S100A7, S100A10, and S100A11 Are Transglutaminase Substrates†

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ABSTRACT: S100 proteins are a family of 10−14 kDa EF-hand-containing calcium binding proteins that function to transmit calcium-dependent cell regulatory signals. S100 proteins have no intrinsic enzyme activity but bind in a calcium-dependent manner to target proteins to modulate target protein function. Transglutaminases are enzymes that catalyze the formation of covalent ϵ-(γ-glutamyl)lysine bonds between protein-bound glutamine and lysine residues. In the present study we show that transglutaminase-dependent covalent modification is a property shared by several S100 proteins and that both type I and type II transglutaminases can modify S100 proteins. We further show that the reactive regions are at the solvent-exposed amino- and carboxyl-terminal ends of the protein, regions that specify S100 protein function. We suggest that transglutaminase-dependent modification is a general mechanism designed to regulate S100 protein function.

Calcium-dependent signal transduction is a complex process that is mediated by calcium binding proteins. The EF-hand-containing proteins represent an important class of calcium-dependent signal transduction mediator (*1*). These proteins contain one or more EF-hand motifs (*1*). S100 proteins comprise a multigene family that are members of this class of protein. There are at least ten S100 family members, each characterized by a high degree of sequence and structural homology (*2*). S100 proteins are thought to exist in cells as homo- or heterodimers that have no intrinsic enzymatic activity of their own (*1*). In response to calcium binding to the EF-hand structures, S100 proteins undergo a conformational change (*3*−*6*). This conformation change exposes regions of the protein which permit the dimer to bind to and alter the activity of specific target proteins. S100 proteins have been shown to participate in a variety of signal transduction pathways (*4*), often in a cell type-specific manner. S100 proteins regulate cell differentiation, cell cycle progression, energy metabolism, kinase activity, and cytoskeletal membrane interactions, and gain or loss of S100 protein expression has been linked to disease states (*1*).

Transglutaminases are calcium-dependent enzymes that function to form interprotein ϵ-(γ-glutamyl)lysine bonds (*7*). Among other proposed functions, type II transglutaminase functions in apoptosis and type I transglutaminase (keratinocyte transglutaminase) is responsible for cornified envelop formation in surface epithelial cells (*7*). Transglutaminases also function as signal transduction proteins (*8*). For example, TG alters the function of CD38, an enzyme that catalyzes the formation of the second messenger cADPR,[1] by covalently cross-linking it to an as yet unidentified 190 kDa protein (*9*). Transglutaminase-dependent cross-linking also modifies epidermal growth factor receptor function (*10*). In addition, type II transglutaminase has been reported to function as a G protein (*8*).

We previously showed that S100A11 is a transglutaminase substrate (*11*, *12*). However, it has been an open issue whether such reactivity would be observed for other S100 proteins and, if other S100 proteins are reactive, whether they would serve equally efficiently as transglutaminase substrates. In this brief report, we show that transglutaminase can covalently modify several S100 proteins and that individual S100 proteins differ markedly in their TG reactivity. We further show that, in each case, the transglutaminase-reactive amino acid residues are located within the solvent-exposed amino- and carboxy-terminal ends of the proteins and that transglutaminases types I and II share, as a substrate, a common glutamine residue in S100A11. We also provide evidence suggesting that S100A7 may be cross-linked in in vivo.

MATERIALS AND METHODS

Preparation of Transglutaminase. Normal human keratinocytes were grown in twenty-five 75 cm^2 dishes on lethally irradiated 3T3 cells in Dulbecco's modified Eagle's medium/

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[1] Abbreviations: cADPR, cyclic ADP-ribose; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; TBS, 10 mM Tris-HCl and 150 mM NaCl, pH 7.4; HPLC, high-pressure liquid chromatography; TFA, trifluoroacetic acid; TG1, type I transglutaminase; TG2, type II transglutaminase; E-FABP, epidermal fatty acid binding protein; HRP, horseradish peroxidase.

