The Light Chain of CD98 Is Identified as E16/TA1 Protein*

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The 80/40-kDa CD98 protein complex was purified using an anti-CD98 heavy chain monoclonal antibody coupled to Sepharose beads. Eluted proteins were subjected to preparative SDS-polyacrylamide gel electrophoresis, and protein corresponding to the 40-kDa CD98 light chain was excised. Following proteolysis with trypsin, a peptide fragment was sequenced by mass spectrometry. The nine residues obtained were identical to established C-terminal sequences of the human E16 and rat TA1 proteins, suggesting that TA1/E16 protein is the CD98 light chain. Consistent with this, anti-TA1/E16 antibodies specifically immunoblotted the 35–40-kDa light chain present upon immunoprecipitation of the human CD98 complex. Furthermore, anti-CD98 heavy chain antibody specifically co-immunoprecipitated hemagglutinin-tagged E16 cDNA. In conclusion, the CD98 light chain is identical to the TA1/E16 protein, based on partial amino acid sequence identity, antibody cross-reactivity, genetic reconstitution evidence, similar molecular size, and comparable cell distribution.

The CD98 (4F2, FRP-1) molecule is a heterodimer of approximately 80 and 40 kDa that was originally reported as a T cell activation antigen (1). It is also expressed on normal proliferating tissue such as the basal layer of squamous epithelia (2, 3), all rapidly growing tumor cells (1, 4, 5), and on cells having secretion or transport functions (6). The CD98 protein may be involved in cell proliferation and activation (4, 7–10), cell surface orientation. Transient transfection of the open reading frame of E16 into 35 kDa was purified to homogeneity using a nickel affinity protein (28); protein (~35 kDa) was purified to homogeneity using a nickel affinity chromatography.

Preparation of E16 Transfectants—The open reading frame of E16 cDNA was N-tagged with an HA tag by polymerase chain reaction and subcloned into pLXIN vector (CLONTECH) in the sense and antisense orientation. Transient transfection of E16 Eco cells was performed by calcium phosphate transfection protocol as described (35). Briefly, 2 × 10⁶ cells/60-mm plate were transfected with 10 mg of DNA. 48 h post-transfection the cells were lysed and used for immunoprecipitation experiments.

Purification, Proteolysis, and Amino Acid Sequencing—40 μg each 24 roller bottles were washed with PBS and lysed for 90 min in 1% Triton X-100 in PBS in the presence of protease inhibitors (10 μg/ml

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**RESULTS AND DISCUSSION**

**Purification of CD98**—The T leukemic cell line Molt4 was chosen as a source for CD98 purification because it contains high levels of cell surface CD98, and it could be grown to high density in suspension culture (~3 × 10^6 cells/ml). Approximately 2 × 10^7 Molt4 cells were lysed in 1% Triton X-100, and then background binding proteins were removed by precipitation with control Sepharose preparations. Next, the lysate was incubated with Sepharose conjugated to anti-CD98 mAb 6B12. Then after washing, purified CD98 protein was eluted at low pH (pH 2.0) and fractions were collected. As indicated (Fig. 1) the majority of silver stained protein appeared in fraction 2.

This material included a prominent protein of ~40 kDa, as expected for the CD98 light chain. Also prominent were proteins corresponding to the CD98 heavy chain (~80 kDa) and possibly unreduced 80/40 complex (~120 kDa).

**Partial Sequencing of CD98 Light Chain**—Numerous attempts to obtain the N-terminal sequence from up to 50 pmol of purified CD98 light chain were unsuccessful. Likewise, we failed in initial attempts to digest CD98 light chain with chymotrypsin, trypsin, thermolysin, or ArgC proteases, even though bovine serum albumin was digested under similar conditions. However, subsequent studies revealed that significant digestion with trypsin could be achieved at pH 7.0 (not shown).

Consequently, purified CD98 light chain was digested with trypsin at pH 7.0, fragments were separated using HPLC, and from one of the purified fractions, an MS/MS ion sequence spectra was obtained that correlated with the sequence "LMQVVPQET" present in the C terminus of the E16 (29) and TA1 (28) protein sequences. Consistent with it being the product of a trypsin digestion, the LMQVVPQET sequence is preceded by lysine in the published sequences. No other sequence identity was found in a GenBank search.

**Confirmation That CD98 Light Chain Corresponds to E16/TA1 Protein**—To confirm the identity of the CD98 light chain, we first tested whether it was recognized by anti-TA1/E16 antibody in an immunoblotting experiment. The CD98 heterodimer was immunoprecipitated from human HT1080 cells, and proteins were resolved using 10% SDS-PAGE. Then, anti-E16/TA1 antibodies were shown to cross-react specifically with a protein of ~35–40 kDa, corresponding to the CD98 light chain (Fig. 2). No E16/TA1 cross-reactivity was observed in immunoprecipitates obtained using a negative control mAb or anti-major histocompatibility class I protein. In a positive control experiment, anti-E16/TA1 antibodies did show reactivity with whole cell lysate from a colon carcinoma but not from normal colon cells (Fig. 2, right panel). This agrees with previous evidence showing that E16/TA1 mRNA and protein were absent from similar specimens of normal colon but greatly up-regulated in colon carcinoma (30). In a separate experiment, immunoprecipitation of rat CD98 (using mAb B3), but not rat CD71, yielded a ~35–40-kDa protein that was again recognized by anti-E16/TA1 antibodies (not shown).

To further establish that E16/TA1 corresponds to the CD98 light chain, E16 cDNA was prepared to include an HA tag, and this cDNA was transiently expressed in human dNX-Eco cells. As indicated (Fig. 3), immunoprecipitation of the CD98 heavy chain, followed by immunoblotting with an anti-HA tag, revealed the association of E16 protein with the heavy chain of CD98. E16 protein was not present in a control immunoprecipitation obtained using anti-β1 integrin antibody A-1A5 (not shown).

The size and distribution of the E16/TA1 gene and/or protein are consistent with the expected size and distribution of the CD98 light chain. The E16/TA1 gene is predicted to code for a protein of 241 amino acids, with a size of 26.5 kDa (28, 29). Anti-TA1 polyclonal antibodies blotted proteins in the 30–40-kDa range (30), which are thus comparable with the 35–40-
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whether another light chain might be found that contributes to CD98-mediated γL transport.

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