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Vesicular transport of Fe and interaction with other metal ions in polarized Caco2 Cell monolayers

MARIA C LINDER, MIZUE MORIYA, ANNE WHON, AZEB KASSA and CYNTHIA GILLEY

Department of Chemistry and Biochemistry, California State University, Fullerton, California 92834-6866, USA

ABSTRACT

Two aspects of the mechanisms by which iron is absorbed by the intestine were studied in the Caco2 cell model, using ^{59}Fe(II)-ascorbate. Data showing the importance of vesicular processes and cycling of apotransferrin (apoTf) to uptake and overall transport of Caco2 cell monolayers (or basolateral ^{59}Fe release) were obtained by comparing effects of: a) adding apoTf to the basal chamber; b) adding vesicular transport inhibitors; or c) cooling to 4ºC. These showed that apoTf may be involved in as much as half of Fe transfer across the basolateral membrane, and that vesicular processes may also play a role in non-apoTf-dependent Fe transport. Studies were initiated to examine potential interactions of other metal ions with Fe(II) via DMT1. Kinetic data showed a single, saturable process for uptake of Fe(II) that was pH dependent and had a Km of 7 μM. An excess of Mn(II) and Cu(I) over Fe(II) of 200: 1 (μM: μM) in 1 mM ascorbate markedly inhibited Fe uptake. The kinetics were not competitive. Km increased and Vmax decreased. We conclude that vesicular transport, involving endo- and exocytosis at both ends of the enterocyte, is a fundamental aspect of intestinal iron absorption and that DMT1 may function as a transporter not just for divalent but also for monovalent metal ions.

Key terms: intestinal iron absorption, Caco2 cells, apotransferrin, vesicular transport, copper, manganese

INTRODUCTION

The intestinal absorption of iron from the diet occurs by processes that are just beginning to be understood at the molecular level. Absorption of inorganic iron is not very efficient, only about 10% of that in the diet normally being absorbed (Linder, 1991). For uptake from the gut lumen, iron must be presented in soluble form. This occurs through the help of dietary agents (such as ascorbate, citrate, fructose and some amino acids) that chelate the iron so it does not precipitate out of aqueous solution. For absorption, iron must first enter the enterocyte by crossing the brush border, then cross the cell to the basolateral membrane, and finally exit to the blood.

Most studies agree that Fe(II) is preferentially absorbed (Andrews, 2000; Conrad and Umbreit, 2000; Roy and Enns, 2000; Rolfs and Hediger, 2001; Morgan and Oates, 2002; Chung and Wessling-Resnick, 2003; Donovan and Andrews, 2004). However, iron administered as Fe(III) is absorbed as well (Linder and Munro, 1977; Raja et al., 1992; Núñez et al., 1994; Han et al., 1995), and a ferrireductase (DCYTB) that may mediate this process has been identified and cloned (Riedel et al., 1995; McKie et al., 2001; Ekmekcioglu et al., 1996). A divalent metal transporter (DMT1), also in the brush border, is likely to be responsible for most of the Fe uptake (Fleming et al., 1997 and 1998; Gunshin et al., 1997). Whether it accounts for all brush border uptake is unknown, and considerable evidence suggests that it is not. From studies with everted gut sacs from rats and mice, there is longstanding evidence that a significant portion of iron uptake is energy dependent (Linder et al., 1975; Linder and

Corresponding Author: Maria C. Linder, Department of Chemistry and Biochemistry, California State University, Fullerton, CA 92834-6866, USA, Tel.: (1-714) 278-3621, Fax: (1-714) 278-5316, E-mail: mlinder@fullerton.edu

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Munro, 1977), e.g., there are energy-dependent and non-energy dependent uptake mechanisms. DMT1 uptake is proton (but not energy) dependent. Moreover, at higher Fe concentrations, other as yet unidentified brush border transporters or uptake mechanisms (perhaps associated with DCYTB) may come into play (Linder and Munro, 1977).

Expression of DMT1 protein (as well as of the ferrireductase) and its deployment are inversely related to levels of iron within the enterocyte (Fleming et al., 1997; Gunshin et al., 1997; Yeh et al., 2000). Together, changes in brush border concentrations of DMT1 and the ferrireductase would thus account, at least in part, for the well-known phenomenon that nutritional iron status is inversely related to the rate of intestinal iron absorption (Linder and Munro, 1977; Andrews, 2000; Roy and Enns, 2000; Chung and Wessling-Resnick, 2003). The mechanisms controlling expression and functions of these proteins are still being studied. In the case of DMT1, there are at least four different mRNA transcripts, two of which contain an iron responsive element (IRE) in the 3'UTR. These might allow the regulation of mRNA levels through stabilization by IRPs, as in the case of transferrin receptor mRNA, which has five IREs in the 3'UTR (Leibold and Guo, 1992; Eisenstein, 2000). However, control by iron deficiency appears to occur mainly via changes in transcription (Donovan and Andrews, 2004; Chung and Wessling-Resnick, 2003). In addition, there is new evidence that iron administration and availability influence deployment of DMT1 at the brush border vs. in endocytic vesicles (Ma et al., 2002).

Despite these important and interesting findings about brush border uptake, there has been evidence for some time that the main control over iron absorption is exerted at the basolateral end of the enterocyte in connection with its transfer to the blood (Linder and Munro, 1977). Thus, there can be considerable apical uptake yet little basolateral transfer, resulting in iron accumulation (in ferritin) within the enterocyte. Such observations led to the early hypothesis that ferritin is a "mucosal block" to iron absorption (Granick, 1946; Linder and Munro, 1977), i.e., that induction of ferritin by incoming iron prevents iron from going further because of incorporation into ferritin. Indeed, since it would appear that DMT1 is required not just for Fe(II) uptake but also for uptake of other divalent metal ions (Gunshin et al., 1997), and since there are non iron-regulated forms of DMT1 mRNA (Lee et al., 1998; Yeh et al., 2000) that must result in expression of the transporter even when iron absorption is not required, large amounts of iron in the diet would be expected to result in a large iron uptake by mucosal cells, even when not needed by the body. This does not mean that most of it will be transferred into the body *per se*, since basolateral transfer is more stringently controlled. However, control is not perfect, and continuous exposure to excess iron in the diet can lead to iron overload (Roy and Enns, 2000; Linder and Munro, 1977).

