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The cell adhesion molecule Cell-CAM 105 is an ecto-ATPase and a member of the immunoglobulin superfamily

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Cell-CAM 105 (C-CAM), a cell adhesion molecule in rat hepatocytes, was digested with trypsin, and peptides were isolated and sequenced by Edman degradation. The sequences of 4 peptides agreed with different regions of rat liver ecto-ATPase. Detailed biochemical analyses confirmed the identity between C-CAM and the ecto-ATPase. C-CAM/ecto-ATPase is a transmembrane protein having 4 immunoglobulin-like domains in the extracellular portion, demonstrating membership of the immunoglobulin superfamily. The ATPase activity suggests that ATP might influence cell adhesion, which would explain the inhibitory effect of exogenously added ATP on adhesion of several cell types.

Cell adhesion; Immunoglobulin superfamily; ATP

1. INTRODUCTION

Intercellular adhesion mediated by cell adhesion molecules (CAMs) is important in formation and maintenance of proper tissue structures. Work in recent years has demonstrated that the majority of CAMs that is involved in tissue formation belongs to two different families. The cadherin family [1] contains calcium-dependent proteins, whereas a number of calcium-independent CAMs are members of the immunoglobulin (Ig) superfamily [2]. Cell-CAM 105 (C-CAM) is a calcium-independent CAM with homophilic binding properties [3]. C-CAM mediates cell-cell adhesion of isolated rat hepatocytes [4] and is present in a variety of epithelia, vessel endothelia, granulocytes and platelets [5]. Whereas detailed chemical characterization has been reported [6], we have had insufficient sequence information to determine the relationship of C-CAM to any established CAM-family. We now report on amino acid sequence determination and chemical characterization demonstrating that C-CAM is identical to an ecto-ATPase in rat liver [7]. The protein contains 4 Ig-domains demonstrating that C-CAM belongs to the Ig-superfamily.

2. MATERIALS AND METHODS

2.1. *Peptide isolation and sequencing*

C-CAM was purified from rat liver and detergent was changed from Triton X-100 to octylglucoside as previously described [6]. The protein was dialysed against 10% 2-propanol/0.1% NH_3, lyophilized and dissolved in 8 M urea/0.1 M NH_4HCO_3. The solution was diluted with 0.1 M NH_4HCO_3 to a final concentration of 2 M urea. Recrystallized, TPCK-treated trypsin was added to an enzyme:protein ratio of 1:50 (w/w), $CaCl_2$ was added to yield 0.1 mM, and the samples were incubated for 24 h at 37°C. The digested material was fractionated by reversed-phase HPLC on a 30 × 2.1 mm Aquapore Butyl column (C_4) (Brownlee Labs Inc.) eluted with a linear 0–70% acetonitrile gradient in water/0.1% trifluoroacetic acid. Six fractions denoted 1–6 were collected and separated on a 30 × 2.1 mm Spheri-5 RP-18 column (C_{18}) (Brownlee Labs Inc.) eluted as the C_4-column. These fractions were denoted by a second number and were, if needed, finally separated on a C_{18}-column eluted with a linear 0–50% acetonitrile gradient in water/0.9% NaCl. These fractions were given a third number. Five major peptides (1.1.2; 1.6.1; 3.4.4; 4.1.5; 4.2) were pure and were sequenced on an Applied Biosystems 470A gas phase protein sequencer utilizing Edman degradation. Released PTH-amino acids were analyzed by HPLC on a Merck Licrospher C_8 column.

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2.2. *Cyanogen bromide cleavage, mild acid hydrolysis, and SDS-PAGE*

Lyophilized C-CAM was dissolved in 70% formic acid. Cyanogen bromide was added to a final concentration of 10 mg/ml. The samples were incubated for 24 h at room temperature; control incubation was done in the absence of cyanogen bromide. Mild acid hydrolysis of C-CAM was performed by incubating the protein in 70% formic acid for 48 h at 37°C. After incubation, 10 vols of water were added, the samples were lyophilized and dissolved in 4% SDS/0.01% Bromophenol blue/10% glycerol/0.0625 M Tris-HCl, pH 8.3. Reduced and alkylated samples were electrophoresed in SDS on 10% polyacrylamide gels, developed by silver staining, as previously described [6].

