were incubated in a buffer solution containing the indicated Zn^{2+} concentrations at either pH 6.0 or pH 7.5, and the assay was performed after 30 s. The values were subtracted from those of control liposomes at pH 6.0 and 7.5. Data are presented as mean ± S.E.M. (n=8–10).

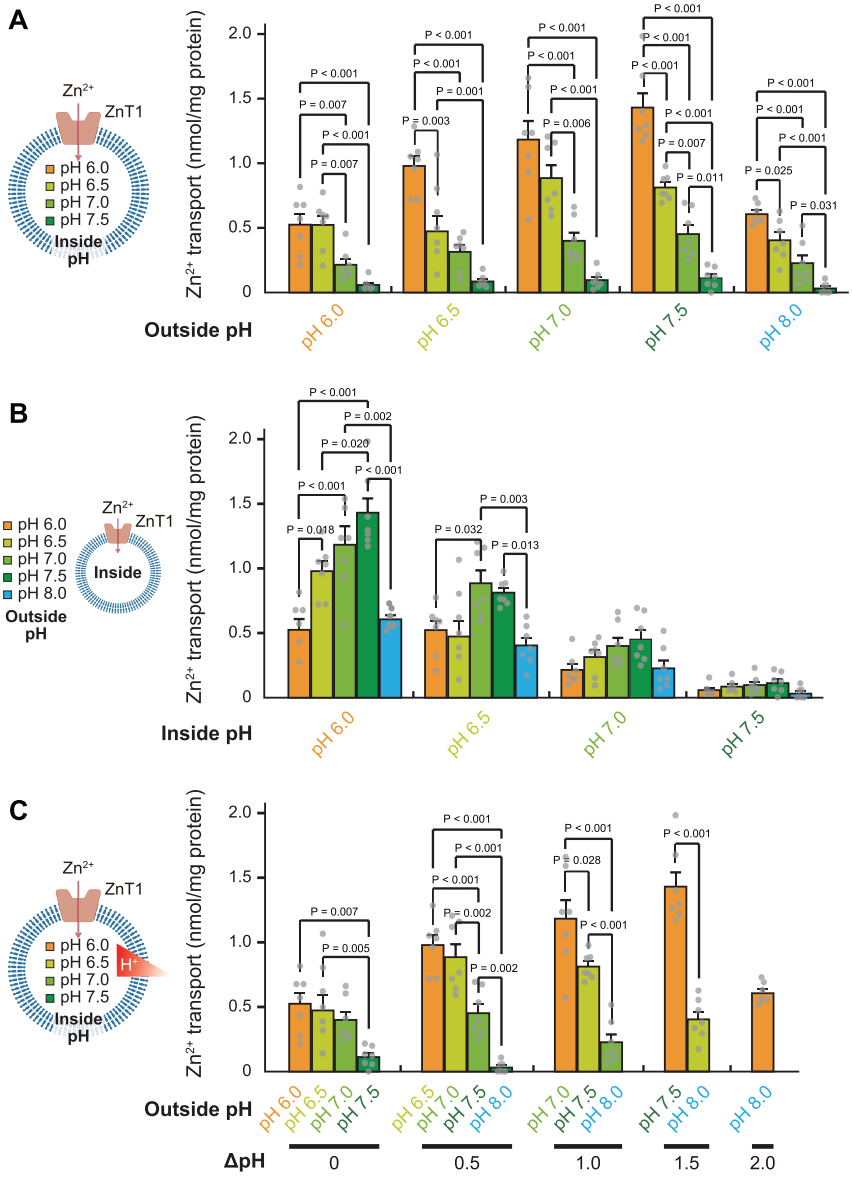

Fig. 3. The Optimum pH Is Important for the Zn^{2+} Transport Activity of ZnT1

(A–C) The effects of pH on Zn^{2+} transport at 100 nM. Proteoliposomes containing purified ZnT1 protein were prepared with inside pH values of 6.0, 6.5, 7.0, and 7.5, incubated in a buffer at outside pH values of 6.0, 6.5, 7.0, 7.5, or 8.0, and assayed after 2 min. The values were subtracted from those of control liposomes at each pH. Each bar means inside pH (A) and outside pH (B). (C) Data are sorted on the horizontal axis according to each ΔpH (ΔpH=0, 0.5, 1.0, 1.5, or 2.0) from Figs. 3A and 3B. Data are presented as mean ± S.E.M. (n=7). The p-values are presented in the figure (one-way ANOVA followed by Tukey's test or two-tailed paired Student's t-test).

