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Biochemistry

cDNA clone for the human invariant γ chain of class II histocompatibility antigens and its implications for the protein structure

(translation *in vitro*/nucleotide sequence)

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ABSTRACT **The invariant γ chain is transitorily associated with class II histocompatibility antigens during intracellular transport. We have isolated and sequenced a cDNA clone corresponding to the human γ chain. mRNA hybridizing to the cDNA clone translated into a 33,000-dalton chain that associated specifically with class II antigen α and β chains. The γ chain consists of 216 amino acids. The two N-linked carbohydrates are attached to asparagines 114 and 120. A continuous stretch of hydrophobic and neutral amino acids occurs in positions 31–56 from the NH_2 terminus. This region seems to constitute the transmembrane portion of the polypeptide chain. The positions of the carbohydrate moieties and the putative transmembrane segment indicate that the NH_2 terminus of the γ chain resides on the cytoplasmic side of the membrane. Cell-free translations in conjunction with radiochemical amino acid sequence analyses suggest that the γ chain lacks an NH_2-terminal signal sequence.**

Human and murine class II antigens of the major histocompatibility complex consist of cell surface-expressed, membrane-integrated α and β subunits (for a review, see ref. 1). The human major histocompatibility complex contains several class II antigen loci (see refs. 2 and 3). During intracellular transport class II antigens are associated with an invariant transmembrane polypeptide (4, 5), provisionally called the γ chain.

Newly synthesized α and β chains form complexes with γ chains in the endoplasmic reticulum (6). After transport of the protein complex to the Golgi apparatus, and concomitant with terminal glycosylation, the γ chain dissociates from the α and β chains (7, 8). At least a fraction of the γ chains subsequently becomes integrated into the plasma membrane independently of the class II antigens (7).

The biological role of the γ chain is as yet unknown. It has been suggested that it may regulate the intracellular transport of the class II antigens (6, 9) and that it may prevent the formation of class II antigen hybrid molecules—i.e., molecules composed of α and β subunits coded for by different loci (7).

We here report on the isolation and characterization of a cDNA clone corresponding to the human γ chain. The nucleotide sequence of this clone, which corresponds to the entire translated portion of the γ chain mRNA suggests that (*i*) the NH_2 terminus of the γ chain resides on the cytoplasmic side of the membrane and (*ii*) the γ chain may be devoid of an NH_2-terminal signal sequence.

MATERIALS AND METHODS

Materials. The reactivities of the antisera employed for immunoprecipitation have been described elsewhere (6, 7).

Methods. The procedures for cultivation and radioactive labeling of cells, immunoprecipitation, electrophoresis, radiochemical amino acid sequence analysis, and endoglycosidase H digestion have been described in detail (10). The preparation and microinjection of oocytes was carried out according to Gurdon (11). The purification and *in vitro* translation of Raji mRNA was performed as described (6). The procedure for selection of mRNA by hybridization on nitrocellulose filters was essentially according to Ricciardi *et al.* (12). Nucleotide sequences were determined with the chemical degradation procedure (13).

Biosafety. All work involving recombinant plasmids was carried out under conditions conforming to the standards outlined in the National Institutes of Health guidelines for recombinant DNA research.

RESULTS

Isolation of a cDNA Clone Corresponding to the γ Chain. At the outset of this study antibodies against the γ chain were not available. Screening was therefore carried out by hybrid selection of mRNA and *in vitro* translation, using the apparent molecular weight as a characteristic for the γ chain. *In vitro* translation of sucrose gradient-fractionated mRNA from the lymphoblastoid cell line Raji in the presence of dog pancreas microsomes showed that mRNA coding for the γ chain occurred in the same fraction as mRNA for α and β chains. As seen in Fig. 1, lane A, a band with a molecular weight of 33,000 appeared to be associated with class II antigen α (M_r, 35,000) and β (M_r, 29,000) chains upon immunoprecipitation of translated products by use of an antiserum to class II antigen (6). In agreement with previous findings, antisera reactive with the individual α (Fig. 1, lane B) and β subunits (Fig. 1, lane C) did not coprecipitate the M_r 33,000 component (6). These data established that the mRNA of the γ chain is similar in size to those of α and β chains. Therefore, a cDNA library constructed from Raji cells, enriched for α and β mRNA (14), was screened for the occurrence of possible γ-chain clones.

