BIOGENESIS OF MEMBRANE-BOUND
AND SECRETED IMMUNOGLOBULINS

II. Two Forms of the Human α Chain Translated In Vitro
and Processed In Vivo as Distinct Polypeptide Chains*

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Immunoglobulins are first synthesized as antigen receptors integrated into the plasma membrane of resting B cells, and, subsequently, as soluble receptors secreted by mature plasma cells. Although these forms, when derived from a single B cell clone, share variable (V) regions (1, 2), their heavy-chain constant (C\text{H}) regions are otherwise functionally and topologically distinct. Recent investigations in both the human and murine systems have demonstrated that the heavy chain of membrane IgM (\(\mu_m\)) and the heavy chain of secretory IgM (\(\mu_s\)) are distinct from one another by a divergence in primary structure at the C terminus (3-7).

The predominance of IgA in external glandular secretions (8) has focused previous attention to the secretory heavy chain of this class. Two distinct human subclasses have been identified (9). The major serum form (IgA1) has been shown by amino acid-sequence analysis to have a hinge region not found in any other Ig class, including IgA2 (10). As dimers in a complex with J chain (11), both subclasses interact with secretory component (12), and are thereby transported across glandular epithelia (13). These interactions, as well as the circulatory patterns and differentiation of surface IgA* B cells, have been reviewed in detail elsewhere (14). In contrast, little is known about the heavy chain of membrane IgA (\(\alpha_m\)).

To investigate whether structural differences might exist between \(\alpha_m\) and the heavy chain of secretory IgA (\(\alpha_s\)), we have in the present study analyzed the primary translation products and in vivo synthesized forms of the α chain, derived from a human B lymphoblastoid cell line positive for both membrane and secretory IgA.

Materials and Methods

Most of the procedures used in this study have been detailed previously, among these are: the maintenance of lymphoblastoid cell lines (15); the preparation and characterization of anti-α chain antibodies (1); immunofluorescent staining for surface and intracellular immunoglobulins (15); extraction of total cellular RNA with sodium dodecyl sulfate (SDS)/phenol/chloroform/isooamylic alcohol and proteinase K (16); and the assay for cell-free protein synthesis in a staphylococcal nuclease-treated wheat germ extract (17).

For in vivo pulses with [\text{3S}]methionine, cells (4 × 10^6/ml) were first incubated for 2 h at 37°C in methionine-free RPMI-1640 supplemented with 5% dialyzed agammaglobulinemic horse serum, and then resuspended at 5 × 10^7/ml with 500 μCi/ml [\text{3S}]methionine for the

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indicated time periods (3-8 min). Products labeled during an 8-min pulse were chased in complete RPMI-1640 medium supplemented with 5% agammaglobulinemic horse serum at a cell concentration of 5 × 10^5/ml. Cells and in vitro translation products were prepared for immunoprecipitation as described elsewhere (3), except that sheep anti-human α chain antibodies were used with protein A-Sepharose CL-4B at pH 8.3.

Immunoprecipitates were washed and then divided for incubation in the presence or absence of endo-β-N-acetylglucosaminidase H (endo H). Each sample was resuspended in 25 μl of 1% SDS, 50 mM dithiothreitol (DTT), 1 mM Tris-HCl (pH 7.6), incubated for 10 min at 37°C and then for 3 min at 100°C, and transferred to an equal vol of 0.5 M Na citrate (pH 5.3) containing 5% Trasylol and 0.5 μg/ml pepstatin. 1 μl of endo H (3 μg/ml) was added to the indicated tubes, after which all tubes were incubated for 16 h at 37°C. Before gel electrophoresis, samples were adjusted directly to 2.5% SDS, 20% sucrose, 0.008% bromphenol blue, 80 mM Tris-HCl (pH 7.0), and 50 mM DTT, reduced for 30 min at 37°C, incubated at 100°C for 3 min, and alkylated in the presence of 250 mM iodoacetamide for 30 min at 37°C. 10% SDS-polyacrylamide slab gels were run at 25 mA, constant current, for 24 h, and then processed for fluorography (18).

