

Human Myeloid Plasma Membrane Glycoprotein CD13 (gp150) Is Identical to Aminopeptidase N

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Abstract

To determine the primary structure of CD13, a 150-kD cell surface glycoprotein originally identified on subsets of normal and malignant human myeloid cells, we isolated the complete sequences encoding the polypeptide in overlapping complementary DNA (cDNA) clones. The authenticity of our cDNA clones was demonstrated by the ability of the coding sequences, subcloned in a retroviral expression vector, to mediate expression of bona fide CD13 molecules at the surface of transfected mouse fibroblasts. The nucleotide sequence predicts a 967 amino acid integral membrane protein with a single, 24 amino acid hydrophobic segment near the amino terminus. Amino-terminal protein sequence analysis of CD13 molecules indicated that the hydrophobic segment is not cleaved, but rather serves as both a signal for membrane insertion and as a stable membrane-spanning segment. The remainder of the molecule consists of a large extracellular carboxyterminal domain, which contains a pentapeptide consensus sequence characteristic of members of the zinc-binding metalloprotease superfamily. Sequence comparisons with known enzymes of this class revealed that CD13 is identical to aminopeptidase N, a membrane-bound glycoprotein thought to be involved in the metabolism of regulatory peptides by diverse cell types, including small intestinal and renal tubular epithelial cells, macrophages, granulocytes, and synaptic membranes prepared from cells of the central nervous system.

Introduction

Normal human myeloid cells and their leukemic counterparts bear unique cell surface differentiation antigens which, by convention, are assigned to cluster differentiation (CD)¹ groups based on their specific reactivity with defined monoclonal an-

tibodies (1). Antibodies used to identify the CD13 cluster group bind a 150-kD cell surface glycoprotein (gp150) that is expressed by committed granulocyte-monocyte progenitors (CFU-GM) and by cells of the granulocytic and monocytic lineages at all morphologically distinct stages of differentiation (2-5). CD13 is also expressed by leukemic blasts from a high percentage of patients with acute myeloid leukemia (2, 6-10) and from a smaller group of patients with acute lymphoid leukemia (10-12) who have an increased risk of treatment failure, especially when the lymphoblasts coexpress CD13 epitopes and B-lineage markers (11). This molecule appears to be highly immunogenic: 11 monoclonal antibodies independently derived from mice immunized with human myeloid cells bound CD13 epitopes in studies reported at a recent international workshop (1, 13). These antibodies are termed MY7, MCS-2, SJ-1D1, WM-15, CLB-mon-gran/2, MoU28, MoU48, DU-HL60-4, 22A5, 72a, and 124a4. CD13-specific antibodies do not bind to normal B or T lymphocytes but do react with nonhematopoietic cells, including fibroblasts, osteoclasts, and cells that line renal proximal tubules and bile duct canaliculi (1).

Biochemical studies have demonstrated that CD13-specific antibodies immunoprecipitate a 130-kD glycoprotein (gp130), as well as gp150, from human myeloid leukemic cell lines (14, 15). Metabolic labeling studies established that gp130 is an intracellular precursor of gp150, differing from the larger cell surface form of the molecule in the composition of its carbohydrate chains. When cells were labeled in the presence of tunicamycin, an antibiotic that blocks the addition of asparagine-linked oligosaccharide chains, a single unglycosylated polypeptide of 110 kD was immunoprecipitated (14, 15). A comparison of labeled tryptic cleavage products of gp130 and gp150 molecules confirmed that these glycoproteins have an identical primary structure and thus are posttranslationally modified products of a single gene (14).

Sequential rounds of DNA-mediated gene transfer and fluorescence-activated cell sorting were used to isolate the gene encoding CD13 in a mouse genetic background (14). Sequences from the CD13 locus that annealed to a human repeated sequence probe were cloned from a tertiary mouse cell transformant that had amplified the transfected human gene (16). Molecular subclones were then used to isolate the complete CD13 gene from a human placental genomic DNA library; the intact gene, assembled from three recombinant phages, encoded authentic gp150 glycoproteins when it was transfected into mouse fibroblasts. Probes prepared from subclones within the CD13 locus were used to identify a 4.0-kb RNA transcript expressed by human myeloid cells and to assign the CD13 gene to the distal long arm of chromosome 15.

