S100A16, a Novel Calcium-binding Protein of the EF-hand Superfamily*

Received for publication, June 16, 2006, and in revised form, September 21, 2006 Published, JBC Papers in Press, October 8, 2006, DOI 10.1074/jbc.M605798200

Emmanuel Sturchler‡, Jos A. Cox‡, Isabelle Durussel‡, Mirjam Weibel‡, and Claus W. Heizmann‡‡

From the ‡Department of Pediatrics, Division of Clinical Chemistry and Biochemistry, University of Zurich, Steinwiesstrasse 75, 8032 Zurich, Switzerland and the ‡‡Department of Biochemistry, University of Geneva, 1211 Geneva, Switzerland

S100A16 protein is a new and unique member of the EF-hand Ca\(^{2+}\)-binding proteins. S100 proteins are cell- and tissue-specific and are involved in many intra- and extracellular processes through interacting with specific target proteins. In the central nervous system S100 proteins are implicated in cell proliferation, differentiation, migration, and apoptosis as well as in cognition. S100 proteins became of major interest because of their close association with brain pathologies, for example depression or Alzheimer’s disease. Here we report for the first time the purification and biochemical characterization of human and mouse recombinant S100A16 proteins. Flow dialysis revealed that both homodimeric S100A16 proteins bind two Ca\(^{2+}\) ions with the C-terminal EF-hand of each subunit, the human protein exhibiting a 2-fold higher affinity. Trp fluorescence variations indicate conformational changes in the orthologous proteins upon Ca\(^{2+}\) binding, whereas formation of a hydrophobic patch, implicated in target protein recognition, only occurs in the human S100A16 protein. In situ hybridization analysis and immunohistochemistry revealed a widespread distribution in the mouse brain. Furthermore, S100A16 expression was found to be astrocyte-specific. Finally, we investigated S100A16 intracellular localization in human glioblastoma cells. The protein was found to accumulate within nucleoli and to translocate to the cytoplasm in response to Ca\(^{2+}\) stimulation.

S100 proteins represent the largest subgroup of Ca\(^{2+}\)-binding proteins of the EF-hand type with 21 identified members (1, 2). The number of S100 genes, their chromosomal localization, transcriptional direction, as well as their expression pattern are highly conserved between human, mouse, and rat (3). Among the different human S100 genes, 17 are clustered on the human chromosome 1q21 (3), a region frequently involved in chromosomal rearrangement in cancers (4–6). Expression patterns of some members, including S100A3, -A8, -A9, -A12, and -B, are tissue-and cell-specific (7–10), whereas S100A2, -A13, and -A16 are expressed in a wide variety of tissues (2, 11–13). S100 proteins play a role in Ca\(^{2+}\)-homeostasis, cell proliferation, migration, differentiation, and apoptosis. A total of ten S100 family members are reported to be expressed in the central nervous system in a temporal, spatial, and cell type-specific manner. In response to Ca\(^{2+}\) oscillation, S100 proteins interact with distinct target proteins and/or relocate to different cellular compartments (for review see Ref. 14). Thereby, they are implicated in multiple intra- as well as extracellular activities. The brain-specific S100B regulates enzyme activity by rendering certain phosphorylation sites inaccessible to their interaction partners as was reported for neurogranin (15), tau protein (16, 17), GFAP (18), and p53 transcription factor (19–22). More recently, S100A10 has been shown to interact with serotonin 1B receptor and to modulate its membrane presence and availability (23). In addition, several S100 proteins have been shown to be secreted (24–27) via different mechanisms, including the classic endoplasmic reticulum-Golgi pathway, and alternative pathways involving cytoskeletal components such as actin and tubulin. S100B secretion from astroglial cells is induced by serotonin 1a receptor agonist binding (28). Moreover, the release of S100 protein from apoptotic cells following brain injury may be sufficient to induce cellular responses. In vitro, extracellular S100B and S100A4 promote neurite outgrowth and neuronal survival by acting through the receptor for advanced glycation end products and heparan sulfate proteoglycans, respectively, both of which are present at the cell surface (29). Moreover, extracellular S100B has been shown to modulate long term neuronal synaptic plasticity (30), glutamate uptake of hippocampal astrocytes (31), and inflammation (32) (for review see Ref. 33). Several S100 proteins have been linked to brain diseases like multiple sclerosis, Down’s syndrome, Alzheimer’s disease, or depression, usually due to altered levels of protein expression.

S100 proteins are small acidic proteins (\(M_r \sim 10–13\) kDa) characterized by distinctive homo- or hetero-dimeric architecture and two highly conserved Ca\(^{2+}\)-binding domains: a classic C-terminal EF-hand with a canonical Ca\(^{2+}\)-binding loop and an S100-specific N-terminal EF-hand (for reviews see Refs. 34–38). In general the affinities of S100 proteins for Ca\(^{2+}\) are low with a \([\text{Ca}^{2+}]_0\) value of 100–500 \(\mu\)M (39). Binding of Ca\(^{2+}\) to S100B induces helix rearrangement within each subunit of the dimer, resulting in the exposure of two hydrophobic surfaces (one in each monomer) (38, 40, 41), which are involved in

* This work was supported by the National Centers of Competence in Research (NCCR) on Neural Plasticity and Repair and the Swiss National Science Foundation (Grant 3100A0-101970). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Dept. of Pediatrics, Division of Clinical Chemistry and Biochemistry, University of Zurich, Steinwiesstrasse 75, 8032 Zurich, Switzerland. Tel.: 41-(44)-266-7541; Fax: 41-(44)-266-7169; E-mail: claus.heizmann@kispi.unizh.ch.

‡‡ The abbreviations used are: GFAP, glial fibrillary acidic protein; TNS, 2-p-toluidinylnaphtalene 6-sulfonate; GFP, green fluorescent protein; NLS, nuclear localization signal; BAPTA-AM, 1,2-bis(2-aminophenoxy)ethane-N,N,N’-tetraacetic acid tetrakis (acetoxymethyl ester); ORF, open reading frame; UTR, untranslated region; DIG, digoxigenin; Ab, antibody; MS, mass spectrometry.
target protein recognition (36, 37). Beside Ca$^{2+}$, a number of S100 proteins can also bind Zn$^{2+}$ or Cu$^{2+}$ (42, 43) that can influence the affinity of Ca$^{2+}$ binding (38). Thus, S100 proteins display variable transition metal-binding properties in agreement with their highly diversified and specialized functions.

