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Evidence for hydrophobic region within heavy chains of mouse B lymphocyte membrane-bound IgM

(membrane immunoglobulin/hydrophobicity/detergent binding/tunicamycin/glycosylation)

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ABSTRACT **The gel filtration behavior, in the presence of detergents, of membrane-bound IgM from normal mouse spleen B lymphocytes was compared to that of secretory IgM from mouse plasma cells. The proteins were labeled either by surface radioiodination or biosynthetically with radioactive amino acids. Cell lysates were fractionated on calibrated Sepharose 6B columns in the presence of the detergents Nonidet P-40 or deoxycholate. Eluted fractions were immunoprecipitated and the reduced or unreduced precipitates were analyzed by sodium dodecyl sulfate gel electrophoresis followed by radioautography. Surface ^{125}I-labeled 8S IgM exhibited a gel filtration pattern in Nonidet P-40 corresponding to much higher apparent molecular weight than that of secretory 8S IgM, a difference that almost disappeared when gel filtration was performed in the presence of deoxycholate, which forms much smaller micelles than does Nonidet P-40. Biosynthetically labeled lymphocytes contain two types of IgM molecules differing in their gel filtration behavior and fate: one identical to secretory 8S IgM of plasma cells and secreted in the medium during chase periods, and the other identical to surface ^{125}I-labeled IgM and remaining cell-associated. Because the surface-bound 8S IgM was not found to be associated with other labeled molecules, it is likely that the detergent-binding behavior of surface IgM is due to a hydrophobic segment carried by these Ig molecules. That lymphocytes synthesize two types of μ chains was also shown by the use of tunicamycin, an inhibitor of glycosylation. In its presence, two unglycosylated μ chains were observed: one identical in size to that made by tunicamycin-treated plasma cells, and the second slightly larger. Gel filtration in Nonidet P-40 of the cell lysates of tunicamycin-treated lymphocytes showed that the nonsecretory 8S IgM contains this second type of μ chains, whereas the IgM molecules of the secretory type contain plasma cell-like μ chains. It is suggested that membrane IgM μ chains contain a hydrophobic segment which is responsible for its association to the membrane.**

In contrast to the 19S IgM molecules circulating in the plasma, the 8S IgM molecules found on the surface of B lymphocytes are in a hydrophobic environment—namely, the cell membrane. Their means of association to the cell membrane is as yet unresolved. Membrane-bound surface IgM might be bound to an integral membrane molecule acting as a carrier, or it might itself possess an extra hydrophobic segment, allowing its association with the membrane (1).

Recent studies, including our own, have shown that membrane-bound μ chains appear to be slightly larger than secreted μ chains, as judged by sodium dodecyl sulfate ($NaDodSO_4$) gel electrophoresis (2–5). This difference might be due to a difference in carbohydrate content or in the polypeptide backbone. Recently, we reported (5) that lymphocytes treated with tunicamycin, an inhibitor of the transfer of "core" sugars to the asparagine residues of glycoproteins (6), synthesize an apparently unglycosylated μ chain that is larger than unglycosylated μ chains from plasma cells, suggesting a difference in the polypeptide chain.

To find out whether surface μ chains have detergent-binding properties suggestive of the existence of a hydrophobic region (7), the gel filtration behavior of surface and secretory 8S IgM molecules in the presence of nonionic and ionic detergents was compared. The effect of tunicamycin treatment on this behavior was also studied. These data strongly suggest that membrane-bound 8S IgM is made up of μ chains that differ from those of secretory IgM and possesses a hydrophobic domain. Evidence for detergent binding of surface IgM has also been reported by others using entirely different techniques (8, 9).