Just how iron crosses the cell after entry and how it is then transferred across the basolateral surface (to the blood) is only beginning to be understood. At least two different mechanisms may be involved. One would be the iron transporter, IREG1/ ferroportin/MTP1, first detected in zebrafish (Donovan et al., 2000) and hypotransferrinemic mice (McKie et al., 2000), and cloned from an IRE binding-enriched mouse cDNA library (Abboud and Haile, 2000). This transporter is present in the basolateral membrane of the intestinal epithelium (McKie et al., 2000) but also has been detected in endosomes (Chen et al., 2003). Second, there is good evidence for an endo/exocytic cycling mechanism involving apotransferrin (Álvarez-Hernández et al., 1994, 1998; Núñez et al., 1999). Based on studies with Caco2 cells, apotransferrin appears to be absorbed from the blood/interstitial fluid and to travel in vesicles to a compartment above the nucleus before returning to the basolateral membrane and exiting the cell. [Iron-containing transferrin can also enter enterocytes from the blood, but it goes to a different vesicular compartment and,

primarily, to crypt cells (Núñez et al., 1999).] Glass and coworkers have shown that during apotransferrin cycling, it colocalizes with DMT1 that has endocytosed in from the brush border (Ma et al., 2002). We postulate that during this endosomal colocalization, and perhaps with the help of hephaestin (a copper-containing ferroxidase mainly in endosomes), Fe(II) that is with DMT1 is oxidized to Fe(III) and transferred to apotransferrin, forming diferric-transferrin which then cycles out to the blood. Hephaestin, originally identified through a sex-linked anemia in mice (Vulpe et al., 1999) and with homology to ceruloplasmin, also may aid in the release of Fe(II) from ferroportin, as some hephaestin may locate to the basolateral membrane [shown in a rat intestinal cell line (IEC-6) and K562 cells (Simovich et al., 2002)].

The studies from the Glass and Núñez groups implicating apotransferrin cycling in intestinal iron absorption interested us particularly not only because apotransferrin is normally abundant in the blood plasma, which has generally been ignored when using cell lines as models for intestinal function; but also because of burgeoning evidence that copper transport across enterocytes involves vesicular processes. Thus, the copper deficiency of Menkes disease is explained by the finding that the ATP7A transporter (MNK), defective in this condition, normally pumps copper (received from a copper "chaperone") into the enterocyte Trans-Golgi network (TGN). This copper probably then enters endosomes for exocytosis to the blood (Linder et al., 2003). Similarly, copper pumped into the TGN by the analogous ATP7 (WND) in hepatocytes finds it way through endosomes either to the bile cannaliculi (formed by hepatocyte membranes) or is incorporated into ceruloplasmin that is secreted through exocytosis to the blood. These considerations prompted our own studies on the effects of apotransferrin and vesicular processes on intestinal iron absorption (Moriya and Linder, 2005), which are summarized in this report.

The other aspect of intestinal iron absorption that interested us particularly was DMT1 and its potential for uptake not just of Fe(II) but also of other divalent metal ions. The original studies of Gunshin et al. (1997) suggested that many divalent metal ions might use this transporter, since they (like iron) produced an inward current. However, other studies showed that certain metals (like Zn) might not use DMT1 (Tallkvist et al., 2000), and those of Arredondo et al. (2003) indicated Cu(I) rather than Cu(II) uptake by DMT1. Our own earlier studies and those of Arredondo et al. (2003) with Caco2 cell monolayers showed that iron deficiency, which increases expression of DMT1, also enhances Cu uptake (Linder et al., 2003; Zerounian et al., 2003) and that reduced expression of DMT1 (through anti-sense oligomer treatment) also reduced uptake of copper (Arredondo et al., 2003). In the studies described here, we took a kinetic approach, reasoning that if Cu and Mn ions compete for uptake with Fe(II), they could reduce initial rates of Fe(II) uptake and that competitive kinetics would be demonstrated. Our results indicate that inhibition does occur but that the kinetics are not competitive.

METHODS

Fe transport in Caco2 cell monolayers

Uptake, retention and overall transport of Fe(II) by Caco2 cell monolayers were measured as described previously (Zerounian and Linder, 2002; Moriya and Linder, 2005). Monolayers were grown in DMEM medium with 10 or 20% fetal bovine serum on collagen-treated filters in Transwells (Corning Costar, Fisher Scientific) to a resistance of 1200-1400 Ohms. Lack of paracellular transport was determined by applying phenol-red, apically. Standard transport over 60-90 min was measured in Hepes buffered saline (130 mM NaCl, 10 mM KCl, 50 mM Hepes, 5 mM glucose, 1 mM $CaCl_2$, 1 mM $MgSO_4$, pH 7.4), with 1μM $^{59}Fe(II)$ in the presence of 1 mM ascorbate applied to the apical chamber, in the presence or absence of apotransferrin (39 μM) in the basal chamber. Cells were incubated in a CO_2

incubator at 37ºC. In some cases, inhibitors of aspects of vesicular transport were also present in the basal and/or apical media, before and/or after addition of the ^{59}Fe(II) substrate. In other cases, Transwell plates were placed on ice during uptake measurements in the CO_2 incubator (kept at room temperature) and compared with uptake at 37ºC. Uptake was calculated as the total radioactivity lost from the apical chamber (that in cells plus the basal chamber). Overall transport was radioactivity appearing in the basal chamber. Cellular iron retention was radioactivity in the washed cell monolayer.