We now report the isolation and characterization of overlapping cDNA clones which together contain all of the CD13 coding sequences. The deduced primary structure and NH₂-

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1. Abbreviations used in this paper: CALLA, common acute lymphoblastic leukemia antigen; CD, cluster of differentiation; gp150, 150-kD cell surface glycoprotein (CD13); gp130, the 130-kD precursor of gp150.

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terminal amino acid sequence of gp150 indicate that it is an integral membrane protein with a large extracellular carboxy-terminal domain, a short intracellular amino-terminal segment, and a hydrophobic signal sequence that is retained and functions as the transmembrane domain. Moreover, the predicted amino acid sequence of CD13 is virtually identical to the recently determined sequence of aminopeptidase N, a prominent intrinsic enzyme of the brush border membranes of the small intestine and renal proximal tubules.

Methods

cDNA cloning and DNA sequence analysis. We previously identified restriction fragments lacking human repetitive sequences that are included in the CD13 genomic locus and that anneal to a 4.0-kb RNA transcript expressed by the HL-60 and KG1 human myeloid leukemic cell lines (16). A mixture of these fragments was nick-translated and used as a probe to screen an oligo-dT primed lambda gt11 cDNA library prepared with polyadenylated RNA from HL-60 cells (a gift of Dr. Michael Holers, Washington University, St. Louis, MO; 17). Additional clones were isolated from a cDNA library prepared in the lambda ZAP vector (Stratagene Corp., La Jolla, CA) with polyadenylated RNA from KG1 cells. First strand synthesis was initiated with random primers and with synthetic oligonucleotides inversely complementary to 5' sequences of clones isolated from the oligo-dT primed library.

Eco RI inserts from the lambda gt11 clones were subcloned into the pBluescript plasmid (Stratagene); lambda ZAP phages were rescued directly as recombinant pBluescript plasmids with procedures recommended by the manufacturer. Restriction endonuclease sites were mapped in the subcloned cDNA inserts, and restriction fragments were nick-translated and used as probes on Southern blots of digested DNA from both cDNA and genomic phage clones (16). In this way, the cDNA clones were oriented relative to each other and to the CD13 genomic locus. To determine the 5' to 3' orientation of the cDNA clones relative to messenger RNA, single-stranded RNA probes were prepared from selected cDNA inserts subcloned in pBluescript. Probes were transcribed from each strand of the cDNA insert with the T3- and T7-specific polymerases that bind specific promoter sequences flanking the cloning sites of this vector, using a kit supplied by Promega Biotec (Madison, WI). Each probe was hybridized to Northern blots, prepared as described previously (16), to determine which probe orientation was complementary to the 4.0-kb CD13 messenger RNA.

Based on an extensive map of restriction endonuclease sites within the inserts of the three longest cDNA clones (λ 6, λ 29 and λ 71), a series of restriction fragments were subcloned into the M13mp18 and M13mp19 vectors for DNA sequence analysis by the Sanger dideoxy chain-termination method (18) using the Sequenase DNA sequencing kit (United States Biochemical Corp, Cleveland, OH). The entire cDNA was sequenced on both strands and each cloning site was resequenced in overlapping subclones. The sequence was verified for each subclone by using fluorescent dye-labeled primers and the 370A automated DNA sequencer (Applied Biosystems). IntelliGenetics (Mountain View, CA) software was used for all sequence manipulation and analysis.

Expression of cDNA sequences cloned in a retroviral vector. Full length CD13 cDNA was reconstructed by subcloning the 5' 1.8 kb Eco RI insert of λ 29 (see Fig. 1) into a pBluescript plasmid containing the 3' 1.6 kb Eco RI insert of λ 6, which had been linearized by partial digestion with Eco RI. The restriction sites in the resulting plasmids were mapped to identify a plasmid that contained both Eco RI cDNA restriction fragments ligated in the correct 5'-3' orientation. Sequencing analysis subsequently disclosed a 139-bp deletion in the insert of λ 29, by comparison with the corresponding sequence of λ 71 and other clones spanning this region. This deletion interrupted the CD13 open reading frame, and presumably represented a cloning artifact. There-