MATERIALS AND METHODS

Spleen cells were obtained from BALB/c, C57, or (C57 × C3H)F_1 mice. Surface radioiodination of spleen cells by the lactoperoxidase method was performed as described (10). Extracellular or intracellular internally labeled plasma cell chains used as markers were obtained from an *Escherichia coli* lipopolysaccharide-stimulated culture of spleen cells used on day 3 of culture, when they contain up to 50% IgM and a few IgG plasma cells (11), or from MOPC 21 myeloma cells (gift from M. Potter, National Institutes of Health) secreting IgG. Purified populations of lymphocytes were obtained by selection on sucrose gradients (12); in each preparation, the IgM plasma cell contamination, studied by immunofluorescence on dense cytocentrifuged smears, was judged to be below 1:10,000 cells. Plasma cells and lymphocytes were labeled at 5×10^6 cells per ml in Dulbecco's modified Eagle's medium lacking methionine and containing 10% dialyzed fetal calf serum (GIBCO) and 50 or 100 μCi (1 Ci = 3.7×10^{10} becquerels) of [^{35}S]methionine (500–1000 Ci/mmol; Radiochemical Center, Amersham, England). For chase periods, the cells were centrifuged and reincubated in a medium containing a 5-fold methionine excess. At the end of all incubation procedures, the cells were washed three times in Hanks' balanced salt solution and lysed in 50 mM Tris·HCl, pH 7.4/25 mM KCl/5 mM $MgCl_2$/0.15 M NaCl/3 mM tosyl-L-lysyl chloromethane·HCl/3 mM tosyl-L-arginine methyl ester·HCl/3 mM phenylmethylsulfonyl fluoride (Sigma)/soybean trypsin inhibitor at 1 μg/ml (Boehringer Mannheim)/10 mM iodoacetamide/0.5% Nonidet P-40 (NP-40) (Fluka AG). The lysate was spun for 30 min at 100,000 × *g*. Details concerning gel fractionation, immunoprecipitation, and polyacrylamide gel electrophoresis are given in the legends of figures. All gels not containing exclusively ^{125}I were treated for fluorography (13).

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Abbreviations: $NaDodSO_4$, sodium dodecyl sulfate; NP-40, Nonidet P-40.

RESULTS

Gel Filtration Profile of Membrane-Bound IgM in Presence of Detergent Suggests Hydrophobic Behavior. To calibrate the column of Sepharose 6B equilibrated in 0.5% NP-40, [^{14}C]leucine-labeled secreted mouse polyclonal IgM and IgG or lysates of IgM plasma cells pulsed with [^{35}S]methionine and containing various forms of intracellular IgM molecules (mainly 19S pentamers, 8S monomers, and half-monomers) were used. Fractions eluted from the column were immunoprecipitated with rabbit IgG anti-mouse Ig or anti-mouse μ chains, and the immunoprecipitates were analyzed, with or without reduction, on NaDodSO$_4$/polyacrylamide gels. The peaks of elution of the [^{14}C]leucine-labeled 19S IgM and IgG corresponded to about fraction 26 and fraction 39, respectively (Fig. 1*A*). ^{14}C-Labeled IgG secreted by myeloma MOPC 21 cells had the same elution pattern as polyclonal IgG but, in reduced precipitates, their L chains had a much retarded migration compared to polyclonal L chains. This characteristic behavior is useful because ^{14}C-labeled MOPC 21 IgG may be added as an internal marker to various labeled cell lysates (see below) and the peculiar mobility of their L chains on reduced gels allows their easy identification among polyclonal Ig molecules. The elution profile of various forms of intracellular IgM was studied with mouse IgM plasma cells pulsed with [^{35}S]methionine under conditions such that various stages in the assembly pathway to 19S pentamers are present within the cells (11). Intracellular 19S IgM eluted similarly to secreted IgM; 8S IgM monomers had a peak of elution close to that of IgG (around fraction 37) and half-monomers eluted around fraction 44 (not seen in the picture) (Fig. 1*B*).

To explore the gel filtration pattern of membrane-bound IgM, the surface of mouse spleen cells was radioiodinated by the lactoperoxidase method, and the cell lysate was placed on the same calibrated Sepharose 6B column. Analysis of the immunoprecipitated unreduced fractions showed that surface IgM 8S monomers have an elution pattern different from that of intracellular secretory 8S IgM, with an elution peak around fraction 30 (Fig. 1*C*). The labeled molecules with a similar pattern of elution but with a NaDodSO$_4$ migration intermediary between 8S IgM and IgG are IgD molecules, as shown by their absence from immunoprecipitates with monospecific anti-μ, anti-α, and anti-γ antibodies.