Fe uptake kinetics

Caco2 cells, and in some cases rat hepatoma cells as well (Tran et al., 1997), were grown to about 80% confluence in T25 flasks. Uptake was measured in the same Hepes buffer (above), usually containing 1 mM ascorbate and a range of concentrations of ^{59}Fe(II), in the absence or presence of Cu(II) or Mn(II)-histidine (1: 10 M ratio). In the presence of 1 mM ascorbate, the Cu(II) was reduced to Cu(I), but the Mn(II) was not changed, based upon considerations of relative reduction potentials and pH (Harold Rogers, personal communication; Buettner and Jurkiewicz, 1993). Initial rates were based on uptake over 20 min, during which accumulation was linear. Rates were corrected for cell number based on protein analysis of cell lysates, using the Bradford method (BioRad reagent), with bovine serum albumin as the standard.

RESULTS

Effects of apotransferrin on iron transport by Caco2 cell monolayers

As indicated in the Introduction, we were intrigued by the observations of Glass and Núñez and their colleagues that apotransferrin (abundant in blood plasma) was entering Caco2 cells at their base (on the "blood side") and cycling within the enterocytes in conjunction with iron absorption across the monolayer. Our own observations confirmed that apotransferrin had a marked effect on iron transport in this intestinal model (Moriya and Linder, 2005). Some of our data on the degree of stimulation are summarized in figure 1. During studies in which 1 μM ^{59}Fe(II) was placed in the apical chamber and apotransferrin in the basal chamber, there was about a doubling of the rate of overall transport, i.e., about twice as much radioactivity was transferred to the basal chamber over 6 h. This implies, first, that vesicular transport is important for intestinal iron absorption and, second, that apotransferrin-mediated transport in intestinal epithelium may account for about half of the iron absorbed. The presence of apotransferrin also resulted in a slightly but significantly greater net *uptake* of the ^{59}Fe (Fig. 1), suggesting some sort of signaling between the cycling of apotransferrin in endosomes to the upper region of the enterocyte and perhaps the endocytic uptake of iron from the brush border (Ma et al., 2005). The effects of apotransferrin on basolateral transport and overall iron absorption through the monolayers was confirmed by studies in which the release of ^{59}Fe from prelabeled monolayers was monitored (Moriya and Linder, 2005).

Effects on iron absorption of inhibiting vesicular transport

If apotransferrin cycling is so important for intestinal iron absorption, one would expect that inhibiting various aspects of vesicle formation and trafficking would interrupt the process. To examine this, we first tested the effects of a variety of substances known to compromise specific aspects of endocytosis and vesicle sorting. These substances and the steps they are thought to target are illustrated in figure 2. FSBA [5'-(4-fluorosulfonylbenzoyl)adenosine hydrochloride] and nocodazole inhibit the first two steps in endocytosis, respectively. The ATP analogue, FSBA, can interfere with sequestration of receptors into clathrin-coated pits by preventing a specific protein phosphorylation step (Olusanya et al., 2001). Nocodazole depolymerizes

microtubules and promotes dispersal and tubulation of the Golgi apparatus (Turner and Tartakoff, 1989). Brefeldin A disrupts the Golgi apparatus (Lippincott-Schwartz et al., 1990) but also has been reported to interfere with normal sorting and recycling of the holotransferrin bound to its receptor in endocytic vesicles (Xia and Shen, 2001; van Dam and Stoorvogel, 2002). Tyrphostin A8 (AG10) inhibits a GTPase (Rab17) necessary for the fusion of vesicles and endosomes in the apical region of polarized epithelial cells (Lütcke et al., 1993; Hunziker and Peters, 1998; Zacchi et al., 1998; Knight et al., 1995; Hughson and Hopkins, 1990). This is where DMT1 (internalized from the brush border) and apotransferrin (in vesicles coming from the basal region) might come together. The effects of these inhibitors on iron transport thus were examined in the standard Caco2 cell monolayer system (Moriya and Linder, 2005). Except in the case of AG10, cells were pretreated with the inhibitor for 60-90 min before ^{59}Fe(II) was added to measure transport. As in studies by other investigators, AG10 was added at the same time as ^{59}Fe. Except for some experiments with AG10, all studies were done with apotransferrin in the basal medium.

The results of these studies are summarized in figure 3. Values for the effectiveness of the inhibitors are given relative to what occurred in untreated (control) monolayers from the same experiment. Consistent with our theories, FSBA and nocodazole each inhibited uptake of iron by about 50% over a range of doses (Fig. 3A). They reduced overall transport of the ^{59}Fe by half as well. Brefeldin A (BFA) had much smaller (but still significant) inhibitory effects on iron uptake and overall transport. AG10 also inhibited iron uptake (Fig. 3B), and this was the case whether or not apotransferrin was present in the basal medium. However, in the presence of apotransferrin, AG10 markedly enhanced the flux of ^{59}Fe from the enterocyte to the basal chamber. This adds further credence to the concept that apotransferrin is important to iron transport by the enterocyte and particularly so with regard to its crossing the basolateral membrane to the blood.

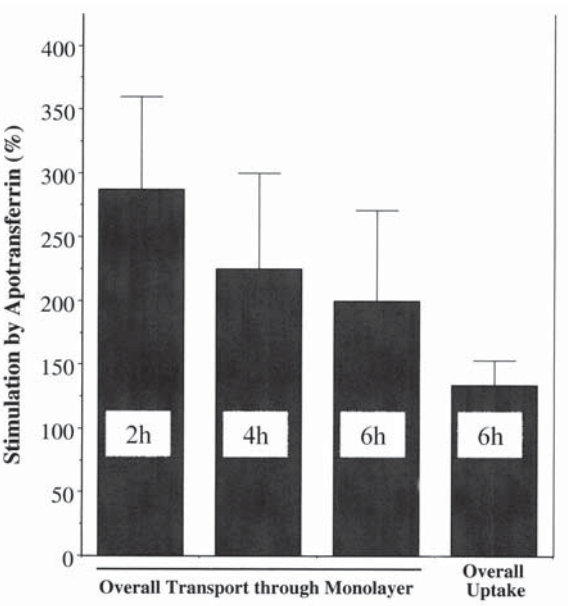

Figure 1. Effects of apotransferrin on uptake and overall transport of iron by Caco2 cell monolayers. Monolayers were incubated for up to 6h with ^{59}Fe(II) (1 μM) added to the apical fluid. The appearance of ^{59}Fe in the basal chamber (Overall Transport) and the net ^{59}Fe removed from the apical chamber (Overall Uptake) were recorded. Data are given as the percent stimulation (Mean±SD, N=5-13) afforded by the addition of apotransferrin to the basal chamber, during absorption. The increases in transport over controls were all significant ($p<0.001$) (partly from Moriya and Linder, 2005).