Several clones likely to contain inserts corresponding to the γ chain were identified. Fig. 1, lane E, shows the translation products of mRNA hybridized to one such clone, denoted pγ-1. No less than five components were visualized. However, it seemed likely that all components were derived from mRNAs that hybridized specifically to pγ-1, since hybridization under identical conditions of the same mRNA preparation to a cDNA clone corresponding to an α chain (15) yielded only α chains as the translation product (Fig. 1, lane F). The most prominent component among the translation products, obtained after hybrid selection using pγ-1, displayed an electrophoretic mobility indistinguishable from that of coprecipitated γ chains (compare

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Abbreviation: bp, base pair(s).

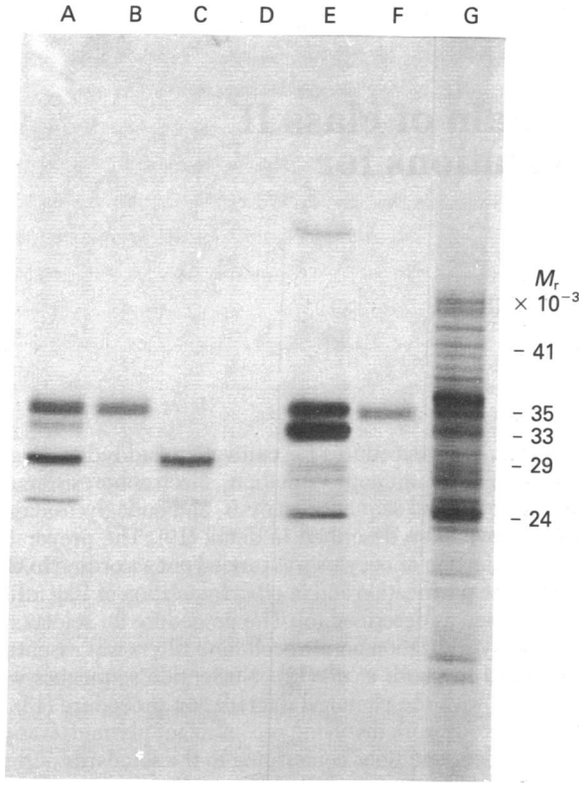

FIG. 1. *In vitro* translation of Raji mRNA. Membrane-bound mRNA was isolated from Raji cells and fractionated by sucrose gradient centrifugation. A fraction enriched for mRNA coding for α, β, and γ chains was either directly (lanes A–D and G) translated in a cell-free system in the presence of dog pancreas microsomes or selected by hybridization to the pγ-1 clone (lane E) and a DR α-chain cDNA clone (lane F) prior to translation. The translation products were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis directly (lanes E–G) or after indirect immunoprecipitation using rabbit antisera against class II antigens (K311, lane A), the α chain (K343, lane B), the β chain (K344, lane C), and a nonimmune serum (lane D). Molecular weights estimated from marker proteins run in parallel are on the right.