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F-12 (3:1) containing 5% delipidized fetal calf serum (*13*, *14*), 100 μM nonessential amino acids, 100 units/mL penicillin, 100 mg/mL streptomycin, 5 μg/mL transferrin, 2 nM T3, and 0.1 nM cholera toxin. At 6 days post-confluence, the cells were washed with phosphate-buffered saline and scraped into homogenization buffer (10 mM HEPES, pH 7.4, 2 mM EDTA, and 2 mM DTT at 1 mL/dish). The cells were lysed by Dounce homogenization (B-pestle) and centrifuged at 100000*g* for 30 min. The pellet was resuspended in 25 mL of homogenization buffer containing 0.2% Triton X-100, homogenized (Dounce, B-pestle), and maintained on ice for 30 min. The extract was centrifuged at 100000*g* for 30 min, and the supernatant containing transglutaminase type I (TG1 activity) was stored as 0.5 mL aliquots at −80 °C. The activity of the preparation was 0.032 unit/mL when measured by monitoring [^{14}C]putrescine incorporation into dimethylcasein (1 unit = 1 μmol of putrescine incorporated/h). Transglutaminase type II (TG2, porcine liver, 1.6 units/mg, Sigma) was purchased from Sigma Chemicals, reconstituted in sterile distilled H_2O, and stored at −80 °C. TG1 and TG2 activities were normalized on the basis of the ability to [^{14}C]-putrescine-label dimethylcasein (Sigma) (*15*).

Production and Purification of Recombinant S100 Proteins. cDNAs for S100 proteins were produced from polyadenylated RNA isolated from normal human keratinocytes. S100 protein-specific primers containing *Nde*I (upstream) and *Bam*HI (downstream) restriction sites were used to amplify S100A10, S100A11, and S100A7 by polymerase chain reaction [S100A7 (upstream primer) 5′-GGC ATA TGA GCA ACA CTC AAG CTG AG, (downstream primer) 5′-TAG GAT CCT GGG TCT CTG GAG GCC CAT TG; S100A10, (upstream primer) 5′-GGC ATA TGC CAT CTC AAA TGG AAC ACG CC, (downstream primer) 5′-TAG GAT CCT TAT CAG GGA GGA GCG AAC TGC; S100A11, (upstream primer) 5′-CAT ATG GCA AAA ATC TCC AGC CC, (downstream primer) 5′-GGA TCC TGA GGT GGT TAG TGT GCT CA] (*11*, *16*, *17*). The S100 protein-encoding cDNAs were then cloned into the pET28a+ bacterial expression vector (Novagen). S100 proteins were expressed in *Escherichia coli* bacterial strain BL21-DE3 transformed with the pET28a+-S100 expression vector using IPTG to induce expression (*18*). The recombinant S100 proteins contains a polyhistidine sequence at the amino terminus that was used for isolation to homogeneity using immobilized metal affinity chromatography. The polyhistidine tract was subsequently removed by thrombin cleavage. Following thrombin cleavage to remove the polyhistidine track, each recombinant S100 protein retains a three amino acid extension (Gly-Ser-His) at the amino terminus.

Synthesis of Biotinylated Amine Acceptor Peptide. An amine acceptor hexapeptide, TVQQGL, was synthesized. The hexapeptide was then biotinylated by incubation with 4 mg of NHS-LC-biotin (Pierce) for 2 h at room temperature in 1 mL of 0.1 M NH_4HCO_3, pH 8. The reaction was terminated by adding 50 μL of 1 M Tris-HCl, pH 7.5, and the product was lyophilized and dissolved in water at a final concentration of 100 mM (*19*, *20*).

In Vitro Cross-Linking of Recombinant S100 Proteins. Recombinant human S100 protein (25 μM) was reacted in vitro, in the presence or absence of [^{14}C]putrescine ([^{14}C]-putrescine, 20 μCi, 0.7 mM; Amersham) as previously described (*11*), or with 2 mM amine acceptor hexapeptide (*20*), in 100 μL reactions containing 50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 3.4 mM DTT, 30 mM NaCl, 0.1% Triton X-100, and 0.002−0.067 unit of type II (guinea pig liver) or type I (keratinocyte) transglutaminase. The activity of each enzyme was normalized on the basis of the ability to incorporate [^{14}C]putrescine into dimethylcasein (*15*). Reactions were initiated by the addition of 10 mM $CaCl_2$, incubated at 37 °C for 1−3 h, and terminated by addition of EDTA to 20 mM on ice. Samples were analyzed by denaturing polyacrylamide gel electrophoresis and/or trichloroacetic acid precipitation onto Millipore type HA filters for scintillation counting. The remainder was dialyzed overnight against Tris-buffered saline (TBS, 10 mM Tris-HCl, 150 mM NaCl, pH 7.4) to remove unreacted putrescine prior to enzymatic digestion.