Additional evidence for the involvement of vesicular transport in iron absorption was obtained by studying the effects of cooling to 4ºC. Vesicular transport of all kinds requires microtubules and energy. Microtubules depolymerize at this low temperature (Jin and Snider, 1993), and the rate of ATP and GTP production (dependent on metabolic pathways) also will decrease. As summarized in figure 4, cooling had a very marked suppressing effect on brush border uptake and basolateral release of iron in the Caco2 cell system (Moriya and Linder, 2005). After 60 min for example, there was 14 times less ^{59}Fe in the cells and

basal chamber when monolayers were held at 4º. ^{59}Fe release to the basal chamber from preloaded cells was reduced to about the same extent, entirely consistent with underlying vesicular transport.

Kinetics of iron uptake by DMT1 and interactions with copper and manganese

It is generally accepted that DMT1 is the primary intestinal brush border transporter for uptake of iron from the diet. Yet, other metal ions may also be transported by DMT1, and if so, they should compete with Fe(II) for uptake. To verify that this might be the case, we began by establishing the kinetics of Fe(II) uptake by Caco2 cell monolayers, measuring initial rates at various concentrations of iron in 1 mM ascorbate. A typical example of the results is given in figure 5A. The curve indicates that a single, saturable transporter (DMT1) is involved. Double reciprocal plots (such as in Fig. 5C) gave Km values for Fe(II) uptake averaging 7±1 μM (Mean±SD; N=6). It is noteworthy that virtually identical results were obtained for the uptake kinetics of Fe(II) with hepatoma cells (Figure 5B), although it is highly doubtful that hepatic cells normally would be exposed to this form of iron. This suggests that DMT1 also is present on the surface of hepatic cells.

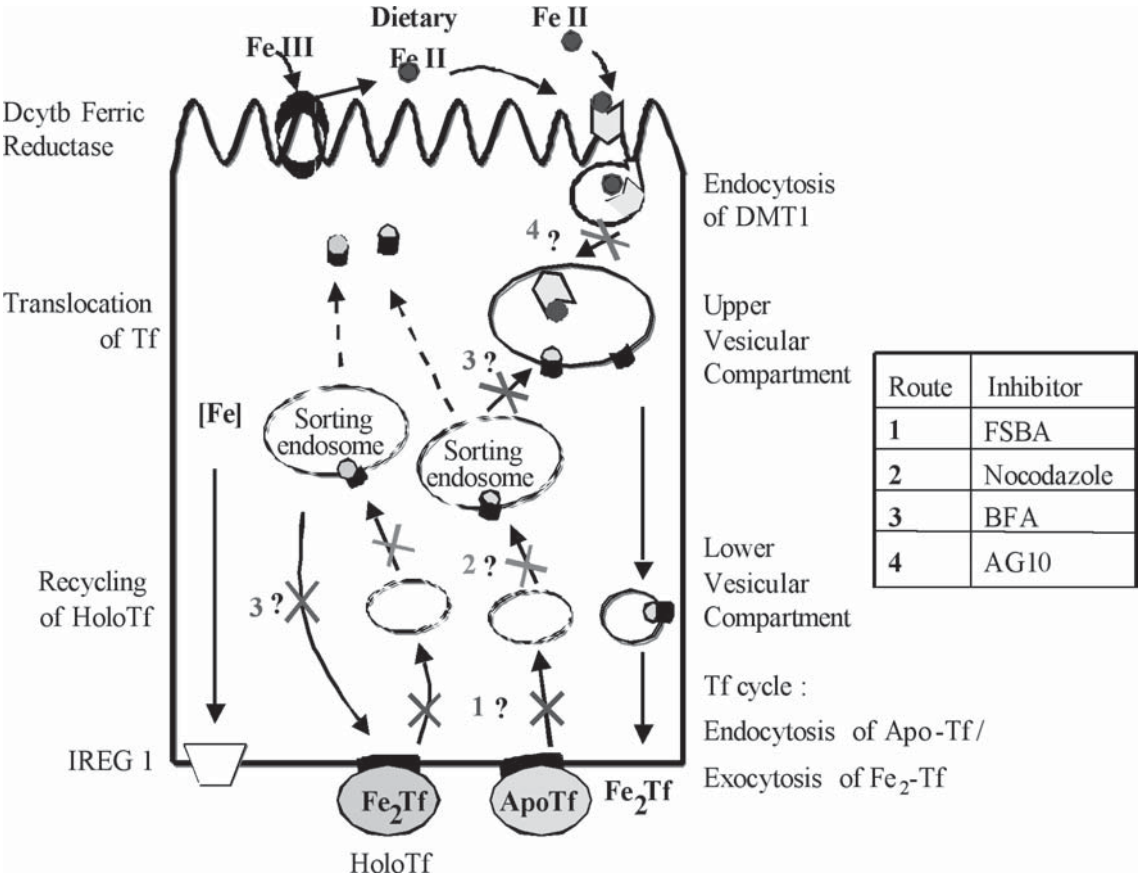

Figure 2. Potential vesicular aspects of iron absorption and potential sites of action of inhibitors. Cartoon detailing hypothesized aspects of the activities of the enterocyte engaged in vesicular Fe transport from the apical brush border (top) to the basolateral surface (bottom), showing binding of Fe(II) to DMT1; its endocytosis and coalescence with endosomes containing apotransferrin (ApoTf) bound to a membrane receptor, and cycling out as Fe_2Tf; in contrast to the cycling in and out of holotransferrin (Fe_2Tf) within the lower region of the enterocyte. Potential sites of action of various inhibitors are shown by number and name.