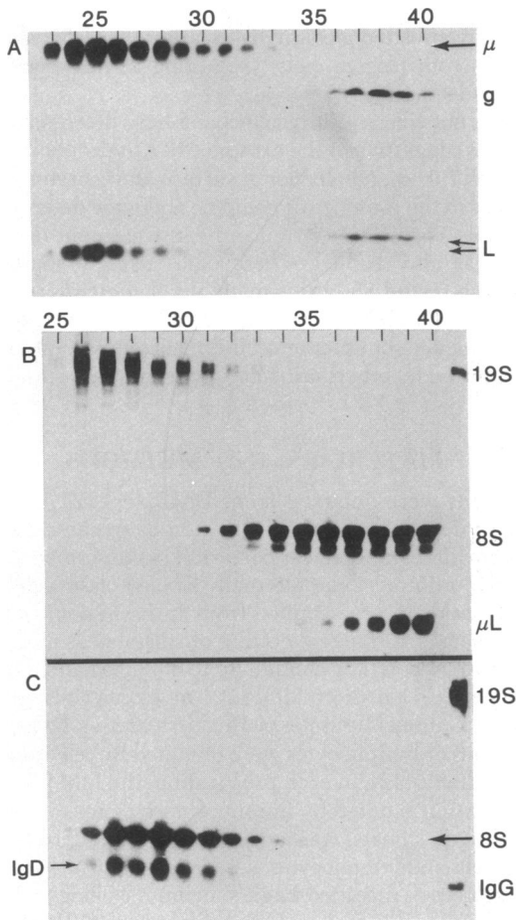

FIG. 1. Radioautography of NaDodSO$_4$ gels of fractions eluted from a column of Sepharose 6B in NP-40. The column (100 × 1.5 cm) was equilibrated in 0.5% NP-40/10 mM Tris·HCl, pH 8.0/1 mM EDTA/0.02% NaN_3 and loaded with 0.4 ml of cell lysate or supernatants in 0.5% NP-40; elution was at a flow rate of 5 ml/hr and 15-min fractions were collected. Fractions were immunoprecipitated with a rabbit anti-mouse Ig or anti-μ chain IgG fraction followed by *Staphylococcus aureus* and treated for gel electrophoresis as described (10). (Fraction numbers are at top.) (*A*) Mixture of polyclonal ^{14}C-labeled secreted IgM and secreted MOPC 21 IgG. Each column fraction was reduced and analyzed on a 17.5% NaDodSO$_4$ gel (14). (*B*) Column loaded with the cell lysate of plasma cells labeled for 10 min with [^{35}S]methionine. Each immunoprecipitated fraction was analyzed in an unreduced form on a 2.5–10% gradient NaDodSO$_4$/borate gel prepared according to a slight modification (11) of the procedure described by Pharmacia. Part of the precipitate of tube 25 was lost. The molecules migrating between 19S and 8S IgM monomers are incomplete polymers, and the molecules migrating immediately below the 8S IgM, observed only after short pulses, are μ_2L molecules (11). (*C*) Column was loaded with the cell lysate of surface ^{125}I-labeled spleen cells and the immunoprecipitates were analyzed without reduction in the gradient gel described above.