HPLC Separation and Microsequencing of Proteolytic Peptides. Recombinant human S100 protein was reacted in vitro in the presence of [^{14}C]putrescine as described above. Cross-linked S100A10 and S100A11 were digested with V8 protease (Worthington) and trypsin (Worthington), respectively, as previously described (*11*). The resulting protein fragments were separated by reverse-phase HPLC using a Waters 600E system equipped with a Waters 484 absorbance detector. Samples were acidified to 0.2% (v/v) with trifluoroacetic acid (TFA) and loaded on a C18 reverse-phase column (Advantage-100, 5 mm, 240 × 4.6 mm; Thompson Liquid Chromatography, Springfield, VA) equilibrated with 0.1% TFA at a flow rate of 1 mL/min. After 15 min, a linear acetonitrile gradient was started and increased at a rate of 0.6%/min. Purified peptides were assayed for radioactivity by scintillation counting. Peaks containing radioactivity were dried, resuspended in 70% acetonitrile (0.1% TFA), spotted to BioBrene Plus-treated (Applied Biosystems) glass fiber filters, and sequenced using a Perkin-Elmer/Applied Biosystems Procise model 494 microsequinator. For each peptide two sequencing runs were completed. The first run determined the sequence of the purified peptide. During the second sequencing run individual cycles were collected and assayed for [^{14}C]putrescine-dependent radioactivity.

Gel Electrophoretic Methods. Samples were boiled in Laemmli sample buffer containing 2% β-mercaptoethanol and electrophoresed on 16% polyacrylamide gels. For gels containing radioactivity, proteins were stained with Coomassie blue, and gels were soaked in Fluor-Hance (RPI, Mount Prospect, IL), dried, and exposed to X-ray film. For immunoblot analysis, proteins were transferred to nitrocellulose membrane. The membrane was blocked with 5% milk, incubated with the appropriate rabbit 1° antisera (anti-S100A7, anti-S100A10, anti-S100A11, or preimmune) at a 1:2500 dilution. The membranes were washed and incubated with a horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (Amersham Corp., 1:10 000 dilution), and antibody binding was visualized using chemiluminesence detection reagents (Amersham Corp.) (*11*). Detection of biotinylated hexapeptide (TVQQGL) incorporation was monitored using horseradish peroxidase conjugated to streptavidin reagent (Vector labs) diluted 1:200.

Preparation of Human Psoriasis Tissue. Uninvolved and involved (active psoriatic plague) epidermis was harvested from psoriatic patients using a dermatome and stored at −80 °C. For analysis, samples of the tissue were homogenized, and equivalent quantities of protein were fractionated on a

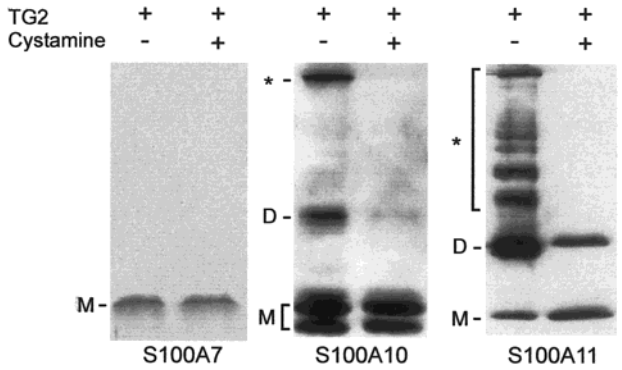

FIGURE 1: S100 proteins as transglutaminase substrates. Equal concentrations (25 μM) of S100A7 (left), S100A10 (center), and S100A11 (right) were incubated with type II transglutaminase (0.005 unit) in the presence or absence of 20 mM cystamine. The products were then electrophoresed on an 16% polyacrylamide gel in denaturing and reducing conditions, transferred to nitrocellulose, and incubated with (left to right) S100A7-, S100A10-, or S100A11-specific antibodies. Binding of the these antibodies was visualized by subsequent incubation with horseradish peroxidase-conjugated goat anti-rabbit IgG, followed by visualization with chemiluminescent reagents. M, D, and *, respectively, indicate the migration of S100 protein monomers, dimers, and multimers. This experiment is representative of three independent experiments. No cross-linking was observed when TG2 was omitted from the reaction mixture.