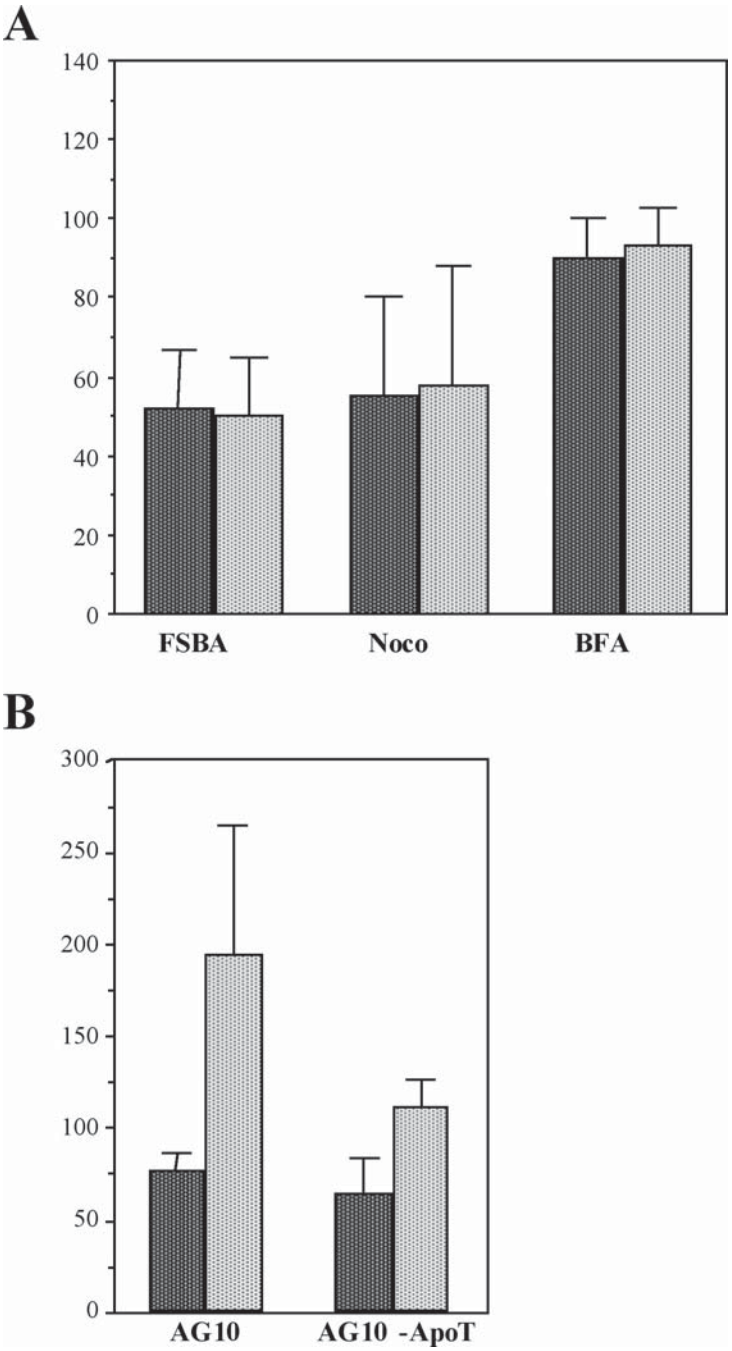

Figure 3. Effects of vesicular transport inhibitors on iron absorption. A. Caco2 cell monolayers were preincubated for 60-90 min with 5'-(4-fluorosulfonylbenzoyl) adenosine hydrochloride (FSBA; 0.1-1 mM) (N=4), nocodazole (5-200 μM) (N=11), or brefeldin A (BFA; 20-50 μg/ml) (N=25) prior to addition of ^{59}Fe(II)-ascorbate (1 μM) to the apical chamber for measurements of uptake (dark bars) or overall transport (light bars) over the next 90-120 min. Reductions in uptake and overall transport are shown relative to control (100%) and are given as Means ± SD (for the N values above). B. ^{59}Fe(II) uptake and overall transport (as in A) were measured in the presence and absence of AG10, with (AG10) and without (AG10-ApoTf) apotransferrin in the basal chamber. Values are given as in A (N=8-10). In both parts, all changes from controls were significant ($p<0.005$) (Data are summarized from Moriya and Linder, 2005)