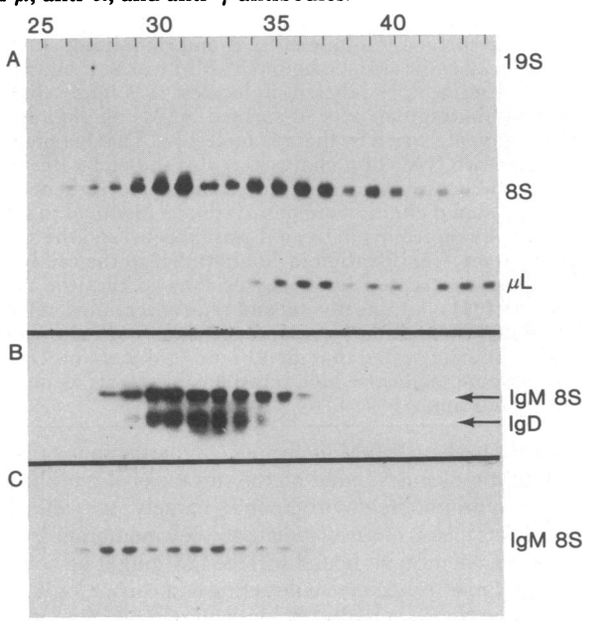

FIG. 2. Radioautography of NaDodSO$_4$/borate gradient gels of immunoprecipitated unreduced fractions eluted from the column of Sepharose 6B in NP-40. (Fraction numbers are at top.) (*A*) Column loaded with a lysate of lymphocytes labeled for 1 hr with [^{35}S]methionine. Fractions were immunoprecipitated with a rabbit anti-μ IgG fraction. (*B*) Column loaded with a lysate of lymphocytes labeled for 2 hr with [^{35}S]methionine and chased for 2 hr in cold medium. Fractions were precipitated with a rabbit anti-mouse Ig IgG fraction. (*C*) Column loaded with a lysate of lymphocytes labeled for 18 hr with [^{14}C]leucine and [^{14}C]valine (at a concentration of 2×10^6 cells and 2 μCi of each precursor per ml) and chased for 2 hr in cold medium. At the end of the incubation, dead cells were removed according to Davidson and Parish (15). No other labeled molecule was found on the lower part of this gel; on the corresponding 17.5% gel analyzing the reduced precipitate, only μ and L chains were seen. Fractions were precipitated with a rabbit anti-μ IgG fraction.

The gel filtration pattern of biosynthetically labeled Ig molecules present in the cell lysate of purified spleen lymphocytes was then examined after various periods of labeling. After precipitation of the various fractions with a rabbit anti-μ IgG fraction, the lysate of lymphocytes labeled for 60 min with [^{35}S]methionine revealed the presence of 8S IgM in a wide range of fractions (Fig. 2*A*). Half-monomers also exhibited a wide distribution. In addition, a small amount of 19S IgM was observed. This elution profile suggests that the lymphocyte populations actually synthesized two types of IgM molecules: one for secretion, whose gel filtration behavior is identical to that of plasma cell IgM, and another one destined for the cell surface, which is apparently indistinguishable from surface ^{125}I-labeled IgM. Gel filtration of lysates of lymphocytes pulsed for 2 hr with [^{35}S]methionine and then chased for 2 hr showed an elution pattern of 8S IgM similar to that observed with ^{125}I-surface labeled cells (i.e., with a peak around fraction 30); in addition, Ig molecules migrating on the gel between 8S IgM and IgG were also detected (Fig. 2*B*). Under these conditions of chase, 19S IgM are recovered in the incubation medium whereas, as shown elsewhere (5), the 8S IgM molecules remaining associated with the labeled lymphocytes are indeed localized on the cell surface because they disappear after Pronase treatment of the intact cells before lysis. Similarly, cell lysates of lymphocytes labeled for 18 hr in the presence of [^{14}C]leucine and [^{14}C]valine, followed by a 2-hr chase, showed, after gel fractionation and precipitation of the fractions with an anti-μ antiserum, an elution pattern consisting of 8S IgM molecules with a peak at fraction 30 (Fig. 2*C*); no other labeled molecules that could be associated with these 8S IgM and thus coprecipitated with the anti-μ antiserum were detected on the radioautography of unreduced (Fig. 2*C*) or reduced (not shown) $NaDodSO_4$ gels, suitable for the detection of small labeled molecules (2000–5000 daltons).