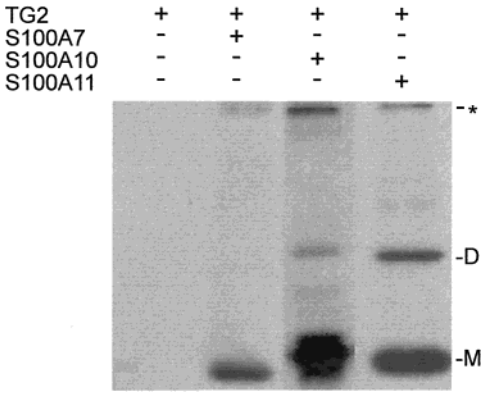

FIGURE 2: [^{14}C]Putrescine incorporation into S100 proteins. Equivalent amounts of S100A7, S100A10, and S100A11 (25 μM) were incubated for 30 min with 880 μM [^{14}C]putrescine, 0.005 unit of TG2, and 20 mM calcium chloride. The resulting cross-linked products were electrophoresed as described in Figure 1, and the gels were fluorographed and exposed on film. The S100A7-, S100A10-, and S100A11-containing lanes were exposed for 4 days, 1 day, and 2 days, respectively. M, D, and *, respectively, indicate migration of monomers, dimers, and multimers. This experiment was repeated three times with similar results. No incorporation of [^{14}C]putrescine was observed when TG2 was omitted from the reaction mixture.

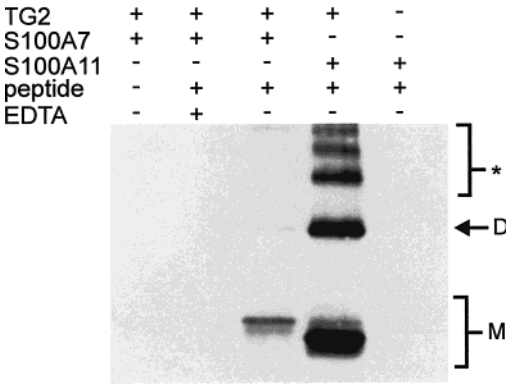

FIGURE 3: Detection of reactive lysines in S100A7. Recombinant human S100A7 (25 μM) or S100A11 (25 μM), 0.005 unit of TG2, and 20 mM $CaCl_2$ were incubated for 60 min at 37 C in the presence or absence of 2 mM biotinylated peptide or 20 mM EDTA. The reaction products were then electrophoresed on a denaturing 16% polyacrylamide gel, transferred to nitrocellulose, and incubated with streptavidin-conjugated horseradish peroxidase (HRP, 5 μg/mL). The blots were washed and incubated to develop the HRP-dependent signal. M, D, and *, respectively, indicate the migration of S100 protein monomers, dimers, and multimers. Similar results were observed in each of four experiments.

12% polyacrylamide gel. The presence of S100A7 was monitored by immunoblot using an S100A7-specific antibody that was generated in rabbits in response to recombinant human S100A7 (Robinson and Eckert, unpublished).

RESULTS

S100 Proteins as Transglutaminase Substrates. To study S100 protein transglutaminase reactivity, we incubated recombinant S100 proteins with transglutaminase and calcium and then measured multimer formation by gel electrophoresis and immunoblot. The results from reaction of S100A7, S100A10, and S100A11 with type II transglutaminase (TG2) are shown in Figure 1. Multimer formation is observed for both S100A10 and S100A11 and is inhibited by the transglutaminase inhibitor cystamine. In contrast to the results with S100A11 and S100A10, multimer formation is not detected with S100A7. These results suggest that S100A10 and S100A11 possess both reactive glutamine and lysine residues and that S100A7 is not able to form intermolecular cross-links. No reaction was observed when transglutaminase was omitted from the reaction (not shown).

