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Rapid report

SLC19A3 encodes a second thiamine transporter ThTr2

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Abstract

Recently, a new family of facilitative carriers has been cloned consisting of the reduced folate (SLC19A1) and the thiamine (SLC19A2) transporters. Despite a high level of sequence identity and similarity there is essentially no functional overlap between these carriers. The former transports folates and the latter thiamine. In this paper we describe the function of SLC19A3, another member of this transporter family most recently cloned, after transient transfection of the cDNA into HeLa cells. Uptake of [^{3}H]thiamine, but not of methotrexate nor folic acid, was enhanced in SLC19A3 transfectants relative to vector control. Similarly, in the transfectants thiamine transport increased with an increase in pH with peak activity at pH $\sim$7.5. While [^{3}H]thiamine uptake was markedly inhibited by nonlabeled thiamine it was not inhibited by several organic cations in 100-fold excess. Hence this carrier has a high degree of specificity for vitamin B_1. The data indicate that SLC19A3 has the characteristics of SLC19A2 (ThTr1) and represents a second thiamine transporter (ThTr2) in this family of facilitative carriers.

Keywords: Thiamine transport; Thiamine transporter; SLC19A3 function; SLC19A family

Recently, a new family of facilitative carriers has been identified consisting of the reduced folate carrier (SLC19A1) cloned in 1995 [1–5] and a thiamine carrier (SLC19A2) cloned in 1999 [6–9]. The human proteins have a sequence identity of 40% and similarity of 55% [8]. However, despite the high degree of structural similarity of these proteins there is essentially no functional overlap. The reduced folate carrier (RFC1) does not transport thiamine and the thiamine carrier, ThTr1, does not transport reduced or oxidized folates [10]. This is consistent with the substantial structural differences between these compounds and the fact that folates are anions (RFC1 is an anion exchanger [11]), and thiamine a cation at physiological pH. Most recently, another transporter with high homology to these carriers has been identified, SLC19A3 [12]. The human gene encodes a protein with a predicted molecular mass of $\sim$56 kDa with 39% sequence identity to RFC1 and 48% identity with ThTr1. The mouse protein is 41% identical to RFC1 and 58% identical to ThTr1. This, along with the Clustal V phylogenetic analyses, indicates that SLC19A3 is more closely related to SLC19A2 than RFC1 [12]. We report here on studies that assess the function of SLC19A3 in HeLa cells transiently transfected with this gene. These studies demonstrate that similar to ThTr1, SLC19A3 is a thiamine transporter without folate transport activity.

HeLa cells (American Type Collection, Manassas,

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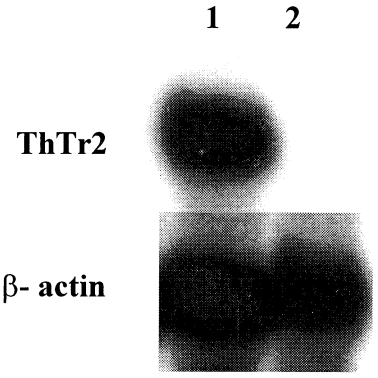

Fig. 1. Expression of ThTr2. Northern blot analysis of transiently transfected HeLa cells. Lane 1, HeLa cells were transiently transfected with ThTr2 cDNA. Lane 2, HeLa cells transiently transfected with the pcDNA 3.1(+) vector alone. Twenty micrograms of total RNA were loaded per lane. Full length ThTr2 cDNA was used as the template for the random primer labeling and subsequently used as probe for the Northern analysis.

VA, USA) were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 4 mM L-glutamine, 4.5 g/l glucose, penicillin (100 U/ml), streptomycin (100 μg/ml), and 10% fetal bovine serum (Gibco BRL). Cells were grown at the bottom of 10 ml glass vials (Research Product International) as previously reported [13]. Transient transfection was achieved using Lipofectamine Plus (Gibco BRL) according to the manufacturer's protocol. Uptake of [^{3}H]thiamine was measured as previously described in adherent cells in the glass vials [13]. Experiments at pH 5.0–6.5 were performed in MBS (20 mM MES, 140 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 5 mM glucose). Experiments at pH 7.0–8.0 utilized an HBS buffer (20 mM HEPES 140 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 5 mM glucose). Radiolabeled compounds (Amersham) were: [^{3}H]thiamine, [3′,5′,7′-^{3}H]methotrexate, and [3′,5′,7′,9-^{3}H]folic acid. All other reagents were obtained in highest purity available from various commercial sources.

