Homo-Oligomerization of Human Corneodesmosin Is Mediated by Its N-Terminal Glycine Loop Domain

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Corneodesmosin (CDSN), a glycoprotein expressed during the late stages of epidermal differentiation, localizes in the extracellular core of upper desmosomes and of corneodesmosomes. Since it displays homophilic adhesive properties, CDSN is thought to reinforce cell–cell cohesion within the upper layers of the epidermis. CDSN presents two serine- and glycine-rich domains in its N- and C-terminus that may fold into highly flexible and adhesive secondary structures called glycine loops. We analyzed the importance of these domains in CDSN homophilic adhesion by producing full-length and truncated recombinant forms of the protein deleted of the N- and/or the C-terminal domain. The adhesive properties of the various proteins were then tested in vitro by overlay binding assays and surface plasmon resonance quantitative analysis. Experiments evidenced the homophilic adhesive properties of the N-terminal glycine loop domain, confirming its involvement in CDSN–CDSN interactions. They further indicated that most of the C-terminal domain is not necessary for the adhesive properties of the protein. The dissociation constant ($K_D$) was calculated to be $1.3 \times 10^{-5}$ M. This interaction strength might allow dynamic regulation of the CDSN–CDSN association to occur in vivo. Moreover, molecular filtration analyses demonstrated for the first time that non-glycosylated CDSN is able to spontaneously form large homo-oligomers in vitro and that the N-terminal glycine loop domain is necessary for the formation of these macromolecular complexes.

Key words: cell adhesion/desmosomes/epidermis/skin/surface plasmon resonance


The human epidermis is a self-renewing stratified epithelium essential for organism defense. The epidermal protective barrier results from metabolic and structural modifications of keratinocytes occurring during their differentiation, from the basal to the upper layers. During this complex program ending in cell death, keratinocytes are transformed into cornocytes: the cytoplasm becomes a cytokeratin-rich fibrous matrix and the plasma membrane is substituted for a strong and water-insoluble cornified envelope (Haftek et al., 1991; Holbrook, 1994; Roop, 1995; Steinert, 2000). At the epidermal surface, the cornocytes constitute the stratum corneum, the highly resistant layer providing vital protection.

In the epidermis, like in other epithelial tissues that experience mechanical stress, intercellular cohesion is mediated by desmosomes. These junctional structures are composed of an extracellular core, and two intracellular plaques that allow anchoring of the cytokeratin filaments to the membrane, forming a resilient supracellular network required for tissue integrity (Simon and Serre, 1995; Burdett, 1998). Cell–cell adhesion is mediated through the extracellular domain, by the transmembrane desmosomal cadherins (Kowalczyk et al., 1999; Green and Gaudry, 2000; Ishii and Green, 2001; Garrod et al., 2002). In the late stages of the epidermal differentiation program, structural rearrangements transform desmosomes into corneodesmosomes. The plaques are included in the cornified envelope, and the density of the core increases, as observed by electron microscopy (Skerrow et al., 1989; Serre et al., 1991; Lundström et al., 1994).

Corneodesmosin (CDSN) is a 52–56 kDa glycoprotein mainly expressed in cornified squamous epithelia. Synthesized by keratinocytes in the upper spinous and lower granular layers, CDSN is secreted and incorporated into desmosomes just before their transformation into corneodesmosomes (Haftek et al., 1991; Serre et al., 1991; Simon et al., 1997; Guerrin et al., 1998). Its presence in the extracellular core of the corneodesmosomes suggests that the protein plays a major role in reinforcing cell adhesion in the stratum corneum. Supporting this hypothesis was the demonstration that CDSN displays homophilic adhesive properties (Jonca et al., 2002). The characteristic of CDSN composition is that serine and glycine constitute almost half of its amino acids. In particular, one domain of the protein, from amino acid 60 to 171, shows tandem repetition of serine- and/or glycine-rich sequences separated by aromatic or aliphatic residues (Guerrin et al., 1998). According to a structural model proposed by Peter Steinert and his collaborators, these are likely to associate and force the intervening serine and glycine residues to fold up into highly flexible structures called “glycine loops”. Mediating inter-

Abbreviations: CDSN, corneodesmosin; GST, glutathione S-transferase; MoAb, monoclonal antibody; PAGE, polyacrylamide gel electrophoresis

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actions with each other, the loops are thought to confer adhesive properties to the proteins (Steinert et al., 1991). In the epidermis, besides CDSN, glycine loop structures have been identified in cytokeratins and in loricrin, one of the major constitutive proteins of the cornified envelope. The loops could mediate molecular interactions essential for skin homeostasis since their mutations in cytokeratins and loricrin lead, in man, to various cutaneous diseases, e.g., ichthyosis hystrix or palmoplantar keratoderma (Maestrini et al., 1996; Terrinoni et al., 2000; Sprecher et al., 2001; Whittock et al., 2002).