fore, a 1.0-kb Nco I restriction fragment containing this region from λ 71 was substituted for the corresponding restriction fragment in the plasmid containing the reconstructed cDNA. The resulting pBluescript plasmid was digested with Sal I, which cut only in the cloning poly-linker adjacent to the 5' end of the cDNA and with Xba I, which cut once in the cDNA insert in the 3' untranslated region upstream of the polyadenylation signal (see Fig. 2). The resulting 3.4-kb restriction fragment, which contained all of the predicted CD13 coding sequences, was inserted into the unique Bam HI site of the murine retroviral vector pZIPneoSV(X)-1 (19) provided by Dr. Richard C. Mulligan, Whitehead Institute, Boston, MA. Blunt end ligation was employed, after the recessed 3' ends of both the insert and the vector had been filled in with the Klenow fragment of *Escherichia coli* polymerase I. Colonies containing recombinant plasmids were identified by hybridization, and a plasmid with the correct 5' to 3' orientation of the cDNA insert relative to the vector was selected on the basis of restriction endonuclease mapping.

This construction linked the CD13 gene to DNA sequences of the vector encoding neomycin resistance (*neo*); the gp150 protein is translated from the unspliced retroviral RNA, whereas the *neo* product is synthesized from a spliced mRNA. The retroviral construct was transfected by the calcium phosphate method into NIH-3T3 cells (14). After transfection, the cells were cultured in medium containing 800 μ g/ml of G418 (Geneticin; Gibco Laboratories, Grand Island, NY), to select for expression of the *neo* gene. G418-resistant cells were sorted by flow cytometry after they had been stained with the monoclonal antibody MY7 (Coulter Immunology, Hialeah, FL), which reacts with an epitope of gp150 (2, 14). Transfected cells isolated according to their reactivity with MY7 were tested by flow cytometry for binding of other monoclonal antibodies that react with epitopes of CD13: SJ-1D1 (20) from Dr. Joseph Mirro of St. Jude Children's Research Hospital, Memphis, TN; and MCS-2 (15) from Dr. Jun Minowada of Loyola University, Chicago, IL. Indirect immunofluorescence labeling, flow cytometric analysis, and cell sorting were performed as previously described (14).

Metabolic labeling and immunoprecipitation. Subconfluent cultures of cells were incubated at 37°C in 5 ml methionine-free DMEM (Gibco Laboratories) containing 5% dialyzed fetal calf serum for 30 min and then labeled for 60 min in 2 ml of the same medium containing 0.25 mCi of [³⁵S]methionine (1,200 Ci/mmol) per ml. An equal volume of complete medium was added, and the incubation was continued for an additional 60 min. The cells were rinsed in ice-cold PBS and lysed in RIPA buffer (50 mM Tris hydrochloride [pH 7.4], 150 mM NaCl, 20 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS) containing 2% aprotinin and 1% PMSF to inhibit proteases. The lysates were immunoprecipitated and separated by SDS-PAGE (14, 21); the mobilities of radiolabeled proteins were determined by autoradiography relative to known polypeptide standards.

Immunoprecipitation of glycoproteins for NH₂-terminal protein sequence analysis. Exponentially growing HL-60 cells (1×10^9) were lysed in RIPA buffer and immunoprecipitated with antiserum specific for CD13 epitopes. For this immunoprecipitation, we used a CD13-specific polyvalent antiserum produced by neonatal (8-d-old) NFS mice that are syngeneic with NIH-3T3 cells. Tumorigenic cells from the NIH-3T3 cell tertiary transformant SJ-150-B (16, 22), which coexpresses high levels of CD13 together with the transforming glycoprotein encoded by the *v-fms* oncogene, were injected subcutaneously into the mice. The animals with tumors were bled, and pooled sera were tested for their ability to react with CD13 in immunofluorescence and immunoprecipitation assays. As we had previously observed for similar antisera produced against epitopes of CD33 (23), pooled CD13-specific antiserum proved superior to monoclonal antibodies for precipitating large quantities of the gp130 and gp150 glycoproteins, presumably because it recognizes multiple epitopes with high affinity. The immunoprecipitates were separated by SDS-PAGE, and gp150 and its gp130 precursor were transferred to Immobilon membranes (Miles Laboratories, Elkhart, IN) following methods of Matsudaira (24), and visualized by Coomassie blue staining. The NH₂-terminal amino acid

sequences of both the gp130 and gp150 forms of the molecule were determined from two independent preparations according to established procedures (24, 25).