Human SLC19A3 cDNA was cloned by RT-PCR. First strand cDNA was synthesized by reverse transcriptase (Superscript II RT, Gibco BRL) and oligo(dT). SLC19A3 cDNA was amplified using specific primers. The primer pairs used were as follows: FP1: 5′-TCGGGATCCTTGGTGAACAGACACT-3′, and RP2: 5′-TCGGAATTCCAAGTTCCACTG-TTGC-3′.

PCR amplification was carried out with pfu Turbo (Clontech). The PCR cycle for amplification encompassed initial denaturation at 95°C for 3 min followed by 30 cycles at 95°C for 1 min, annealing at 55°C for 1 min and elongation at 72°C for 2 min. The final extension was performed at 72°C for 10 min. The 1.8 kb amplified PCR fragment was cloned in the pcDNA3.1(+) (Invitrogen) expression vector at *Bam*HI-*Eco*RI sites.

Northern blot hybridization was carried out using total RNA from the transiently transfected HeLa cells. The RNA samples were size-fractionated on a denaturing formaldehyde-agarose gel and probed with ^{32}P labeled human SLC19A3 cDNA.

Human SLC19A3 cDNA was cloned from human kidney total RNA by RT-PCR and transiently transfected into HeLa cells. Fig. 1 is a Northern blot that demonstrates high level expression of SLC19A3 in the cDNA transfected HeLa cells. No message was detected in the vector-only transfected cells consistent with a low level of endogenous expression. Fig. 2 illustrates that uptake of [^{3}H]thiamine (1 μM) was increased ~3.5-fold in the SLC19A3 transfected cells as compared to the vector-only transfectant but there was no change in influx of equimolar methotrexate or folic acid. The pH profile of thiamine uptake mediated by SLC19A3 (Fig. 3) was similar to that reported for ThTr1 [6]. Peak transport activity was pH ~7.4 and influx at this pH was ~3.5-fold higher than at pH 5.0. Increasing pH beyond 7.4 decreased the activity. In the vector-only transfected

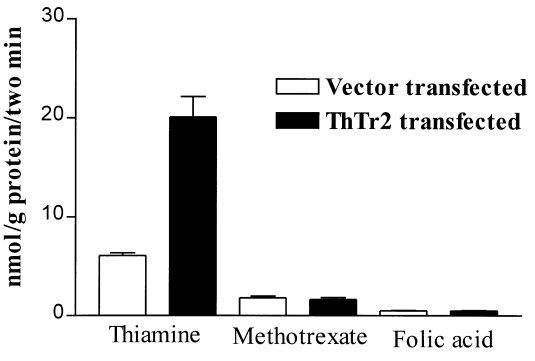

Fig. 2. Functional activity of ThTr2. HeLa cells transiently transfected with ThTr2 cDNA were assessed for transport activity. Uptake was measured over 2 min as described in the text with 1 μM of [^{3}H]thiamine, [^{3}H]methotrexate or [^{3}H]folic acid. Results are the averages of three separate experiments ± S.E.M.

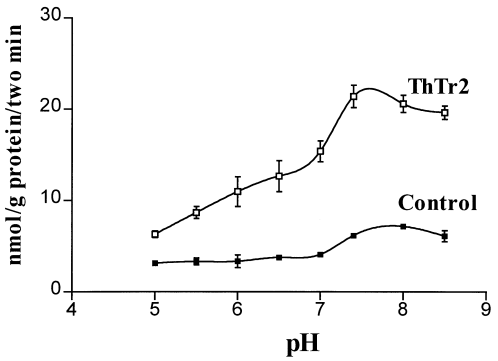

Fig. 3. pH profile of ThTr2 activity. Uptake of 1 μM [^{3}H]thiamine over 2 min was assessed in transiently transfected HeLa cells at the indicated pH. Results are averages of three separate experiments ± S.E.M.

