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Human cystine/glutamate transporter: cDNA cloning and upregulation by oxidative stress in glioma cells

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Abstract

A human cDNA for amino acid transport system x_C^- was isolated from diethyl maleate-treated human glioma U87 cells. U87 cells expressed two variants of system x_C^- transporters hxCTa and hxCTb with altered *C*-terminus regions probably generated by the alternative splicing at 3′-ends. Both hxCTa and hxCTb messages were also detected in spinal cord, brain and pancreas, although the level of hxCTb expression appears to be lower than that of hxCTa in these tissues. When expressed in *Xenopus* oocytes, hxCTb required the heavy chain of 4F2 cell surface antigen (4F2hc) and exhibited the Na^+-independent transport of L-cystine and L-glutamate, consistent with the properties of system x_C^-. In agreement with this, 137 kDa band was detected by either anti-xCT or anti-4F2hc antibodies in the non-reducing condition in western blots, whereas it shifted to 50 kDa or 90 kDa bands in the reducing condition, indicating the association of two proteins via disulfide bands. We found that the expression of xCT was rapidly induced in U87 cells upon oxidative stress by diethyl maleate treatment, which was accompanied by the increase in the L-cystine uptake by U87 cells. Because of this highly regulated nature, xCT in glial cells would fulfill the task to protect neurons against oxidative stress by providing suitable amount of cystine to produce glutathione.

Keywords: Transporter; Cystine; Glutamate; Alternative splicing; Oxidative stress; Glioma cell; Human

1. Introduction

It has been proposed that the transport of cystine through the plasma membrane is crucial to maintain intracellular glutathione (GSH) levels [1]. GSH is a tripeptide radical scavenger synthesized intracellularly from glutamate, cysteine and glycine. Because cysteine is easily oxidized to form cystine in the extracellular environment, cystine transport mechanisms are essential to provide cells with cysteine for GSH synthesis [1]. The amino acid transport system

Abbreviations: GSH, glutathione; 4F2hc, 4F2 heavy chain; xCT, x_C-type transporter

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x_C^- has been proposed to be responsible for the cystine transport through the plasma membrane. System x_C^- mediates an amino acid exchange and prefers cystine and glutamate as its substrates [1].

Recently, a cDNA which encodes a transporter subserving the system x_C^- has been isolated from mouse peritoneal macrophages treated with diethyl maleate and lipopolysaccharide [2]. The transporter designated as xCT requires the heavy chain of 4F2 cell surface antigen (4F2hc) for its functional expression in *Xenopus* oocytes [2] and belongs to the family of amino acid transporters which associates with the type II membrane glycoproteins such as 4F2hc and rBAT (related to $b^{0,+}$ amino acid transporters) [3–8]. It has been shown that xCT is upregulated on the GSH depletion by diethyl maleate treatment in the mouse peritoneal macrophages [2,9,10]. In brain, it has been proposed that system x_C^- is upregulated in glial cells upon the oxidative stress and plays an essential roles to protect neurons against oxidative stress [11–13]. In this study, we have isolated a cDNA for xCT from diethyl maleate-treated human glioma U87 cells and demonstrated that the expression of xCT is induced by oxidative stress in the glioma cell line.

2. Materials and methods

2.1. cDNA cloning

By means of guanidinium isothiocyanate method using cesium-trifluoroacetic acid (Pharmacia), total RNA was prepared from human glioma U87 cells treated with 0.1 mM diethyl maleate for 8 h in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum [14]. The oligo dT-primed cDNA library was prepared from U87 cell poly(A)$^+$ RNA selected by oligo(dT) cellulose chromatography (Pharmacia) using Superscript Choice System (Gibco BRL) [4,15]. The synthesized cDNA was ligated to λZipLox *Eco*RI arms (Gibco BRL). Screening cDNA library and isolation of positive plaques were performed as described elsewhere [14]. A cDNA fragment corresponding to 897–1107 bp of mouse xCT (GenBank/EMBL/DDBJ Data Bank accession number AB022345) [2] was amplified by reverse transcription–polymerase chain reaction (RT–PCR) on the mouse brain poly(A)$^+$ RNA using synthetic oligonucleotide primers 5′-TCCTAACCTTTTGCAAGCTCACA-3′ and 5′-TCAGGGTTGTCTACTTCTTCAG-3′ (corresponding to nucleotides 897–919 and 1086–1107 of mouse xCT cDNA, respectively). The PCR product was used as a probe for screening the cDNA library. cDNAs in positive λZipLox phages were rescued into plasmid pZL1. The cDNA was sequenced in both direction by dye terminator cycle sequencing method (Perkin Elmer and Applied Biosystems). Transmembrane regions were predicted on the human xCT amino acid sequence based on TopPred algorithm [16,17].