Using protein–protein interaction assays on membranes, we previously demonstrated that not only the entire CDSN molecules but also the N-terminal glycine loop domains alone actually display homophilic interactions (Jonca et al., 2002). However, when this domain was deleted from the entire protein, interactions were only reduced but not completely abolished. This suggested that another region of CDSN also imparts adhesive properties.

In the present study, the adhesive characteristics of full-length and truncated recombinant CDSN forms deleted of the N-terminal glycine loop domain and/or the C-terminus were tested in vitro by overlay binding assays, surface plasmon resonance, and molecular filtration chromatography.

**Results and Discussion**

The N-terminal glycine loop domain is essential for, whereas a putative C-terminal glycine loop domain weakly contributes to, the adhesive properties of CDSN. In the CDSN amino-acid sequence, outside the N-terminal glycine loop domain, another part of the molecule, from amino acid 375 to 476, is highly serine- and glycine-rich, these two amino acids representing more than 50% of the residues (Fig 1a, b), with interspaced hydrophobic residues. Therefore, this domain may also fold into glycine loops and contribute to the homophilic interactions of CDSN, even if it does not perfectly fulfill one of the criteria proposed earlier (Steinert et al., 1991), i.e., there are not two consecutive hypothetical glycine loops formed on association of aromatic residues.

To characterize the relative contribution of the two hypothetical glycine loop domains in CDSN adhesive properties using macromolecular interaction assays, various recombinant CDSN forms fused to GST were produced in *E. coli* (Fig 1c). The GST–CDSN33 protein corresponded to the full-length CDSN lacking the only signal peptide. The GST–CDSNΔ61–171, GST–CDSNΔCOOH and GST–CDSNΔ61–171/COOH) proteins lacked the N-terminal glycine loop domain, most of the C-terminal glycine loop domain (except the 14 most N-terminal residues) or both, respectively. The GST–CDSN60–171 protein contained only the N-terminal glycine loop domain. The recombinant proteins were purified by affinity chromatography using glutathione sepharose 4B and immunodetected by an anti-GST antibody (Fig 1d). Several peptides were co-purified with the expected entire CDSN–GST fusion proteins. These peptides were immunodetected by anti-GST or anti-CDSN antibodies (data not shown) and therefore correspond to recombinant protein fragments. Despite the careful use of
protease inhibitors, they were always observed whatever conditions we tested for the protein production. The fragments probably arose from the codon bias problem. It is known that in *E. coli*, efficient production of heterologous proteins is frequently limited by the availability of some tRNA species that are less abundant in bacteria than in other organisms. Indeed, the level of the different tRNAs is correlated with the preferential usage of their corresponding codons (Kane, 1995; Nakamura et al., 2000). Frequently, some tRNA insufficiency leads to an interrupted translation of recombinant proteins that results in the production of truncated forms. The derivative BL21-CodonPlus-RIL (DE3 + ) *E. coli* strain, containing extra copies of argU, ileY and leuW tRNA genes and lacking the Lon and the Ompt protease systems, was used but did not solve the problem. Unfortunately, no *E. coli* strains exist carrying extra copies of rarely used gly and ser tRNA genes.

The adhesive properties of the various recombinant CDSN forms were analyzed by overlay binding assays on membranes and by surface plasmon resonance. In the overlay binding assays, the GST–CDSN60–171 protein, which contains only the N-terminal glycine loop domain of CDSN, and the GST used as a control target were resolved on SDS-PAGE and transferred onto nitrocellulose membranes. The membranes were then incubated with the other CDSN recombinant forms, used as probes. Binding of the probes onto the adsorbed targets was specifically revealed with the MoAb G36–19, which recognizes all the forms except the targets. The GST–CDSN COOH protein was shown to interact strongly with GST–CDSN60–171, as strongly as GST–CDSN 63, whereas weak or no signals were observed when GST–CDSN Δ61–171 or GST–CDSN (61–171/COOH) were used as probes (Fig 2). Furthermore, GST–CDSN COOH but not GST–CDSN Δ61–171/COOH was shown to interact with the GST–CDSN 93 fusion protein, a CDSN form lacking the signal peptide and the following 30 amino acids, used as target (data not shown). None of the incubated CDSN forms bound to either the GST or bovine serum albumin, showing the specificity of the interactions. These results confirm that the N-terminal glycine loop domain is sufficient to promote homophilic interactions, and further indicate that the C-terminus of CDSN, including most of its C-terminal glycine loop domain, is not necessary for the adhesive properties of the protein.