Endo H was the kind gift of Dr. P. W. Robbins, Massachusetts Institutes of Technology. Wheat germ was obtained from General Mills, Inc., Minneapolis, Minn.; [35S]methionine (700 Ci/mmol) from New England Nuclear, Boston, Mass.; RPMI-1640 medium from Microbiological Associates Boprodacts, Walkersville, Md.; agammaglobulinemic horse serum from Grand Island Biological Co., Grand Island, N. Y.; protein A-Sepharose CL-4B from Pharmacia Fine Chemicals, Inc., Uppsala, Sweden; and Trasylol from FBA Pharmaceuticals, Inc., New York.

Results and Discussion

The synthesis of the IgA heavy chain (α chain) was initially studied in two types of cells, which were chosen by fluorescence for their differential expression of membrane and intracellular (secretory) IgA: 32a.1, a human Epstein-Barr virus-transformed B lymphoblastoid cell line, made both forms of IgA; plasma cells from patient Va with IgA multiple myeloma, exhibited predominantly intracellular staining. Each cell type was pulsed in vivo for 8 min with [35S]methionine, lysed in 1% Triton X-100, and immunoprecipitated with affinity-purified sheep anti-human α chain antibodies. SDS-polyacrylamide gel electrophoresis (PAGE) of reduced and alkylated immunoprecipitates demonstrated that 32a.1 cells synthesized three discrete forms of the α chain (Fig. 1, lane A4). When precipitates of Va plasma cells were run on SDS-PAGE, two forms of the α chain were found: a dominant band migrating near the upper band of the 32a.1 forms and a minor band with a slightly faster mobility (data not shown). These results were suggestive of structural differences between αm and αs.

However, the distinction between αm and αs may be a result of a difference in polypeptide structure and/or in post-translational processing. To resolve this, total cellular RNA was prepared from the cell line 32a.1 and translated in vitro in a wheat germ cell-free system. Under these conditions, polypeptide chains are synthesized without modifications. The [35S]methionine-labeled primary translation products were immunoprecipitated with sheep anti-human α chain antibodies, in the presence or absence of SDS-denatured human IgA, and were then resolved on SDS-PAGE. In 32a.1, two discrete primary translation products of the α chain were immunoprecipitated (Fig. 1, lane A1); the precipitation of each was inhibited by the addition of cold IgA (data not shown). If these two forms of the α chain are analogous to the two in vitro translated forms of μ chain (3, 5, 6), they might be pre-αm and pre-αs, and the difference in molecular weight between them must reside in the primary structure of their polypeptide chains.
Further experiments were carried out in vivo. Cells from 32a.1 were pulsed with $[^{35}S]$methionine for 3, 5, or 8 min to identify the earliest in vivo synthesized forms of the α chain (Fig. 1A, lanes 2–4). In addition, cells were pulsed for 8 min and then chased in medium containing cold methionine for 1, 3, or 10 h to analyze later biosynthetic forms (Fig. 1A, lanes 5–7). When anti-α chain immunoprecipitates were resolved by 10% SDS-PAGE (Fig. 1A), it was found that after a 3-min pulse, only two labeled forms of the α chain resolved (lane A2). This was also the case when cells were pulsed for 5 min (lane A3). However, after an 8-min pulse, three discrete species were present (lane A4). These three forms of the α chain remained associated with the cell pellet for chase periods of up to 3 h (lanes A5 and A6). By 10 h (lane A7), only one form was detectable. The relative mobility of the two primary translation products of α chain is shown for comparison (lane A1). The results of these pulse-chase experiments indicated that the early biosynthetic forms of α chain consist of two chains that are subsequently modified to yield three.

Posttranslational proteolysis, as well as modifications of N- or O-linked oligosaccharides, might have been the basis for this apparent conversion. To discriminate between these possibilities, each of the above immunoprecipitates was digested with endo H. Remaining after such treatment would be polypeptide chains stripped of sensitive N-linked oligosaccharides, with any O-linked oligosaccharides left intact (19). The results are shown in Fig. 1B. The relative mobilities of the primary translation products were not affected by this treatment and are included for comparison (lane A1 and lane B1). It was found that, at all pulse and chase periods up to 3 h, the intermediates of α chain synthesis in vivo were reduced to a doublet. This doublet had a faster mobility than their untreated counterparts, which is consistent with the removal of N-linked oligosaccharides, as well as a faster mobility than the two primary translation products, which is consistent with the removal in vivo of a signal peptide. The relative mobility of this doublet was the same after a 3-min pulse (lane B2), when two α chain intermediates were present (lane A2), as it was after a 3-h chase (lane B6), when three
α chain intermediates were present (lane A6). This comparison of the α chain forms in the presence or absence of endo H treatment demonstrates that the three forms of α chain do not arise as a function of post-translational proteolysis; otherwise, three forms of α chain would be generated by endo H treatment. Nor are they generated as a consequence of detectable O-glycosylation; otherwise, at longer chase periods, endo H treatment would have shifted the α chain forms to a doublet with a different mobility than that found at earlier pulse periods (20). It appears, instead, that these three forms represent two different α chain polypeptide chains with endo H-sensitive oligosaccharides of varying compositions. The data would suggest that the lower band of the earliest biosynthetic forms (lanes A2 and A3) chased to the band of intermediate mobility at later time points (lanes A4-6). It is not clear what type of modification of an N-linked oligosaccharide would produce such a shift in relative mobility.