2.2. Northern analysis

For Northern blot analysis, the *Hpa*I/*Nco*I fragment corresponding to 196–954 bp of hxCTb cDNA was labeled with [^{32}P]dCTP using ^{T7}Quick prime kit (Pharmacia). Human Multiple Tissue Northern Blots (Clontech) were hybridized with the probe and processed following the manufacturer's instructions.

2.3. Detection of spliced variants by RT–PCR

To examine the expression of spliced variants, PCR primers were designed which are unique to each spliced fragment, 5′-TCCCTATTTTGTGTCTCCCC-3′ (antisense primer A) and 5′-GCCCATAAACACCATCTGGC-3′ (antisense primer B) designed on C-terminus regions of hxCTa (corresponding to nucleotides 1776–1795 of GenBank/EMBL/DDBJ Data Bank accession number AB026891) and hxCTb (corresponding to nucleotides 1689–1708 of GenBank/EMBL/DDBJ no. AB040875), respectively. The sense primer (primer C) which is common to hxCTa and hxCTb was designed: 5′-CACCTTCCAGAAATCCTCTC-3′ corresponding to the nucleotide sequences of 1283–1302 of hxCTa and 1183–1202 of hxCTb. The first-strand cDNA was synthesized from human brain, human spinal cord and human pancreas poly(A)$^+$ RNA (Clontech) and poly(A)$^+$ RNA of diethyl maleate-treated U87 cells (see above) using oligo dT_{12-18} as a primer. PCR amplification of the cDNA was performed as described elsewhere [15] using the prim-

er sets: sense primer C and antisense primer A, or sense primer C and antisense primer B.

2.4. Anti-peptide antibody

Oligopeptides [MVRKPVVATISKGGYC] corresponding to amino acid residues 1–15 of mouse xCT [2] and [EPHEGLLLRFPYAAC] corresponding to amino acid residues 516–529 of human 4F2hc (GenBank/EMBL/DDBJ accession no. AB018010) were synthesized. Only a single amino acid is altered in human xCT in the region (Ser8 for human xCT vs. Ala8 for mouse xCT). The C-terminus cysteine residues were introduced for conjugation with keyhole limpet hemocyanin. The anti-peptide antibodies were generated as described elsewhere [5,8,18]. Antisera were affinity-purified as described [8,19].

2.5. Western blot analysis

For Western blot analysis, U87 cell membranes were prepared as described elsewhere [8,20], with minor modifications. Briefly, U87 cells treated with 0.1 mM diethyl maleate for 16 h as described above were homogenized in 9 volumes of 50 mM Tris–HCl (pH 7.5), 25 mM KCl, 1 mM $MgCl_2$, 1 mM phenylmethylsulfonyl fluoride and 0.25 M sucrose, with 15 strokes of a Dounce homogenizer. The homogenate was centrifuged for 10 min at $8000 \times g$, and the supernatant was centrifuged further for 1 h at $100\,000 \times g$. After centrifugation, the membrane pellet was resuspended in 0.25 M sucrose, 100 mM KCl, 5 mM $MgCl_2$ and 50 mM Tris (pH 7.4). The protein samples were heated at 100°C for 5 min in sample buffer either in the presence or absence of 5% 2-mercaptoethanol and subjected to sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis. The separated proteins were transferred electrophoretically to a Hybond-P PVDF transfer membrane (Amersham). The membrane was then treated with non-fat dried milk and diluted affinity-purified anti-xCT antibody (1:1000) or anti-h4F2hc antiserum (1:20 000). The membrane was then treated with horseradish peroxidase-conjugated anti-rabbit IgG as a secondary antibody (Jackson Immuno Research Laboratories). The signals were detected with an ECL Plus system (Amersham) [5,8]. To verify the specificity of immunoreactions by absorption experiments, the membranes were treated with primary antibodies in the presence of antigen peptides (50 μg/ml) [5,8].