3. RESULTS AND DISCUSSION

C-CAM was purified from rat liver membranes by a procedure that by all applied criteria has been demonstrated to yield a pure protein [6]; see also Fig. 1, lane 1. Such purified C-CAM consists of two peptide chains of apparent molecular masses of 105000 and 110000, respectively, that by peptide mapping were

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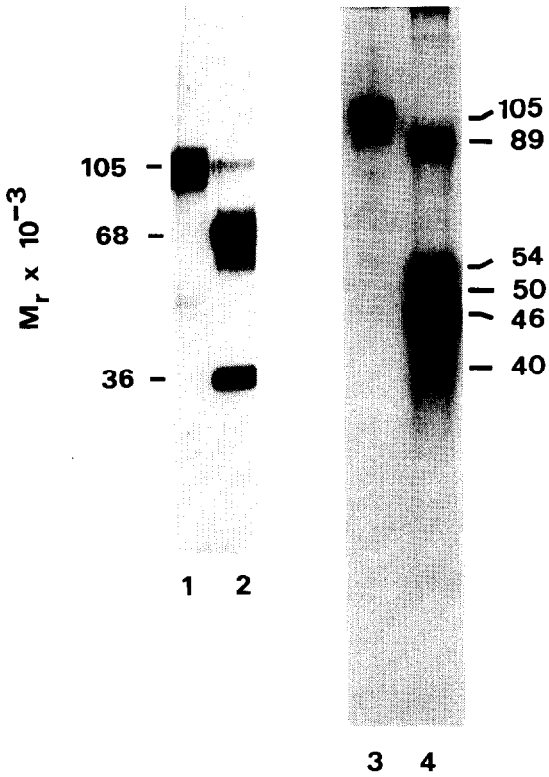

Fig. 1. SDS-PAGE of peptides derived by cyanogen bromide cleavage or mild acid treatment of purified C-CAM. The molecular masses indicated to the left refer to lanes 1 and 2, and those to the right refer to lanes 3 and 4. (Lane 1) C-CAM incubated with 70% formic acid for 24 h at room temperature. The 105 kDa component represents uncleaved C-CAM. (Lane 2) C-CAM incubated with cyanogen bromide (10 mg/ml) in 70% formic acid for 24 h at room temperature. Two major peptides of 68 kDa and 36 kDa were formed. The minor band just below the 68 kDa peptide has the expected size that would result from a partial cleavage of the Met–Cys bond at position 166–167 in the 68 kDa peptide. (Lane 3) Purified, non-treated C-CAM. (Lane 4) C-CAM incubated with 70% formic acid for 48 h at 37°C. Five major bands with mean molecular masses of 89 kDa, 54 kDa, 50 kDa, 46 kDa and 40 kDa were observed.

shown to be structurally similar [6]. The pure C-CAM was digested with trypsin and 5 peptides were isolated and sequenced. None of the sequences was found in a computer search of the NBRF protein sequence data base. Recently, however, Lin and Guidotti [7] published the deduced amino acid sequence of a cloned rat liver ecto-ATPase having biochemical properties strikingly similar to those of C-CAM. When we analyzed this sequence we found the complete sequences of 4 of the 5 C-CAM peptides represented in different parts of the 519 amino acid long ecto-ATPase (Table I). On further comparison of C-CAM and the ecto-ATPase we made the following observations, which strengthened the conclusion that these proteins are identical.

(1) Both C-CAM and the ecto-ATPase are localized to the bile canalicular membranes in adult rat liver [5,8]. (2) Both proteins have the same apparent molecular mass [6,7]. (3) C-CAM contains 33% N-linked carbohydrate [6] and chemical determination indicated that there should be approximately 15 oligosaccharides per peptide chain [9]. The ATPase has 16 potential N-glycosylation sites [7]. (4) The two peptide chains of C-CAM have apparent molecular masses of 54000 and 58000 after deglycosylation [6]. The molecular mass of the peptide chain of the ATPase is 57388 and 53410 if the leading signal sequence is removed [7]. (5) The amino acid composition of C-CAM [6] is almost identical to that calculated from the ecto-ATPase sequence. The only significant exception is glycine which amounted to 12.3 mole% in C-CAM, but only to 5.8 mole% in the ATPase. Glycine is, however, a common contaminant in amino acid determinations. (6) The amino acid composition suggests that C-CAM has 5–6 methionine residues per peptide chain [6], which would yield a maximum of 6–7 peptides after CNBr-cleavage. However, only two major peptides with apparent molecular masses of 68000 and 36000 were obtained (Fig. 1). This fits well with the amino acid sequence of the ecto-ATPase. The ATPase has 5 methionine residues including one in the N-terminal position. Of these only the Met–Glu bond at position 157–158 should be cleaved efficiently by CNBr in 70% formic acid [10]. The other bonds, a Met–Cys at position 166–167 and especially the Met–Ser at position 88–89 and the Met–Thr at position 368–369 are cleaved very inefficiently under these conditions [10]. Cleavage of the Met–Glu bond should yield two peptides with molecular masses of approximately 70000 and 30000 when glycosylation is taken into account. (7) A consistent feature of purified C-CAM is that it is readily cleaved to several fragments by mild acid treatment. After hydrolysis in 70% formic acid at 37°C for 48 h C-CAM was completely degraded, and in SDS-PAGE we observed 5 major, incompletely resolved