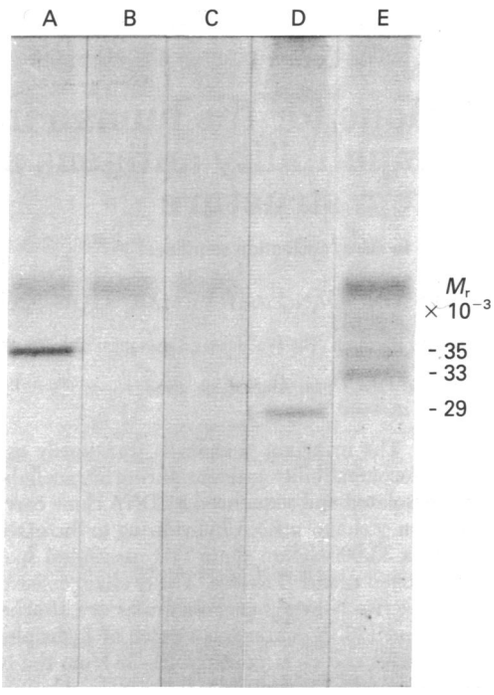

FIG. 2. Translation of hybrid-selected mRNA in microinjected oocytes. Hybrid-selected mRNA of Raji cells was isolated from plasmid DNA immobilized on nitrocellulose filters. The eluted mRNA was microinjected into *Xenopus laevis* oocytes. Translation products were obtained by immunoprecipitation and then subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis and fluorography. The plasmids used to select the mRNA were lane A, an α-chain clone; lane B, pγ-1; lanes C and D, a β-chain clone; and lane E, a mixture of α- and β-chain clones and pγ-1. Translation products were immunoprecipitated with antisera specific for the α chain (lanes A–C and E) and the β chain (lane D). The numbers denote the positions of marker α (M_r, 35,000), β (M_r, 29,000), and γ (M_r, 33,000) chains.

lanes A and E in Fig. 1). Thus pγ-1 was chosen for further characterization.

The γ chain was originally identified by its association with class II antigens (4, 5). Consequently, by use of the frog oocyte system (11), we examined whether mRNA obtained by hybrid selection using the pγ-1 clone and mRNA coding for α and β subunits, respectively, might produce complexes consisting of α, β, and γ subunits. The three mRNA preparations were microinjected individually and after mixing. After translation the oocytes were lysed and subjected to immunoprecipitation. The immunoprecipitates were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis and the results are summarized in Fig. 2. As expected, after separate microinjections of the three individual mRNA fractions, an antiserum against the α chain precipitated only this subunit (lane A) and not γ (lane B) or β subunits (lane C), whereas an antiserum against the β chain precipitated only this subunit (lane D). After microinjection and translation of a mixture of the three mRNA preparations the α-subunit antiserum not only precipitated α chains but also coprecipitated γ as well as β chains (lane E). It should be pointed out that the antiserum used coprecipitates γ chains after translation *in vivo* (16) but not *in vitro* (6). Thus, the coprecipitation with class II antigens of the most prominent of the five components obtained after hybrid selection using pγ-1 strongly suggested that this clone encodes the γ chain.

Characterization of a γ-Chain cDNA Clone. A nick-translated restriction fragment of the pγ-1 clone was used in colony hybridization. Almost 1% of the cDNA clones of the cDNA library hybridized to the probe. The cDNA clone with the longest insert was chosen for further characterization.

A restriction map of this clone, pγ-2, was constructed (Fig. 3), and the complete nucleotide sequence of the 1,287-base-pair (bp) insert was determined (Fig. 4). Only one open reading frame could account for the predicted size of the γ chain (Fig. 4). The putative initiation codon for this reading frame is located 95 bp from the left-hand end of the insert (see Fig. 3). However, another ATG codon in the same frame precedes the putative initiation codon by 45 bp. This cannot be the initiation codon because it is present in a portion of the 5′ end of the insert that consists of a 28-bp inverted repeat of the 3′ end (see Fig. 4). It seems likely that the inverted repeat is an artifact of the snapback type, generated during cDNA synthesis (17, 18), particularly since a DR α clone of the same library also displays a similar inverted repeat (K. Gustafsson, personal communication). Moreover, the second, but not the first, ATG codon is flanked by nucleotides identical to those of the consensus sequence for initiation sites (19).

The precise length of the untranslated 3′ region of the insert could not be assessed because the 540 bp ascribed to this region contains neither a poly(A) addition signal nor a poly(A) stretch (Fig. 4). Thus, like other cDNA clones of the same library (15, 20), the pγ-2 clone seems to be truncated in its 3′ end.