2.6. Functional characterization

Functional characteristics of hxCTb were examined by *Xenopus* oocyte expression. cRNAs for hxCTb and human 4F2hc were obtained by in vitro transcription using SP6 RNA polymerase for hxCTb in plasmid pZL1 linearized with *Rsr*II and T7 RNA polymerase for human 4F2hc in pZL1 linearlized with *Hin*dIII as described elsewhere [14]. *Xenopus* oocyte expression studies and uptake measurements were performed as described [14,21]. hxCTb cRNA (12.5 ng) and human 4F2hc cRNA (12.5 ng) were solely injected or co-injected to each oocyte. The uptake of L-[^{14}C]cystine or L-[^{14}C]glutamate (New England Nuclear) was measured 3 days after injection of the cRNAs. Groups of 6–8 oocytes were incubated in 500 μl of standard uptake solution (NaCl 100 mM, KCl 2 mM, $CaCl_2$ 1 mM, $MgCl_2$ 1 mM, HEPES 10 mM, Tris 5 mM, pH 7.4) or Na^+-free uptake solution in which NaCl in the standard uptake solution was replaced by choline-Cl, containing 0.25 μCi of radiolabeled compounds. The uptakes were measured for 30 min and the values were expressed as pmol/oocyte per minute. Each data point in the figures represents the mean ± S.E.M. of uptake values ($n = 6$–8).

2.7. Evaluation of the effect of diethyl maleate treatment

In order to examine the effect of diethyl maleate treatment, total RNA was prepared using a RNA preparation kit Isogen (Nippon-Gene, Japan) from U87 cells incubated with 0.1 mM diethyl maleate in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum for 4, 8, 16 and 24 h [2,10]. The total RNA (10 μg/lane) was separated on a 1% agarose gel in the presence of 2.2 M formaldehyde and was blotted onto a nitrocellulose filter (Schleicher and Schuell) [14]. The filter was hybridized with ^{32}P-labeled hxCTb cDNA fragment described above for 20 h at 42°C. The hybridization solution contained 5×SSC, 3×Denhardt's solution, 0.2% SDS,

10% dextransulfate, 50% formamide, 0.01% Antifoam B (sigma), 0.2 mg/ml denatured salmon sperm DNA (Wako, Japan), 2.5 mM sodium pyrophosphate and 25 mM MES, pH 6.5. The filters were washed in 0.1×SSC/0.1% SDS at 65°C [14]. After stripping, the filter was rehybridized with human β-actin probe (Clontech). Based on the densitometric analysis of the hybridization using Bio-Imaging Analyzer System BAS2000 (FujiX), the levels of hxCT mRNA expression were normalized by those of the β-actin mRNA expression.

The effect of diethyl maleate treatment was further evaluated on the L-[^{14}C]cystine uptake by U87 cells. U87 cells were seeded on 24-well plates (1×10^5/well) in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. After 2 days of culture, the cells were incubated with 0.1 mM diethyl maleate in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum for 4, 8, 16 and 24 h. For the measurement of L-[^{14}C]cystine uptake, the cells were washed twice with regular uptake solution (Dulbecco's modified phosphate-buffered saline (Dulbecco's PBS) supplemented with 5 mM glucose) after the removal of growth medium, and preincubated for 10 min at 37°C. Then, the medium was replaced by the regular uptake solution containing L-[^{14}C]cystine (100 μM). The uptake was measured for 2 min and expressed as pmol/mg protein per minute. The uptake reaction was terminated by removing uptake medium followed by washing three times with ice-cold regular uptake solution. Then, cells were solubilized with 0.1 N NaOH and radioactivity was counted [8].