Among the S100 protein family, the S100A16 protein is a novel member. S100A16 gene was isolated from astrocytoma and is located in the S100A cluster on human chromosome 1q21. Interestingly, the mouse S100A16 protein differs from its human orthologue by the presence of five additional QQE(C/S) cluster on human chromosome 1q21. Interestingly, the mouse S100A16 protein differs from its human S100A16 protein in that it has an additional arginine residue at position 326 and two additional histidine residues at positions 110 and 127. These additional residues provide the mouse S100A16 protein with additional metal-binding properties.

EXPERIMENTAL PROCEDURES

His Tag S100A16 Constructs—The human and the mouse S100A16 cDNA were obtained from the IMAGE consortium. The coding region of both human and mouse S100A16 was amplified by PCR using specific oligonucleotides containing, respectively, a 5'-BamHI site (5'-GGTGGATCCATGGCTGACTGCTGCTAC-3') and a HindIII site (5'-GGTGGATCCATGGCTGACTGCTGCTAC-3'). PCR products were cut with the corresponding restriction enzymes and cloned into the pET20-nHisT vector downstream of the His tag.

Generation of S100A16-GFP Fusion Construct—The coding region of human S100A16 cDNA was amplified by PCR using oligonucleotides containing a 5'-Xho site (5'-CTCGAGATGTGACGACTGCTAC-3') and a BamHI site (5'-GGATCCGGCGTGCTGCTTCTGTG-3'). PCR products were cut with the corresponding restriction enzymes and cloned into the pEGFP-N1 vector (Clontech). Plasmids were sequenced by responding restriction enzymes and cloned in-frame into the TGCTGCTCTGCTG-3' vector.

Expression and Purification of the Human and Mouse Recombinant S100A16 Proteins—The S100A16-pET20-nHisT constructs were transformed into the BL21(DE3)pLysS strain. After induction of the expression with 1 mM isopropyl-1-thio-β-D-galactopyranoside and cell lysis, the purification of the protein was performed on a nickel-nitrilotriacetic acid column (Invitrogen) according to the manufacturer's protocol. The His-tagged S100A16 fusion proteins were then cleaved with thrombin (Roche Diagnostics, Mannheim, Germany) overnight at room temperature. Recombinant S100A16 was further purified by using a HiTrap benzamidine FF (high sub) column (Amersham Biosciences) to remove thrombin. Fractions were collected, and the presence of S100A16 proteins was identified by Western blotting and mass spectrometry. The flow-through of the HiTrap benzamidine column containing the human S100A16 protein was dialyzed against 50 mM Tris-HCl, pH 7.5, 500 mM KCl, 1 mM β-mercaptoethanol, 50 μM EGTA. The mouse S100A16 protein was dialyzed against 50 mM Tris-HCl, pH 7.5, 150 mM KCl, 1 mM β-mercaptoethanol, 50 μM EGTA. The protein concentrations were determined by spectrophotometry using the extinction coefficients ε$_{278}$ nm of 11590 M$^{-1}$cm$^{-1}$ for the human S100A16 and 11960 M$^{-1}$cm$^{-1}$ for the mouse S100A16, both based on their Trp and Tyr content.

Metal Removal and Cation Binding—Human S100A16 and mouse S100A16 were dialyzed against 50 μM EGTA or precipitated with 3% trichloroacetic acid to reduce the Ca$^{2+}$ contamination and passed through a 40 X 1 cm Sephadex G-25 column equilibrated in 50 mM Tris-HCl, pH 7.5, 150 mM KCl (buffer A) for mouse S100A16 or 50 mM Tris-HCl, pH 7.5, 500 mM KCl (buffer B) for human S100A16. Typically, the contamination represents <2% of the total binding capacity. Total Ca$^{2+}$ was determined with a PerkinElmer Life Sciences 2380 atomic absorption spectrophotometer.

Flow dialysis on 100–200 μM S100A16 were carried out on mouse S100A16 in buffer A and on human S100A16 in buffer B, both in the absence or presence of 50 μM Zn$^{2+}$ at 25 °C according to the modified method of Colowick and Womack (44). Treatment of the raw data and evaluation of the binding parameters was done as previously described (45).

Direct Zn$^{2+}$ binding was determined with the method of equilibrium gel filtration on a Sephadex G-25 (column of 40 × 1 cm) equilibrated in buffer A containing a fixed concentration of Zn$^{2+}$. The protein concentration was determined by spectrophotometry, and the free and protein-bound Zn$^{2+}$ were quantified with atomic absorption.

Trp Fluorescence—Emission fluorescence spectra were taken with a PerkinElmer LS-5B spectrofluorometer on 12 μM of the metal-free form in buffer A or B at 25 °C with excitation at 278 nm and slits of 5 nm. 20 μM EGTA, or 1 mM Ca$^{2+}$, or 20 μM Zn$^{2+}$, or 4 μM guanidine-HCl were added to monitor the effect of the respective cations or to obtain the spectrum of the denatured protein. Solution backgrounds were subtracted.

Interaction with Hydrophobic Probes—The Ca$^{2+}$-dependent changes in the hydrophobic core of the S100A16 proteins were followed by monitoring the fluorescence properties of TNS as described previously (46). Briefly, the final solutions contained 40 μM TNS and 8 μM protein in buffer A (mouse S100A16) or B (human S100A16). The Ca$^{2+}$ and Zn$^{2+}$ titrations were carried out on 6–8 μM metal-free protein in buffer A or B.

Thiol Reactivity—The metal dependence of the single thiol in human S100A16 and of the four thiols in mouse S100A16 was monitored after the protein samples had been thoroughly reduced by overnight incubation at room temperature with 100 Nomenclature (see Ref. 3) used was updated in Marenholz, I., Lovering, R. C., and Heizmann, C. W., Biochim Biophys Acta (2006) Vol. 1763, in press.
Brain Distribution and Ca²⁺-dependent Translocation

mm dithiothreitol in the presence or absence of 8 m urea. The reducing agent and urea were removed by gel filtration on Sephadex G-25 equilibrated in buffer A, which was freshly degassed and saturated with nitrogen. The kinetics of the thiols was initiated by mixing 12.5 μM mouse S100A16 in buffer A or 25 μM human S100A16 in buffer B with 5,5’-dithiobis(2-nitrobenzoic acid) at 100 μM final concentration, and the absorption was continuously monitored at 412 nm (47). The pseudo first order rate constants and t₁/₂ values were extracted by standard procedures.