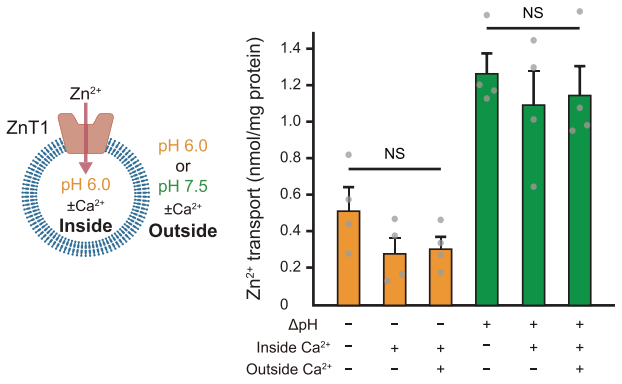

Fig. 4. ZnT1 Transports Zn^{2+} in a Ca^{2+}-Independent Manner

The Ca^{2+} effect on Zn^{2+} transport at 100 nM in ZnT1 proteoliposomes. Proteoliposomes containing ZnT1 or liposomes (negative control) at pH 6.0 were incubated in the presence or absence of 1.8 mM Ca^{2+} at either pH 6.0 or pH 7.5 and assayed after 2 min. Data are presented as mean ± S.E.M. (n = 4). NS: not significant (one-way ANOVA followed by Tukey's test).

hepatocellular carcinoma.[18] As endosomes are maintained at a weakly acidic state, and ZnT1 can transport low concentrations of Zn^{2+} in a pH-dependent manner (Fig. 2), endosomes may exhibit stronger ZnT1-mediated Zn^{2+} transport activity than plasma membranes, even at low Zn^{2+} concentrations.[30] Conversely, the cancer microenvironment possesses an acidic extracellular space and is associated with cancer invasion and suppression of immune response.[31,32] In the cancer microenvironment, ZnT1 is thought to be activated in a pH-dependent manner in the plasma membrane and intracellular organelles. As Zn^{2+} is essential for the activation of the tumor suppressor p53, decreasing intracellular Zn^{2+} levels leads to cancer progression.[33] Of note, ZnT1 reduces intracellular Zn^{2+} levels in prostate cancer,[34] and an increase in ZnT1 expression reduces survival in patients with esophageal carcinoma, pancreatic adenocarcinoma, rectal adenocarcinoma, stomach adenocarcinoma, and thymoma.[16] As ZnT1 inhibition in cancer cells normalizes intracellular Zn^{2+} levels and reduces cancer progression, ZnT1 is expected to be a potential therapeutic target for these cancers because it restores tumor suppression. Further studies exploring potent and selective ZnT1 inhibitors are currently underway.

ZnT1 may be localized not only in endosomes but also in other intracellular organelles, depending on the cell type. Other isoforms of ZnT are localized to various intracellular organelles. For example, ZnT2 and ZnT4 are also localized to endosomes that contain Zn^{2+}-requiring structural and catalytic proteins.[8] These knockout mice possessed decreased Zn^{2+} in breast milk but did not exhibit a severe phenotype, suggesting that ZnT1 acts as an alternative mechanism, similar to ZnT2 or ZnT4. Future investigations into the localization of ZnT1 to intracellular organelles in various cell types are expected to clarify the novel roles of ZnT1 and the physiological significance of its pH dependence of ZnT1.

In summary, we determined the kinetic parameters and optimal pH conditions for ZnT1-mediated Zn^{2+} transport and demonstrated that human ZnT1 transports Zn^{2+} with high affinity in pH-dependent and pH-independent manners. As the intracellular and extracellular pH is almost neutral, our findings strongly suggest that ZnT1 is important in the export of excess intracellular Zn^{2+}, even without ΔpH, in the physiological range of Zn^{2+} concentrations. The physiological and pathological significance of pH-dependent Zn^{2+} transport may be involved in intracellular organelles of normal cells and plasma membranes of cancer cells.

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DECLARATIONS

Author Contributions T.M. designed the study. Y.Y. performed all experiments. Y.Y. and T.M. analyzed the data. Y.Y. and T.M. wrote the manuscript.