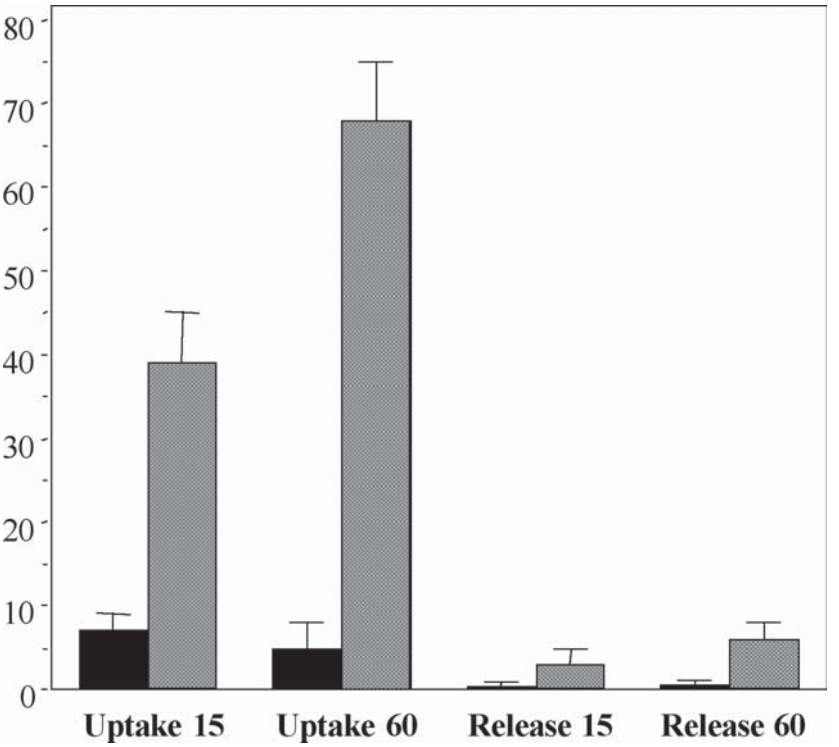

Figure 4. Effects of cooling to 4ºC on iron transport in Caco2 cell monolayers. Uptake of ^{59}Fe (1 μM in apical chamber) and its release into the basal chamber measured over 15 and 60 min are given for monolayers that were incubated at 37ºC (light bars) or cooled to about 4ºC by placing them on ice (dark bars). Data are given as percent of dose (Mean±SD; N=4). All differences due to cooling were significant ($p<0.001$). (Summarized from Moriya and Linder, 2005)

Transport by DMT1 is known to be at least partly dependent upon the presence of protons (Gunshin et al., 1997; Rolfs and Hediger, 2001). Consistent with that, we found that uptake of Fe(II) by the Caco2 cell monolayers was considerably greater at pH 5 and 6 than at pH 7 and 8 (Fig. 6). Similar pH dependence curves were obtained when cells were exposed to 5 and 50 μM iron. (It should be noted that two different sets of cells were used for the 5 and 50 μM uptake studies.) Similar pH profiles for uptake at different iron concentrations is again consistent with the presence of a single Fe(II) uptake mechanism.

To begin to explore whether other metal ions are using the same transporter and in what ionic state they might be doing so, we first looked for inhibition of Fe(II) uptake by large concentrations of Mn and Cu ions. Some of our findings are given in figure 7A. Here, 200 μM Mn or Cu ions, or both, were added to the apical fluid during uptake of 1 μM ^{59}Fe(II), in the presence of fresh 1 mM ascorbate. This concentration of ascorbate reduced the Cu to Cu(I) but did not alter the valence state of Mn(II) (Methods). Under these conditions, both metal ions markedly inhibited uptake of divalent iron (Fig. 7A). Lower concentrations of both ions (5 and 200 μM) resulted in lesser, dose-dependent inhibition (data not shown). The monovalent form of Cu was very effective, implying that this is the form taken up by DMT1. Follow-up studies (not shown) indicated that inhibition also occurred in the presence of 1 mM mannitol, which was added to scavenge any radicals that might form and injure DMT1. The effects of Cu(I) and Mn(II) were not additive, implying that they were using the same mechanism/site of inhibition.