Quantitative PCR Analysis—Two-month-old mouse brain RNA was extracted using TRIzol reagent (Invitrogen) and treated with DNase (Roche Diagnostics) for 30 min at 37 °C. S100 mRNAs, converted to cDNA, were quantified by real-time PCR using the ABI/PRISM 7700 sequence detection (Applied Biosystems). Analyses were performed using primers, internal fluorescence probes specific to each S100 mRNA, and the TaqMan Gene Expression assays kit (Applied Biosystems). PCR contained 0.4 μM primers and 0.2 μM TaqMan MGB probe and consisted of a 10-min denaturation step at 95 °C followed by 40 cycles of 15 s at 95 °C and 60 s at 60 °C. Mix of unlabeled primers and TaqMan MGB probes were obtained from Applied Biosystems (Assays-on-demand™ Gene Expression Products). Normalized value for S100 mRNAs expression in each sample was calculated as the relative quantity of S100 divided by the relative quantity of S100B.

RNA Probe Design and Labeling—Two different antisense mouse S100A16 probes corresponding either to the open reading frame (ORF) or to the 3’-untranslated region (3’UTR) sequence of the gene were obtained by PCR using the mouse S100A16 cDNA clone as template (IMAGE consortium, ID IMGp998199175). The primers used for the amplification of mouse S100A16-ORF and mouse S100A16-3’UTR were, respectively, 5’-GGGATTCATATGCTGACTGCTATAC-3’/5’-CGGGATCTAGCTGACTGCTATAC-3’/5’-CGGGATCTAGCTGACTGCTATAC-3’/5’-CGGGATCTAGCTGACTGCTATAC-3’/5’-CGGGATCTAGCTGACTGCTATAC-3’/5’-CGGGATCTAGCTGACTGCTATAC-3’.

In situ Hybridization—Briefly, 7- to 9-week-old C57/BL6 mice were perfused intracardially with 250 ml of cold 4% paraformaldehyde solution in 0.1 M phosphate buffer, pH 7.4. The brains were removed, post-fixed for 4 h, placed in 20% sucrose overnight, and stored at −80 °C until use. Slices were obtained on a freezing microtome at 20-μm thickness. Sections were treated with proteinase K (0.5 μg/ml), washed in phosphate-buffered saline, acetylated, washed in distilled water and pre-hybridized for 4 h at 56 °C. Each slide was then hybridized overnight in hybridization buffer containing 200 ng/ml DIG-labeled cRNA. Slides were washed under stringent conditions (56 °C, 2× SSC, 2× 50% formamide, 0.2× SSC, 50% formamide, 0.1× SSC). The in situ signal was visualized using alkaline phosphatase conjugated to anti-digoxigenin antibody (Roche Diagnostics) diluted 1:2000 in phosphate-buffered saline, 1% blocking reagent, and developed with nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate substrate (Roche Diagnostics). Sections were examined and photographed using a Zeiss Axioskop light microscope (Zeiss, Germany).

Production and Purification of Polyclonal Antibodies—Antisera against human recombinant S100A16 were produced in rabbits by DakoCytomation (Glostrup, Denmark). The titers of the antisera were determined using 1 μg of recombinant protein on Western blots as described by Ilg et al. (12). An aliquot of antiserum was affinity-purified by using a HiTrap protein A column (Amersham Biosciences) following the manufacturer’s protocol. This antibody (hS100A16 Ab) was used for further studies.

Western Blot Analysis—Total cell extracts were prepared as described previously (12). Nuclear protein fractions were obtained from extracts of U373 MG cells by centrifugation at 1000 × g, 10 min at 4 °C.

Total mouse brain tissue, cortical, hippocampal, and cerebellar regions were lysed in 50 mM Tris-HCl, pH 7.5, 300 mM NaCl, 1% Triton-X supplemented with Complete Proteinase Inhibitor Mixture (Roche Diagnostics). The lysates were passed through a 23-gauge needle and centrifuged at 13,000 × g at 4 °C for 15 min, and supernatants were analyzed. Subcellular fractionation (nuclei, cytosol, and membrane) from mouse cortex were performed as described in Ref. 48.

Protein concentration of the lysates was measured by use of the BCA Protein Assay Reagent (Pierce). Per sample, 20–50 μg of total protein were separated with 10% SDS-PAGE and blotted onto nitrocellulose (Schleicher and Schuell, Dassel, Germany). The membrane was incubated for 1 h with hS100A16 (1:5,000), with the mouse monoclonal human anti-β-tubulin (1:10,000) (Sigma) or with anti-nuclear laminin (1:1,000, Abcam, Cambridge, UK). The blot was incubated with anti-IgG conjugated with peroxidase (1:10,000, Sigma). The bands were detected using ECL solution (Amersham Biosciences).

Cell Cultures and Transient Transfections—SKN-LE and SKN-BE neuroblastoma (a gift from Dr. A. Fontana, University Hospital, Zurich) and U87 MG and U373 MG glioblastoma (ATCC, Manassas, VA) cells were cultivated in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum, 2 mm L-glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin. SHSY-5Y (ATCC) neuroblastoma cells were maintained in RPMI supplemented with 10% fetal calf serum, 2 mm L-glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin.

U87 MG and U373 MG cells were transfected with human S100A16-GFP construct using Lipofectamine 2000 (Invitrogen) following the manufacturer’s instructions and incubated for 16 h to allow gene expression. Cells were stimulated from 1 to 30 min with 1 μM thapsigargin (Sigma), 1 μM ionomycin (Sigma) in medium supplemented with 1.5 mM CaCl₂, 2 mM EGTA, or BAPTA-AM.

Immunocytochemistry—For GFP fluorescence and immunostaining, U87 MG and U373 MG cell lines were transfected with human S100A16-GFP construct using Lipofectamine 2000 (Invitrogen) following the manufacturer’s instructions and incubated for 16 h to allow gene expression. Cells were stimulated from 1 to 30 min with 1 μM thapsigargin (Sigma), 1 μM ionomycin (Sigma) in medium supplemented with 1.5 mM CaCl₂, 2 mM EGTA, or BAPTA-AM.
Brain Distribution and Ca$^{2+}$-dependent Translocation

A kDa

1 2 3 4 5 6 7 8

37 25 15 10

B

FIGURE 1. Expression and purification of human and mouse recombinant S100A16. A, SDS-PAGE of protein extracts of human S100A16 transformed Escherichia coli/BL21 culture before (lane 1) and after induction with isopropyl 1-thio-β-D-galactopyranoside (lane 2). Human (Lane 3) and mouse (Lane 6) His-tagged S100A16 proteins were purified on a nickel-nitrilotriacetic acid-agarose column. Purified human (lane 4) and mouse (lane 7) S100A16 protein after His tag removal runs as a monomer. S100A16 dimers where present even after treatment with 250 mM di-thiothreitol (lane 5 and 8).