Results

Isolation of CD13 cDNA clones. Unique sequence probes derived from the CD13 genomic locus were used to screen cDNA libraries that were prepared with polyadenylated RNA extracted from human myeloid cell lines that express CD13. 12 strongly hybridizing clones were isolated from a cDNA library primed with oligo-dT, and 10 additional clones were obtained from a library primed with a mixture of random primers and specific oligonucleotides representing sequences inversely complementary to 5' CD13 sequences. Restriction endonuclease cleavage sites were determined for the three clones with the largest inserts; defined restriction fragments were then used as probes in Southern blotting experiments to align the cDNA clones and to establish their orientation relative to previously isolated genomic clones (Fig. 1). Together, the overlapping cDNA clones spanned 3.5 kb of the CD13 messenger RNA, which was estimated to be 4.0 kb in length as judged from Northern blots hybridized with the same genomic probes that were used to isolate the cDNA clones (16).

When used as probes for Southern blots of restriction digests of previously isolated genomic clones, the cDNA restriction fragments hybridized to a region of ~ 20 kb (Fig. 1). Genomic clones containing this region mediate expression of

gp150 when appropriately ligated together and transfected into mouse fibroblasts (16). The 5' to 3' orientation of the cDNA clones shown in Fig. 1 was determined by hybridizing strand-specific RNA probes prepared from cDNA restriction fragments subcloned in the pBluescript transcription vector to Northern blots of polyadenylated RNA from myeloid cell lines. RNA probes prepared from restriction fragments cloned in an antisense orientation hybridized to the 4.0 kb CD13 messenger RNA (data not shown).

CD13 cDNA sequence. The complete nucleotide sequence of overlapping CD13 cDNA inserts contained in the λ 6, λ 29, and λ 71 clones extends for 3,494 nucleotides and is flanked by a poly(A) sequence at its 3' end (Fig. 2). The longest predicted open reading frame of 2901 residues (nucleotides 121-3021) is preceded by 120 nucleotides of 5' untranslated sequence and terminates with 473 nucleotides of 3' untranslated sequence. A polyadenylation signal (AATAAA) (26) is located 13 nucleotides upstream from the poly(A) sequence at the 3' end of the cDNA. The first ATG codon, at position 121, matches the consensus sequence for a translation initiation site (27). Seven residues separate the initiator methionine from a hydrophobic region of 24 amino acids, which is likely to represent a signal peptide sequence necessary for transport of the nascent polypeptide chains into the lumen of the endoplasmic reticulum. The deduced amino acid sequence of the CD13 polypeptide predicts a total of 967 amino acids with a molecular mass of 110 kD, in agreement with the apparent mass of the unglycosylated CD13 polypeptide synthesized by cells grown in the presence of tunicamycin (14, 15). 11 canonical sites (Asn-X-Ser/Thr) for the addition of asparagine-linked oligosaccharide chains are present 3' to the putative membrane-spanning segment. Nearly all of these potential sites may be utilized, because each high-mannose chain has an estimated mass of 2.5 kD, and immature gp130 precursor molecules differ by ~ 20 kD from the mass of the unglycosylated polypeptide. Five cysteine residues are distributed over the polypeptide sequence. Because the CD13 mRNA is estimated to be 4.0 kb by Northern blot analysis, and our cDNA clones span 3494 nucleotides, the native transcripts most likely contain additional untranslated nucleotides at their 5' ends.