Evidence for Reactive Glutamine Residues. As a more sensitive method of detecting reactive glutamines, S100A7, S100A10, and S100A11 were incubated with transglutaminase and calcium in the presence of the amine donor [^{14}C]-putrescine (*11*). As shown in Figure 2, all three proteins incorporated [^{14}C]putrescine, indicating that each protein contains TG-reactive glutamine residue(s). S100A10 and S100A11 incorporated [^{14}C]putrescine into monomers, dimers, and multimers, while S100A7 incorporated radioactivity only into monomers. To estimate the relative reactivity of each substrate, equivalent amounts of each S100 protein were incubated with a fixed concentration of TG2 and assayed for [^{14}C]putrescine incorporation. Total incorporation was measured by monitoring radioactivity in trichloroacetic acid-precipitable S100 protein. S100A11 and S100A10 incorporated 6 and 19 times more [^{14}C]putrescine, respectively, compared to S100A7.

Reactive Lysine Residues in S100A7. Since S100A7 encodes a reactive glutamine residue (Figure 2) but does not form multimers (Figure 1), it is possible that S100A7 lacks a reactive lysine residue. To test this possibility, S100A7 protein was incubated with transglutaminase and the biotinylated hexapeptide, TVQQEL, which functions as an amine acceptor (*20*). The reaction products were electrophoresed and transferred to nitrocellulose, and incorporation of the hexapeptide was monitored by streptavidin blot analysis (*19*, *20*). As shown in Figure 3, the amine acceptor peptide was covalently linked to the S100A7 monomer. However, no labeled multimers were detected. The presence of labeled

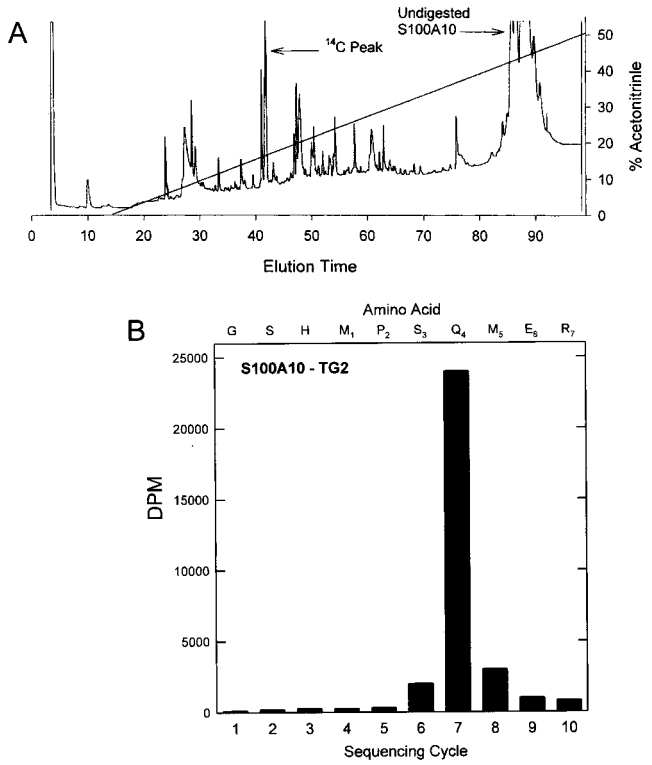

FIGURE 4: Identification of the TG2-reactive glutamine residues in S100A10. S100A10 was cross-linked for 60 min in the presence 20 mM calcium chloride, 880 μM [^{14}C]putrescine, and 0.005 unit of type II transglutaminase. The resulting cross-linked products were V8 protease-digested, and the protease-cleavage products were chromatographed using a reverse-phase HPLC column (A). V8 protease-digested peptides were eluted using an acetonitrile gradient (diagonal line), and peptide elution was detected using a Waters 484 absorbance detector. The radioactivity, which eluted as a single peak (^{14}C peak), was collected for microsequencing. As shown in panel B, the radioactivity was released in cycle 7 corresponding to amino acid Q_4.

monomers suggests that S100A7 contains a reactive lysine residue. In contrast to the low amount of hexapeptide linked to S100A7 monomer, high-level incorporation of hexapeptide was observed into S100A11 monomers, dimers, and multimers (Figure 3).

Identification of TG2-Reactive Glutamine Residues in S100A10 and S100A7. S100A11 is known to contain only two transglutaminase-reactive residues: K_3 at the amino terminus and Q_{102} at the carboxyl terminus (*11*). As S100 proteins share a common structure, it could be expected that TG reactive residues in other S100 proteins would be located within corresponding domains. To evaluate this possibility, we incubated S100A10 with TG2 in the presence of [^{14}C]-putrescine. Following cross-linking the products were digested with V8 protease, and the resulting peptide fragments were separated by HPLC. The single radioactive peak, eluting at 42.5 min, was collected for sequencing (Figure 4A). Microsequencing of this peptide identified labeling at the S100A10 amino terminus. The [^{14}C]putrescine label eluted in cycle 7, corresponding to cleavage of residue Q_4 of the S100A10 protein (Figure 4B).