For immunostaining of tissue sections, 7- to 9-week-old C57/BL6 mice were perfused intracardially with 250 ml of cold 4% paraformaldehyde solution in 0.1 M phosphate buffer, pH 7.4, containing 1% picric acid. The brains were removed, post-fixed for 4 h, placed in 20% sucrose overnight, and stored at −80 °C until use. Coronal sections (20 μm) were pretreated (10 min at 90 °C in citrate buffer) and incubated with hS100A16 Ab (1:1000) and with a chicken anti-mouse Gfap antibody (1:2000, Abcam) overnight at 4 °C, with secondary fluorophore-conjugated antibodies for 2 h at room temperature, counterstained with 4′,6-diamidino-2-phenylindole (1:2000, Sigma) and mounted in eukit mounting media (Merck, Darmstadt, Germany). Sections were imaged by using a Zeiss Axioskop light microscope.

RESULTS

Expression and Purification of Human and Mouse Recombinant S100A16—Human and mouse recombinant S100A16 proteins were expressed in BL-21 bacteria and purified. Expression of recombinant mouse and human S100A16 was facilitated by the presence of a His tag at the N-terminal end (Fig. 1A, lanes 1 and 2). The human and the mouse His-tagged proteins were purified by passing the bacterial crude lysate onto a nickel-nitrilotriacetic acid column (Fig. 1A, lanes 3 and 6). About 10 mg of each protein could be expressed in 500 ml of LB medium. After cleavage of the His tag and thrombin removal using a benzamidine FF column, purity and concentration of both human and mouse S100A16, were examined by SDS gel electrophoresis (Fig. 1A, lanes 4 and 7). S100A16 proteins migrate in two bands of −12 and 24 kDa for the human protein and 15 and 30 kDa for the mouse S100A16 (Fig. 1A, lanes 4 and 7) suggesting that each protein can form covalent dimers as it was already reported for most of the other members of this family (34–38). Furthermore, even harsh treatment with 250 mM di-thiothreitol could not completely reduce either the human or the mouse dimers to the monomeric form (Fig. 1A, lanes 5 and 8). The correct synthesis and molecular mass of both recombinant proteins were verified by MALDI-TOF mass spectrometry (matrix-assisted laser desorption/ionization-time of flight). The spectra show a peak with a molecular mass of 11951 Da for the human recombinant protein (Fig. 1B) and 14464 Da for the mouse recombinant protein (data not shown), corresponding to the theoretically calculated molecular masses plus one additional serine and one additional glycine. In addition, higher order oligomers, namely dimers (23884 Da), trimers (35805 Da), and even tetramers (47706 Da) were also observed in both spectra as shown for human S100A16 (Fig. 1B).

Biophysical Characterization—The recombinant human S100A16 appeared to be poorly soluble compared with the mouse protein, likely due to its more basic character (pI 6.3 versus pl 5.6, respectively). The additional Q/QE/C/S repeats present in the C-terminal part of mouse S100A16 may also account for the improved solubility of the mouse protein. In physiological conditions, the mouse S100A16 is soluble to up to 5–10 mg/ml protein, whereas the human S100A16 is only soluble to ∼0.1 mg/ml. Higher concentrations of human S100A16 can only be kept in solution of high ionic strength, i.e. in the presence of 0.5 M KCl. The onset of human S100A16 precipitation takes several hours, which allows performing short lasting experiments with clear solutions. The addition of Zn$^{2+}$ (≥200 μM) to both proteins facilitates their precipitation.

Direct Cation Binding Studies—Flow dialysis experiments on apo mouse S100A16 (50–90 μM) in buffer A (50 mM Tris-HCl, pH 7.5, 150 mM KCl) yielded a simple isotherm (Fig. 2, red) with saturation at one Ca$^{2+}$ per monomer and a $K_d$ of ∼750 μM and $n_{1/2}$ close to 1. Thus, the affinity for Ca$^{2+}$ is very low and there is only one binding site per monomer. Similar experiments on human S100A16 in buffer B (50 mM Tris-HCl, pH 7.5, 500 mM KCl) yielded also one Ca$^{2+}$-binding site per monomer (Fig. 2, blue) with a $K_d$ of 430 μM and $n_{1/2}$ close to 1. In the presence of 100 μM Zn$^{2+}$ the two proteins precipitate at the protein concentrations employed. Because this may alter the diffusion properties of the membrane, Ca$^{2+}$ titrations in the presence of Zn$^{2+}$ were not performed. The main difference between the S100A16 proteins and other S100 proteins is the presence of only one functional Ca$^{2+}$-binding site (per monomer) in S100A16. This is predicted from the S100A16 amino acids sequences, which both show a Glu to Ser substitution in the critical Z position of the N-terminal EF-hand. The human protein differs from the mouse one...
Brain Distribution and Ca\textsuperscript{2+}-dependent Translocation

FIGURE 2. Ca\textsuperscript{2+}-binding to the S100A16 proteins. Direct Ca\textsuperscript{2+} binding was monitored by flow dialysis at 25 °C in 50 mM Tris-HCl, pH 7.5, 150 mM KCl (buffer A) for mouse S100A16 (red) and in 50 mM Tris-HCl, pH 7.5, 500 mM KCl (buffer B) for human S100A16 (blue). The protein concentration is 50 \muM (circles) or 90 \muM (rectangles). The solid lines are the theoretical isotherm calculated with the Adair equation for one site.

mainly in its higher affinity for Ca\textsuperscript{2+}, i.e. 430 \muM, which approximates the value in several other S100 proteins (39). Direct Zn\textsuperscript{2+} binding measurements were performed with mouse S100A16 by equilibrium gel filtration at low Zn\textsuperscript{2+} concentration (30 \muM) to minimize protein precipitation. In these conditions ~0.47 atom of Zn\textsuperscript{2+} was bound per monomer. This suggests the presence of one Zn\textsuperscript{2+}-binding site in the monomer with a \(K_d\) of ~25 \muM (see below). At higher free Zn\textsuperscript{2+} concentrations the protein remains precipitated on the Sephadex G-25 column and could only be eluted with EGTA.

**Trp Fluorescence**—Both mouse S100A16 and human S100A16 contain a single Trp-80 located in the second helix of the C-terminal EF-hand. In the native apo proteins the Trp is well protected from the solvent, because addition of 4 M guanidine-HCl leads to a red shift from 331 to 351 nm and a slight decrease of the fluorescence intensity (Fig. 3). In mouse S100A16 in buffer A the Trp fluorescence intensity is not affected by Ca\textsuperscript{2+} up to 2 mM, but there is a 4 nm red shift of the maximum. Zn\textsuperscript{2+} (40 \muM) increases the fluorescence slightly (1.1-fold) without a wavelength shift (Fig. 3A), but strongly increases the light scattering measured at 285–290 nm, meaning that the protein aggregates (Fig. 3A). This protein precipitation starts at 10 \muM Zn\textsuperscript{2+} and is very marked at 250 \muM. Ca\textsuperscript{2+} amplifies the extent of Zn\textsuperscript{2+}-induced aggregation (Fig. 3A, red dotted versus green solid line).