After a 10-h chase, the major cell-associated form of the α chain (lane A7) was reduced by endo H treatment to a single band, comigrating with the lower band of the doublets in panel B (lane B7). The presence of only one band indicates that the two α chain intermediates differ not only in molecular weight, but in intracellular turnover rate. The finding that this band was the lower molecular weight form of the endo H-treated intermediates was unexpected. Its slow turnover rate is consistent with that of a membrane, as opposed to a secretory, protein; in this case, αm would appear to be of a lower molecular weight than αs. The predominance in Va plasma cells of a higher molecular weight form would support this interpretation. However, this experiment does not rule out the possibility that this form in fact represents αs; in this case, αm would appear to have a faster turnover rate than αs. Either possibility would differ from observations with μm and δm (the heavy chain of membrane IgD). For each of these Ig heavy chain classes, the membrane chains have a higher molecular weight and a slower turnover rate than the corresponding secretory chains, μs and δs (the heavy chain of secretory IgD; 3, 21).

This study indicates that αm and αs are distinct from one another on the basis of a difference in molecular weight, both in vitro as primary translation products and as intermediates of in vivo biosynthesis. This difference is most likely reflective of a difference in amino acid sequence of the respective α chains. An analogous situation has been observed with other immunoglobulin heavy chain classes. μm and μs are distinguished from one another by different C-terminus tails (3-7); δm and δs are likewise encoded as distinct polypeptide chains (21). The C-terminal tail of αs is similar to that of μs, in both amino acid sequence (22) and in the ability to bind J chain (11). It is probable that αm possesses, like μm, a different C-terminal tail that is hydrophobic in nature and necessary for membrane integration. These different C-termini might be generated as a result of differential processing of a single nuclear RNA transcript, as seems to be the case for μm, μs, δm and δs. It is likely that these analogies extend to all immunoglobulin heavy chain classes.

Some processing events appear, however, to be class specific. The finding that the α chain of lowest molecular weight has the slowest intracellular turnover rate is clearly different from the results for μ or δ chains. This finding may be reflective of a second point of variance between the polypeptide chains of αm and αs or, alternatively, of a post-translational processing event unique for α chain. Further analysis of these chains will be informative on this point.
Summary

Structural differences between $\alpha_m$ (the heavy chain of membrane IgA) and $\alpha_s$ (the heavy chain of secretory IgA) were investigated. Messenger RNA from the human B lymphoblastoid line 32a.1, expressing both membrane and secretory IgA, was translated in a wheat germ cell-free system, resulting in the synthesis of two primary translation products for the $\alpha$ chain, that differed in molecular weight. In vivo pulse and pulse-chase experiments demonstrated that two early biosynthetic forms of the $\alpha$ chain were subsequently modified to yield three intracellular forms. As shown by endo-$\beta$-N-acetylglucosaminidase H (endo H) treatment, these forms represent two $\alpha$ polypeptide chains, with varying compositions of $N$-linked oligosaccharides. Of the two forms of the $\alpha$ chain remaining after endo H treatment, only the form with the lowest molecular weight was associated with cells after long chase periods. The possible significance of this difference from the results with $\mu$ and $\delta$ chains is discussed. These results indicate that $\alpha_m$ is distinguished from $\alpha_s$ by a difference in both primary structure and intracellular processing. The functional consequences of this distinction, previously shown for the heavy chain of membrane IgM ($\mu_m$) and heavy chain of secretory IgM ($\mu_s$), may reflect a principle common to the secretory and membrane forms of all immunoglobulin heavy chain classes.

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