Amino Acid Sequence of the Alpha Subunit of Human Leukocyte Adhesion Receptor Mol (Complement Receptor Type 3)

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Abstract. Mol (complement receptor type 3, CR3; CD11b/CD18) is an adhesion-promoting human leukocyte surface membrane heterodimer (alpha subunit 155 kD [CD11b] noncovalently linked to a beta subunit of 95 kD [CD18]). The complete amino acid sequence deduced from cDNA of the human alpha subunit is reported. The protein consists of 1,136 amino acids with a long amino-terminal extracytoplasmic domain, a 26-amino acid hydrophobic transmembrane segment, and a 19-carboxyl-terminal cytoplasmic domain. The extracytoplasmic region has three putative Ca²⁺-binding domains with good homology and one with weak homology to the "lock washer" Ca²⁺-binding consensus sequence. These metal-binding domains explain the divalent cation-dependent functions mediated by Mol. The alpha subunit is highly homologous to the alpha subunit of leukocyte p50,95 and to a lesser extent, to the alpha subunit of other "integrin" receptors such as fibronectin, vitronectin, and platelet IIb/IIIa receptors in humans and position-specific antigen-2 (PS2) in Drosophila. Mol, like p50, contains a unique 187-amino acid stretch NH₂-terminal to the metal-binding domains. This region could be involved in some of the specific functions mediated by these leukocyte glycoproteins.

The ability of human granulocytes and monocytes to adhere in a divalent cation-dependent manner to particles coated with the activated complement C3 fragment, iC3b, is mediated by a surface glycoprotein heterodimer, Mol (complement receptor type 3 [CR3]) (Arnaout et al., 1983; Dana et al., 1984). Mol also mediates other divalent cation-dependent granulocyte adhesion functions such as binding to activated endothelium (Wallis et al., 1985), homotypic cell adhesion (Arnaout et al., 1985), phagocytosis (Arnaout et al., 1983), and chemotaxis (Beatty et al., 1983; Dana et al., 1986). Mol consists of two noncovalently associated subunits, each the product of a single gene (Kishimoto et al., 1987; Law et al., 1987; Arnaout et al., 1988). The alpha subunit has an apparent molecular mass of 155-165 kD (CD11b) and associates in a divalent cation-independent manner with a beta subunit of 95 kD (CD18) (Todd et al., 1982). The beta subunit is common to two other leukocyte surface glycoproteins, LFA-1 (alpha subunit 177 kD) and p150,95 (alpha subunit 130-150 kD) (Trowbridge and Omary, 1981; Sanchez-Madrid et al., 1983; Lanier et al., 1985). In addition to sharing the property of binding to the same beta subunit, Mol, LFA-1, and p150,95 (leukocyte adhesion molecules [Leu-CAM]) require divalent cations to mediate their adhesion functions, have homologous NH₂ termini (Pierce et al., 1986; Miller et al., 1987), and presumably arose by gene duplication events (Arnaout et al., 1988).

One mechanism by which Mol and p150,95 mediate enhanced adhesiveness of activated phagocytes is through increased expression of these glycoproteins on the surface of activated cells. In granulocytes this occurs primarily by translocation of these antigens from intracellular storage pools present in secondary and tertiary granules (Arnaout et al., 1984) to the cell surface. The biologic importance of Leu-CAM is underscored by the finding that their inherited deficiency in humans impairs leukocyte adhesion-dependent inflammatory functions and predisposes to life-threatening bacterial infections (Dana et al., 1984). Leu-CAM are also members of a larger family of cell adhesion heterodimeric receptors "integrins", which include platelet IIb/IIIa, fibronectin and vitronectin receptors in humans, and position-specific adhesion receptors in Drosophila (Argraves et al., 1987; Bogaert et al., 1987; Corbi et al., 1987; Hynes, 1987; Kishimoto et al., 1987; Law et al., 1987; Fitzgerald et al., 1987; Ponecz et al., 1987; Arnaout et al., 1988).

Recombinant DNA cloning studies revealed significant homologies among the beta subunits of Leu-CAM, fibronectin receptor, and platelet IIb/IIIa. Additional studies, includ-
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**Figure 1.** cDNA nucleotide sequence and deduced amino acid sequence of the open reading frame of human Mol. 1 Peptide sequences (including NH2-terminal sequence) determined from the purified antigen are underlined. Potential N-linked glycosylation sites (*) are indicated. The overlined regions represent, respectively, the 5' hydrophobic leader sequence and the putative transmembrane hydrophobic domain. Three putative metal-binding domains and a fourth weak site are underlined by broken lines. The 378-bp Mol clone used to isolate the rest of the cDNA, corresponds to nucleotides X07421 and M18044, respectively. These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession numbers X07421 and M18044.4, respectively.
ing isolation of a partial human Molα cDNA clone (Arnaout et al., 1988), characterization of the alpha subunit of pl50,95 (Corbi et al., 1987) and of Drosophila PS-2 antigen (Bogaert et al., 1987), confirmed the structural relatedness and overall homology of the alpha subunits of integrins. The specific functions mediated by these adhesion receptors will be better understood once the complete amino acid sequence of each of these molecules is elucidated. In this report, we describe the complete primary structure of the alpha subunit of human Molα and elucidate its homologous as well as its distinctive structural features in relation to the other alpha subunits in the integrin supergene family.