Upon addition of 2 mM Ca\textsuperscript{2+} to human S100A16 in buffer A, there was very limited aggregation (Fig. 3B, red solid line in the 285–290 nm region) and no precipitation, but the fluorescence intensity decreased to half its initial value (Fig. 3B, red arrow). This step was titrated at 331 nm (Fig. 3B, red arrow) and yielded a \(K_d\) of 220 \muM in buffer B (Fig. 3C) and of 180 \muM in buffer B (not shown). In human S100A16 (12 \muM) in buffer A, Zn\textsuperscript{2+} provoked a mild decrease of the Trp fluorescence again accompanied with protein aggregation (Fig. 3B). In conclusion, Trp-80 in the C-terminal EF-hand is quite strongly influenced by the C-terminal poly QQE(C/S) sequence, which is present in mouse but not in human. Whereas Ca\textsuperscript{2+} binding to this EF-hand lowers the fluorescence in human, the C-tail prevents this phenomenon in mouse. This segment of 21 amino acids, which has equal propensity to form coil and helix, may interact intramolecularly with the Ca\textsuperscript{2+}-binding core.

**Interaction with the Hydrophobic Probe TNS**—Metal-free mouse S100A16 in buffer A enhances the fluorescence of the hydrophobic probe TNS 5-fold with a maximum at 426 nm (Fig. 4A). Upon further addition of up to 2 mM Ca\textsuperscript{2+} this fluorescence emission does not change (red versus blue solid line), suggesting that Ca\textsuperscript{2+} binding does not induce a hydrophobic surface. In contrast, binding of Zn\textsuperscript{2+} leads to a 2-fold increase in fluorescence intensity over that of the apo form (green versus blue solid line). The signal change is complete at 250 \muM Zn\textsuperscript{2+} and the \([Zn^{2+}]_{0.5}\) is estimated at 25 \muM, both in the presence and absence of 2 mM Ca\textsuperscript{2+} (Fig. 4C) This value is in agreement with the direct Zn\textsuperscript{2+}-binding data for mouse S100A16. Note that the presence of Ca\textsuperscript{2+} does not modify the interaction of mouse S100A16 with Zn\textsuperscript{2+}. The Zn\textsuperscript{2+} effect is mostly reversible upon addition of 0.35 M KCl corresponding to buffer B (Fig. 4A, green dotted line), which solubilizes (visual observation) the Zn\textsuperscript{2+}-induced protein precipitate, suggesting that the aggregation creates hydrophobic sites. Cu\textsuperscript{2+} up to 100 \muM does not affect the TNS fluorescence enhancement by mouse S100A16 (data not shown) and, indirectly, Cu\textsuperscript{2+} has also no effect on the Zn\textsuperscript{2+} enhancement, suggesting that Cu\textsuperscript{2+} does not interact with the protein.

FIGURE 3. Conformational changes measured by intrinsic Trp fluorescence. A, mouse S100A16 in buffer A, red; human S100A16 in buffer A, green; mouse S100A16 in buffer B, dotted red; human S100A16 in buffer B, dotted blue; A, apo; B, Ca\textsuperscript{2+} bound. C, titration of apo human S100A16 in buffer A with Ca\textsuperscript{2+}.
Metal-free human S100A16 in buffer A enhances the basic TNS fluorescence 3-fold with a maximum at 428 nm. One mM Ca\(^{2+}\) induces a 1.7-fold increase in fluorescence enhancement (Fig. 4C, red arrow), and the fluorescence change was monitored at 435 nm; the estimated \([Ca^{2+}]_{0.5}\) is 150 \(\mu M\), and the curve displays pronounced positive allostery with \(n_H\) equal to 1.7 (Fig. 4D). Zn\(^{2+}\) addition to apo human S100A16 promotes a 2.1-fold enhancement and a \([Zn^{2+}]\) of 5 \(\mu M\) (Fig. 4C). Again, Cu\(^{2+}\) has no effect, neither on the fluorescence of the apo state, Ca\(^{2+}\), or Zn\(^{2+}\), or Ca\(^{2+}\)/Zn\(^{2+}\)-state. The difference between the mouse and the human protein in Ca\(^{2+}\)-dependent hydrophobic exposure suggests that the extended C terminus in the mouse neutralizes the hydrophobic patch.

Conformational Changes Measured by Thiol Reactivity—Mouse S100A16 possesses four Cys residues, one at position 4 from the N-terminal end, one in the middle of the C-terminal EF-hand, and two in the unusual C-terminal tail containing the QQE(C/S) repeats. After extensive reduction only two thiols were instantaneously and completely titrated in both the apoprotein and in the Ca\(^{2+}\)-saturated form of mouse S100A16. In the presence of high concentrations of Zn\(^{2+}\) only one of these two thiols reacted instantaneously, whereas the second was partly protected. Half-maximal protection of the latter thiol showed an estimated \([Zn^{2+}]_{0.5}\) of 170 \(\mu M\) (see Table 1).

The two remaining Cys were either oxidized to a disulfide bridge or in a thiol form, therefore inaccessible to 5,5′-dithiobis(2-nitrobenzoic acid). We estimate that the non-reactive thiols in mouse S100A16 are located in the C-terminal tail. Human S100A16 has only one Cys residue in position 4, and this thiol reacted instantaneously in apo and Ca\(^{2+}\) form and was protected by Zn\(^{2+}\) in a similar way as the Zn\(^{2+}\)-sensitive thiol in mouse S100A16. It is thus likely that in mouse S100A16 also the Zn\(^{2+}\)-sensitive thiol is Cys-4.

S100A16 mRNA Expression and Distribution in Mouse Brain—Previous studies reported low S100A16 gene expression in human brain (2). We first compared the relative expression of S100A16 and other S100 protein genes to the expression of S100B, a brain-specific calcium-binding protein, using real-time PCR assay. Total RNA was extracted from adult mouse brain and mRNA quantification revealed a 10-fold lower expression of S100A16 compared with S100B (Fig. 5A). Furthermore, S100A16 transcript levels were also below those of
S100A1, S100A6, and S100A13, whereas S100A16 mRNA was ten times more abundant than S100A4 (Fig. 5A).