The translated amino acid sequence, encompassing a total of 216 residues, consists of an NH_2-terminal stretch of 30, mostly

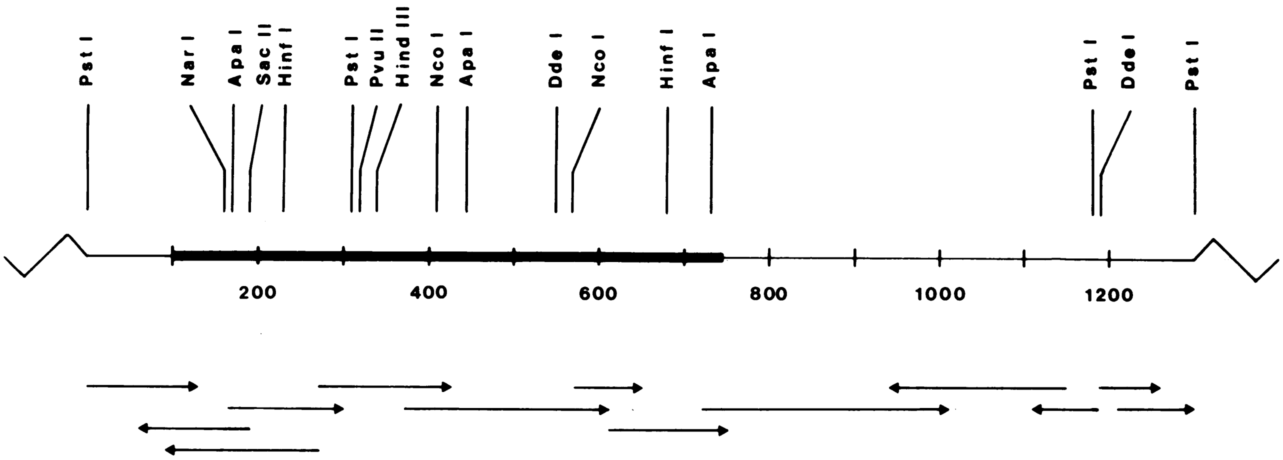

FIG. 3. Restriction map of the pγ-2 insert. The 5′ end of the coding strand is close to the *Eco*RI site in pBR322. Both *Pst* I sites were reconstituted. The thick line represents the translated portion. Arrows denote the Maxam–Gilbert sequencing strategy. The 1,287-bp sequence was confirmed by sequencing the complementary strand and by repeated sequencing of the coding strand from different restriction sites (not shown).

hydrophilic, amino acids. They are succeeded by a segment of 26 residues devoid of charged amino acids (Fig. 4). Another hydrophobic region (amino acids 93–116) also occurs in the γ chain. However, residues 31–56 most probably represent the transmembrane portion of the molecule. This can be inferred from the observation that γ chains contain two asparagine-linked carbohydrate moieties (7). Since one of the two putative carbohydrate addition sites (Asn-114) is included in the second hydrophobic stretch, it is unlikely that this region serves as the transmembrane segment. The second asparagine-linked carbohydrate must occur on Asn-120, since the protein only contains two carbohydrate addition sequences (21).

The amino acid sequence predicted from the nucleotide sequence is unusually rich in proline and methionine residues, which at least partly may explain why the γ chain becomes so much more intensely labeled than class II antigen α and β chains when [^{35}S]methionine is used (8). The sequence contains a single cysteine residue, which may be important in the interactions with proteins other than α and β subunits. The cysteine may also play a role in homodimer formation (22).

The predicted amino acid sequence of the γ chain was compared with 1,664 other protein sequences by use of the computer program SEARCH (see ref. 15). No statistically significant homology between the γ chain and any other protein sequence was observed. In separate analyses the γ-chain sequence was compared to the sequences of α and β chains of class II antigens. Also, these proteins failed to reveal any obvious relatedness to the γ chain.