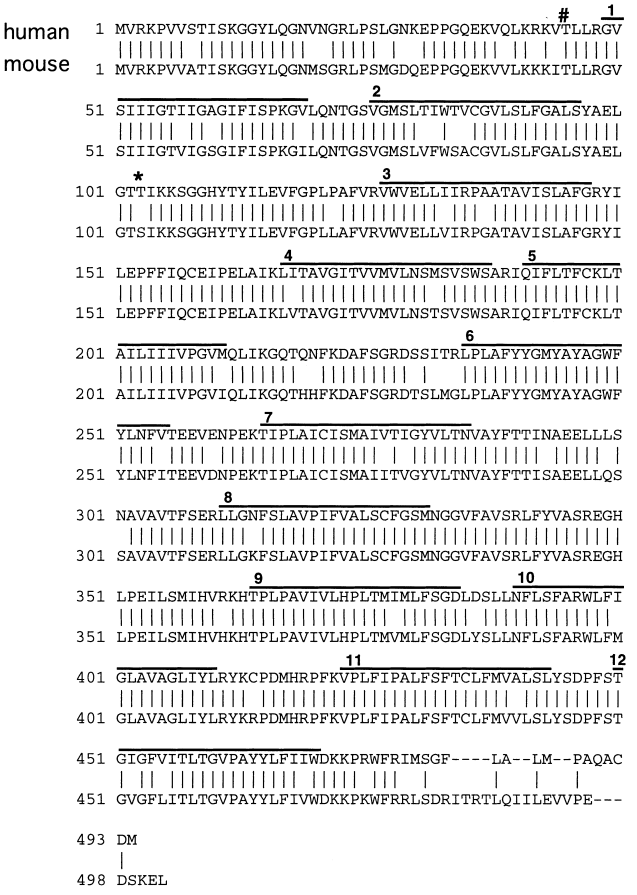

Fig. 1. Structural features of a human xCT. The deduced sequence of hxCTb is shown aligned with that of mouse xCT [2]. Membrane spanning domains predicted for hxCTb are indicated by lines numbered 1–12. Potential cAMP-dependent phosphorylation sites are predicted at residues 45 and 346, in which Thr 45 is predicted to be located intracellularly (labeled with #). Potential protein kinase C-dependent phosphorylation sites are predicted at residues 65, 103, 187, 226 and 308, among which Thr 103 is located in the predicted intracellular domain (labeled with *).

3. Results

3.1. Structural properties of hxCT

A cDNA clone with 2000 bp insert isolated from diethyl maleate-treated human glioma U87 cells contained an open reading frame from nucleotide 136 to 1617 encoding a putative 495-amino-acid protein designated as hxCTb (human x_C-type transporter b) which exhibited 86% amino acid sequence identity to mouse xCT [2] (Fig. 1). As shown in Fig. 1, 12 transmembrane regions were predicted on hxCTb amino acid sequence. There is a conserved cysteine residue (hxCTb amino acid residue 158) in the putative extracellular loop between predicted transmembrane domains 3 and 4, which is supposed to link to 4F2hc via a disulfide bond [22]. A cAMP-dependent phosphorylation site (Thr45) and a protein kinase C-dependent phosphorylation site (Thr103) are predicted in the intracellular domains (Fig. 1).

We found that the C-terminus sequence of hxCTb is different from that of the already published human xCT [23,24] (Fig. 2A), suggesting the alternative splicing at the 3′-ends. Because of this alteration, C-terminus 22 amino acid residues of the human

xCT protein reported by Sato et al. and Bridges et al. [23,24] (referred to as hxCTa in this paper) were replaced by 13 amino acid residues in hxCTb (Fig. 2A,B). Three polyadenylation sites (AATAAA) were identified in the 3′-untranslated region of hxCTb, whereas they were not found in hxCTa (GenBank/EMBL/DDBJ Data Bank accession number AB026891) (Fig. 2B).

3.2. Tissue distribution of expression

The distribution of expression of xCT in human tissues was analyzed by Northern blot. The cDNA fragment corresponding to the region of mRNA common to both hxCTb and hxCTa was used as a probe. The probe hybridized to the long message at 8.5 kb in brain, spinal cord and pancreas (Fig. 3A).

In order to examine the expression of spliced variants, RT–PCR was performed using primers specific to each spliced variant (Fig. 2B). As shown in Fig. 3B, PCR products corresponding to both hxCTa and hxCTb were detected in diethyl maleate-treated U87 cells, human brain, human spinal cord and human pancreas, indicating that both spliced variants were expressed in these cells and tissues. The level of expression of hxCTb, however, appeared to be lower than that of hxCTa particularly in brain, spinal cord and pancreas (Fig. 3B).

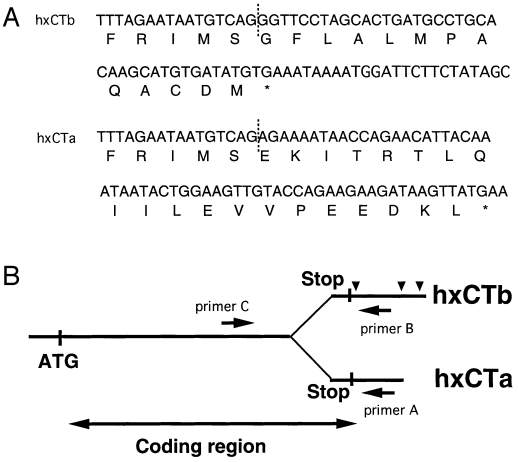

Fig. 2. Spliced variants of human xCT. (A) Comparison of nucleotide sequences corresponding to C-termini of hxCTb and hxCTa [23,24]. Deduced amino acid sequences are attached to the nucleotide sequences. The broken lines indicate the predicted points of alternative splicing. *, Stop codon. (B) Structures of human xCT mRNAs corresponding to hxCTb and hxCTa. Polyadenylation sites (AATAAA) are identified at 1621, 1798 and 1956 of the hxCTb nucleotide sequence and indicated by arrowheads. PCR primers labeled with A, B and C are indicated at the positions on which they are designed. ATG, start codon; Stop, stop codon; left, 5′-end; right, 3′-end.