Conflict of Interest The authors declare no conflict of interest.

REFERENCES

1) Fukada T, Yamasaki S, Nishida K, Murakami M, Hirano T. Zinc homeostasis and signaling in health and diseases: Zinc signaling. *J. Biol. Inorg. Chem.*, **16**, 1123–1134 (2011).
2) Wessels I, Maywald M, Rink L. Zinc as a gatekeeper of immune function. *Nutrients*, **9**, 1286 (2017).
3) Andreini C, Banci L, Bertini I, Rosato A. Counting the zinc-proteins encoded in the human genome. *J. Proteome Res.*, **5**, 196–201 (2006).
4) Prasad AS. Discovery of human zinc deficiency: its impact on human health and disease. *Adv. Nutr.*, **4**, 176–190 (2013).
5) Chen B, Yu P, Chan WN, Xie F, Zhang Y, Liang L, Leung KT, Lo KW, Yu J, Tse GMK, Kang W, To KF. Cellular zinc metabolism and zinc signaling: from biological functions to diseases and therapeutic targets. *Signal Transduct. Target. Ther.*, **9**, 6 (2024).
6) Kumari A, Singh KP, Mandal A, Paswan RK, Sinha P, Das P, Ali V, Bimal S, Lal CS. Intracellular zinc flux causes reactive oxygen species mediated mitochondrial dysfunction leading to cell death in *Leishmania donovani*. *PLoS One*, **12**, e0178800 (2017).
7) Kim YH, Eom JW, Koh JY. Mechanism of zinc excitotoxicity: a focus on AMPK. *Front. Neurosci.*, **14**, 577958 (2020).
8) Kambe T, Taylor KM, Fu D. Zinc transporters and their functional integration in mammalian cells. *J. Biol. Chem.*, **296**, 100320 (2021).
9) Palmiter RD, Findley SD. Cloning and functional characterization of a mammalian zinc transporter that confers resistance to zinc. *EMBO J.*, **14**, 639–649 (1995).
10) Palmiter RD. Protection against zinc toxicity by metallothionein and zinc transporter 1. *Proc. Natl. Acad. Sci. U.S.A.*, **101**, 4918–4923 (2004).

