Protein Expression and Purification 21, 343–351 (2001)
doi:10.1006/prep.2000.1382, available online at http://www.idealibrary.com on IDEAL®

# Purification and Characterization of Human Cell–Cell Adhesion Molecule 1 (C-CAM1) Expressed in Insect Cells[1,2]

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Received November 3, 2000, and in revised form December 1, 2000

The cell–cell adhesion molecule 1 (C-CAM1) plays an important role as a tumor suppressor for prostate cancer. Decreased expression of C-CAM1 was detected in prostate, breast, and colon carcinoma. Reexpression of C-CAM1 in prostate and breast cancer cell lines was able to suppress tumorigenicity *in vivo*. These observations suggest that C-CAM1 may be used as a marker for cancer detection or diagnosis. To generate monoclonal antibodies specific to C-CAM1, we have overexpressed full-length human C-CAM1 in Sf9 cells using a baculovirus expression system. The protein was purified 104-fold using nickel affinity chromatography. About 0.4 mg purified C-CAM1 was obtained from 200 mg of infected cells. When the purified protein was digested with peptidyl-*N*-glycosidase, the apparent mobility of the protein on SDS–PAGE changed from 90 to 58 kDa, which is close to the molecular weight predicted from the cloned cDNA sequence. This observation suggests that C-CAM1 was glycosylated on asparagine residues when expressed in Sf9 cells. Western blotting and internal protein sequencing analysis confirmed that the purified protein is human C-CAM1. Biochemical and functional assays indicate that this protein expressed in Sf9 cells displays characteristics similar to those of native protein, including adhesion function and glycosylation modification. Using this protocol, sufficient quantity of this protein can be produced with purity suitable for monoclonal antibody generation and biochemical study. 

C-CAM1[4] was originally identified as an adhesion molecule that mediated hepatocyte aggregation (1). Recently, it was shown that C-CAM1 may play critical roles in prostate cancer initiation and progression because loss of C-CAM1 is an early event in prostate carcinogenesis (2). Decreased expression of C-CAM1 was also detected in endometrial (3), breast (4, 5), and colon carcinoma (6–8). These observations suggest that C-CAM1 may be used as a marker for cancer detection or diagnosis.

In addition, it was also shown that reintroduction of C-CAM1 into prostate cancer cells can reverse their cancerous growth and that reduction of C-CAM1 expression, by C-CAM1 antisense transfection, in nontumorigenic prostate epithelial cells induces their tumorigenicity in a nude mouse xenograft model (9). These results indicate that C-CAM1 is a tumor suppressor. Based on the accumulated evidence of C-CAM1's antitumor activity, C-CAM1 should be a promising agent for

[1] This work was performed during the Cold Spring Harbor Laboratory "Protein Purification and Characterization" course on April 5–18, 2000. The authors were course participants and contributed equally to the work.

[2] The work was supported by Grant 2 R25 CA09481 from the National Institutes of Health.

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[4] Abbreviations used: C-CAM1, cell–cell adhesion molecule 1; Ad-CAM1, adenoviral vector containing C-CAM1; PBS, phosphate-buffered saline; PNGase F, peptidyl *N*-glycosidase F.

1046-5928/01 $35.00

prostate cancer therapy. Preclinical studies using adenoviral vector containing C-CAM1 (Ad-CAM1) to deliver the C-CAM1 gene as a therapeutic agent for prostate cancer have shown promising antitumor effect *in vivo* in that direct injection of Ad-CAM1 into DU145 tumors in nude mice significantly suppressed their growth (10, 11). These results suggest that Ad-CAM1 has potential use in prostate cancer therapy.

C-CAM1 belongs to the CEA family group of proteins having immunoglobulin (Ig)-like structure (12). CEA was originally identified as a tumor antigen because its expression is significantly increased in colon carcinoma. CEA has been used as a marker to monitor postoperative tumor recurrence. The structural similarity between C-CAM1 and CEA has made it difficult to generate C-CAM1-specific antibodies. Available monoclonal antibodies against C-CAM1 proteins are found to have varied degrees of cross-reactivity with other CEA family members. Antibodies that can specifically detect C-CAM1 protein are needed for monitoring the expression and distribution of C-CAM1 before Ad-CAM1 can be used in clinical trial. To generate C-CAM1 antibodies with specificity, we need a good source of C-CAM1 with sufficient purity.

To date, no recombinant expression of human C-CAM1 has been reported. C-CAM1 is glycosylated in mammalian tissues and glycosylation makes up about 50% of the apparent molecular weight. Such massive glycosylation suggests that to generate antibodies that can recognize native protein, we may need the glycosylated form of the C-CAM1 molecule. We therefore chose an insect cell/baculovirus expression system to express C-CAM1 as a membrane-bound protein. In this report, we showed that C-CAM1 can be expressed in Sf9 cells and C-CAM1 thus expressed retains its cell adhesion function. In addition, we showed that the expressed C-CAM1 protein can be purified from the insect cell membrane by solubilization and one-step metal binding affinity chromatography.