The expression pattern of S100A16 in adult mouse brain was also investigated by in situ hybridization. To analyze the S100A16 mRNA distribution, two specific probes corresponding to the ORF and to the 3'UTR were generated. The different types of anesthesia, tissue preparation, or specific S100A16 antisense probes did not influence the appearance of the staining pattern. Moreover, sections hybridized with sense probes did not reveal any positive signal (data not shown). In adult mouse brain, expression of S100A16 gene was observed in the cerebral cortex at all levels of the mouse brain (Fig. 5B). We found an intense staining throughout layer I to layer VI (CL 1–6), in the parietal (Par), occipital (Oc), and temporal cortex (Te). Moreover, lower, but unambiguous S100A16 mRNA levels were detected in cells resident in cortical layer 1 (Cl 1). In the hippocampal formation (Fig. 5C), hybridization signal was detected in the Ammon’s horn fields 2 (CA2) and 3 (CA3) and in the hilus of the dentate gyrus (DG). Higher magnification imaging revealed S100A16 mRNA labeling in cell bodies present around neurons of the pyramidal cells layer. In the cerebellum, hybridization performed at the cellular level, showed S100A16 expression in cells that surround Purkinje cells (Fig. 5D). These results indicate that S100A16 gene is widely expressed in the cortical regions, whereas its expression is restricted to discrete regions and specific cell types in both the hippocampus and the cerebellum.

Characterization of hS100A16 Ab—The human recombinant protein was used to produce specific rabbit polyclonal antisera. Affinity-purified polyclonal anti-human S100A16 antibodies (hS100A16 Ab) were first tested for their cross-reactivity against other S100 proteins by Western blot analysis. hS100A16 Ab recognized specifically the corresponding antigen (Fig. 6) and did not cross-react with the other family members. Furthermore, hS100A16 Ab also recognized the mouse S100A16 recombinant protein (Fig. 6). In addition to the monomer, hS100A16 Ab recognized two additional protein bands of ~22 kDa and 35 kDa, which correspond to the dimeric and trimeric forms of human and mouse recombinant S100A16 (Fig. 6) in agreement with the MS data presented in Fig. 1B.

Expression and Immunolocalization of S100A16 in Mouse Brain—To examine S100A16 protein expression, 50 μg of total protein extracts from different brain regions were analyzed by Western blot analysis. As shown in Fig. 7A, hS100A16 Ab detected a single protein band in cortical, hippocampal, and cerebellar extracts, with a relative molecular mass of ~15 kDa, consistent with the theoretically calculated molecular weight, and our MS analysis. Immunoblotting of the 20-μg membrane, cytosolic, and nuclear fractions of cortical extract revealed the presence of S100A16 protein in the cytoplasm and a high amount in the nucleus of cells (Fig. 7A, Cy and Nu).

We next performed immunohistochemistry using hS100A16 Ab to evaluate the distribution and the intracellular localization of S100A16 in mouse brain. The expression of S100A16 protein was relatively prominent in layers 1–6 of all cortical regions (Fig. 7B). To further investigate cell type specificity of S100A16 protein expression in the cortex, double-label immunofluorescence staining was performed. S100A16 labeling was mainly associated with nuclei and somata of cells positive for Gfap, an astrocyte marker (Fig. 7C). In the cerebellum (Fig. 7D), we found prominent staining at the level of Purkinje neurons. However, higher

**TABLE 1**

<table>
<thead>
<tr>
<th>[Zn(^{2+})] (μM)</th>
<th>Mouse S100A16</th>
<th>Human S100A16</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>ND(^a)</td>
</tr>
<tr>
<td>40</td>
<td>6</td>
<td>ND</td>
</tr>
<tr>
<td>50</td>
<td>7</td>
<td>ND</td>
</tr>
<tr>
<td>100</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>150</td>
<td>14</td>
<td>ND</td>
</tr>
<tr>
<td>200</td>
<td>27</td>
<td>25</td>
</tr>
<tr>
<td>250</td>
<td>19</td>
<td>ND</td>
</tr>
<tr>
<td>400</td>
<td>33</td>
<td>70</td>
</tr>
<tr>
<td>500</td>
<td>25</td>
<td>ND</td>
</tr>
</tbody>
</table>

\(^a\) ND, not determined.
Brain Distribution and Ca\textsuperscript{2+}-dependent Translocation

resolution images of co-immunostaining revealed labeling in somata and neurites of cells co-expressing Gfap (Fig. 7D, right panel). Moreover, only a few cells in the molecular and in the granular layer were also positive for S100A16 (Fig. 7D). In the hippocampal formation (Fig. 7, E–G), hS100A16 Ab consistently labeled cells in the molecular cell layer of the CA2–CA3 subfields (Fig. 7E). A more prominent staining was detected in the polymorphic cell layer of the dentate gyrus (Fig. 7F), where S100A16 was present in cell bodies and neurites spreading into the granular cell layer. In this brain structure, S100A16 and Gfap staining overlap (Fig. 7G). There was virtually no labeling of dendate granule cells (Fig. 7F) or pyramidal neurons in CA1–CA3 regions (Fig. 7E). Altogether, these data confirm in situ hybridization observation and provide first evidence for the occurrence of S100A16 protein in nuclei, somata, and neurites of astrocytes in the adult mouse brain.

Intracellular Localization of S100A16 in Human U373 MG and U87 MG Glioblastoma Cells—Western blot analysis, using hS100A16 Ab, revealed a single band of ~12-kDa in total protein extract of human U87 MG and U373 MG glioblastoma cell lines (Fig. 8A). A previous study identified S100A16 protein in nucleolar fraction of HeLa cells (49). Because S100 protein expression and subcellular localization might differ between each member of the family and between different tissues and cell types, we examined intracellular distribution of endogenous S100A16 in both glioblastoma cell lines as well as in HeLa cells. Cells were fixed and co-stained with hS100A16 Ab and with anti-nucleolin, a nucleoli marker.

We found intense staining in the nucleus of U373 MG (Fig. 8, B and C, arrowheads) and U87 MG cells (data not shown). Confocal microscopy revealed a clear punctuated peripheral cytoplasmic S100A16-staining in both U373 MG (Fig. 8, B–D, arrows) and U87 MG cells (data not shown). Moreover, S100A16 nuclear labeling overlaps with nucleolin staining, defining submicron-sized subdomains within the nucleolus in glioblastoma cells (Fig. 8, E–G) and in HeLa cells (Fig. 8I), in accordance with previous MS data (49).