The γ Chain Is Devoid of an NH_2-Terminal Signal Sequence. The predicted amino acid sequence of the γ chain derived from the nucleotide sequence of the pγ-2 clone did not contain any sequence stretch that could be easily identified as the signal sequence (for a review, see ref. 23). To further explore this issue we subjected a mRNA fraction, enriched for γ-chain mRNA by sucrose-gradient centrifugation, to cell-free translation in the presence and absence of microsomes. The

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                                                                                       Met Asp Asp Gln Arg     5
CTGCAGGGGGGGGGGGGGGGGCGGGGGG[ACATTGGCTCTTCCTTGGGGAGTGATGC]ACAGGAGGAGAAGCAGGAGCTGTCGGGAAGATCAGAAGCCAGTC [ATG] GAT GAC CAG CGC   114

Asp Leu Ile Ser Asn Asn Glu Gln Leu Pro Met Leu Gly Arg Arg Pro Gly Ala Pro Glu Ser Lys Cys Ser Arg Gly Ala Leu Tyr Thr    35
GAC CTT ATC TCC AAC AAT GAG CAA CTG CCC ATG CTG GGC CGG CGC CCT GGG GCC CCG GAG AGC AAG TGC AGC CGC GGA GCC CTG TAC ACA   204

Gly Phe Ser Ile Leu Val Thr Leu Leu Leu Ala Gly Gln Ala Thr Thr Ala Tyr Phe Leu Tyr Gln Gln Gln Gly Arg Leu Asp Lys Leu    65
GGC TTT TCC ATC CTG GTG ACT CTG CTC CTC GCT GGC CAG GCC ACC ACC GCC TAC TTC CTG TAC CAG CAG CAG GGC CGG CTG GAC AAA CTG   294

Thr Val Thr Ser Gln Asn Leu Gln Leu Glu Asn Leu Arg Met Lys Leu Pro Lys Pro Pro Lys Pro Val Ser Lys Met Arg Met Ala Thr    95
ACA GTC ACC TCC CAG AAC CTG CAG CTG GAG AAC CTG CGC ATG AAG CTT CCC AAG CCT CCC AAG CCT GTG AGC AAG ATG CGC ATG GCC ACC   384

Pro Leu Leu Met Gln Ala Leu Pro Met Gly Ala Leu Pro Gln Gly Pro Met Gln [Asn Ala Thr] Lys Tyr Gly [Asn Met Thr] Glu Asp His   125
CCG CTG CTG ATG CAG GCG CTG CCC ATG GGA GCC CTG CCC CAG GGG CCC ATG CAG AAT GCC ACC AAG TAT GGC AAC ATG ACA GAG GAC CAT   474

Val Met His Leu Leu Gln Asn Ala Asp Pro Leu Lys Val Tyr Pro Pro Leu Lys Gly Ser Phe Pro Glu Asn Leu Arg His Leu Lys Asn   155
GTG ATG CAC CTG CTC CAG AAT GCT GAC CCC CTG AAG GTG TAC CCG CCA CTG AAG GGG AGC TTC CCG GAG AAC CTG AGA CAC CTT AAG AAC   564

Thr Met Glu Thr Ile Asp Trp Lys Val Phe Glu Ser Trp Met His His Trp Leu Leu Phe Glu Met Ser Arg His Ser Leu Glu Gln Lys   185
ACC ATG GAG ACC ATA GAC TGG AAG GTC TTT GAG AGC TGG ATG CAC CAT TGG CTC CTG TTT GAA ATG AGC AGG CAC TCC TTG GAG CAA AAG   654

Pro Thr Asp Ala Pro Pro Lys Glu Ser Leu Glu Leu Glu Asp Pro Ser Ser Gly Leu Gly Val Thr Lys Gln Asp Leu Gly Pro Val Pro   215
CCC ACT GAC GCT CCA CCG AAA GAG TCA CTG GAA CTG GAG GAC CCG TCT TCT GGG CTG GGT GTG ACC AAG CAG GAT CTG GGC CCA GTC CCC   744

Met                                                                                                                 216
ATG [TGA] GAGCAGCAGAGGCGGTCTTCAACATCCTGCCAGCCCCACACAGCTACAGCTTTCTTGCTCCCTTCAGCCCCCAGCCCCTCCCCCATCTCCCACCCTGTACCTCATCCCATGA   862

GACCCTGGTGCCTGGCTCTTTCGTCACCCTTGGACAAGACAAACCAAGTCGGAACAGCAGATAACAATGCAGCAAGGCCCTGCTGCCCAATCTCCATCTGTCAACAGGGGCGTGAGGTCC   982