## EXPERIMENTAL PROCEDURE

*Cloning of full-length C-CAM1 cDNA into the baculoviral expression vector.* The full-length human C-CAM1 cDNA with 7 histidine residues attached to the C-terminus was constructed by polymerase chain reaction (PCR) using oligonucleotides, oligo T7 (5′-TAATACGACTCACTATAGGG-3′), which contains a portion of the pSK plasmid sequence that flanks the C-CAM1 cDNA, and oligo 131 (5′-GCGGCCGCTTAATGATGATGATGATGATGCTGCTTTTTTACTTCTGAATA-3′), which is complementary to the 3′ end C-CAM1 coding sequence plus 7 histidine codons. Plasmid pSK-CD66a-L (11), which contains the full-length human C-CAM1 cDNA, was used as the template. The PCR were performed for 35 cycles; each cycle consists of 0.5 min at 94°C to denature the template DNA, 1 min at 60°C to anneal the primers, and 2 min at 72°C for DNA extension. The 1.6-kb PCR product was subcloned into plasmid pCR2.1-TOPO (Invitrogen) to produce pCR-CD66-His, and the nucleotide sequence of the double-stranded DNA was determined to confirm the sequence. For the construction of the baculoviral vector containing C-CAM1, plasmid pCR-CD66-His was digested with *Eco*RI and *Not*I. The resulting 1.6-kb fragment was then subcloned into pVL1393 at the *Eco*RI/*Not*I sites to generate plasmid pVL-CD66a-His.

*Expression of human C-CAM1 in insect cells.* Recombinant baculovirus was generated by cotransfection of the baculoviral vector (pVL-CD66a-His) with Baculogold (PharMingen, San Diego, CA). Sf9 (*Spodoptera frugiperda*) cells were grown in SF900II medium (Gibco BRL). Sf9 cells in 10-cm tissue culture plates ($1 \times 10^7$ cells/plate) were infected with C-CAM1 baculovirus for 66 h. The infected cells were harvested by centrifugation at 3000g for 5 min and the cell pellets were frozen at −80°C.

*Immunostaining.* Sf9 cells grown on coverslips were infected with C-CAM1 or control (TA1) recombinant virus. After 48 h, the cells were fixed with 4% formaldehyde in phosphate-buffered saline (PBS) for 10 min at room temperature. The cells were then washed with PBS three times. The cells on coverslips were incubated with anti-CEA antibody at 1:1000 dilution in PBS containing 1% bovine serum albumin for 1 h at room temperature. After three washes with PBS, FITC-labeled goat anti-rabbit secondary antibody was added and incubated at room temperature for 1 h. Fluorescence photomicrographs were taken using the Nikon fluorescence microscope.

*Adhesion assays.* Sf9 cells in monolayer culture were infected with C-CAM1 or control (TA1) recombinant virus. Formation of cell aggregates was examined at 48 h after infection. Adhesion was also quantified as decreases in the number of single cells. The virus-infected Sf9 cells were cultured in a test tube with constant mixing at room temperature. At various intervals, aliquots of control Sf9 cells or virus-infected Sf9 cells were removed and the numbers of single cells in these aliquots were counted using a hemocytometer. The number of single cells was expressed as percentage of the total number of cells used for infection at time 0.

*Purification of human C-CAM1 from Sf9 cells.* Recombinant C-CAM1 baculovirus-infected Sf9 cells harvested and stored at −80°C as described above were used for C-CAM1 purification. Cell pellets (200 mg) were resuspended in 5 ml of cold (4°C) buffer A [50 mM $NaH_2PO_4$, pH 8.0, 300 mM NaCl, 10 mM imidazole, Protease Complete EDTA-free inhibitor cocktail (Roche, Indianapolis, IN)]. Twenty milliliters of 10% Triton X-100 was added to resuspended cells and the cells were

solubilized on ice for 30 min. The solubilized lysate was diluted 1:1 with buffer A and centrifuged at 22,000*g* for 5 min at 4°C. The supernatant was transferred to a fresh tube and 1 ml of a 50% slurry of Ni-NTA agarose (Qiagen, Valencia, CA) was added and mixed at room temperature for 15 min. The agarose beads were pelleted by centrifugation at 120*g* for 1 min at 4°C. The supernatant was transferred to a fresh tube containing an additional 1 ml of 50% Ni-NTA slurry and mixed at room temperature for 15 min. The agarose beads were pelleted by centrifugation and combined with the previous beads. Beads were resuspended with 40 ml of buffer A and incubated on ice for 1 h. The supernatant was discarded and the beads were transferred to a Poly-Prep Chromatography Column (Bio-Rad Laboratory, Hercules, CA). Beads were washed with an additional 5 ml of buffer A. Bound proteins were eluted in a stepwise manner with 3 ml each of buffer A containing increasing amounts of imidazole (50, 100, and 250 mM) and collected as 0.5-ml fractions. Finally, materials on the column, not yet removed by imidazole elutions, were stripped by using 3 ml buffer A containing 50 mM EDTA.

*Protein determination.* Protein assays were carried out using the Coomassie blue Plus protein assay reagent kit (Pierce, Rockford, IL) according to the manufacturer's procedures. BSA standards were used for calibration.

*SDS–PAGE.* Eluted fractions were analyzed by SDS–PAGE using 4–12% gradient NuPage gels (Novex, San Diego, CA) after samples were heated at 70°C in the sample buffer (0.075 M Tris–HCl, pH 6.8, 1.5% SDS, 1.5% 2-mercaptoethanol, 15% glycerol) for 5 min. Gels were run for 150 V h and stained with Gelcode blue stain reagent (Pierce, Rockford, IL) according to the manufacturer's procedures.