To identify the reactive glutamine in S100A7, the protein was incubated with [^{14}C]putrescine, TG2, and calcium for 1 h at 37 °C. A single radioactive product was observed upon gel electrophoresis that corresponded to the S100A7 monomer. This product was collected and sequenced. The radioactivity was released in sequencing cycle 8, corresponding to the release of S100A7 amino acid Q_5.

Identification of S100 Proteins as Type I Transglutaminase Substrates. The above studies demonstrate that S100 proteins are substrates for type II (TG2) transglutaminase. Transglutaminase type I (TG1) is a second type of transglutaminase. TG1 is involved in assembly of cross-linked structures during terminal keratinocyte differentiation (*7*, *21*, *22*). To determine whether S100 proteins are TG1 substrates, we incubated S100A11 with TG1 and measured multimer formation and [^{14}C]putrescine incorporation. As shown in Figure 5, TG1 promotes S100A11 dimer and multimer formation (left panel). Moreover, the reaction is inhibited by the transglutaminase inhibitor, cystamine. The opposite panel shows the TG1-dependent incorporation of the amine donor, [^{14}C]putrescine, and that the incorporation is inhibited by EDTA-dependent chelation of the required transglutaminase cofactor, calcium. To identify the reactive glutamine residue, the TG1-cross-linked S100A11 product was digested with trypsin, and the tryptic fragments were separated by HPLC (not shown). A single radioactive peak was identified. Microsequencing the labeled peptide identified the radioactivity as being associated with residue Q_{102}.

We also examined the TG1 reactivity of S100A10. As shown in Figure 6, incubation of S100A10 with TG1 resulted in a low quantity of dimer and multimer formation, which was inhibited by addition of cystamine. However, the low level of incorporation in this case did not permit sequence determination. S100A7 did not appear to function as a substrate for TG1 in this in vitro system.

S100A7: In Vivo Reactivity. The above studies indicate that S100A7 does not form homomultimers in vitro and has low TG reactivity. However, it is possible that S100A7 may become cross-linked to non-S100 proteins in vivo. S100A7 is markedly overexpressed in psoriatic epidermis (*23*, *24*). We therefore harvested involved and uninvolved epidermis from several psoriasis patients and assayed for S100A7 by immunoblot. As shown in Figure 7, S100A7 levels are markedly elevated in actively scaling psoriatic epidermis (involved) as compared to nonscaling epidermis (uninvolved). In addition to the presence of S100A7 monomer, high molecular weight anti-S100A7 immunoreactivity bands are observed in both involved and uninvolved samples.

DISCUSSION

Covalent Modification of S100 Proteins. S100 proteins exist in cells as antiparallel, noncovalently associated homo- and heterodimers (*25*, *26*). Dimer formation relies on the interaction of hydrophobic globular domains derived from helices I and IV from each monomer (*27*, *28*). This pairing forms a highly stable structure that coordinates zinc and calcium ions (*27*, *28*). The assembly of these domains leads to the formation of the target protein binding cleft. In response to increased intracellular calcium, there is a conformational change in the dimeric unit to expose hydrophobic surfaces. Exposure of these surfaces permits interaction of the binding cleft with target protein(s) (*29*). Ultimately, this interaction results in a change in distribution and/or activity of the target protein. Because of the potential importance of covalent modification on the structure of the binding cleft and, as a result, on the ability of S100 proteins