11) Nishito Y, Kambe T. Zinc transporter 1 (ZNT1) expression on the cell surface is elaborately controlled by cellular zinc levels. *J. Biol. Chem*., **294**, 15686–15697 (2019).
12) Andrews GK, Wang H, Dey SK, Palmiter RD. Mouse *zinc transporter 1* gene provides an essential function during early embryonic development. *Genesis*, **40**, 74–81 (2004).
13) Lovell MA, Smith JL, Xiong S, Markesbery WR. Alterations in zinc transporter protein-1 (ZnT-1) in the brain of subjects with mild cognitive impairment, early, and late-stage Alzheimer's disease. *Neurotox. Res.*, **7**, 265–271 (2005).
14) Zhang LH, Wang X, Stoltenberg M, Danscher G, Huang L, Wang ZY. Abundant expression of zinc transporters in the amyloid plaques of Alzheimer's disease brain. *Brain Res. Bull.*, **77**, 55–60 (2008).
15) Lyubartseva G, Smith JL, Markesbery WR, Lovell MA. Alterations of zinc transporter proteins ZnT-1, ZnT-4 and ZnT-6 in preclinical Alzheimer's disease brain. *Brain Pathol.*, **20**, 343–350 (2010).
16) Lehvy AI, Horev G, Golan Y, Glaser F, Shammai Y, Assaraf YG. Alterations in ZnT1 expression and function lead to impaired intracellular zinc homeostasis in cancer. *Cell Death Discov.*, **5**, 144 (2019).
17) Rege J, Bandulik S, Nanba K, Kosmann C, Blinder AR, Plain A, Vats P, Kumar-Sinha C, Lerario AM, Else T, Yamazaki Y, Satoh F, Sasano H, Giordano TJ, Williams TA, Reincke M, Turcu AF, Udager AM, Warth R, Rainey WE. Somatic *SLC30A1* mutations altering zinc transporter ZnT1 cause aldosterone-producing adenomas and primary aldosteronism. *Nat. Genet.*, **55**, 1623–1631 (2023).
18) Yang D, Tian T, Li X, *et al*. ZNT1 and Zn^{2+} control TLR4 and PD-L1 endocytosis in macrophages to improve chemotherapy efficacy against liver tumor. *Hepatology*, **80**, 312–329 (2024).
19) Shusterman E, Beharier O, Shiri L, Zarivach R, Etzion Y, Campbell CR, Lee IH, Okabayashi K, Dinudom A, Cook DI, Katz A, Moran A. ZnT-1 extrudes zinc from mammalian cells functioning as a Zn^{2+}/H^{+} exchanger. *Metallomics*, **6**, 1656–1663 (2014).
20) Cotrim CA, Jarrott RJ, Whitten AE, Choudhury HG, Drew D, Martin JL. Heterologous expression and biochemical characterization of the human zinc Transporter 1 (ZnT1) and its soluble C-terminal domain. *Front Chem.*, **9**, 667803 (2021).
21) Gottesman N, Asraf H, Bogdanovic M, Sekler I, Tzounopoulos T, Aizenman E, Hershfinkel M. ZnT1 is a neuronal Zn^{2+}/Ca^{2+} exchanger. *Cell Calcium*, **101**, 102505 (2022).
22) Sun C, He B, Gao Y, Wang X, Liu X, Sun L. Structural insights into the calcium-coupled zinc export of human ZnT1. *Sci. Adv.*, **10**, eadk5128 (2024).
23) Mitani-Ueno N, Yamaji N, Huang S, Yoshioka Y, Miyaji T, Ma JF. A silicon transporter gene required for healthy growth of rice on land. *Nat. Commun.*, **14**, 6522 (2023).
24) Juge N, Yoshida Y, Yatsushiro S, Omote H, Moriyama Y. Vesicular glutamate transporter contains two independent transport machineries. *J. Biol. Chem.*, **281**, 39499–39506 (2006).
25) Kato Y, Ohsugi K, Fukuno Y, Iwatsuki K, Harada Y, Miyaji T. Vesicular nucleotide transporter is a molecular target of eicosapentaenoic acid for neuropathic and inflammatory pain treatment. *Proc. Natl. Acad. Sci. U.S.A.*, **119**, e2122158119 (2022).
26) Outten CE, O'Halloran TV. Femtomolar sensitivity of metalloregulatory proteins controlling zinc homeostasis. *Science*, **292**, 2488–2492 (2001).
27) Long Y, Zhu Z, Zhou Z, Yang C, Chao Y, Wang Y, Zhou Q, Wang MW, Qu Q. Structural insights into human zinc transporter ZnT1 mediated Zn^{2+} efflux. *EMBO Rep.*, **25**, 5006–5025 (2024).
28) McMahon RJ, Cousins RJ. Regulation of the zinc transporter ZnT-1 by dietary zinc. *Proc. Natl. Acad. Sci. U.S.A.*, **95**, 4841–4846 (1998).
29) Cousins RJ, McMahon RJ. Integrative aspects of zinc transporters. *J. Nutr.*, **130** (Suppl.),1384S–1387S (2000).
30) Cain CC, Sipe DM, Murphy RF. Regulation of endocytic pH by the Na^{+}, K^{+}-ATPase in living cells. *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 544–548 (1989).
31) Estrella V, Chen T, Lloyd M, Wojtkowiak J, Cornnell HH, Ibrahim-Hashim A, Bailey K, Balagurunathan Y, Rothberg JM, Sloane BF, Johnson J, Gatenby RA, Gillies RJ. Acidity generated by the tumor microenvironment drives local invasion. *Cancer Res.*, **73**, 1524–1535 (2013).
32) Boedtkjer E, Pedersen SF. The acidic tumor microenvironment as a driver of cancer. *Annu. Rev. Physiol.*, **82**, 103–126 (2020).
33) Puca R, Nardinocchi L, Porru M, Simon AJ, Rechavi G, Leonetti C, Givol D, D'Orazi G. Restoring p53 active conformation by zinc increases the response of mutant p53 tumor cells to anticancer drugs. *Cell Cycle*, **10**, 1679–1689 (2011).
34) Franz MC, Anderle P, Bürzle M, Suzuki Y, Freeman MR, Hediger MA, Kovacs G. Zinc transporters in prostate cancer. *Mol. Aspects Med.*, **34**, 735–741 (2013).