*Western blot analysis.* Eluted fractions were analyzed by SDS–PAGE using 4–12% gradient gels as described above. The proteins were transferred onto nitrocellulose (Schleicher and Schuell, Hamburg, Germany) using Tris–glycine transfer buffer (Novex) containing

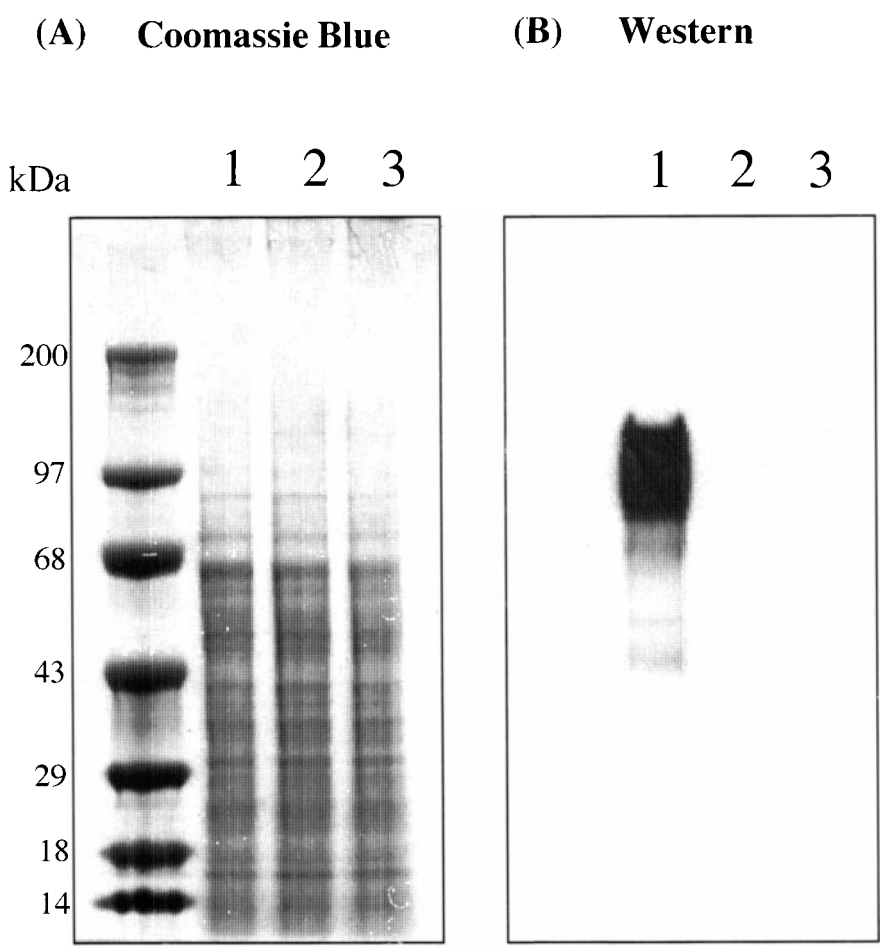


FIG. 1. Immunoblot analysis of C-CAM1 protein expressed from Sf9 cells. Cell lysates (5 $\mu$g) from C-CAM1 baculovirus (lane 1), control baculovirus (lane 2), or no virus (lane 3)-infected cells were analyzed by SDS–PAGE and Western immunoblot using anti-CEA antibody. (A) Proteins were stained with Coomassie blue; (B) anti-CEA antibody in 1:1000 dilution was used.

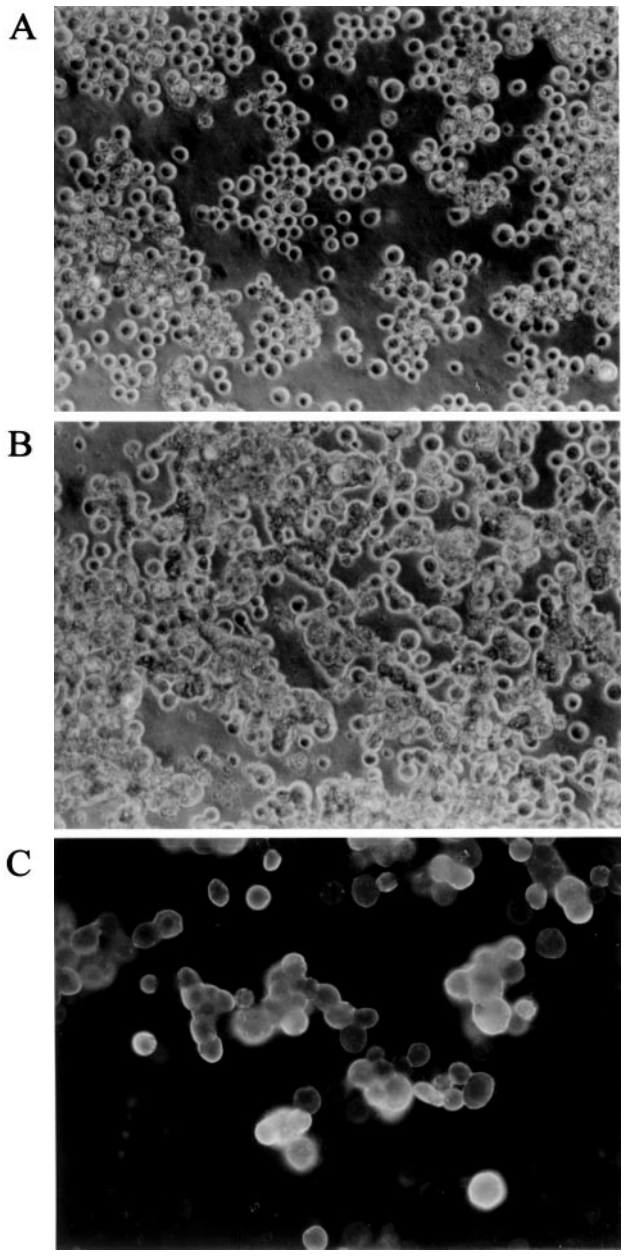


FIG. 2. Morphology of (A) Sf9 cells or (B) C-CAM1-expressing Sf9 cells at 48 h after infection (100×). (C) Immunostaining of C-CAM1 recombinant virus-infected insect cells (100×). (D) Adhesion activity in suspension culture. Sf9 cells cultured in suspension were infected with control virus (filled circle), C-CAM1 recombinant baculovirus (open triangle), or no virus (open square). At various times, aliquots of samples were removed for microscopic examination and the number of single cells was determined. The number of cells is presented as percentage of the cell numbers used for infection at time 0.