Table I

Comparison of the amino acid sequences of C-CAM peptides with rat liver ecto-ATPase

Peptide 4.2	
	- X - L - P - Q - E - F - Q - V - F - Y -
(57)-	N - L - P - Q - E - F - Q - V - F - Y - (66)
Peptide 4.1.5	
	- A - Y - T - L - S - V - F - D - Q - Q - F - N - P -
	- I - Q - T - X - V - Q - F -
(119)-	A - Y - T - L - S - V - F - D - Q - Q - F - N - P -
	- I - Q - T - S - V - Q - F - (138)
Peptide 3.4.4	
	- X - L - F - N - S - Q - X - L - Q - L - T -
(355)-	W - L - F - N - S - Q - S - L - Q - L - T - (365)
Peptide 1.6.1	
	- A - Y - F - L - Y -
(444)-	A - Y - F - L - Y - (448)

The sequences are written in the single letter code for amino acids. The sequence of the respective C-CAM peptide is shown in bold letters in the upper row. X indicates that the amino acid in this position could not be identified. The sequence of the ecto-ATPase [7] is given in the lower row. The amino acid positions in the 519 amino acid long ecto-ATPase are indicated on the left and right margins

bands having mean molecular masses of 89 kDa, 54 kDa, 50 kDa, 46 kDa and 40 kDa (Fig. 1). Three Asp–Pro bonds, which are sensitive to mild acid hydrolysis [10], are present in the ATPase. Cleavage of these bonds would be expected to give rise to maximally 6 glycosylated peptides with predicted molecular masses of approximately 89 kDa, 86 kDa, 53 kDa, 48 kDa, 41 kDa and 38 kDa, and 3 non-glycosylated peptides of smaller size (15 kDa, 12 kDa and 4 kDa). Given the uncertainty in the calculated molecular masses due to the glycosylation there is thus good agreement between the peptide patterns observed for C-CAM and predicted for the ATPase. (8) C-CAM can be phosphorylated on serine residues [6]. The ATPase has one potential cAMP-dependent phosphorylation site [7].

The most plausible explanation for not finding the sequence of the fifth C-CAM peptide (1.1.2) in the ecto-ATPase sequence is that this peptide was derived from the larger of the two C-CAM chains. The published ecto-ATPase sequence would then correspond to the smaller of the C-CAM chains, which is the most abundant [6]. Given the structural similarities between the two C-CAM chains it is possible that the other 4 C-CAM peptide sequences also occur in the larger C-CAM chain. If true, C-CAM would thus be analogous to the cell adhesion molecules MAG and N-CAM, which both belong to the immunoglobulin superfamily. As a result of alternative splicing 2 slightly different chains of MAG [11] and at least 5 different forms of N-CAM [2] have been found to exist.

The ecto-ATPase is the rat homologue of human biliary glycoprotein 1 (BGP1) [12]. These proteins are members of the carcinoembryonic antigen (CEA) gene family, which is a subfamily in the Ig-superfamily [13]. The ecto-ATPase (C-CAM) and BGP1 contain four Ig-domains, one V-domain and three C2-domains [7,12]. CEA has seven Ig-domains, one V-domain and six C2-domains [12]. It has recently been shown that CEA can serve as a calcium-independent cell adhesion molecule with homophilic binding properties [14]. Proteins in the CEA gene family thus seem to constitute a novel subfamily of cell adhesion molecules in the Ig-superfamily.

What is the role of the ATPase activity of C-CAM? An interesting possibility is that it might regulate the adhesive function of the molecule. It has in fact been demonstrated that exogenously added ATP inhibits cell adhesion of mouse liver cells [15] and chick embryo fibroblasts [16], whereas ADP has a stimulating activity. The triggering effect of ADP on platelet aggregation is well-known. More recently, it has been demonstrated that exogenously added ATP leads to adhesion-stimulated phosphorylation of two external proteins on HeLa cells [17], one of which has an apparent molecular mass of 105000. Furthermore, N-CAM on brain cells is probably a substrate for ATP-dependent phosphorylation by ecto-kinases [18], and contactin, a 130 kDa glycoprotein in chick interneuronal contacts, has been shown to contain a potential nucleotide-binding site in the extracellular domain [19]. It has also been demonstrated that several cell types such as platelets, granulocytes and neurons can secrete ATP [18,20,21]. In view of these observations, it seems plausible that cells might control their adhesive interactions in a dynamic manner by localized, regulated ATP-secretion. We accordingly propose that C-CAM is one of the actors in ATP-regulated cell adhesion. Interactions between the Ig-domains of C-CAM may be the structural basis for the specific homophilic binding. Binding and hydrolysis of ATP may then modify this binding in a manner similar to the ATP-regulated binding between actin and myosin. It will be interesting to see if cell adhesion molecules other than C-CAM have ecto-ATPase activity.

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