Cell lysates from plasma cells pulsed with [^{35}S]methionine (containing, as shown above, 19S IgM, 8S monomers, and half-monomers) and from ^{125}I-surface labeled spleen cells were then fractionated on a column of Sepharose 6B in the presence of 0.5% deoxycholate. Comparison of Fig. 3 *A* and *B* shows that ^{125}I-labeled surface 8S IgM (and ^{125}I-labeled IgD) have a gel filtration behavior close to that of secretory 8S IgM. To emphasize that ^{125}I-labeled surface 8S IgM (and ^{125}I-labeled IgD) have a gel chromatographic behavior in deoxycholate different from that found in NP-40, cell lysates of surface ^{125}I-labeled cells were mixed before gel filtration with small amounts of [^{14}C]leucine-labeled "internal" markers, either 19S IgM or 8S IgG. On the NP-40 column, the ^{125}I-labeled 8S IgM eluted slightly later than 19S IgM (Fig. 4*A*) and markedly ahead of IgG (Fig. 4*C*), whereas on the deoxychloate column, the ^{125}I-labeled 8S IgM eluted markedly later than 19S IgM (Fig. 4*B*) but slightly ahead of IgG (Fig. 4*D*). Fig. 4*C* shows that trace amounts of ^{125}I-labeled IgG can probably be detected under the IgD molecules; the reduced gel (not shown) indicated that this is not due to some aggregated MOPC 21 IgG molecules, because these molecules can be easily identified by the retarded migration of their L chains.

Two Unglycosylated Lymphocyte μ Chains Are Synthesized in the Presence of Tunicamycin and Are Assembled into IgM Molecules with Different Gel Filtration Behavior in Detergent. We have reported (5) that murine polyclonal plasma cells are very sensitive to the action of the drug [i.e., they

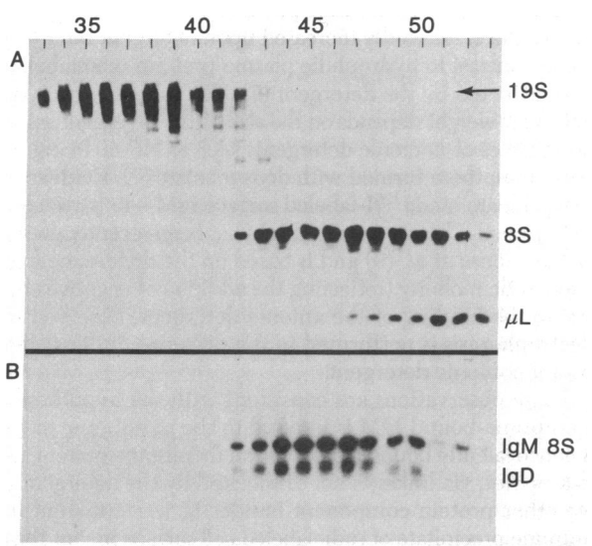

FIG. 3. Radioautography of $NaDodSO_4$/borate gradient gels of fractions eluted from a column of Sepharose 6B in 0.5% deoxycholate. A column (100 × 1.5 cm) of Sepharose 6B, equilibrated in 0.5% deoxycholate/10 mM Tris·HCl, pH 8.0/1 mM EDTA/0.02% NaN_3/0.15 M NaCl was loaded with 0.4 ml containing 0.2 ml of cell lysate in 0.5% NP-40 and 0.2 ml of 5% deoxycholate. Elution, immunoprecipitation with rabbit anti-mouse Ig IgG fraction, and gel electrophoresis were as described in Fig. 1. (*A*) Column loaded with the cell lysate of plasma cells pulsed for 10 min with [^{35}S]methionine followed by a 10-min chase in unlabeled medium. (*B*) Column loaded with the cell lysate of surface ^{125}I-labeled spleen cells.