The entire CD13 cDNA and protein sequences were used to search for similarity with other sequences available from the NIH DNA sequence library (GenBank; Release 56), the European Molecular Biology Laboratory DNA sequence library (EMBL; Release 14), and the National Biomedical Research Foundation protein sequence library (NBRF; Release 16). These searches did not disclose statistically significant similarity between the sequence obtained for CD13 and other previously sequenced proteins or DNA sequences. We did, however, identify a region spanning amino acid residues 388-392 that conforms to a pentapeptide consensus sequence, His-Glu-[Ile, Leu, Met]-X-His, which is essential for zinc coordination and the catalytic activity of metalloproteases (27-31).

Sequence comparisons with known enzymes of this class revealed virtual identity between our CD13 sequence and the recently determined sequence of aminopeptidase N (32). Our CD13 cDNA clone includes 96 nucleotides at its 5' end and 10 nucleotides plus the poly(A) sequence at its 3' end that are missing from the aminopeptidase N clone. Within the sequenced regions of both cDNA clones, there were 10 nucleotide mismatches, only four of which result in predicted amino

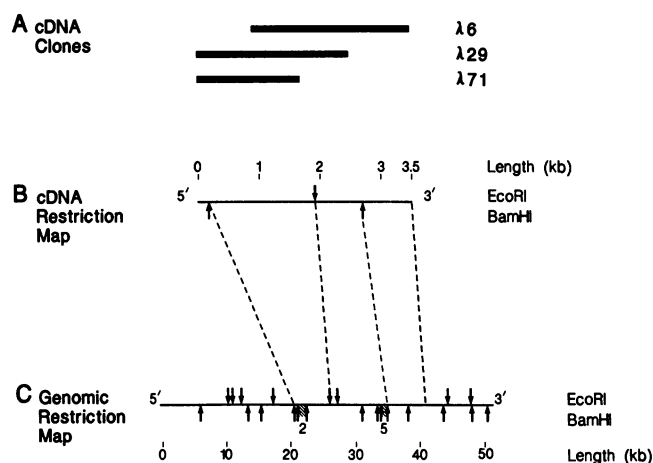


Figure 1. Restriction map of CD13 cDNA clones (A and B), in relation to the restriction map of the previously isolated genomic locus (C). Clones λ 6 and λ 29 were isolated from an oligo-dT primed cDNA library made from HL-60 cell mRNA, using a probe prepared from a mixture of two unique sequence genomic restriction fragments (labeled 2 and 5, C) that had been previously shown to hybridize with the 4.0 kb CD13 mRNA. Clone λ 71 was isolated from a cDNA library prepared from KG1 cell mRNA by using a mixture of random primers and oligonucleotides inversely complementary to 5' sequences from λ 29. The inserts of these clones contained one internal Eco RI site and two Bam HI sites, with positions as shown in B. The corresponding restriction sites in the CD13 genomic map (dashed lines connecting B with C) were identified with cDNA probes from Bam HI-Eco RI restriction fragments of the inserts of λ 6 and λ 29 to probe Southern blots of restriction digests of previously isolated phage clones that span the CD13 genomic locus (16).