To confirm S100A16 subcellular localization, glioblastoma cells were transfected with a GFP-S100A16 construct. The exogenous fusion protein exhibited similar intracellular distri-

![Figure 6. Specificity of hS100A16 Ab. hS100A16 Ab was tested against other human S100 proteins and mouse S100A16 protein. Western blot analysis against indicated recombinant proteins (2 μg each) revealed the specificity of the antibody and cross-reaction with the mouse S100A16 protein. The proteins were blotted onto nitrocellulose membrane and stained with Ponceau S as a loading control (bottom), and immunoreactivity was visualized using horseradish peroxidase-coupled antibody.](http://www.jbc.org/content/281/50/38912/F6)

![Figure 7. S100A16 protein levels in different parts of the mouse brain. A, top, Western blot analysis using hS100A16 Ab revealed different levels of the protein in the cortex (Cx), hippocampus (Hip), and cerebellum (cer). Immunoblotting of subcellular fractions of cortical tissue in membrane (Mb), cytoplasmic (Cy), and nuclear (Nu) extracts (A, bottom). Immunolocalization of S100A16 in mouse brain. Coronal sections were immunostained with hS100A16 Ab. DNA is stained with 4',6-diamidino-2-phenylindole (blue). B, in cortex, S100A16-positive cells (red) were uniformly distributed throughout cortical layers 1–6 (C1 1–6). C, in cortical layer 2, S100A16 (green), was detected in the cytoplasm and nuclei (arrowheads) of cells co-expressing Gfap (red). D, in cerebellum, intense S100A16 staining (red) was observable in the vicinity of Purkinje cells and in single cells of the molecular layer (CbM) and granular layer (CbG). S100A16 labeling was not observed in cerebellar white matter (CbW). E, in the hippocampal formation, S100A16 immunoreactivity was mainly localized in the molecular layer (HmoL) of the CA2 field (Ca2) and in the polymorphic cell layer (Po) of the dentate gyrus (F). G, at higher magnification, endogenous S100A16 (green) was distributed throughout neurites. Note the absence of in pyramidal neurons (Py) and in granule cell neurons (Gr). Scale bars = 100 μm; 10 μm in C, D (right), and G.](http://www.jbc.org/content/281/50/38912/F7)
Brain Distribution and Ca^{2+}-dependent Translocation

Consistently, S100A16 was found in nucleus and cytoplasm of astrocytes in vivo (Fig. 7).

Ca^{2+}-dependent Nuclear Export/Import of S100A16 in U373 MG Cells—S100 proteins have been reported to relocate within the cells in response to [Ca^{2+}], oscillation. Therefore, we investigated Ca^{2+}-dependent translocation of S100A16 in U373 MG cells exposed to either ionomycin, a Ca^{2+} ionophore supplemented with extracellular Ca^{2+}, or thapsigargin, an endoplasmic reticulum Ca^{2+}-ATPase inhibitor. Cells were treated for different periods of time, fixed, and co-stained for hS100A16 Ab and anti-nucleolin. Overall, in presence of 1.5 mM or 5 mM extracellular Ca^{2+}, the response of U373 MG cells to both drugs was similar. No immediate cytotoxicity was noted in U373 MG cells during the observation periods in response to the different treatments. Both ionomycin and thapsigargin treatments resulted in increased S100A16 fluorescence in both the nucleoplasm and perinuclear area (Fig. 9, A and B). Higher magnification images revealed that a fraction of S100A16 was still associated with the nucleoli after 5 min of treatment (Fig. 9, A and B). Up to 10 min of exposure to 1 mM ionomycin resulted in the total loss of S100A16 immunoreactivity within nucleoli (Fig. 9, B and C, bottom), and a punctate cytoplasmic S100A16-staining clearly appeared after 15 min of treatment (Fig. 9C).

Ca^{2+}-dependent cytoplasmic translocation was then confirmed using the S100A16-GFP fusion protein. As shown in Fig. 9G, 15 min after ionomycin addition fluorescence was only observed in the cytoplasm. In contrast, addition of a membrane-permeable Ca^{2+} chelator, EGTA or BAPTA-AM, induced S100A16 perinuclear accumulation (Fig. 9, D and E) and nuclear entry (Fig. 9, E and F). Typically, intense nuclear staining and loss of S100A16 immunoreactivity in the cytoplasm was observed 30 min after EGTA addition (Fig. 9F). Furthermore, an intense S100A16 fluorescence was observed within nucleoli 20–30 min after treatment (Fig. 9, E and F, bottom). Western blot analysis using nuclear extracts of U373 MG cells confirmed the Ca^{2+}-dependent nuclear export/import of S100A16 (Fig. 9H). Together, these results indicate that
[Ca$^{2+}$], regulates S100A16 subcellular localization; i.e. 1) a high intracellular Ca$^{2+}$ level induces S100A16 nucleolar exit and nucleocytoplasmic transport, whereas 2) lowering intracellular Ca$^{2+}$ concentration leads to S100A16 nuclear translocation and accumulation within specific region of nucleoli.

**DISCUSSION**

The present study firmly establishes that both human and mouse S100A16 proteins have only one functional Ca$^{2+}$-binding site located in the C-terminal EF-hand, as was already expected from the sequences (2). The C-terminal EF-hand is canonical and should bind Ca$^{2+}$, whereas the N-terminal EF-hand diverging from the corresponding motif in most S100 proteins, should not bind Ca$^{2+}$. Comparatively, the S100-specific EF-hand of S100A7 displays the same Glu to Ser substitution in position Z (50) and does not bind Ca$^{2+}$. Surprisingly, the affinity of the C-terminal EF-hand for Ca$^{2+}$ in mouse S100A16 is very low; the Ca$^{2+}$ affinity of this site in human S100A16 is 2-fold higher. Because high ionic strength buffer can depress the Ca$^{2+}$ affinity 2- to 3-fold, we speculate that at physiological ionic strength the Ca$^{2+}$ affinity of human S100A16 would be higher and would correspond to a $K_d$ of 100–200 $\mu$M, i.e. very similar to that of many other S100 proteins. Indeed, the dissociation constant for Ca$^{2+}$ measured by the Trp fluorescence changes in buffer A was 220 $\mu$M (Fig. 4D). The weak binding of Ca$^{2+}$ to the C-terminal EF-hand of the mouse protein either 1) does not lead to the exposure of a hydrophobic patch as it usually occurs in many other S100 proteins, with the notable exception of S100A13 (13) or 2) leads to the formation of a hydrophobic patch, which is then occupied by the C-tail. Surprisingly, Ca$^{2+}$ binding to human S100A16 leads to an enhancement of the TNS fluorescence and thus to hydrophobic exposure. The major difference in the C-terminal EF-hand loops of the two proteins is a Cys in position Y in mouse S100A16 only. Possibly, the very short C-tail of...
human S100A16 is not capable of neutralizing the hydrophobic patch and thus leaves room for TNS binding.