CDSN homophilic interactions were confirmed and kinetic parameters of the interactions were calculated using surface plasmon resonance on a Biacore 3000 instrument. Further purification of the recombinant forms, however, was necessary in order to eliminate the co-purified fragments. Immunoprecipitation with a serum directed to amino acids 472–486 in the C-terminal region of CDSN failed to eliminate most of the fragments because they still retained adhesive properties and co-immunoprecipitated with the full-length recombinant CDSN (data not shown). Thus, we used SDS-PAGE as a preparative purification tool. Purity of the proteins was checked by electrophoresis and immunoblotting (Fig 3a), and adhesive properties by overlay binding assays, as described above (data not shown). Surface plasmon resonance confirmed the homophilic association of GST–CDSN Δ33 and showed a typical concentration-dependent response curve between 9 and 144 nM (Fig 3b).

The complexes formed spontaneously dissociated from the surface at the end of the injection, the binding curves returning to baseline. Calculation of rate constants yielded a *K* of 11.3 μM (k on and k off of 1.11 × 10 3 ± 2.81 per M per s and 1.26 × 10−2 ± 4.05 × 10−6 per s (means ± SEM), respectively). This interaction strength is similar to either the interactions reported between the extracellular domains of two desmosomal cadherins, desmoglein 2 and desmocollin 2 (K D of 23.4 μM, as determined using surface plasmon resonance), or to the homo-dimerization of desmocollin 2 (K D of 4.2 μM, as determined using sedimentation equilibrium; Syed et al., 2002). Moreover, the affinity of the CDSN–CDSN interaction is higher than that observed for classical cadherins (around 100–200 μM; Baumgartner et al., 2000). The interaction, however, is rather weaker as compared to the binding of erbin to plakophilin-4 (K D = 88 nM; Jaulin-Bastard et al., 2002) or the binding of tight junction protein ZO-3 to claudin-1 (K D = 18 nM; Itoh et al., 1999). The relative weakness of the CDSN interactions can be attributed to a relatively high k off, as compared for instance to the dissociation of interleukin-1 from its receptor (around 10−4 per s; Van der Merwe and Barclay, 1994) or α-catenin from the vinculin head (8 × 10−4 per s; Weiss et al., 1998).

Interactions between the immobilized GST–CDSN Δ33 and the various truncated CDSN forms, used as analytes, were then tested over the same concentration range. Clear interactions were observed between GST–CDSN Δ33 and GST–CDSN COOH (Fig 3c; 700 RU (resonance units) at 144 nM). The interactions were concentration-dependent (data not shown) with K D values of 7.3 μM, of the same

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**Figure 2**
The N-terminal glycine loop domain of CDSN is sufficient to promote homophilic interactions. GST–CDSN60–171 or GST alone (1 μg per lane) were separated by SDS-PAGE and transferred onto a nitrocellulose membrane. The membranes were incubated for 1 h with buffer alone (-), or with the different GST–CDSN recombinant forms indicated on the top, diluted to 50 μg per ml. Proteins interacting with the transferred targets were specifically revealed with the anti-CDSN MoAb G36–19 that did not recognize the targets. On the right of the figure, the transferred GST–CDSN60–171 and GST were visualized with an anti-GST MoAb. The position of molecular mass standards (kDa) is indicated on the left.
were interpreted taking into account this basal interaction. Revealing its dimerization (data not shown). All the results between 500 and 25 kDa. The 28 kDa GST, used as a powerful technique to assess the dimeric or multimeric nature of CDSN association. The Superose 12 column used allowed the separation of proteins with a molecular mass of CDSN forms deleted of the N-terminal domain (Fig 2; Jonca et al., 2002). A very weak interaction (28 RU) was observed between GST–CDSNΔ33 and GST–CDSNΔ61–171 (Fig 3c) and no interactions were obtained using GST–CDSNΔ(61–171/COOH) as the analyte (data not shown). These results confirm that the N-terminal glycine loop domain of CDSN is crucial for its homophilic interactions, whereas its putative C-terminal glycine loop domain seems to only contribute to the interactions weakly or not at all. In addition, the difference between the GST–CDSNΔCOOH and the GST–CDSNΔ60–171 responses (700 vs 190 RU) suggests a role for the central region (amino acids 172–388) in the adhesive properties of CDSN. It could display binding properties by itself, but only in the presence of the N-terminal loop domain since the central domain alone was not observed to promote association, either in vitro (GST–CDSNΔ(61–171/COOH) analyzed using surface plasmon resonance) or in vivo (Simon et al., 2001). More probably, it could cooperatively stabilize the N-terminal interactions, e.g., by maintaining a proper conformation of the loops.