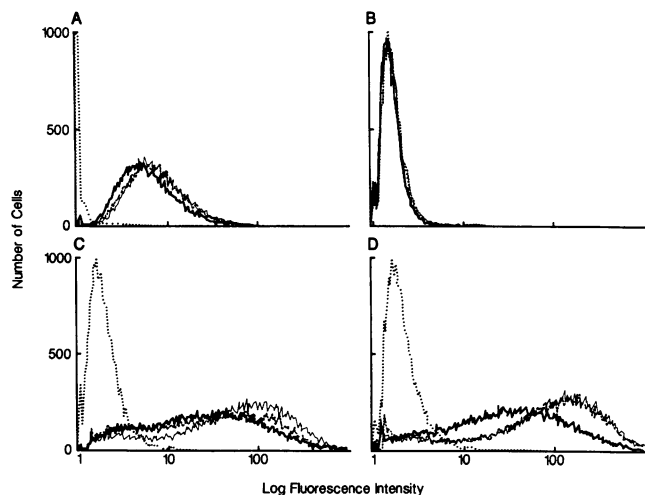


Figure 3. Expression of CD13 on the surface of NIH-3T3 cells transfected with CD13 cDNA sequences cloned in a retroviral vector. The flow cytometric profiles were obtained after binding of monoclonal antibodies specific for CD13 epitopes to HL-60 human myeloid cells (A), untransfected NIH-3T3 cells (B), and cells from two representative NIH-3T3 cultures that had been transfected with complete CD13 cDNA coding sequences (C and D) reconstructed from portions of the inserts of three overlapping cDNA clones. Transfected cells were grown in G418 to select for cells expressing the *neo* gene of the retroviral vector and then sorted once for the 2% of cells with the brightest fluorescence after staining with the CD13-specific monoclonal antibody, MY7. Each panel shows fluorescence profiles that resulted from testing the cells with the CD13-specific monoclonal antibodies MY7 (dark solid line), SJ-1D1 (dashed line), and MCS.2 (light solid line), compared with results with a mouse myeloma protein control (dotted line).

cules, we assembled the intact coding sequences from overlapping clones and inserted them into the murine retroviral vector pZIPneoSV(X)-1 (19). The retroviral construct was transfected into NIH-3T3 cells, and the cells were cultured in medium containing G418 to select for transfectants that expressed the *neo* gene contained in the vector. Analysis of these cells by flow cytometry after immunofluorescence labeling with the CD13-specific monoclonal antibody MY7 disclosed that over half the cells expressed high levels of gp150 at the cell surface. Cells that bound the highest levels of antibody were isolated by cell sorting, and cultured for further analysis. Fig. 3 shows the results of flow cytometric analysis of cells from two independently transfected cultures stained with three different CD13-specific monoclonal antibodies. The expression of gp150 epitopes by cells transfected with the retroviral vector was approximately tenfold higher than that observed for the HL-60 human myeloid leukemia cell line.

To characterize the biochemical properties of CD13 molecules expressed by cells transfected with the retroviral construct, we metabolically labeled cells with [35 S]methionine, and immunoprecipitated detergent lysates with the MY7 monoclonal antibody. High levels of polypeptides with the mobilities of gp130 and gp150 were detected in lysates from control HL-60 cells, in a tertiary mouse cell transformant (SJ-150-B) known to express high levels of gp150 (16, 22), and in three NIH-3T3 cultures that had been transfected with the CD13 retroviral vector (Fig. 4). No specifically precipitable polypeptide was observed in NIH-3T3 cells that were either

untransfected or transfected with a plasmid containing the *v-fms* oncogene. Immature gp130 molecules had identical apparent mobilities in human HL-60 cells and in transfected mouse cells; the more rapid mobility of the mature cell surface form of the molecule in mouse cells, as compared with HL-60 cells, has been observed previously (14, 16) and may reflect differences in glycosylation in the different cell types.

Topology of CD13. The hydrophobicity profile predicted by the algorithm of Kyte and Doolittle (33) showed a 24 amino acid hydrophobic region, starting nine amino acids from the amino terminus, that could function as both a signal peptide and a transmembrane domain (Fig. 1). No other hydrophobic region that would be of sufficient length to span the plasma membrane was identified. This suggested that CD13 might be a member of a small family of integral membrane proteins, in which the signal sequence is not removed after translocation of the NH_2 -terminus into the lumen of the endoplasmic reticulum, but rather is retained to serve as a transmembrane anchor (34). Proteins of this type are oriented with their amino terminus in the cytoplasm and their carboxyl terminus outside the cell.

To test this hypothesis, we determined the NH_2 -terminal peptide sequence of both the gp130 and gp150 forms of the molecule, which were immunoprecipitated with specific antisera from lysates of HL-60 cells. The yields indicated that $\sim 10\%$ of the molecules were susceptible to Edman degradation, and the first 20 residues matched amino acids 2-21 of the