CAGGAAGTGGCCAAAAGCTAGACAGATCCCCGTTCCTGACATCACAGCAGCCTCCAACACAAGGCTCCAAGACCTAGGCTCATGGACGAGATGGGAAGGCACAGGGAGAAGGGATAACCC   1102

TACACCCAGACCCCAGGCTGGACATGCTGACTGTCCTCTCCCCTCCAGCCTTTGGCCTTGGCTTTTCTAGCCTATTTACCTGCAGGCTXAGCCACTCTCTTCCCTTTCCCCAGCATCACT   1222

CCCCAAGGAAGAGCCAATGTTTTCCACCCATCCCTCCCCCCCCCCCCCCCCCCCCCCCCCTGCAG   1287
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FIG. 4. Nucleotide sequence of the pγ-2 insert and its predicted amino acid sequence. The nucleotide sequence contains a 28-bp inverted repeat (within brackets) of the 3′ end. Boxes, in order of appearance, denote the putative initiation codon, the two carbohydrate addition sites, and the stop codon. Amino acid residues forming the putative transmembrane region are underlined. The middle base of the *Dde* I site, position 1,191, has subsequently been determined to be G.

translation products were immunoprecipitated by using a recently established monoclonal antibody against the γ chain (unpublished results), mock-incubated or treated with endoglycosidase H, and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis. As a control in these experiments a mRNA fraction enriched for class II antigen α chains was examined under identical conditions.

Fig. 5 shows that the antibodies precipitated a core-glycosylated γ chain with an apparent molecular weight of 33,000 after translation in the presence of microsomes (lane A). This molecular weight was reduced to approximately 26,000 after endoglycosidase H treatment (lane B). Fig. 5 demonstrates that core-glycosylated α chains (lane E) also display a diminished apparent molecular weight after endoglycosidase H digestion (lane F). Translation in the absence of microsomes yielded α chains slightly larger than the endoglycosidase H-treated counterparts (compare lanes F and G), consistent with the signal sequence being cleaved off by the microsomes (24). In contrast to the α chains, γ chains translated in the absence of microsomes (lane C) are smaller than the endoglycosidase H-digested γ chains (lane B). This result is compatible with γ chains being devoid of an NH_2-terminal signal sequence that is cleaved off in microsomes. However, to account for the increased electrophoretic mobility of the γ chain after translation in the absence of microsomes, one would have to assume that the γ chain becomes post-translationally modified also in other respects than the asparagine-linked glycosylation. In fact, preliminary data suggest that γ chains undergo *O*-glycosylation (7), which decreases their electrophoretic mobility. Whether such modifications can be accomplished by the crude microsomal fraction used is a matter of conjecture.

To further examine whether γ chains lack an NH_2-terminal signal sequence, γ chains were labeled with [^{35}S]methionine and [^{3}H]leucine, respectively, by cell-free translation in the presence and absence of microsomes. After immunoprecipitation and $NaDodSO_4$/polyacrylamide gel electrophoresis the isolated γ chain was subjected to automatic amino acid sequence analysis. Regardless of whether the γ chain had been translated in the presence or absence of microsomes, [^{35}S]methionine occurred in positions 1 and 16 and [^{3}H]leucine in positions 7, 14, and 17 (not shown), in complete agreement with the amino acid sequence predicted from the nucleotide sequence (see Fig. 4). Consequently, γ chains translated in the presence and absence of microsomes display identical NH_2-terminal sequences. This suggests that the γ chain is devoid of the usual type of NH_2-terminal signal sequence that is cleaved off during transfer across the membrane of the endoplasmic reticulum.