In both proteins, Zn\(^{2+}\) binding leads to enhancement of the TNS fluorescence, and one should consider the question if this represents the solvent exposure of a hydrophobic surface. An independent way to prove hydrophobic exposure on the surface in Ca\(^{2+}\)-binding proteins consists of phenyl-Sepharose chromatography. Unfortunately, this experiment could not be performed, because after binding of the Zn\(^{2+}\)-saturated protein to the surface, Zn\(^{2+}\) sequestration to restore the apo form did not allow elution of the protein. Either the apoprotein is hydrophobic enough to stick to the column, or EGTA does not efficiently remove Zn\(^{2+}\) from the protein.

Human S100A16 favors TNS fluorescence enhancement upon binding of Ca\(^{2+}\) or Zn\(^{2+}\) and binding of both cations leads to additive fluorescence enhancement, thus indicating that Ca\(^{2+}\) and Zn\(^{2+}\) do not bind to the same sites and that the hydrophobic patches are different. We favor the idea that the Ca\(^{2+}\)-dependent patch is entirely made up of elements within each subunit, \textit{i.e.} the hydrophobic residues in helix 3 and 4 and in the linker 3–4. These are thus independent patches in each dimer, and likely these surfaces are also instrumental for the binding of two natural target peptides (36, 51). In contrast, the Zn\(^{2+}\)-dependent patch may result from oligomerization with exposure of hydrophobicity at the interfaces of the oligomers. Two arguments point in this direction: 1) the kinetic effect in the fluorescence experiments with TNS and 2) EGTA cannot easily reverse the effect of Zn\(^{2+}\). In several S100 proteins with resolved three-dimensional structure, such as S100A7 and S100B, the two Zn\(^{2+}\) sites are located at the ends of the antiparallel helices IV and IV\(^{'}\) in the dimer. A possible explanation is the exposure of hydrophobic sites upon Ca\(^{2+}\) binding.

S100A16 has previously been reported in a wide spectrum of adult human tissues including brain (2). Among the S100 protein examined, the relative expression of S100A16 was low in adult mouse brain (Fig. 5A) as previously described in the human adult brain (2). However, S100A16 transcript levels were ten times higher when compared with S100A4 mRNA levels, a S100 protein involved in neuronal differentiation as well as in the response of astrocytes to degeneration of myelinated axons (52, 53). Moreover, as reported for other members of the S100 protein family, brain expression gradually decreases with age (54). Indeed, S100 proteins exhibit specific spatiotemporal patterns of expression in agreement with the different roles they play during brain development (S100B, S100A6, S100A4, S100A5, and S100A13), or in cell cycle (S100B and S100A4) (54–57). In the present study, the expression and distribution of S100A16 was examined in the adult mouse cortex, hippocampus, and cerebellum using \textit{in situ} hybridization and immunohistochemistry. Our results showed that expression of S100A16 mRNA and protein is restricted to cells of the molecular layer in the CA2 and CA3 field of the hippocampus (Figs. 5C and 7E). An S100A16 positive signal was stronger in the polymorphic layer of the dentate gyrus, throughout the whole cortex and in cells located in the vicinity of Purkinje neurons in the cerebellum (Fig. 7, B, D, and F).

At the cellular level, \textit{in situ} hybridization revealed an S100A16 mRNA expression in cells of the cortical layer I and of

---

**Brain Distribution and Ca\(^{2+}\)-dependent Translocation**

---

Ca\(^{2+}\) regulates a wide range of cytoplasmic and nuclear events in a spatial and temporal manner. Intracellular translocation of S100 protein in response to [Ca\(^{2+}\)]\(_{\text{cyt}}\), variation has been reported to play an important role in the regulation of signaling complexes activating specific cellular pathways (14, 33). In the present study, an increase in [Ca\(^{2+}\)]\(_{\text{cyt}}\), stimulated the export of endogenous S100A16 from the nucleolus to the cytoplasm (Fig. 9). [Ca\(^{2+}\)]\(_{\text{cyt}}\), increase appears to be critical for S100A16 relocation. Our biochemical data indicate that, upon Ca\(^{2+}\) binding, S100A16 undergoes conformational changes leading to the exposure of hydrophobic patches (Figs. 3 and 4) implicated in S100 protein translocation (27). As nuclear and cytoplasmic Ca\(^{2+}\) signaling are regulated independently (61), S100A16 Ca\(^{2+}\)-dependent translocation might be regulated by the recently described nucleoplasmic reticulum, which terminates near the nucleoli and is responsible for the release of free Ca\(^{2+}\) into localized subnuclear regions (62). In contrast, opposite Ca\(^{2+}\)-dependent translocation was described for other members of
the EF-hand protein family. Indeed, S100B, S100A11, and calmodulin translocate from the cytosol to the nucleus in response to Ca\(^{2+}\) stimulation. However, in the present study, the decrease in [Ca\(^{2+}\)]\(_i\) resulted in the nuclear import of S100A16 and its accumulation within the nucleoli, re-establishing the initial situation (Figs. 8E and 9). Like other members of the family, S100A16 lacks the canonical nuclear localization signal. Nuclear import might then require interaction with transporter proteins as it was described for S100A11 (63) or might occur via alternative facilitated-diffusion pathways as observed for calmodulin (64). Furthermore, phosphorylation has been reported to be essential for S100A11 nuclear import (49, 63). Interestingly, a recent study identifies phosphorylated S100A16 protein in nucleoli of HeLa cells (49), indicating a possible function of phosphorylation in S100A16 nuclear import. However, further studies are required to elucidate the roles of S100A16 in the nucleoli of cells, and the origin and functional significance of S100A16 Ca\(^{2+}\)-dependent nuclear export.

Acknowledgments—We are indebted to Drs. R. Lang, D. P. Wolfer, E. Leclerc, D. Boller, and A. Galichet for continuous support. We thank H. Winter (DakoCytomation) for the help in producing the antibodies, P. Kleinert for the MS analyses, and Dr. M. Gruetter for providing the pET20-nHisT vector.

REFERENCES

Brain Distribution and Ca\(^{2+}\)-dependent Translocation

S100A16, a Novel Calcium-binding Protein of the EF-hand Superfamily
Emmanuel Sturchler, Jos A. Cox, Isabelle Durussel, Mirjam Weibel and Claus W. Heizmann

doi: 10.1074/jbc.M605798200 originally published online October 8, 2006

Access the most updated version of this article at doi: 10.1074/jbc.M605798200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 60 references, 21 of which can be accessed free at
http://www.jbc.org/content/281/50/38905.full.html#ref-list-1