20% methanol for 90 V h. Blots were first blocked in 5% dry milk in Tris-buffered saline containing 0.1% Triton X-100 for 1 h and then washed three times for 5 min each with Tris-buffered saline containing 0.1%

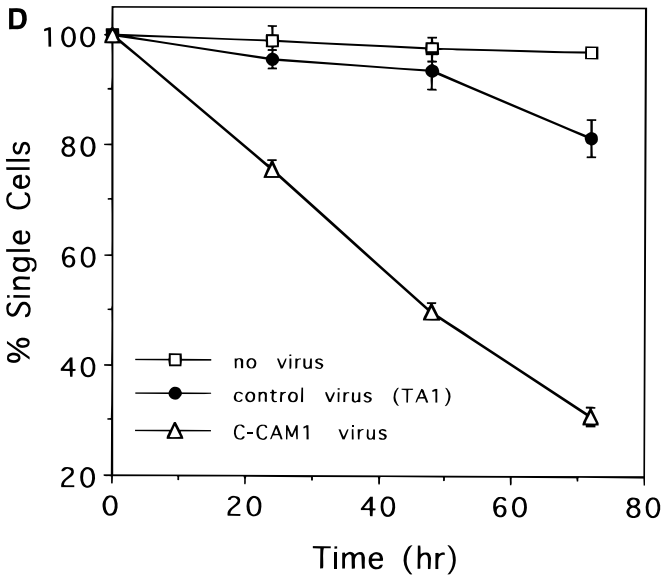


FIG. 2—*Continued*

Triton X-100. Blots were probed with anti-CEA polyclonal antibody (DAKO, Carpinteria, CA) at a 1:1000 dilution in Tris-buffered saline containing 0.1% Triton X-100. Peroxidase-labeled anti-rabbit antibody (Amersham Pharmacia Biotech, Piscataway, NJ) was used as a secondary antibody and proteins were visualized using ECL Western blotting detection reagent (Amersham Pharmacia Biotech) according to the manufacturer's procedures.

*Deglycosylation.* Three micrograms of protein obtained from the 100 mM imidazole elution was denatured with 0.5% SDS containing 1% 2-mercaptoethanol and heated at 100°C for 10 min. The denatured samples were then treated with 1.5 $\mu$l each of peptidyl *N*-glycosidase F (PNGase F) (500 U/ml) (New England Biolabs, Beverly, MA) in 50 mM sodium phosphate, pH 7.5, containing 1% NP-40. Deglycosylation was allowed to proceed at 37°C for 1 h. The samples were then subjected to SDS–PAGE and Western blot analysis as described above.

*N-terminal sequencing.* Purified C-CAM1 was blotted onto PVDF membrane and stained with Gelcode Blue reagent (Pierce, Rockford, IL) according to the manufacturer's procedures. The PVDF membrane was then allowed to dry at room temperature. The most prominent band from the 100 mM imidazole elution was excised from the membrane and analyzed with an Applied Biosystems Protein Sequencer (Foster City, CA) at the Cold Spring Harbor Laboratories.

*Internal protein sequencing.* Purified C-CAM1 (3 $\mu$g) was deglycosylated by digesting with PNGase F as described above and separated on SDS–PAGE. The Gelcode blue-stained protein spot was excised and used

for internal protein sequence analysis. An *in situ* digestion of the protein was performed with 1 $\mu$g modified trypsin (Promega, Madison, WI) in 100 mM ammonium bicarbonate at 37°C overnight. The peptides produced were subjected to mass spectrometry analysis using API 3000 (PE/SCIEX) equipped with a nanospray source (Protana). Selected peptide fragments were analyzed by the tandem MS.

## RESULTS AND DISCUSSION

### *Expression of Human C-CAM1 in Insect Cells*

The expression of human C-CAM1 in insect cells was examined in Western immunoblots using polyclonal anti-CEA antibodies, which cross-react with C-CAM1. As shown in Fig. 1, anti-CEA antibodies detected a protein expressed in Sf9 cells infected with the C-CAM1 recombinant virus but not in the control baculovirus (TA1)-infected cells, suggesting that the C-CAM1 protein had been expressed. However, the C-CAM1 protein was not abundantly expressed in Sf9 cells because there was no detectable difference in the protein profile of total cell lysates prepared from C-CAM1 and control recombinant baculovirus-infected cells (Fig. 1A). The expressed protein has an apparent molecular weight of around 90 kDa on SDS–PAGE (Fig. 1). Without the signal sequence and glycosylation, the full-length C-CAM1 is predicted to be a protein of 53 kDa. This result suggests that C-CAM1 expressed in insect cells is post-translationally modified.

Immunostaining of Sf9 cells infected with C-CAM1 recombinant virus showed strong staining of C-CAM1 protein on the plasma membrane of these cells (Fig. 2C), suggesting that the expressed C-CAM1 protein is properly targeted to the cell surface. In addition, the C-CAM1-expressing insect cells in culture dishes showed strong interaction with adjacent cells and formed multiple cell aggregates with continuous contours (Fig. 2B). This aggregation phenotype was not observed in Sf9 cells (Fig. 2A) or Sf9 cells infected with control recombinant baculovirus (data not shown). To better quantify the extent of aggregation, Sf9 cells grown in suspension were infected with C-CAM1 or control recombinant baculovirus for various lengths of time and the numbers of single cells were determined. Cell aggregation occurred with Sf9 cells infected with C-CAM1-recombinant baculovirus but not control virus (Fig. 2D). These observations indicate that the Sf9 cell-expressed C-CAM1 protein is properly targeted to the cell membrane and promotes cell–cell adhesion, both properties of the native protein.