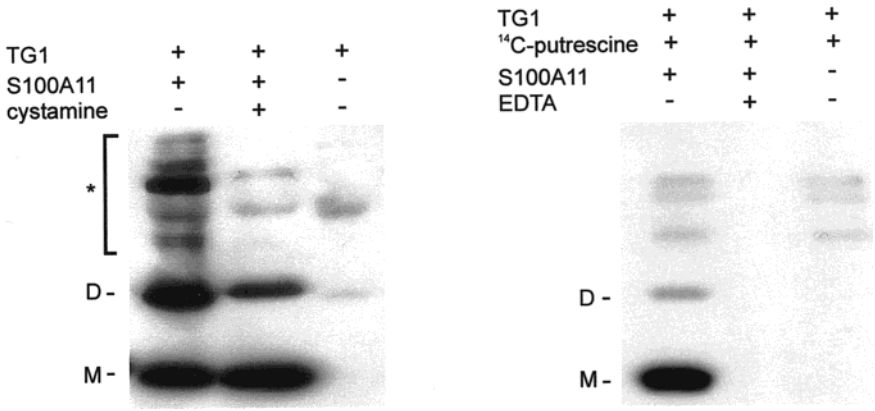

FIGURE 5: S100A11 is a substrate for type I transglutaminase. Recombinant human S100A11 (25 μM) was incubated with type I transglutaminase and calcium in the presence or absence of 20 mM cystamine, 20 mM EDTA, or 880 μM [^{14}C]putrescine for 60 min at 37 °C. The resulting cross-linked products were electrophoresed on a denaturing 16% polyacrylamide gel. Presence of the S100A11 protein was detected by immunoblot using a rabbit anti-human S100A11 antibody (left) or by detecting [^{14}C]putrescine incorporation by fluorography (right). M, D, and *, respectively, indicate the migration of S100 protein monomers, dimers, and multimers. This experiment is representative of four independent experiments.

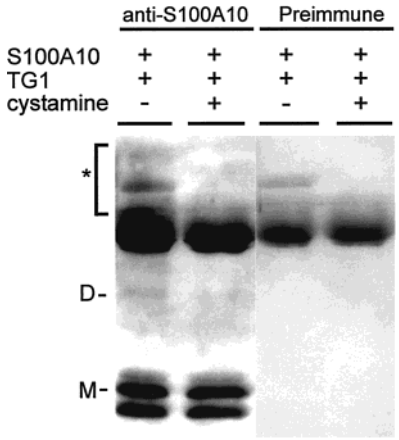

FIGURE 6: TG1-dependent cross-linking of S100A10. Recombinant human S100A10 (25 μM) was incubated with 20 mM calcium chloride and 0.005 unit of TG1 for 60 min at 37 °C in the presence or absence of 20 mM cystamine. The reaction products were then electrophoresed in parallel sets of lanes. One set was incubated with rabbit anti-human S100A10 and the other set with preimmune serum. Binding of the primary antibodies was detected by subsequent incubation with horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody. M, D, and *, respectively, indicate migration of S100A10 monomers, dimers, and multimers. Similar results were observed in each of four separate experiments.

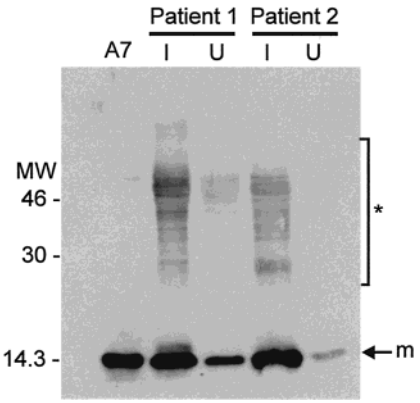

FIGURE 7: Detection of S100A7 in psoriatic epidermis. Epidermis was harvested from psoriasis patients (1 and 2) from both involved (I) and uninvolved (U) regions. Protein extracts were prepared, and the equivalent amount of protein was electrophoresed, transferred to nitrocellulose, and blotted with a rabbit anti-human S100A7 antibody. The letter m indicates migration of S100A7 monomers, while the asterisk indicates migration of higher molecular weight immunoreactive S100A7 bands that may be cross-linked. Recombinant human S100A7 was electrophoresed in the lane labeled A7. This experiment was repeated four times with similar results.

to regulate target protein function, it is important to identify posttranslational modifications that may modify S100 protein structure. S100A8 and S100A9, for example, are phosphorylated, and this modification is thought to enhance S100-associated calcium binding and association with the plasma membrane (*30*, *31*).