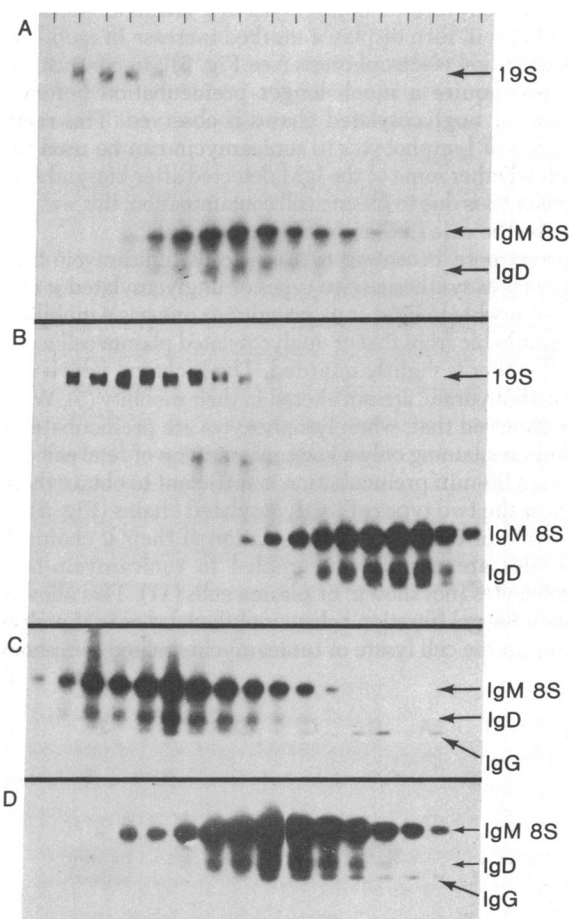

FIG. 4. Radioautography of $NaDodSO_4$/borate gradient gels of fractions eluted from Sepharose 6B columns in NP-40 (*A* and *C*) or deoxycholate (*B* and *D*) and immunoprecipitated with a rabbit anti-mouse Ig IgG fraction. In all cases, cell lysates from ^{125}I-labeled spleen cells were placed on the column, mixed with internal markers (in *A* and *B*, ^{14}C-labeled secreted polyclonal IgM; in *C* and *D*, ^{14}C-labeled secreted MOPC 21 IgG).

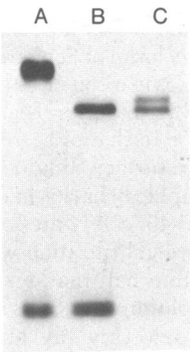

FIG. 5. Autoradiography of $NaDodSO_4$ gels (17.5%) of immunoprecipitated and reduced mouse μ chains. Lane A: supernatant from plasma cells labeled with [^{35}S]methionine for 2 hr, showing the mobility of secreted μ and L chains. Lane B: lysate from plasma cell preincubated for 60 min with tunicamycin (1 μg/ml; lot 361-26 E-117, gift of R. Hamill, Eli Lilly) followed by 2-hr incubation with [^{35}S]-methionine and tunicamycin. This shows the mobility of unglycosylated μ chains and the unchanged mobility of L chains. Lane C: lysate from lymphocytes preincubated for 90 min with tunicamycin (1 μg/ml) in the presence of 0.1% fetal calf serum, then incubated for 2 hr with [^{35}S]methionine in the same conditions. The faster of the two μ chains had a mobility identical to that of tunicamycin-treated plasma cells.

require only a short preincubation with tunicamycin before biosynthetically labeled unglycosylated μ chains are made, and these chains in turn display a marked increase in mobility on $NaDodSO_4$ gel electrophoresis (see Fig. 5)]; in contrast, lymphocytes require a much longer preincubation before the synthesis of unglycosylated chains is observed. This relative resistance of lymphocytes to tunicamycin can be used to ascertain whether some of the IgM detected after biosynthesis by lymphocytes is due to plasma cell contamination; this was found not to be the case in the present experiments.

Furthermore, in contrast to plasma cells, tunicamycin-treated lymphocytes synthesize two types of unglycosylated μ chains that are unable to bind concanavalin A: one has a mobility indistinguishable from that of unglycosylated plasma cell μ chains and the other is slightly retarded. The L chains, which do not bear carbohydrate, are not altered in their mobility (5). We now have observed that, when lymphocytes are preincubated in a medium containing only a low concentration of fetal calf serum (0.1%), a 90-min preincubation is sufficient to obtain the synthesis of the two types of unglycosylated chains (Fig. 5).