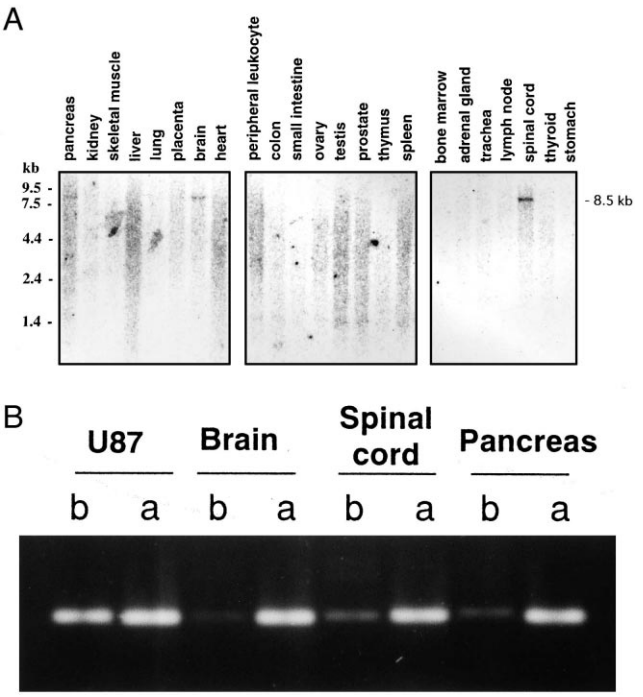

Fig. 3. Tissue distribution of expression. (A) High-stringency Northern blot analysis of xCT expression in humans. The 8.5 kb band was detected in brain, spinal cord and pancreas. The cDNA fragment corresponding to the region of mRNA common to both hxCTb and hxCTa was used as a probe. (B) RT–PCR of poly(A)$^+$ RNAs from U87 cells, human brain, human spinal cord and human pancreas using primers specific to hxCTb (primers B and C) and hxCTa (primers A and C). The figure shows the ethidium bromide-stained agarose gel electrophoresis of the PCR products using the primers B and C (labeled as 'b'), and the primers A and C (labeled as 'a').

3.3. The association of hxCT and 4F2hc

The antibody raised against N-terminus region of xCT recognized a prominent band at 137 kDa and a faint band at 50 kDa in the non-reducing condition, whereas the 137-kDa band disappeared and shifted to 50 kDa in the reducing condition (Fig. 4). Anti-4F2hc antibody also recognized 137 kDa band in the non-reducing condition. In the reducing condition, 90-kDa band was detected by the anti-4F2hc antibody (Fig. 4). All these bands were not detected in

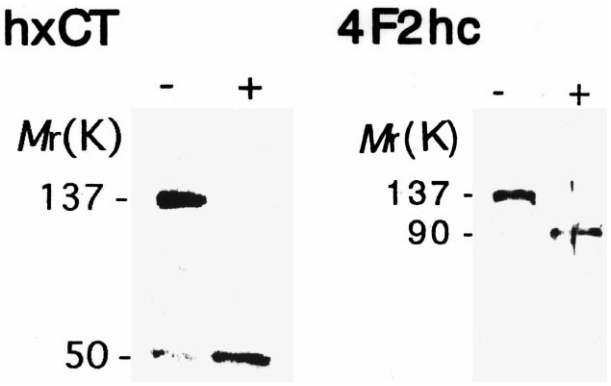

Fig. 4. Western blot analyses under reducing and non-reducing conditions. Western blot analyses were performed using an anti-xCT antibody (labeled as 'hxCT') and an anti-4F2hc antibody (labeled as '4F2hc') on the membrane fractions prepared from human glioma U87 cells in the presence (labeled as '+') or absence (labeled as '−') of 2-mercaptoethanol.

the absorption experiments in which the membranes were treated with anti-xCT or anti-4F2hc antibodies in the presence of antigen peptides, confirming the specificity of the immunoreactions (data not shown).