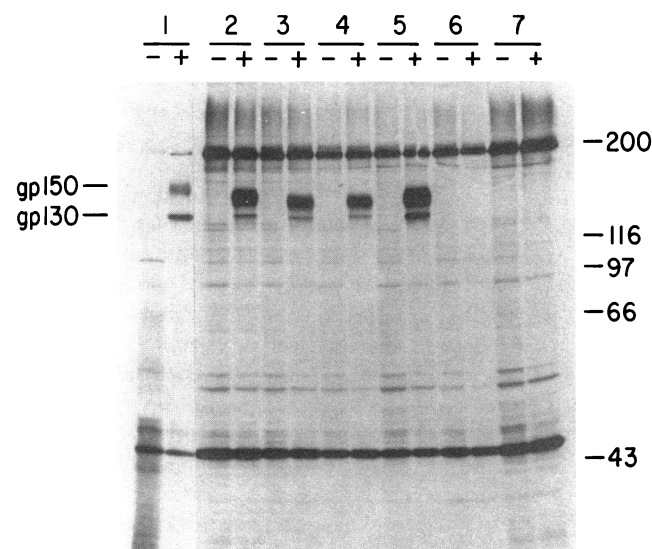


Figure 4. Biochemical characterization of gp130 and gp150 glycoproteins expressed by NIH-3T3 cells transfected with CD13 cDNA sequences cloned in a retroviral vector. Cells were metabolically labeled with [35 S]methionine for 1 h and incubated an additional hour with complete medium; then, detergent lysates were immunoprecipitated with the MY7 CD13-specific monoclonal antibody (+) or with a control monoclonal antibody (-). Labeled proteins in immune complexes were analyzed in polyacrylamide gels containing SDS. Results are shown for HL-60 cells (lane 1); the tertiary transformant SJ-150-B (16, 22), derived from serial transfections of genomic DNA with the *v-fms* oncogene as a selectable marker (2); NIH-3T3 cells from three cultures independently transfected with complete CD13 coding sequences cloned in a retroviral vector (3, 4, and 5); untransfected NIH-3T3 cells (6); and NIH-3T3 cells transfected with the *v-fms* oncogene (7).

sequence predicted from the CD13 cDNA nucleotide sequence (Ala-Lys-Gly-Phe-Tyr-Ile-Ser-Lys-Ser-Leu-Gly-Ile-Leu-Gly-Ile-Leu-Leu-Gly-Val-Ala; Fig. 2). The N-terminal amino acid sequence is consistent with translation being initiated at the ATG codon predicted from the nucleic acid sequence, and indicates that the signal sequence is not cleaved after insertion of the molecules into membranes of the endoplasmic reticulum. This result is typical of integral membrane proteins whose signal sequences are retained as a transmembrane anchor. In such proteins, the amino-terminal methionine is characteristically removed, and the second residue is acetylated, leaving only a minority of unblocked amino termini that remain susceptible to Edman degradation (35–37).

Discussion

The cellular distribution and biochemical properties of CD13 glycoproteins suggested that they might have important physiologic roles, not only on myeloid cells but also on cells from the diverse tissues that bind CD13-specific monoclonal antibodies (1, 14, 15). We undertook the molecular cloning of CD13 cDNA with the idea that the deduced primary structure of the polypeptide would provide insight into the functions of this glycoprotein on normal and malignant cells. The translated amino acid sequence predicts a polypeptide of 967 amino acids with 11 potential sites of asparagine-linked oligosaccharide addition, accounting for the 110-kD molecular mass of the unglycosylated polypeptide and the additional mass attributable to the carbohydrate moiety of the cotranslationally modified glycoprotein (14, 15). Aminoterminal protein sequence analysis indicated that CD13 molecules are synthesized with an uncleaved signal sequence and that proteolytic processing at the amino-terminus is limited to removal of the initiator methionine. Because the retained signal sequence is the only potential membrane-spanning segment apparent from hydrophobicity analysis, CD13 glycoproteins are likely to be oriented with their amino terminus inside and their carboxyl terminus outside the cell. Integral membrane proteins with this topology include a small group of molecules that function predominately as receptors or membrane-bound enzymes (34–40). The extracellular carboxyterminal CD13 domain contains a pentapeptide signature sequence that serves as the catalytic site of zinc-binding metalloproteases, suggesting that CD13 is a membrane-bound enzyme of this class (27–31). Comparison of the recently published sequence of a cDNA clone encoding aminopeptidase N (32), a prominent metalloprotease, with our CD13 cDNA sequence disclosed that the two molecules are identical.