CDSN forms large homo-oligomers, as estimated by molecular filtration Molecular filtration constituted a powerful technique to assess the dimeric or multimeric nature of CDSN association. The Superose 12 column used allowed the separation of proteins with a molecular mass between 500 and 25 kDa. The 28 kDa GST, used as a control, was eluted in a single peak as a protein of 50 kDa, revealing its dimerization (data not shown). All the results were interpreted taking into account this basal interaction.

When GST–CDSNΔ33, purified on glutathione sepharose, was analyzed by gel filtration under native conditions, the elution profile mainly consisted of two peaks (Fig 4a). The full-length form, with an apparent molecular weight of 76,000 after SDS-PAGE, was eluted in the first peak, with most fragments of 45–70,000 and part of the shorter fragments of 40,000. Eluted close to the void volume, they formed large molecular complexes with an estimated molecular mass higher than 500 kDa. The remaining shorter fragments were eluted later, constituting the second peak. These findings provide the first evidence that CDSN is engaged in homo-multimerization, the molecular mass of the complex corresponding to at least three subunits. In an attempt to disrupt the complex and evaluate the association strength, GST–CDSNΔ33 was dialyzed against 8 M urea and analysed by gel filtration on the same column previously calibrated and equilibrated in 8 M urea-containing buffer (Fig 4b). In these conditions, most of the GST–CDSNΔ33 protein fragments were shifted to the second peak, showing their dissociation. The full-length form and the largest fragments, however, remained eluted in the first peak as a large complex. Similar results were obtained when we used 6 M guanidine-HCl to dissociate GST–CDSNΔ33 complexes (data not shown). The fact that denaturing conditions led to only partial oligomer disruption attests to the relative stability of the complex.

The N-terminal glycine loop domain of CDSN is sufficient to promote oligomerization When GST–CDSNΔ60–171 was analyzed by gel filtration in native conditions, the chromatogram showed the complete absence of a peak just behind the void volume (Fig 4c). Immunodetection indicated that the full-length and the different co-purified fragments were eluted progressively as a function of their decreasing molecular mass. This order of size as that calculated above. A clear but lower interaction was also observed between GST–CDSNΔ33 and GST–CDSNΔ60–171 (Fig 3c; 190 RU at 144 nM). Consistent with the absence or low membrane-overlay fixation of CDSN forms deleted of the N-terminal domain (Fig 2; Jonca et al., 2002), a very weak interaction (28 RU) was observed between GST–CDSNΔ33 and GST–CDSNΔ61–171 (Fig 3c) and no interactions were obtained using GST–CDSNΔ(61–171/COOH) as the analyte (data not shown). These results confirm that the N-terminal glycine loop domain of CDSN is crucial for its homophilic interactions, whereas its putative C-terminal glycine loop domain seems to only contribute to the interactions weakly or not at all. In addition, the difference between the GST–CDSNΔCOOH and the GST–CDSNΔ60–171 responses (700 vs 190 RU) suggests a role for the central region (amino acids 172–388) in the adhesive properties of CDSN. It could display binding properties by itself, but only in the presence of the N-terminal loop domain since the central domain alone was not observed to promote association, either in vitro (GST–CDSNΔ(61–171/COOH) analyzed using surface plasmon resonance) or in vivo (Simon et al., 2001). More probably, it could cooperatively stabilize the N-terminal interactions, e.g., by maintaining a proper conformation of the loops.

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indicates that the recombinant CDSN form deleted of the N-terminal glycine loop domain was unable to become organized into multimers. Therefore, the N-terminal glycine loop domain is not only necessary for the adhesive properties of CDSN but also for its oligomerization.