Materials and Methods

Protein Purification and Sequencing

Human Molα was purified from granulocytes by affinity chromatography using lentil-lectin Sepharose and anti-Molα monoclonal antibody-linked Sepharose as previously described (Pierce et al., 1986; Arnaout et al., 1987). Molα-derived tryptic peptides were separated by reverse-phase HPLC on a C18 column and eluted with a gradient of acetonitrile in 0.1% TFA (Pierce et al., 1986). Individual peptides were subjected to microsequencing on gas phase sequencer (Applied Biosystems Inc., Foster City, CA).

Screening a Human Phorbol–Ester–induced HL-60 cDNA Library

A human cDNA library constructed from poly(A)+ messenger RNA derived from PMA-differentiated promyelocytic cell line HL-60 was a generous gift from Drs. Brian Seed and David Simmons, Massachusetts General Hospital, Boston, MA. This library was constructed in the Bst XI-cloning site of plasmid pEM3 and placed after transcription into Escherichia coli MC1061/p3 host strain (Seed and Aruffo, 1987). I-million colonies plated on 150-mm plates containing LB-agar, 7.5 µg/ml ampicillin, and 12.5 µg/ml tetracycline were screened according to Maniatis and co-workers (Maniatis et al., 1982). A human Molα cDNA probe, 378 bp long (Arnaout et al., 1988) and several synthesized oligonucleotides (17-mers) were random hexanucleotide–32P labeled or 5’-end 32P labeled, respectively, and used to probe nitrocellulose replicates using standard hybridization and washing conditions. Positive colonies were purified and plasmid DNA isolated as described (Maniatis et al., 1982). The dideoxy method for DNA sequencing (Sanger et al., 1977) was carried out using plasmid DNA or cDNA fragments cloned into M13 vectors and, respectively, plasmid vector- or M13-specific primers as well as primers derived from the generated sequences. Both strands of the isolated cDNA inserts were sequenced. Oligonucleotides were synthesized using a 380 DNA synthesizer (Applied Biosystems Inc.).

Sequence Analysis and Homologies

Sequence data analysis was performed using BIONET System of Intelligenetics, Inc. (Palo Alto, CA). Sequence alignment was done using ALIGN and GENALIGN programs from BIONET. Protein homology was sought using the PIR data base and sequence similarities determined by using the FASTP program of Lipman and Pearson (1985).