Aminopeptidase N (EC 3.4.11.2) is an important enzyme of the brush border membranes of the small intestine, renal proximal tubules, and placenta (41–43). Its expression has also been documented on synaptic membranes of the central nervous system (44) and on the surface of macrophages (45) and granulocytes (46, 47). This enzyme catalyzes the removal of NH₂-terminal amino acids from peptides, with a preference for neutral residues, but with broad specificity in the cleavage of basic and acidic residues as well (48). The natural substrates appear to be peptides rather than proteins, but the enzyme is more effective in the removal of residues from oligopeptides than from dipeptides (48). In the intestinal brush border where aminopeptidase N constitutes 8% of the total protein, the carboxyterminal enzymatic domain faces the lumen and most

likely plays an important role in the final stages of the digestion of small peptides (41, 42). In other tissues, the enzyme has been postulated to function in the hydrolytic inactivation of regulatory peptides, including enkephalins, that are involved in signal transduction at the cell membrane (44, 45, 49).

The synthesis and posttranslational processing of aminopeptidase N have been characterized biochemically in epithelial cells from the small intestine and renal proximal tubules of the pig (50, 51). Pulse-chase experiments have demonstrated that newly synthesized molecules have an apparent molecular mass of ~ 140 kD and contain cotranslationally added asparagine-linked oligosaccharide chains that are rich in mannose. Within 30 to 60 min of synthesis, the side chains are remodeled to complex oligosaccharides, resulting in the 160 kD mature form of the molecule that is expressed at the cell surface. Endoglycosidase H treatment of the immature form of the molecule removes the high-mannose oligosaccharide chains, and produces a polypeptide backbone of 115 kD. These results obtained by two different laboratories studying epithelial cells from separate organs of the pig, are perfectly consistent with our studies of CD13 synthesis and processing by human myeloid leukemia cell lines (14). In addition, aminopeptidase N has been shown to be expressed as a homodimer on the surface of intestinal epithelial cells of several species, including rat, pig, and human, but as a monomer in the rabbit (42, 43).

The gene encoding the common acute lymphoblastic leukemia antigen (CALLA or CD10) was recently cloned (31, 35), and its cDNA sequence was shown by Letarte and co-workers (31) to be identical to that of neutral endopeptidase (EC 3.4.24.11), a membrane-associated enzyme also known as metalloendopeptidase or enkephalinase (28, 52, 53). The predicted amino acid sequences of CD13/aminopeptidase N and CD10/neutral endopeptidase do not show significant overall similarity; however, both molecules are integral membrane metalloproteases with the characteristic zinc-binding motif in their extracellular carboxyterminal domains (27–31). Neutral endopeptidase hydrolyzes peptide bonds at the amino side of hydrophobic amino acids (54), and is also thought to inactivate regulatory peptides at the cell surface (55–61). Both of these enzymes are expressed on epithelial cells of the renal proximal tubule and small intestine, granulocytes, stromal cells, and synaptic membranes in the central nervous system (1, 55–72). The two membrane-bound enzymes collaborate in the hydrolysis of oligopeptides in the small intestine (41, 42) and appear to act in concert to inactivate opioid peptides and enkephalins in the brain (44, 49), as well as tuftsin (46, 47) and the chemotactic peptide FMLP by neutrophils (60, 61). On hematopoietic cells, there are clear differences in their patterns of expression: CD10/neutral endopeptidase is uniquely expressed by early lymphoid precursors (62–65), while monocytes and committed myeloid progenitors express only CD13/aminopeptidase N (1–10). Neither enzyme is expressed by mature B or T lymphocytes. The reactivity profiles of CD13- and CD10-specific antibodies with human leukemic cells rarely overlap, mirroring the lineage-specific binding pattern of these reagents to normal hematopoietic cells. CD13 is expressed on the leukemic blasts from the majority of patients with myeloid leukemia (1–10), whereas CD10 is expressed on lymphoid leukemias with the phenotype of early B cell progenitors (62–65). The identification of these differentiation antigens as membrane-bound metalloproteases suggests new avenues for testing their physiologic roles on myeloid and lym-

phoid progenitors, and for determining their possible influence on normal and malignant hematopoietic cell development.

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Note added in proof: The CD13/aminopeptidase N cDNA sequence has been submitted to Gen Bank, accession number M22324.

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