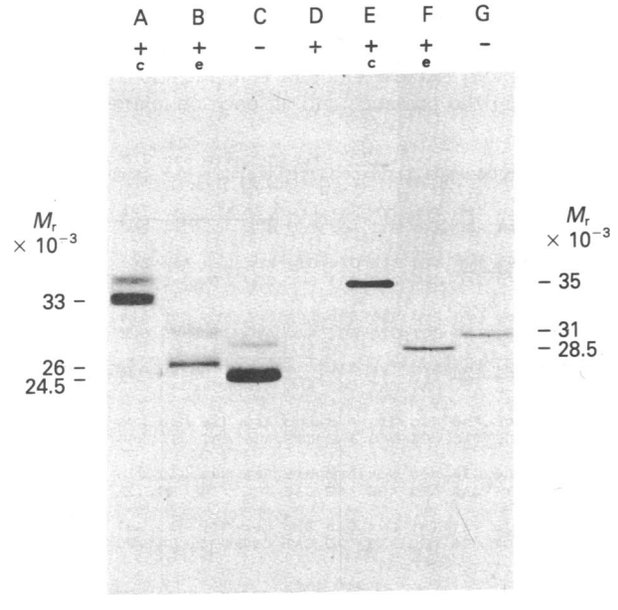

FIG. 5. *In vitro* translation of mRNA in the presence and absence of dog pancreas microsomes. Sucrose gradient-fractionated mRNA was translated in the presence (+) and absence (−) of dog pancreas microsomes. The translation products were immunoprecipitated by using the monoclonal antibody against the γ chain (lanes A–C), nonimmune rat immunoglobulin (lane D), and rabbit antiserum against the α chain (lanes E–G). Prior to $NaDodSO_4$/polyacrylamide gel electrophoresis and fluorography, immunoprecipitates of proteins translated in the presence of microsomes were mock-incubated (c) or incubated with endoglycosidase H (e).

DISCUSSION

Several lines of evidence demonstrate that the isolated cDNA clones pγ-1 and pγ-2 correspond to the human γ chain. First, hybridization to pγ-1 selects mRNA species that upon translation give rise to a prominent component whose electrophoretic behavior is indistinguishable from that of the γ chain. However, other mRNA species coding for polypeptide chains with apparent molecular weights of 24,000, 29,000, 35,000, and 41,000 also hybridized to pγ-1. The appearance of the latter molecules may have been the result of cross-hybridization or, less likely, contamination. Similar components have been shown to coprecipitate with murine (22) and human (unpublished observation) γ chains. The relatedness of these extraneous molecules to α, β, and γ chains requires further investigation.

Second, mRNA selected by hybridization using the pγ-1 clone translated into a M_r 33,000 protein that specifically associated with class II antigen α and β chains. It should be noted that the M_r 33,000 γ chain was the only one of the five translation products generated from the hybrid-selected mRNA that could be coprecipitated with class II antigens.

Third, the pγ-2 clone could be specifically hybridized to an independently isolated cDNA clone corresponding to the murine invariant chain. Thus, the data obtained clearly identify pγ-1 and pγ-2 as cDNA clones corresponding to the γ chain.

It was previously shown that the γ chain is a transmembrane protein and that approximately 3,000 daltons of its polypeptide chain reside on the cytoplasmic side of the membrane (6). Unlike α and β chains, the γ chain does not contain a stretch of hydrophobic amino acids in the COOH-terminal region. In fact, the only stretch of hydrophobic amino acid residues likely to serve as transmembrane segment occurs close to the NH_2 terminus of the protein. Because this segment of 26 residues is preceded by 30 mostly hydrophilic amino acids—i.e., the expected size of the cytoplasmic portion of the γ chain (6)—it seems highly likely that the γ chain has its NH_2 terminus on the cytoplasmic side of the membrane. This information, together with the distribution of the asparagine-linked carbohydrate moieties, suggests that the γ chain has a reversed membrane orientation as compared to class II antigen α and β chains (see Fig. 6). The proposed orientation of the γ chain is not unique. For example, isomaltase, a transmembrane protein, displays the identical orientation (23).