HeLa cells the highest thiamine uptake activity was also at pH ~7.4 reflecting the properties of the endogenous transport process(es). Thiamine is an organic cation but this transport system does not have significant affinity for other organic cations since neither 100 μM choline chloride, cimetidine, nor tetraethylammonium bromide inhibited influx of 1 μM [^{3}H]thiamine. Two other B vitamins, pyridoxine (a cation) and niacin (a zwitterion), whose specific transporters are not as yet identified [14,15] also did not inhibit [^{3}H]thiamine uptake. On the other hand, 100 μM nonlabeled thiamine inhibited radiotracer uptake by 95% under these conditions (Fig. 4). These data demonstrate the high degree of specificity of this carrier for thiamine. This is unlike a number of other carriers that accept a variety of organic cations as substrates [16].

These studies are consistent with the conclusion that SLC19A3 is a second thiamine transporter (to be termed ThTr2) that, like SLC19A2, has high sequence identity with RFC1 but essentially no functional overlap. ThTr1 has been cloned by several groups [6–9] and identified as the locus of the genetic defect associated with thiamine-responsive megaloblastic anemia (TRMA). Mutations in the ThTr1 gene have been shown to be the cause of the defect in transporter function in this syndrome [7]. Of particular interest is that while TRMA patients manifest diabetes, deafness and megaloblastic anemia, they usually do not exhibit other neurological or cardiac signs of thiamine deficiency [17]. Further, these patients have normal thiamine blood levels [18]. These observations suggest that there must be other route(s) by which thiamine is transported in human cells [17]. ThTr1 is widely expressed in human tissues but is most abundant in skeletal muscle, followed by heart, placenta, kidney and liver [6–9]. ThTr2 is also expressed in many human tissues but is most abundant in placenta followed by liver, kidney and heart [12]. The pattern of expression of ThTr2 relative to ThTr1 may account for the sparing of thiamine-dependent tissues under conditions in which thiamine transport mediated by ThTr1 is impaired, as in TRMA. It will be important to clarify the specific location of these transporters in enterocytes and their role in intestinal absorption of thiamine [19], in particular the vectorial flows of thiamine across the apical brush border and basolateral membranes. Clearly, further understanding of the role of these two thiamine transporters in various human tissues will await the development of antibodies that will permit assessment of protein expression and localization.

Finally, there is another potential route for transport of thiamine into human cells, RFC1. Studies from this laboratory have demonstrated that whereas thiamine is not a substrate for RFC1, thiamine py-

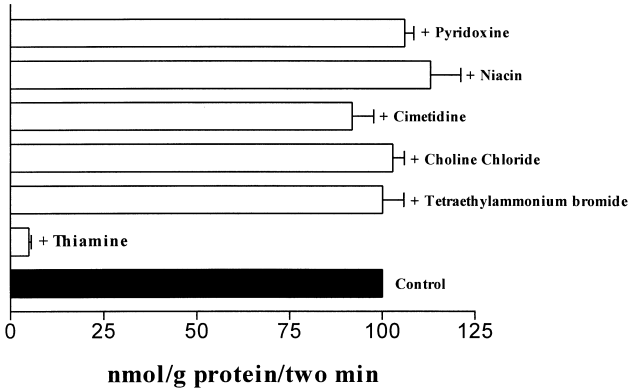

Fig. 4. Specificity of ThTr2 uptake. Uptake of 1 μM [^{3}H]thiamine over 2 min was assessed in the presence of 100 μM nonlabeled organic cations or niacin in transiently transfected HeLa cells. Thiamine uptake in the absence of a competitor was the control. ThTr2 specific uptake in each case was calculated by subtracting the endogenous thiamine (transport of thiamine in vector-only transfected cells) uptake from the total uptake. Transport activity is expressed as the percentage of control. The results are averages of three separate experiments ± S.E.M.

rophosphate is a relatively good substrate [10]. While thiamine pyrophosphate is found almost exclusively in cells where it is synthesized by thiamine pyrophosphokinase, the monophosphate is present in the blood of man at levels nearly comparable to thiamine [20]. Hence, in cells in which transport mediated by the thiamine carriers is impaired, or these carrier are not normally expressed, RFC1 is a potentially important alternative pathway by which cells can meet their metabolic needs for vitamin B_1.

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