### *Purification of Human C-CAM1*

We have developed a one-step purification for human C-CAM1 expressed in Sf9 cells. To find the best conditions to solubilize C-CAM1, eleven different detergents

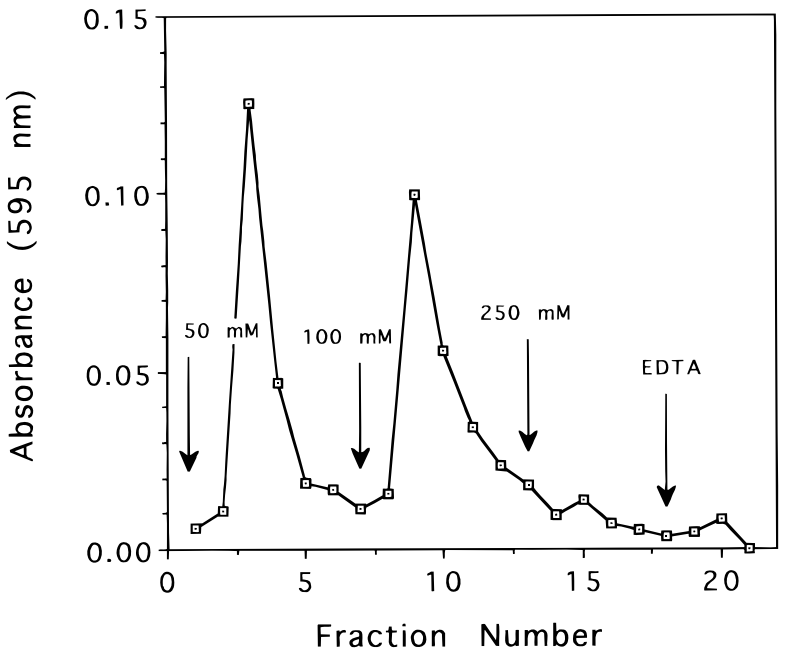


FIG. 3. Purification of C-CAM1 on Ni-NTA column. Elution of proteins from Ni-NTA agarose was performed using imidazole in buffer A. Half-milliliter fractions were collected and 5 $\mu$l from each fraction was used for protein determination by Coomassie blue Plus protein assay. Fractions 1–6, 7–12, and 13–17 were eluted with 50, 100, and 250 mM imidazole, respectively. Fractions 18–21 were eluted with 50 mM EDTA.

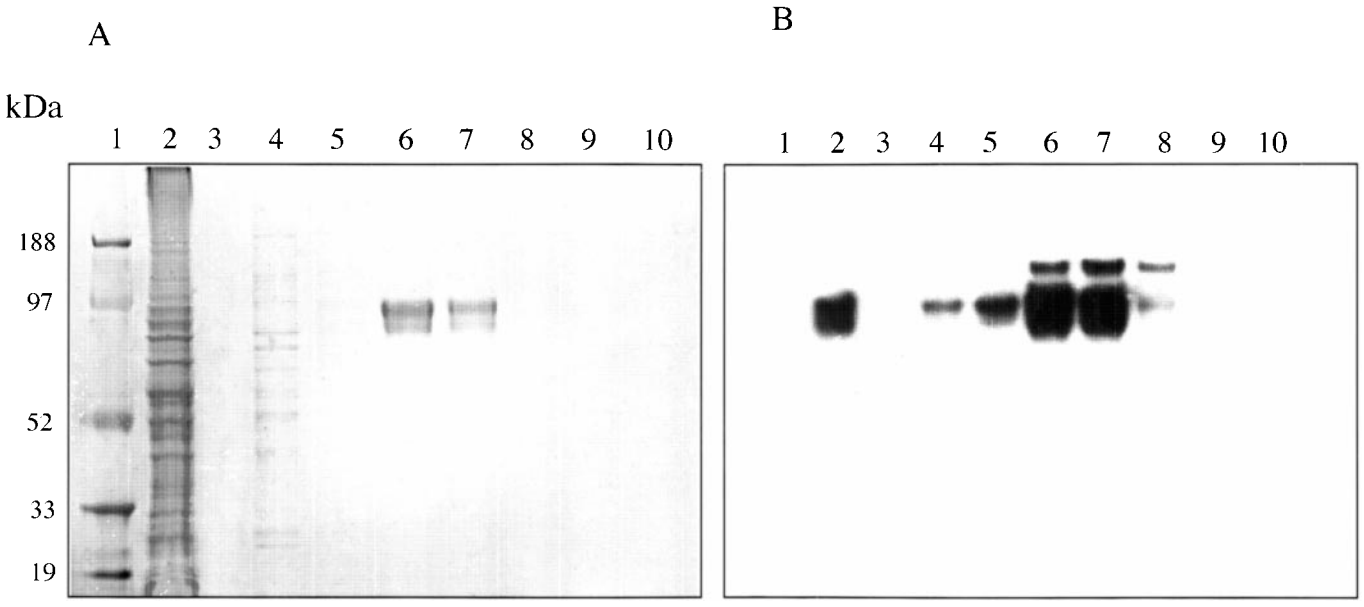


FIG. 4. Analysis of fractions eluted from Ni-NTA agarose. (A) SDS–PAGE analysis. Sample (10 $\mu$l) were analyzed on 4–12% gradient SDS–PAGE gels and stained with Gelcode blue. Detergent solubilized Sf9 lysates (lane 2), fractions 3 and 4 from the 50 mM imidazole elution (lanes 4 and 5), fractions 9 and 10 from the 100 mM imidazole elution (lanes 6 and 7), fractions 15 and 16 from the 250 mM imidazole elution (lanes 8 and 9), and fraction 20 from the 50 mM EDTA (lane 10). Molecular weight markers (lane 1) are indicated on the left. (B) Western blot analysis. Samples (1 $\mu$l) were used for SDS–PAGE analysis and C-CAM1 protein was detected with anti-CEA antibodies.