When GST–CDSN60–171 was analyzed, the chromatogram obtained was very similar to that obtained with GST–CDSNΔ33 (Fig 4d). Whereas the full-length GST–CDSN60–171 presents an apparent molecular mass of 40 kDa after SDS-PAGE, it was eluted in the first peak as a large molecular complex with an apparent size of 500 kDa. This indicates that the N-terminal glycine loop domain alone is able to form large complexes engaging at least six monomers. Interestingly, co-purified fragments were found
EBNA-293 cell line, and shown to bind GST–CDSN i.e., affinity of binding and/or oligomerization. This will be carbohydrate moieties modify CDSN properties as a glycoprotein, we cannot exclude the fact that the la ted recombinant CDSN forms whereas epidermal CDSN is homophilic interactions nor its oligomerization, at least in vitro.

As a whole, the results described here confirm that the N-terminal glycine loop domain of CDSN displays adhesive properties, strongly suggest that CDSN is able to engage in homo-oligomers, and evidence the necessity of this domain for CDSN oligomerization. The central region of the molecule may also be important in stabilizing the interactions, but this assumption remains to be experimentally confirmed. Since these results were obtained using unglycosylated recombinant CDSN forms whereas epidermal CDSN is a glycoprotein, we cannot exclude the fact that the carbohydrate moieties modify CDSN properties in vivo, i.e., affinity of binding and/or oligomerization. This will be investigated in future work using a glycosylated recombinant form of CDSN produced by the human embryonic EBNA-293 cell line, and shown to bind GST–CDSN33 in overlay binding assays (unpublished preliminary data). Moreover, cis- and trans-interactions of either desmosomal or classical cadherins were consistently observed in vitro using unglycosylated E. coli-expressed proteins, as well as in various in vivo models (Chitaev et al, 1997; Haussinger et al, 2002; Syed et al, 2002). These data support the idea that the CDSN binding mode described here really occurs in vivo. The moderate CDSN interaction strength in the micromolar range deduced from our experiments suggests a transient oligomerization, reinforcing the model previously proposed (Steinert et al, 1991). Considering the location of CDSN in the extracellular part of corneodesmosomes, such interactions could reinforce corneocyte adhesion and stratum corneum cohesion, but respect the elastic properties of the epidermis, glycine loops being able to disrupt and to re-form reversibly. If this is true, CDSN would be a member of a new superfamily of glycine- and/or serine-rich proteins with elastic properties. This family includes proteins of calcium-carbonate-containing sea urchin skeletal elements and mollusc shell and pearls, which have been proposed to undergo reversible unfolding and refolding under force extension, thereby conferring fracture-resistant properties to mineralized structures (Shen, 1997; Wustman et al, 2002). Bombyx mori silk sericin, protein glue that ensures the cohesion of the cocoon threads, and cuticle proteins may also belong to this family (Smith et al, 1999; Altman et al, 2003).

Several genetic approaches have emphasized the great importance of CDSN in epidermis physiology. Indeed, the CDSN gene, located in PSORS1, the major susceptibility locus for psoriasis type I, is a strong candidate for this disease (Allen et al, 1999; Jenish et al, 1999; Tazi-Anhini et al, 1999; Orru et al, 2002; Capon et al, 2003). Particular psoriasis-associated polymorphisms encoding variants of the glycine loops may alter CDSN adhesive properties and be involved in the pathophysiology of the disease. Moreover, nonsense mutations in the CDSN gene cause hypotrichosis simplex of the scalp, an autosomal dominant form of alopecia. Interestingly, the described mutations lead to the expression of a truncated CDSN form, which practically corresponds to the N-terminal glycine loop domain. In patients, aggregates of this form, suspected to be toxic, have been observed in the dermis (Levy-Nissenbaum et al, 2003). In agreement, we show that GST–CDSN60–171 is able to form hexamers in vitro.

Further research into the functions of CDSN and its involvement in various pathogenic processes will be necessary for a better understanding of the physiology of stratum corneum and its disorders in dermatological diseases.