Class II antigen α and β chains contain NH_2-terminal regions that include signal sequences that serve to direct the synthesis of the nascent chain across the membrane of the endoplasmic reticulum (ref. 25; for a review see ref. 24). Usually, the signal sequence is cleaved off by an enzyme residing on the lumenal side of the membrane of the endoplasmic reticulum (24). Provided that the 30 most NH_2-terminal residues of the γ chain occur on the cytoplasmic side of the membrane, it is hard to conceive how they could encompass the signal sequence. In

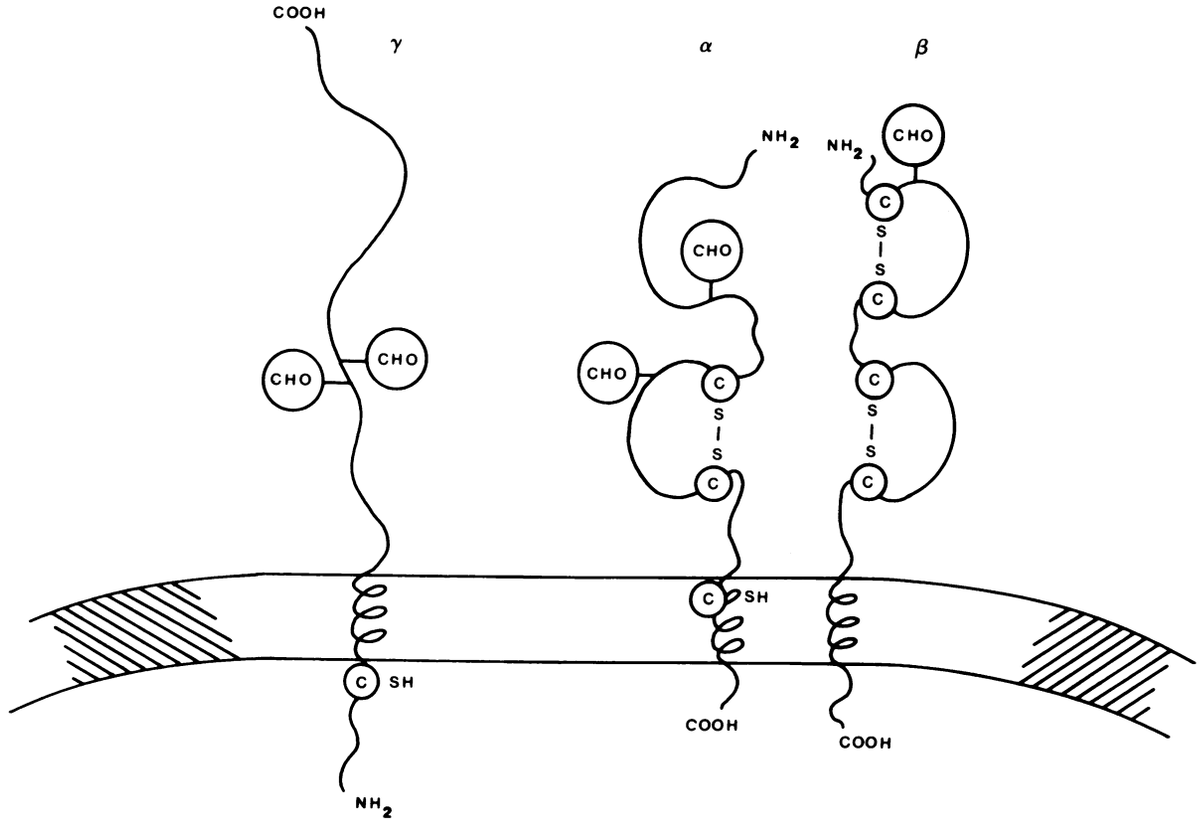

FIG. 6. Proposed membrane orientation of α, β, and γ chains. Cysteines (C) and asparagine-linked carbohydrate moieties (CHO) are indicated. The nonglycosylated tails reside on the cytoplasmic side of the membrane.

fact, cell-free translation of the γ chain in the presence and absence of microsomes in conjunction with radiochemical amino acid sequence analyses strongly indicate that the γ chain does not contain an NH_2-terminal signal sequence. This is not unprecedented, since Lingappa *et al.* (26) demonstrated that ovalbumin, a secreted protein, contains a signal sequence that is not NH_2 terminal but is an integral part of the mature protein. In view of the hydrophobic nature of signal sequences in general, it is suggested that the transmembrane segment of the γ chain may serve two functions: (*i*) it anchors the protein in the lipid bilayer, and (*ii*) it serves as a signal sequence.

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