In spite of the lack of glycosylation of their μ chains, IgM molecules are normally assembled in tunicamycin-treated lymphocytes (not shown) or plasma cells (11). This allows one to study the gel filtration behavior of the labeled IgM molecules present in the cell lysate of tunicamycin-treated lymphocytes

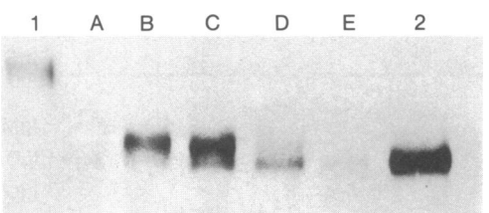

FIG. 6. Autoradiography of $NaDodSO_4$ gels (17.5%) of reduced anti-mouse μ chain immunoprecipitates obtained from fractions of the Sepharose 6B NP-40 column loaded with a cell lysate from [^{35}S]methionine-labeled tunicamycin-treated lymphocytes. Lanes: 1 and 2, "markers," representing secreted μ chains and unglycosylated μ chains from tunicamycin-treated plasma cells, respectively; A–E, fractions eluted from the column pooled as described in the text.

incubated for 2 hr with [^{35}S]methionine. The unreduced immunoprecipitates of each fraction showed a pattern similar to that illustrated in Fig. 2A; however, these molecules displayed a somewhat faster mobility on the gel than did their normal counterpart, due to the lack of glycosylation of their μ chains. An aliquot of each of these precipitates was saved, pooled in five fractions, and, after reduction, subjected to $NaDodSO_4$ gel electrophoresis and compared to reduced precipitates of labeled IgM obtained from normal or tunicamycin-treated lymphocytes (Fig. 6). Fraction A (corresponding to fractions 24–27, containing 19S IgM) showed unglycosylated μ chains of the "secretory" plasma cell type; fraction B (pool of fractions 28–32, with 8S monomers) showed only the more retarded unglycosylated μ chains; fraction C (pool of fractions 33–37, with 8S monomers and half-monomers) showed a mixture of the two chains, with a predominance of the more retarded species; fractions D (pool of fractions 38–41, with 8S and half-monomers) and E (pool of fractions 42–44, with half-monomers only) showed only the unglycosylated μ chains of the secretory plasma cell type (Fig. 6). These data are compatible with the assumption that the lymphocyte membrane-bound 8S IgM monomers, which elute very early in gel filtration in the presence of NP-40, are made of species of μ chains distinct from that of the IgM molecules characterized by an elution pattern similar to that of plasma cell intracellular 8S IgM.

DISCUSSION

The data presented here show that (*i*) the gel filtration behavior of surface ^{125}I-labeled normal mouse spleen cell 8S IgM in the presence of the nonionic detergent NP-40 is different from that of plasma cell intracellular 8S IgM, thus indicating that membrane-bound IgM displays under these conditions a much higher apparent molecular weight than does secretory IgM; (*ii*) in the presence of the ionic detergent deoxycholate, this difference in apparent molecular weight almost entirely disappears. This situation is reminiscent of protein molecules containing a hydrodrophobic domain (7), such as histocompatibility antigens, integral membrane proteins whose apparent molecular weight is markedly increased upon detergent binding (16, 17), in contrast to hydrophilic plasma proteins whose behavior is not affected by the detergent (7). The increase in apparent molecular weight depends on the size of the detergent micelles, the micelles of nonionic detergents such as NP-40 being much larger than those formed with deoxycholate (7). Evidence that deoxychloate binds ^{125}I-labeled surface IgM—in particular the Fc fragment—but not secreted IgM has been recently presented by Parkhouse *et al.* (9) and is based on the difference in electrophoretic mobility (reflecting the addition of negative charges due to the binding of the anionic detergent) observed when electrophoresis is performed in the presence of deoxycholate or of a nonionic detergent.