3.4. Functional properties

In order to determine the functional properties of hxCTb, hxCTb was expressed in *Xenopus* oocytes. As shown in Fig. 5A, hxCTb required 4F2hc for its functional expression. Although hxCTb by itself did not exhibit amino acid transports, the co-expression of hxCTb and 4F2hc resulted in the induction of transport activity for L-[^{14}C]glutamate and L-[^{14}C]cystine. Thus, the functional properties of hxCTb were examined by coexpressing hxCTb with 4F2hc.

The uptake of L-[^{14}C]glutamate mediated by hxCTb was not dependent on Na^+ (Fig. 5B). The substrate selectivity of hxCTb was investigated by inhibition experiments in which 50 μM L-[^{14}C]-glutamate uptake was measured in the presence of 5 mM non-labeled amino acids. As shown in Fig. 5C, hxCTb-mediated glutamate uptake was inhibited at high levels by L-glutamate and L-cystine and at a lower level by L-aspartate, whereas D-glutamate, D-aspartate, L-alanine, L-leucine and L-lysine did not inhibit hxCTb-mediated glutamate uptake.

3.5. Induction of hxCT expression by oxidative stress

In order to examine the effect of diethyl maleate treatment on the expression of xCT mRNA in U87 cells, Northern blot analysis was performed on the

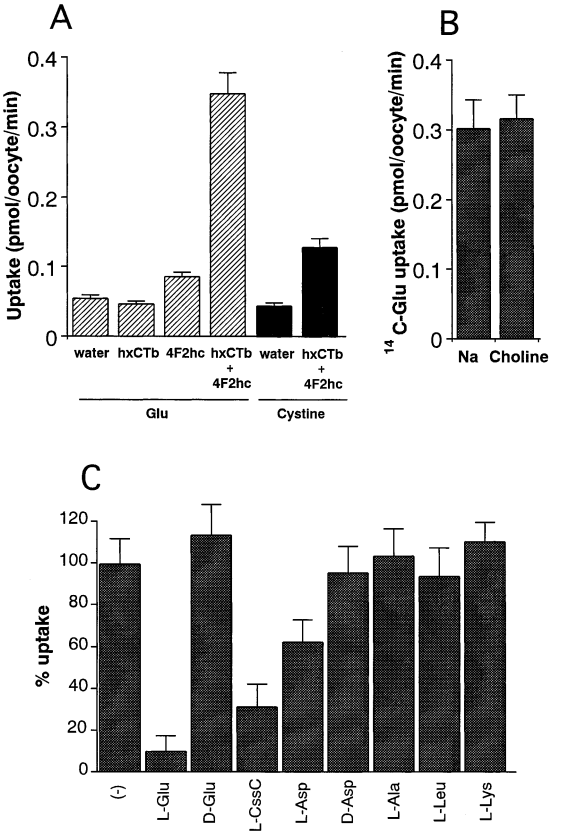

Fig. 5. Functional properties of hxCTb. (A) Functional expression of hxCTb in *Xenopus* oocytes. The uptake of L-[^{14}C]glutamate (shaded column) and L-[^{14}C]cystine (solid column) was measured at the concentration of 100 μM in Na^+-free uptake solution on the *Xenopus* oocytes injected with water (labeled as 'water'), hxCTb cRNA ('hxCTb'), 4F2hc cRNA ('4F2hc') or both hxCTb cRNA and 4F2hc cRNA ('hxCTb+4F2hc') 3 days after injection. The co-expression of hxCTb and 4F2hc resulted in the induction of activity for the uptake of L-glutamate and L-cystine. (B) Na^+-dependence of hxCTb-mediated L-[^{14}C]glutamate transport. The hxCTb-mediated uptake of 100 μM L-[^{14}C]glutamate measured in the regular-uptake solution (labeled as 'Na') was not altered in the uptake medium in which Na^+ was replaced by choline ('choline'). (C) Inhibition of hxCTb-mediated uptake of L-[^{14}C]glutamate by non-labeled amino acids. The LAT1-mediated L-[^{14}C]glutamate uptake (50 μM) was measured in the presence of 5 mM non-radiolabeled L-amino acids and expressed as percent of the control L-leucine uptake in the absence of inhibitors ('(−)'). The L-glutamate uptake was inhibited by L-glutamate, L-cystine and L-aspartate. L-CssC, L-cystine.