each at three different detergent-to-protein ratios (5:1, 10:1, and 20:1) were tested for their solubilization efficiencies (data not shown). Most detergents tested had similar extraction efficiencies; Triton X-100 at a 10:1 detergent-to-protein ratio was chosen for preparative purpose. Once the proteins were solubilized and the lysates were collected, the histidine-tagged C-CAM1 was purified on a Ni-NTA agarose affinity column in a batch mode. Two factors were found to be crucial to the purification of C-CAM1 from the lysates. First, inclusion of 10 mM imidazole in the binding buffer resulted in decreased nonspecific binding to the Ni-NTA beads. Secondly, once the beads were pelleted from the lysates, incubation of these beads in the washing solution containing 10 mM imidazole for 1 h was found to further reduce contaminant proteins in the eluted fractions.

After washing, beads were loaded onto a column and elution of proteins from the Ni-NTA column was performed by a stepwise imidazole gradient. A solution with fixed concentration of imidazole was used to elute the column until proteins were no longer detected by Coomassie blue Plus protein assay. Once proteins were no longer detected, the imidazole concentration in the elution solution was increased. In this study, imidazole concentrations of 50, 100, and 250 mM were used to elute C-CAM1 from the Ni-NTA agarose column. Finally, a solution containing 50 mM EDTA was added to strip the column of any remaining proteins. The elution profile is shown in Fig. 3. Selected fractions plus the initial Sf9 lysates were analyzed by SDS–PAGE and the proteins were detected by Coomassie blue staining using Gelcode (Fig. 4A). A prominent band with an apparent molecular mass of approximately 90 kDa was eluted with the 100 mM imidazole buffer. This fraction is essentially free of contaminant proteins.

Fractions eluted from Ni-NTA agarose were also analyzed by Western blot using an anti-CEA polyclonal antibody, which cross-reacts with human C-CAM1.

TABLE 1

Purification of Human C-CAM1 Expressed in Sf9 Cells

| Purification step | Total protein (mg) | Total activity (arbitrary units) | Specific activity (unit/mg) | Fold purification | Yield (%) |
|---|---|---|---|---|---|
| Cell lysate | 200 | 25,000 | 125 | 1 | 100 |
| Ni-NTA agarose | 0.43 | 5,600 | 13,000 | 104 | 22.4 |

While all fractions show reactivity with the anti-CEA antibody, only the 100 mM imidazole fraction showed a prominent signal (Fig. 4B). In contrast, minimal amounts of reactive protein were detected in the 50 and 250 mM imidazole fractions. This result suggests that the 90-kDa protein eluted from Ni-NTA agarose by 100 mM imidazole is the C-CAM1 protein. Interestingly, a protein band with apparent molecular weight of 180 kDa on SDS–PAGE was also detected in 100 mM imidazole elutions. This 180-kDa protein may be the dimerized form of C-CAM1. The dimerization most likely arises from Ni-ion-mediated chelation, a phenomenon commonly observed with His-tagged proteins that have been purified from Ni-affinity column.

The extents of C-CAM1 protein purification and recovery were estimated by quantitative Western blot analysis. Various amounts of solubilized cell lysate and purified C-CAM1 were loaded on gel and analyzed with anti-CEA antibody. The amount of cell lysate that gave similar intensity with a specific amount of purified C-CAM1 was used to derive the relative activity. Table 1 summarizes the result of the purification based on this calculation. Thus, C-CAM1 protein was enriched about 100-fold by the one-step Ni-NTA agarose purification with a total recovery of about 22%.

*Glycosylation of Human C-CAM1 in Sf9 Cells*

The cDNA coding for C-CAM1 predicts a protein of 53 kDa. There are 21 potential N-linked glycosylation