Materials and Methods

Construction of expression vectors Plasmids pGEX-CDSN33 encoding glutathione S-transferase (GST) fused to CDSN amino acids 34–529, pGEX-CDSN61–171 encoding GST fused to CDSN amino acids 34–60/172–529, and pGEX-CDSN60–171 encoding GST fused to amino acids 60–171 (corresponding to the N-terminal glycine loop domain of CDSN) were produced as previously described (Jonca et al, 2002). Plasmids pGEX-CDSN33COOH and pGEX-CDSN33(COOH) were produced by digestion with StyI of pGEX-CDSN33 and pGEX-CDSN61–171, respectively. Plasmid pGEX-CDSN33(COOH) encodes GST fused to CDSN amino acids 34–388, a frame shift due to the sub-cloning replacing CDSN amino acids 389–529 by the following amino acids: GSYSTVHKST-GIHHRD. Plasmid pGEX-CDSN61–171(COOH) encodes a protein corresponding to CDSN(COOH) further deleted of amino acids 61–171. Each plasmid insert was verified by sequencing.

Protein purification The Escherichia coli strain BL21-Codon Plus (DE3 ¼ )-RIL (Stratagene, La Jolla, California) was transformed with the various recombinant expression plasmids. Bacteria were grown in 2-YT broth medium (Gibco Invitrogen Corporation, Carlsbad, California) supplemented with glucose (5%), ampicillin (50 µg per mL), and chloramphenicol (34 µg per mL). Cultures were incubated at 37 °C until cell density reached an OD600 nm of 0.6–0.8. Then, the bacteria were induced to express GST-CDSN forms for 1 h by the addition of 2 mM isopropyl-β-D-galactoside to the culture medium. Cultures were centrifuged for 10 min at 6000 7 g and the bacterial pellet was placed overnight at −20 °C. Recombi nant proteins were extracted with B-Per extraction buffer (Pierce, Rockford, Illinois) and affinity purified from bacterial lysates using Glutathione Sepharose 4B (Amersham Bioscience, Buckinghamshire, UK). Protein expression was verified by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and immunodetection as described below.

Protein electrophoresis and immunodetection Proteins were separated by SDS-PAGE on 10% acrylamide gel. After electrophoresis, they were electrotransferred onto nitrocellulose membranes and stained with Ponceau red. Membranes were probed with antibodies as previously described (Simon et al, 1997). Monoclonal antibodies (MoAbs) G36-19 (anti-CDSN; Serre et al, 1991) and anti-GST (Pierce) were used at 0.1 µg per mL. They were detected with peroxidase-conjugated goat anti-mouse IgG (Zymed, South San Francisco, California) diluted to 1/10,000. Immunoreactivities were revealed with the ECL western blotting kit (Amersham Bioscience, Buckinghamshire, UK).
Overlay binding assays Overlay binding assays were performed as previously described (Jonca et al, 2002). GST and GST–CDSN60–171 were separated by SDS-PAGE and transferred onto nitrocellulose membranes. The membranes were incubated for 1 h at room temperature with buffer alone, or with the different GST–CDSN recombinant forms diluted to 50 μg per mL. Proteins either transferred to or bound to the membranes were specifically revealed with the MoAbs anti-GST or G36-19, respectively, both used at 0.1 μg per mL.

Purification from SDS gels Recombinant proteins were separated by SDS-PAGE. Gels were washed briefly in deionized water and stained for 1 min in Coomasie blue diluted in water. The bands showing the expected molecular sizes were cut off and gel pieces. Proteins were eluted overnight at 4 °C with mechanical agitation in 500 μL of the extraction buffer containing 0.2% SDS, 5 mM DTT, 150 mM NaCl, 50 mM Tris–HCl (pH 7.4), and 5 μL of a protease inhibitor cocktail (Sigma, St. Louis, Missouri). The mixture was centrifuged briefly to pellet the crumbled gel. The supernatant was then concentrated about 4- to 5-fold using Y-30 centrifuges (Amicon, Bedford, Massachusetts). Removal of SDS from solutions was then performed using SDS-OUT sodium dodecyl sulfate precipitation reagent (Pierce) and centrifugation for 10 min at 10,000 x g. Finally, the purified proteins were dialyzed overnight at 4 °C against PBS.

Surface plasmon resonance analysis Surface plasmon resonance measurements were carried out at 25 °C using a Biacore 3000 apparatus (Biacore AB, Uppsala, Sweden). GST–CDSNΔ33 were immobilized on separate channels of a CM5 sensor chip (approximately 14 ng per mm²) using traditional amine coupling chemistry (Jonhsson et al, 1991). After purification from SDS-PAGE, the various GST–CDSN forms used as analytes were transferred to or bound to the membranes were specifically detected by the MoAb anti-GST, as described above.

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