Results and Discussion

A 378-bp human Molα cDNA clone (Arnaout et al., 1988), was used to isolate three additional cDNA clones that together contained the 3,408-base nucleotide coding sequence of Molα gene (Fig. 1). The coding sequence is preceded by a single translation initiation methionine. The translation product of the single open reading frame began with a 16-amino acid hydrophobic peptide representing a leader sequence, followed by the NH2-terminal phenylalanine residue (Pierce et al., 1986). The translation product also contained all eight tryptic peptides isolated from the purified antigen, the amino-terminal peptide, a 26-amino acid hydrophobic domain representing a potential transmembrane (TM) region, and a short 19-amino acid carboxyl-terminal cytoplasmic domain (Fig. 1). The coding region of Molα (1,136 amino acids) is eight amino acids shorter than the alpha subunit of pl50,95 (1,144 amino acids) (Corbi et al., 1987). This suggests that the larger apparent molecular mass of Molα (155-165 kD by PAGE versus 130-150 kD for the alpha subunit of pl50,95) is largely due to the greater number of N-glycosylation sites (18 potential sites in Molα versus 10 in pl50). The cytoplasmic region of Molα contains one serine residue that could serve as a potential phosphorylation site. The cytoplasmic region is also relatively rich in acidic residues and in proline (Fig. 1). It is interesting to note that the cytoplasmic domain of endocytic receptors (Goldstein et al., 1985) and some integral lysosomal membrane receptors (e.g., cation-dependent and cation-independent mannose-6-phosphate receptors [Dahms et al., 1987; Lobel et al., 1987]) are also rich in acidic residues and in proline. Since Molα is involved in the process of phagocytosis and is also targeted to intracellular storage pools (Arnaout et al., 1984), these residues may be important in mediating these functions. Although the TM region is highly homologous to the TM region in other integrins (Fig. 2) and could therefore serve additional common functions such as in the formation or stabilization of the αβ complex. The long extracytoplasmic amino-terminal region has several interesting features. First, it contains four putative metal-binding domains (outlined by broken lines in Fig. 1) that are similar to Ca2+-binding sites (consensus sequence: DXDXXDXXE) found in calmodulin (Williams, 1986), intestinal vitamin D–binding protein (Fullmer and Wasserman, 1981), parvalbumin (Coffee and Bradshaw, 1973), myosin light-chain (Reinach et al., 1986), troponin (Kretsinger, 1976), thrombospondin (Lawler and Hynes, 1986) with two exceptions: (a) the predicted secondary structure (Chou and Fasman, 1978) shows that the metal-binding sites are surrounded by beta structure rather than by alpha helical segments (not shown); (b) the invariant glutaminy1 residue at position 12 of the metal-binding domains of EF-hand-loop proteins is not present in Molα domains or in metal-binding sites of other integrins. This finding suggests that each Molα–metal-binding site may be composed of two noncontiguous peptide segments (lock-washer configuration) (Vyas et al., 1987). The two peptide segments could lie within the receptor protein itself, as occurs in galactose-binding protein (Vyas et al., 1987) or between the receptor and a natural ligand, accounting for some of the metal-dependent adhesion-promoting functions of Leu-CAM and the cation-dependent formation of αβ complex in other integrins. Secondly, the extracytoplasmic region contains a unique 187-amino acid sequence (beginning at R150) which is not present in the homologous alpha subunits of fibronectin, vitronectin, or platelet IIb/IIIa receptors (Fig. 2) or in PS-2 antigen in Drosophila (Bogaert et al., 1987). Interestingly, this sequence is present in the highly homologous alpha subunit of leukocyte pl50,95 with 57% of the amino acids identical and 34% representing conserved substitutions (Fig. 2). It is known that both Molα and pl50,95 have a binding site for complement fragment iC3b (CR3 activity) (Arnaout et al., 1983; Micklem et al., 1984). It is tempting to speculate that this unique region is involved in iC3b binding. Even

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Figure 2. Alignment of the alpha subunits of human integrins Mol, p150,95 (Corbi et al., 1987), fibronectin receptor (Argraves et al., 1987), vitronectin receptor (Suzuki et al., 1987), and IIb (Poncz et al., 1987). Identical amino acids are boxed. The 187-amino acid sequence unique to leukocyte p150 and Mol is apparent. The proteolytic cleavage sites occurring in the alpha subunits of fibronectin receptor, vitronectin receptor, and IIb are indicated (arrowheads).
more intriguing is the possibility that this region may be involved in some heterotypic or homotypic adhesive interactions mediated by Leu-CAM. This is suggested by the fact that this region of Molα (residues 150–337) has significant homology (17.1% identity and 52.9% conserved substitutions) to the collagen/heparin/platelet GpI binding region (A domain) (residues 530–713) of the mature von Willebrand factor (Verweij et al., 1986; Girma et al., 1987). Thirdly, the region just NH2-terminal to the TM site contains the consensus (CXXXXXCCXX) that is conserved in all alpha chains of integrins (Fig. 2 and Bogaert et al., 1987). In these integrins, it is proposed that these cysteines are involved in the covalent binding of the heavy and light chains of the alpha subunit that are generated by proteolytic cleavage at usually dibasic sites just NH2-terminal to this sequence. It is interesting to note that no such cleavage occurs in the alpha subunit of Mol despite occurrence of a dibasic cleavage site at amino acid positions 999 and 1000. The large deletion introduced in this region to maximize homology to other integrins (Fig. 2) suggests that differences in secondary structure may explain lack of susceptibility of Mol and other Leu-CAM to proteolytic cleavage. This region (Q900–E1025) also contains an epitope—recognized by a monoclonal antibody (M26) directed against guinea pig Molα, which binds to precursor or mature monomeric Molα but does not bind to the alpha subunit after formation of the αβ complex (Remold-O’Donnell and Savage, 1988; Arnaout et al., 1988). This suggests that the M26 epitope becomes inaccessible to the antibody in the αβ complex or is involved in its formation. Fourthly, internal homology blot (not shown) (Dayhoff et al., 1983) revealed four internal tandem repeats (amino acid residues: 358–412; 426–483; 487–553; 554–614), each containing one of the four putative metal-binding sites and their flanking regions. This suggests that these regions of the alpha subunit might have arisen by tandem duplication events.

Future studies using site-directed mutagenesis should further elucidate the structural features that underlie the specific functions mediated by Mol and the other evolutionary related integrins. Given the recent findings that anti-Molα monoclonal antibodies block leukoaggregation (Arnaout et al., 1985) and reduce the size of myocardial infarct in dogs by 50% (Simpson et al., 1988), a detailed structure-function analysis of Mol may provide useful reagents for controlling disease processes characterized by neutrophil-mediated tissue injury.

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