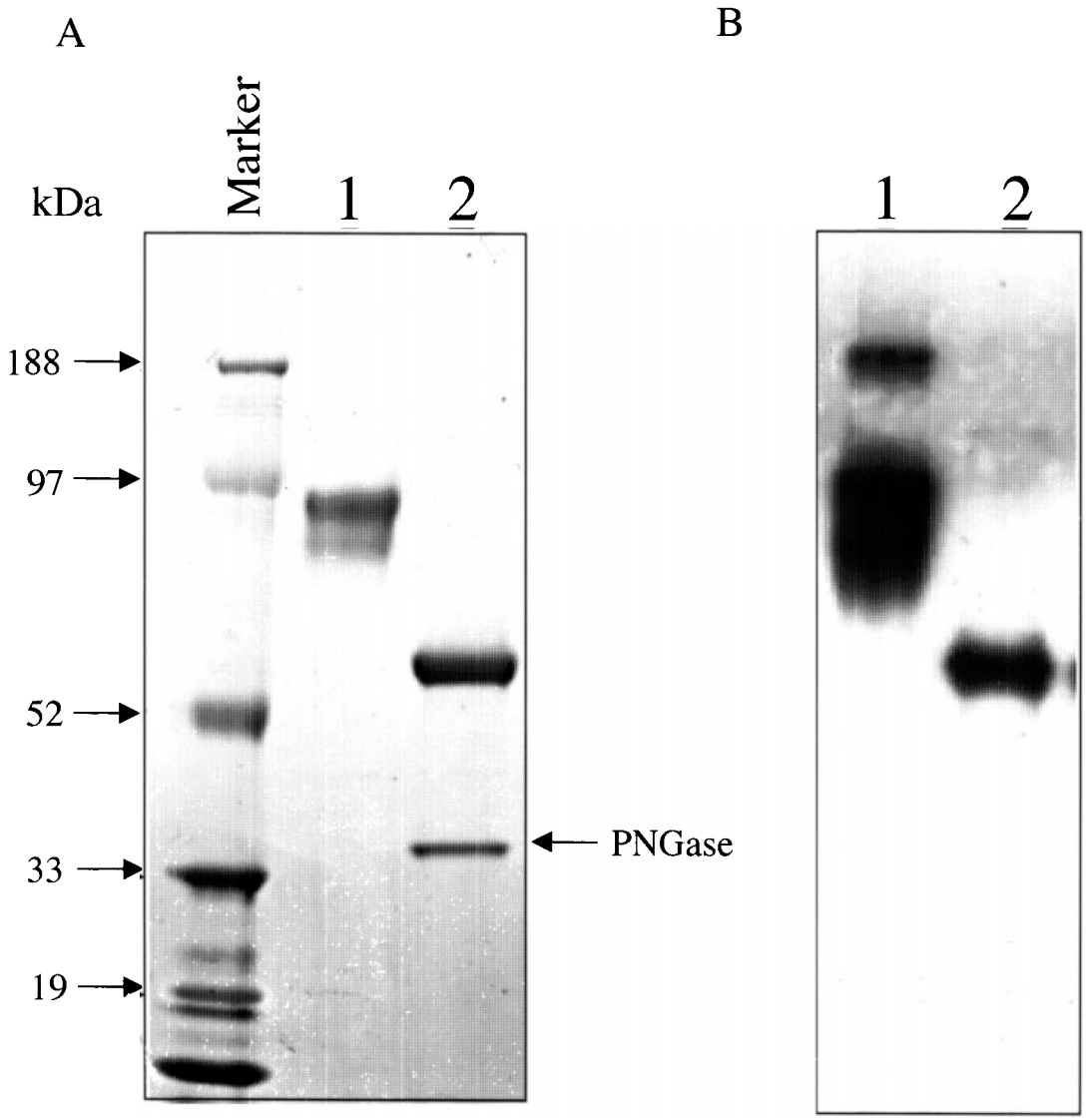


FIG. 5. PNGase F digestion of purified human C-CAM1. (A) Purified C-CAM1 (3 $\mu$g) was digested without (lane 1) and with PNGase F (lane 2) and analyzed on SDS–PAGE. The proteins were stained with Gelcode blue. MW standards are listed to the left. PNGase F is indicated as an arrow; (B) Western blot analysis. C-CAM1 was detected using anti-CEA polyclonal antibody.

sites predicted from sequence analysis. That the apparent molecular weight of C-CAM1 is 90 kDa and the protein band appears diffused in SDS–PAGE suggests that C-CAM1 is most likely glycosylated in Sf9 cells. To determine whether C-CAM1 is indeed glycosylated in Sf9 cells, the purified C-CAM1 protein was incubated with peptidyl *N*-glycosidase F (PNGase F), which cleaves the bond between the asparagine residue and *N*-acetylglucosamine of an N-linked oligosaccharide. Figure 5A shows an increased migration and a narrowing of the protein band after PNGase F digestion. Western blot analysis indicates that the PNGase F-digested protein band is reactive to anti-CEA antibodies (Fig. 5B). The apparent molecular mass of this protein changed from 90 to around 58 kDa, which is close to the molecular weight predicted from cDNA sequence. This result suggests that C-CAM1 contain mainly N-linked glycosylation when expressed in Sf9 cells. It is interesting to note that the 180-kDa band present in the undigested sample is absent in the digested sample (Fig. 5B). This is probably due to the conditions used for PNGase F digestion, which involves treating the protein sample at 100°C for 10 min before enzyme digestion at 37°C. Such conditions probably cause the dissociation of C-CAM1 dimer.

### *Protein Analysis*

N-terminal sequence analysis was performed on the 90-kDa protein eluted by 100 mM imidazole. No sequence could be obtained by Edman degradation. This observation suggests that the N-terminus of the 90-kDa protein is probably blocked because the predicted N-terminal amino acid of the mature C-CAM1 is glutamine which is often converted into pyroglutamate. Because the N-terminus of this protein is blocked, internal sequencing was performed. Analysis of the peptide fragments obtained from trypsin digestion of the 90-kDa protein by MALDI-TOF revealed only 4 tryptic fragments with correct masses as predicted by cDNA. This is most likely due to glycosylation of C-CAM1 protein in insect cells. The purified C-CAM1 protein was therefore deglycosylated with PNGase F and the 58-kDa deglycosylated protein was then used for trypsin digestion followed by internal sequence analysis using nanospray MS. Three peptide sequences were obtained. Two of these, TLTLLSVTR and TIIVTELSPVVAK, correspond to the 199–207 and 315–327 regions of the C-CAM1 sequence (13). The third peptide with the sequence of LSQGDTTLSINPVK corresponds to the 374–387 region, but with a single amino acid substitution (D vs N) at position 378. This substitution could be due to a true amino acid substitution, deamidation during digestion/recovery of peptide fragments, or conversion of N to D by PNGaseF as a result of deglycosylation at that position. Since the cDNA used for the generation of C-CAM1 recombinant baculovirus encodes an N at position 378, which is located in a consensus sequence for N-linked glycosylation, i.e., NTT at 378–380, this observation suggests that the N at position 378 was glycosylated in Sf9 cells.