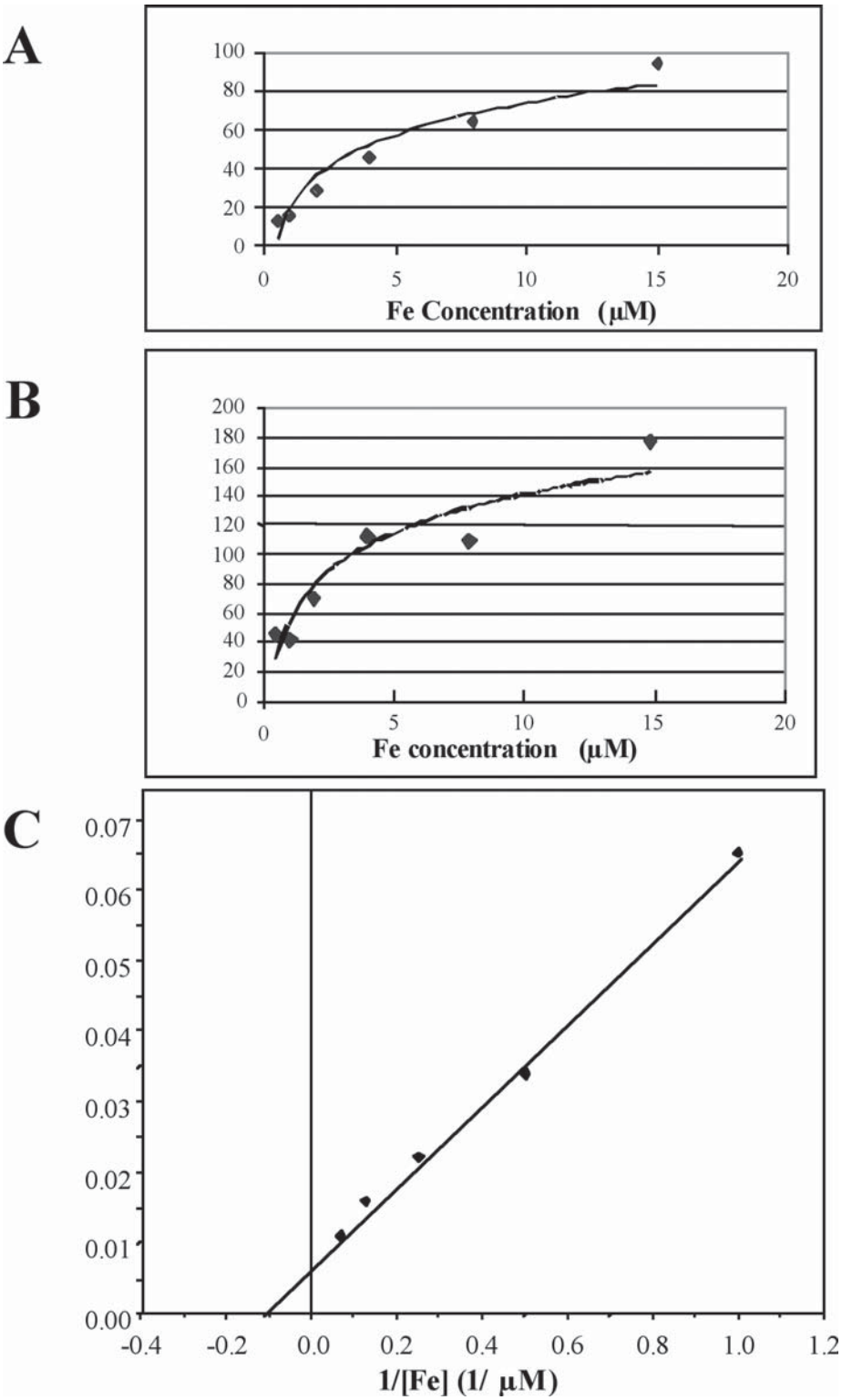

Figure 5. Kinetics of Fe(II) uptake by Caco2 cells and rat hepatoma cells. Representative examples of studies in which initial rates of uptake (pmol/20 min/mg cell protein) were compared at various concentrations of ^{59}Fe(II)-ascorbate are shown in A and B, for Caco2 cells and hepatoma cells, respectively. C. Double reciprocal plot of the data in A.

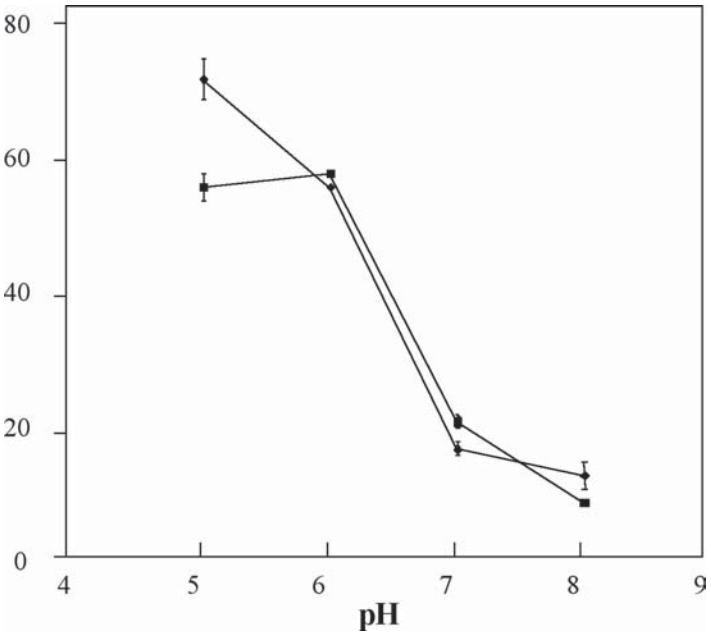

Figure 6. pH dependence of Fe(II) uptake by Caco2 cell monolayers. Net uptake of 5 (circles) and 50 μM (squares) ^{59}Fe(II) from ^{59}Fe-ascorbate in the apical chamber, over 90 min, was compared at the different pHs of the apical solution indicated. The 5 and 50 μM uptake studies were carried out with different batches of cells, so absolute values cannot be compared. Data are Means±SD for N=3, as percent of control.

The kinetics of Fe(II) uptake in the presence of Mn were then examined. The results of one such study, with and without 50 μM Mn(II), are given in figure 7B in the form of a double reciprocal plot. Both Km and Vmax were altered by having Mn(II) present. This suggests that Mn(II) is binding to a portion of DMT1 that does not directly compete with the Fe(II) portal or binding site but reduces the ability of the transporter to utilize Fe(II). Alternatively and/or in addition, Mn(II) may be utilizing another part of DMT1 for entry into the enterocytes, and this interferes with its ability to transport Fe(II) through another channel.

DISCUSSION

The results summarized and presented here add to and reinforce aspects of the picture emerging on the details of how iron is absorbed by the intestine and how this relates to the absorption of other metal ions. At the brush border, Fe(II) is taken up by DMT1 by a mechanism (or mechanisms) that can be inhibited by Cu(I) and Mn(II). Our evidence suggests that this inhibition occurs through the binding of these metal ions to a site on DMT1 different from that for Fe(II): Cu(I) and Mn(II) appear to bind to the same site since their inhibitory effects are not additive; and the effects on the kinetics of Fe(II) absorption are not competitive. Thus, DMT1 has binding sites for several metal ions and not only for divalent metal ions. This is consistent with the findings of Arredondo et al. (2003), that Cu(I) transport by Caco2 cells is altered when DMT1 expression is manipulated through anti-sense technology. We have tended to think of DMT1 as being a large transmembrane protein with one or more channels that allows transfer of such metal ions. That may be the case, but we must also take into account the facts that in response to Fe(II), DMT1 endocytoses fairly rapidly from the brush border membrane into vesicles (Jonathan Glass, personal communication), and that it appears to co-localize with apotransferrin in

endosomes (Ma et al., 2002), which stimulates iron efflux to the blood (by exocytosis). Here, if we are correct, it would appear that DMT1 is acting not like a metal ion channel but more like a metal ion binding site or receptor that allows receptor-mediated endocytosis of the metal ions. The metal ions then are transferred to proteins in the endosome or transported across the endocytic membrane to the cytoplasm. For now, we cannot be sure that either transporter or receptor functions (or both) actually are involved, but these are possibilities.