Transglutaminase enzymes catalyze the formation of covalent interprotein ϵ-(γ-glutamyl)lysine bonds (*32*−*35*). In a previous report, we identified S100A11 as a transglutaminase substrate (*11*, *12*). However, it was not clear that transglutaminase reactivity would be a property shared by other S100 proteins. In the present study we compare the transglutaminase reactivity of S100A10, S100A11, and S100A7. As summarized in Table 1, although all S100 proteins are substrates, each displays substantial differences in reactivity. In the case of S100A10 and S100A11, transglutaminase promotes the formation of covalently linked homomultimers. The major product formed in this reaction is a dimer with lesser quantities of higher multimers. Since each S100 protein appears to contain a single reactive glutamine and a single reactive lysine residue (*11*), we suspect that these homomultimers consist of head-to-tail linked monomers. In contrast, S100A7 does not form homomultimers in vitro, suggesting that, with respect to

Table 1: Summary—Transglutaminase Modification of S100 Proteins

S100 protein	TG substrate	reactive residues	homo-multimer formation	in vivo reactivity
S100A7	yes (TG1[a] and -2)	Q_5, $K_?$[b]	no	yes (epidermis)[a]
S100A10	yes (TG1 and -2)	Q_4, $K_?$	yes	yes (epidermis) (*11*, *12*)
S100A11	yes (TG1 and -2)	Q_{102}, K_3	yes	yes (epidermis) (*11*, *12*)

[a] S100A7 appears in psoriatic epidermal extracts as monomers and higher molecular weight bands. We hypothesize that these are cross-linked products; however, this must be directly confirmed. [b] $K_?$ indicates that a reactive lysine is present but that the specific residue is not known.

transglutaminase reactivity, S100A7 is fundamentally different from S100A10 and S100A11. Additional studies, using small molecular probes, indicate that S100A7 does in fact contain transglutaminase-reactive glutamine and lysine residues. The low reactivity of S100A7 may be explained by the sequence of the S100A7 protein. The single lysine in the carboxy-terminal domain, K_{88}, is located relatively close to the central globular EF-hand domain, which may reduce its reactivity. It is interesting that psoriatic epidermis contains putative S100A7 multimers, consistent with the hypothesis that S100A7 functions as a type I transglutaminase substrate in tissue. If these forms are cross-linked, it would suggest that S100A7 does not cross-link to itself but does become cross-linked to non-S100 proteins. A candidate for a cross-linking partner is epidermal fatty acid binding protein (E-FABP). E-FABP has been reported to form a noncovalent complex with S100A7 (*36*, *37*). Additional studies will be necessary to determine whether S100A7 and E-FABP become covalently associated via a transglutaminase-dependent mechanism.

Location of Transglutaminase-Reactive Amino Acids. As noted above, S100 proteins share a common structure (*29*). Thus, it was of interest to identify the TG-reactive amino acids. These include Q_5 in S100A7, Q_4 in S100A10, and Q_{102} and K_3 in S100A11. It is noteworthy that all of these residues are localized in the solvent-exposed amino- and carboxy-terminal S100 protein flanking domains. This is consistent with the idea that transglutaminases prefer to modify highly accessible solvent-exposed glutamine and lysine residues (*7*, *38*−*41*). It has important functional implications that these residues are located within regions that are required for S100 protein interaction with target proteins (*29*). Since the carboxyl terminus of each S100 protein is located fairly close to the homodimeric fold (target protein binding site) and since a conformational change is required for target protein binding, we postulate that transglutaminase-dependent covalent modification at the carboxyl terminus will cause inappropriate S100 protein function. Thus, we postulate that transglutaminase-dependent modification may be a general mechanism designed to inactivate S100 protein function.

Transglutaminase exists in several forms (*7*, *21*, *22*, *42*). Each form is expressed in specific tissues and appears to have a distinct function. TG1, for example, is expressed in keratinocytes and plays a major role in assembling the keratinocyte cornified envelop (*43*, *44*). TG3 also has a role in covalent assembly of structures during keratinocyte differentiation (*42*). TG2 (tissue-type transglutaminase) is a ubiquitously expressed, soluble enzyme that functions to regulate receptor function (*8*, *10*), apoptosis (*45*, *46*), cross-linking of extracellular matrix (*47*), and other cellular processes (*8*, *10*). The above results show that S100 proteins are both TG1 and TG2 substrates. In addition, our results suggest that both TG1 and TG2 modify the same sites on S100A11 (i.e., Q_{102}) and the rank order of reactivity of the three S100 proteins is similar regardless of which TG is involved. On the basis of these results, we hypothesize that TG-dependent modification of S100 proteins may be a general mechanism that terminates S100 action. Studies are currently underway to test this hypothesis.

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