These observations are consistent with the hypothesis that membrane-bound IgM is integral to the membrane or that it is noncovalently bound to an integral membrane protein, acting as a carrier, via linkages not dissociated by the detergent used. No other protein component besides Ig was apparent in the immune precipitate of radiolabeled cell surface Ig, but this does not rule out the possibility that carrier molecules may not be accessible to radioiodination. Therefore, gel fractionation in the presence of detergent was also performed with the lysates of biosynthetically labeled cells. These experiments led to two conclusions. (*i*) Lymphocytes synthesize two types of 8S IgM, one intended for secretion, which has the characteristic elution pattern of plasma cell 8S IgM, and the other appearing to be associated to the membrane (5), which has the elution pattern of ^{125}I-labeled surface 8S IgM. (*ii*) Because prolonged periods

of labeling with the representative nonpolar amino acids leucine and valine did not reveal any labeled proteins associated with membrane IgM, the hypothesis that membrane IgM is held on the membrane by an integral membrane protein carrier seems unlikely.

Strong support for the contention that membrane-bound 8S IgM contains a hydrophobic domain and thus is different from secreted IgM is provided by the gel filtration pattern of 8S IgM molecules made by tunicamycin-treated lymphocytes. We have reported that, in contrast to plasma cells which, under the influence of tunicamycin synthesize only one type of apparently completely unglycosylated μ chains, lymphocytes subjected to appropriate tunicamycin treatment synthesize two types of μ chains, one of which is similar to unglycosylated plasma cell μ chains and one slightly retarded when compared to the first one (Fig. 5). A similar observation has been made by using an entirely different procedure to remove the asparagine-linked sugars—namely, by treating pulse-labeled lymphocytes (unpublished experiments) or plasma cell (11) lysates with the enzyme endoglucosaminidase H, which cleaves the asparagine-linked "core" sugars (18). Because the lack of glycosylation of μ chains does not interfere with IgM assembly, gel filtration analysis of the lysate of tunicamycin-treated lymphocytes was possible and revealed that the IgM species with the elution pattern characteristic of membrane-bound IgM contains this second type of μ chains and the IgM molecules eluting as the "secretory" chains correspond to the unglycosylated chains of the plasma cells. Taken together, the data provide strong evidence that membrane-bound IgM is made of a different μ chain than secreted IgM and that these surface μ chains have a longer polypeptide backbone, which may result from the insertion of a hydrophobic region. Another possibility might be that the membrane IgM contain some serine-linked carbohydrate side chains whose transfer to the μ chain may not be prevented by tunicamycin. However, it would be difficult to understand how such a situation might lead to the peculiar gel filtration behavior in the presence of detergents as observed in the present experiments.

We have reported preliminary results of peptide analysis of mouse spleen B lymphocyte surface μ chains as compared to plasma cell secreted μ chains, showing differences in peptide mapping (5). However, both types of chains were found to have the same COOH-terminal sequence Cys-Tyr (5). Similar results were reported by others with lymphoma cells (4, 19). This does not favor the possibility that membrane μ chains might differ from secreted μ chains by the existence of an extra hydrophobic tail. The recent demonstration that the genes coding for heavy chains are made up of a succession of coding sequences (20, 21), corresponding to each domain and to the hinge region, separated by noncoding, intervening sequences (20), leads us to speculate that a μ chain with a hydrophobic region can be generated by a genetic process allowing the insertion of this region between two μ chain domains.

Finally, it should be pointed out that membrane-bound IgD molecules also have a behavior compatible with a hydrophobic domain and that the same appears to be true for surface IgG molecules as well, when they are detectable (Fig. 4). Under certain conditions of hyperimmunization, IgG appears to become a predominant surface Ig molecule, showing a characteristic hydrophobic behavior in the presence of detergent, in contrast to that of secreted IgG. It seems likely that all surface Ig might be characterized by the existence of a hydrophobic region in the heavy chains, thus allowing anchorage in the plasma membrane.

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