In conclusion, we have developed a method for expression and purification of human C-CAM1 from Sf9 cells using a baculoviral expression system. Human C-CAM1 protein can be purified from Sf9 cell lysate using one-step affinity chromatography. Although C-CAM1 protein was not abundantly expressed in Sf9 cells (about 1% of total protein), the one-step affinity process allows for a rapid and efficient purification of the C-CAM1 protein without the inconvenience of traditional purification techniques. Biochemical and functional studies of the expressed C-CAM1 indicate that this protein exhibits similar characteristics as those of the native protein, e.g., adhesion function and glycosylation modification. Using the method reported here, sufficient quantity C-CAM1 protein can be produced with a purity suitable for monoclonal antibody generation.

## ACKNOWLEDGMENTS

We thank Drs. Richard Burgess, Sheenah Mische, and Albert Courey, instructors of the protein purification and characterization course at the Cold Spring Harbor Laboratories, for their encouragement. The assistance of Farzin Gharahdaghi and Denise Meagher in protein sequence analysis is highly appreciated. We also thank Weiping Luo for the generation of recombinant baculovirus.

## REFERENCES

1. Ocklind, C., and Obrink, B. (1982) Intercellular adhesion of rat hepatocytes: Identification of a cell surface glycoprotein involved in the initial adhesion process. *J. Biol. Chem.* 257, 6788–6795.
2. Pu, Y. S., Luo, W., Lu, H.-H., Gingrich, J., Greenberg, N., and Lin, S.-H. (1999) Differential expression of C-CAM protein in prostate cancer progression in a transgenic adenocarcinoma mouse model. *J. Urol.* 162, 892–896.
3. Bamberger, A. M., Riethdorf, L., Nollau, P., Naumann, M., Erdmann, I., Gotze, J., Brummer, J., Schulte, H. M., Wagener, C., and Loning, T. (1998) Dysregulated expression of CD66a (BGP, C-CAM), an adhesion molecule of the CEA family, in endometrial cancer. *Am. J. Pathol.* 152, 1401–1406.
4. Huang, J., Simpson, J. F., Glackin, C., Riethorf, L., Wagener, C., and Shively, J. (1998) Expression of biliary glycoprotein (CD66a) in normal and malignant breast epithelial cells. *Anticancer Res.* 18, 3203–3212.
5. Riethdorf, L., Lisboa, B. W., Henkel, U., Naumann, M., Wagener, C., and Loning, T. (1997) Differential expression of CD66a (BGP), a cell adhesion molecule of the carcinoembryonic antigen family, in benign, premalignant, and malignant lesions of the human mammary gland. *J. Histochem. Cytochem.* 45, 957–963.
6. Neumaier, M., Paululat, S., Chan, A., Matthaes, P., and Wagener, C. (1993) Biliary glycoprotein, a potential human cell adhesion molecule, is down-regulated in colorectal carcinomas. *Proc. Nat. Acad. Sci. USA* 90, 10744–10748.
7. Nollau, P., Scheller, H., Kona-Horstmann, M., Rohde, S.,

Hagenmuller, F., Wagener, C., and Neumaier, M. (1997) Expression of CD66a (human C-CAM) and other members of the carcinoembryonic antigen gene family of adhesion molecules in human colorectal adenomas. *Cancer Res.* 57, 2354–2357.

8. Nollau, P., Prall, F., Helmchen, U., Wagener, C., and Neumaier, M. (1997) Dysregulation of carcinoembryonic antigen group members CGM2, CD66a (biliary glycoprotein), and nonspecific cross-reacting antigen in colorectal carcinomas. Comparative analysis by northern blot and *in situ* hybridization. *Am. J. Pathol.* 151, 521–530.
9. Hsieh, J. T., Luo, W., Song, W., Wang, Y., Kleinerman, D. I., Van, N. T., and Lin, S. H. (1995) Tumor suppressive role of an androgen-regulated epithelial cell adhesion molecule (C-CAM) in prostate carcinoma cell revealed by sense and antisense approaches. *Cancer Res.* 55, 190–197.
10. Lin, S.-H., Pu, Y.-S., Luo, W., Wang, Y., and Logothetis, C. (1999) Schedule dependence of C-CAM1 adenovirus gene therapy in a prostate cancer model. *Anticancer Res.* 19, 337–340.
11. Luo, W., Talposky, M., Earley, K., Wood, C., Wilson, D., Logothetis, C. J., and Lin, S.-H. (1999) The tumor suppressive activity of CD66a in prostate cancer. *Cancer Gene Ther.* 6, 313–321.
12. Thompson, J. A., Grunert, F., and Zimmermann, W. (1991) Carcinoembryonic antigen gene family: Molecular biology and clinical perspectives. *J. Clin. Lab. Anal.* 5, 344–366.
13. Hinoda, Y., Neumaier, M., Hefta, S. A., Drzeniek, Z., Wagener, C., Shively, L., Hefta, L. J. F., Shively, J. E., and Paxton, R. (1988) Molecular cloning of a cDNA coding biliary glycoprotein. I. Primary structure of a glycoprotein immunologically cross-reactive with carcinoembryonic antigen. *Proc. Natl. Acad. Sci. USA* 85, 6959–6963.