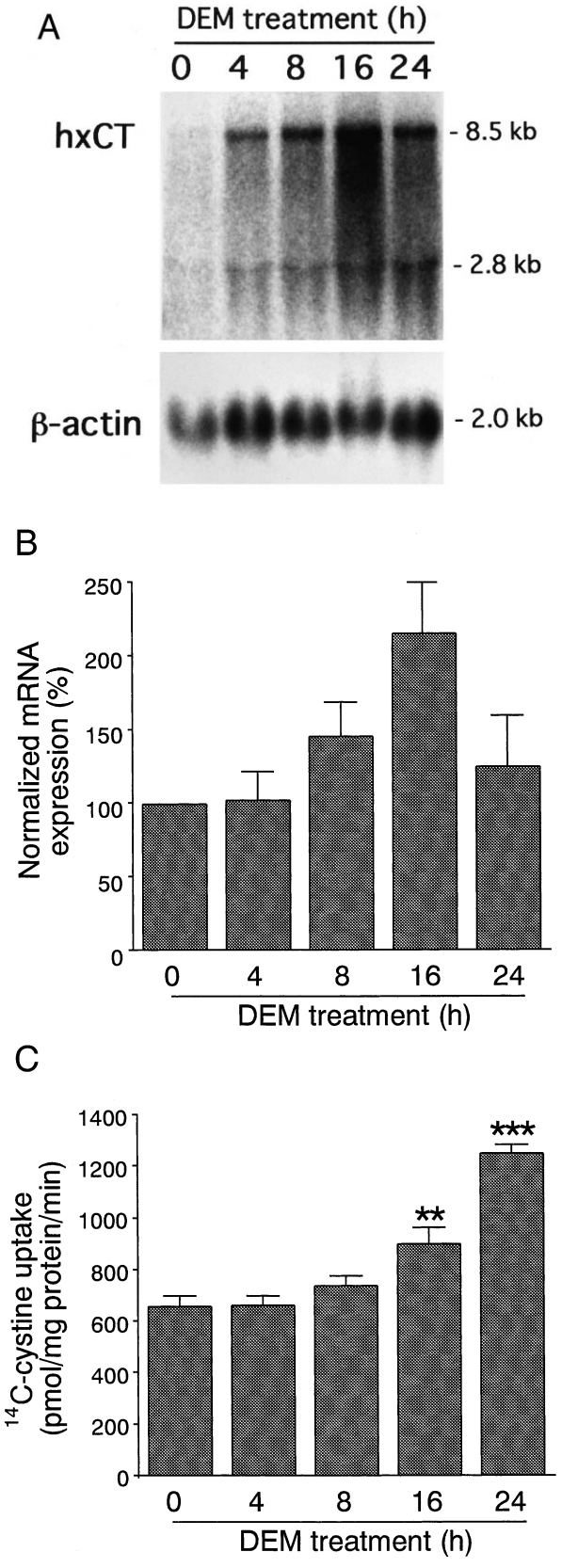

Fig. 6. Diethyl maleate-induced increase in the level of human xCT mRNA. (A) Northern blot analyses were performed using a ^{32}P-labeled human xCT probe on the total RNA separated from human glioma U87 cells treated with 0.1 mM diethyl maleate for 4, 8, 16 and 24 h. '0 hr' indicates U87 cells without diethyl maleate treatment. The same blots were rehybridized with human β-actin probe (bottom). (B) The levels of human xCT mRNA at 8.5 kb are shown by normalizing with the levels of β-actin mRNA for each sample. The ordinate indicates the value of the normalized human xCT mRNA level at each time point compared, in the same series of experiments, with that at 0 h (100%). The values are expressed as mean ± S.E.M. of three separate experiments. (C) The effect of diethyl maleate treatment on L-[^{14}C]cystine uptake by U87 cells. U87 cells were treated with diethyl maleate in the same condition as that for A and B. Each value represents mean ± S.E.M. ($n=4$). Asterisks indicate statistical significance between the uptake at 4, 8, 16 or 24 h and that at 0 h (**$P<0.01$, ***$P<0.001$, Student's unpaired t-test).
←

total RNA prepared from U87 cells treated with 0.1 mM diethyl maleate for 4, 8, 16 and 24 h. As shown in Fig. 6A,B, the levels of xCT mRNA increased during diethyl maleate treatment with the peak value at 16 h.

The effect of diethyl maleate treatment was also examined on the L-[^{14}C]cystine uptake by U87 cells. As shown in Fig. 6C, L-[^{14}C]cystine uptake was increased at 16 and 24 h.