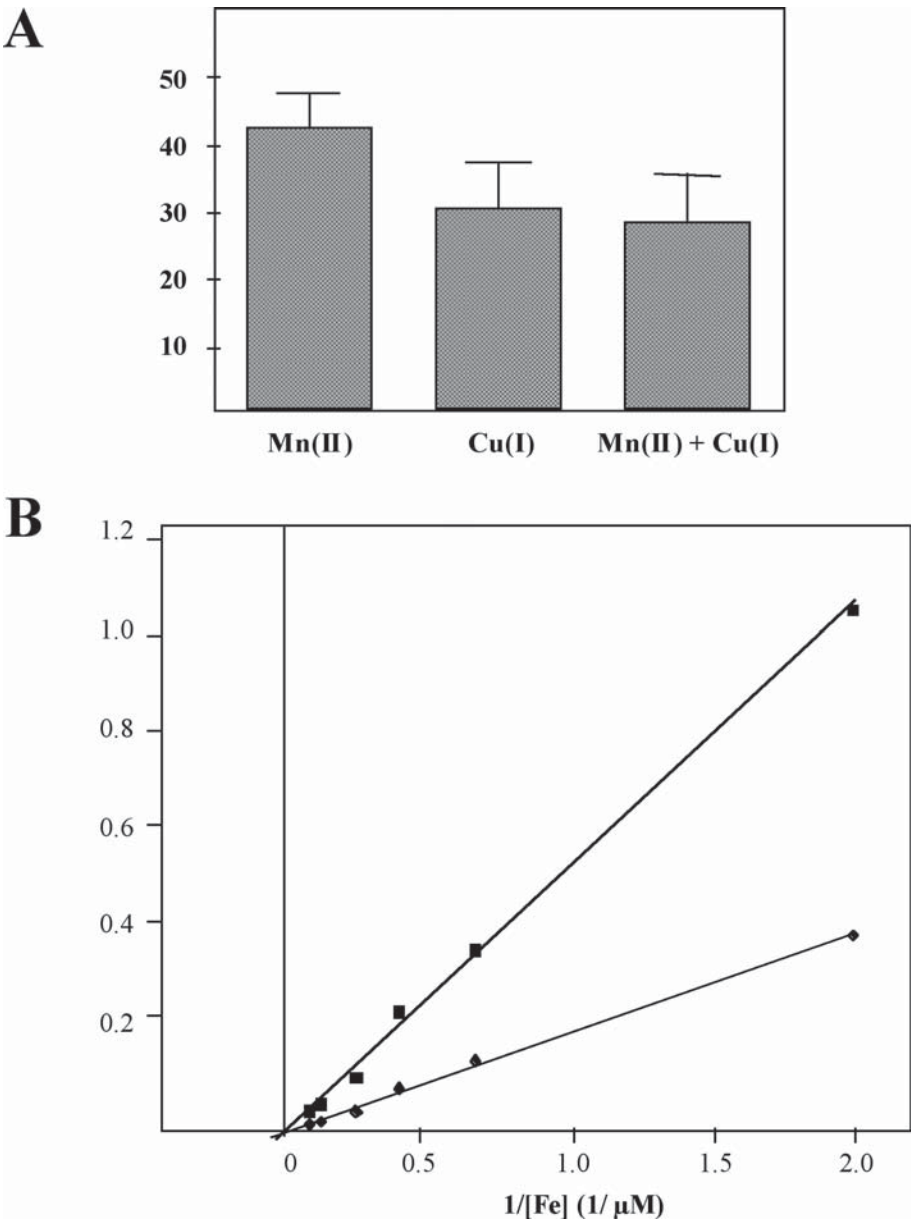

Figure 7. Effects of Mn and Cu ions on uptake of Fe(II) by Caco2 cells. Initial rates of uptake of ^{59}Fe(II)-ascorbate were monitored as indicated for Figure 5 in the presence and absence of Cu(I) or Mn(II) (1 µM Fe, 200 µM Cu or Mn) (A), or in the presence and absence of 50 µM Mn(II) at different Fe concentrations (B). A shows the uptake (Means±SD for N=4) as percent of control (100%; bar); B is a double reciprocal plot of the kinetic data from a representative experiment.

Our findings that the availability of apotransferrin at the basolateral surface of the enterocyte monolayer markedly enhances the release of iron across the basolateral membrane but also somewhat stimulates uptake of iron at the brush border (Moriya and Linder, 2005) lends further credence to the extensive work of Glass and Núñez and their colleagues on the importance of apotransferrin to intestinal Fe absorption (Álvarez-Hernández et al., 1994, 1998; Núñez et al., 1999; Ma et al., 2002). Consistent with previous work, we found there was a doubling of the rate of basolateral iron transfer when apotransferrin was available from the "blood" side, as it would be in the case of the intestine of the whole animal. That is a profound impact. We also found a small but significant stimulatory effect on apparent uptake, as measured by the net ^{59}Fe remaining in the apical fluid after 60-360 min of incubation (Moriya and Linder, 2005). Especially in conjunction with the confocal microscopy studies showing apotransferrin and DMT1 co-localizing in endosomes in the apical region of the Caco2 cells (Ma et al., 2002, 2005), these effects of apotransferrin imply that vesicular trafficking is an important feature in iron transport by the enterocyte. All of this helps to explain much earlier (and partly controversial) reports that transferrin is present in intestinal mucosal cells (particularly in iron deficiency) and plays a role in iron absorption (Moriya and Linder, 2005; Linder et al., 1975; Linder and Munro, 1977).

Our results showing marked inhibition of iron transport by Caco2 cell monolayers in the presence of inhibitors of vesicular transport or upon cooling to 4ºC (Moriya and Linder, 2005) lend further strong support to the concept that endo- and exocytosis are central to intestinal iron absorption. Overall, our work with the Caco2 cell model (with apotransferrin) suggests that about half of iron absorption may be dependent upon vesicular trafficking. However, our evidence also indicates that vesicular transport is involved not just in connection with the in and out cycling of apotransferrin but also with some other aspects of iron transport. This is consistent with DMT1 binding Fe and undergoing endocytosis from the brush border. It also would be consistent with endocytosis of ferroportin (Ireg1) from the basolateral membrane (Jonathan Glass, personal communication), perhaps in preparation for transport of Fe to the blood. These concepts must be further explored.

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