4. Discussion

The human system x_C^- transporter hxCTb identified in the present study in glioma U87 cells exhibits an altered C-terminus sequence compared with that of mouse xCT isolated from macrophages [2] because of the proposed alternative splicing at the 3′-ends. In contrast, the other spliced variant hxCTa identified in human fibroblasts and human retinal pigment epithelial cell line ARPE-19 possesses the identical C-terminus to that of the mouse xCT [23,24]. In human tissues, xCT is expressed in spinal cord, brain and pancreas, consistent with the result from mouse Northern blot in which xCT message was detected only in brain [2] (Fig. 3A). In brain, spinal cord and pancreas, both spliced variants were demonstrated to be expressed as well as in U87 glioma cells, although the level of expression of hxCTb seems lower than that of hxCTa in brain, spinal cord and pancreas

(Fig. 3B). Because typical polyadenylation sites are not identified in the 3′-untranslated region of hxCTa while the 3′-untranslated region of hxCTb contains polyadenylation sites, it is reasonable to assume that hxCTa and hxCTb correspond to the long message (8.5 kb) and the short message (2.8 kb) detected in U87 cells, respectively (Fig. 6A). This is consistent with the observation that the long message was predominant in brain, spinal cord and pancreas in which the expression of hxCTa seems much higher than that of hxCTb (Fig. 3A,B).

It is notable that the C-terminus of hxCTa contains many charged residues whereas that of hxCTb is rather hydrophobic, as is observed for the spliced variants of mouse glutamate transporter GLT-1 [15]. In mouse GLT-1, the coexpression of two spliced variants with altered C-termini resulted in the increase in the V_{max} value of the transport. It would be interesting to know whether the two hxCT variants are expressed in the same cells and the ratio of expression of each variant affects the transport activity.

Because the members of LAT family except BAT1/$b^{0,+}$AT are associated with the 4F2hc, they are proposed to be the light chains of 4F2 heterodimeric cell surface antigen [3–8,25–27]. Among the members of LAT family, system y^+L transporter y^+LAT1, system asc transporter Asc-1 and system L transporter LAT1 have been demonstrated to be linked to 4F2hc via a disulfide bond [5,25,26]. In the present study, using specific antibodies raised against xCT and 4F2hc, we showed that the bands at 137 kDa detected in the non-reducing condition corresponding to the heterodimeric complex of xCT and 4F2hc shift to 50 kDa and 90 kDa corresponding to the monomers of xCT and 4F2hc, respectively, in the reducing condition, which indicates the association of xCT and 4F2hc via a disulfide bond (Fig. 4). This is consistent with the results from the functional expression indicating that xCT requires 4F2hc for its functional expression (Fig. 5A and reference [2,23,24]). In the presence of 4F2hc, hxCTb exhibits the Na^+-independent transport of L-glutamate and L-cystine with the properties of system x_C^- (Fig. 5), consistent with hxCTa and mouse xCT [2,23,24].

The expression of xCT has been shown to be inducible in mouse macrophages, human fibroblasts and ARPE-19 human retinal pigment epithelial cells [2,23,24]. In the present study, U87 glioma cells are examined as an additional human cell model on which the responses to lipopolysaccharide and oxidative stress have been well studied [28–30]. The expression of xCT in U87 glioma cells is highly inducible and upregulated with the peak value at 16 h upon the oxidative stress caused by diethyl maleate treatment (Fig. 6A,B). This is consistent with the observations for mouse peritoneal macrophages, human fibroblasts and ARPE-19 human retinal pigment epithelial cells in which xCT is upregulated on the oxidative stress or the cell activation by lipopolysaccharide [2,23,24]. In the present study, we further demonstrated that the diethyl maleate treatment also elevated the L-[^{14}C]cystine uptake by U87 cells (Fig. 6C). This effect is, however, with slower onset and lasting longer compared with the effect on the mRNA level (Fig. 6C), which is reasonable when considering that functional xCT proteins are increased following the mRNA rise and stay longer than the messages.

In the central nervous system, it has been proposed that glial cells play critical roles to maintain neuronal GSH levels by providing neurons with cysteine because neurons have less activity in system x_C^- [31,32]. Glial cells fulfill this task by producing GSH and releasing it to the extracellular space in the vicinity of neurons [32,33]. Although system x_C^- is essential to provide cells with cystine for GSH synthesis, the over-function of system x_C^- would be damaging neurons by increasing extracellular glutamate concentration because this exchanger releases glutamate through the cystine/glutamate exchange mechanism [34]. Therefore, the system x_C^- activity in glial cells needs to be regulated to meet just the requirement. In the present study, we demonstrated that the xCT mRNA level is transiently increased in U87 glioma cells by the oxidative stress. It would be an important issue to examine whether xCT is regulated in the same manner in glial cells in vivo to protect neurones against oxidative stress.

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