Glycosylation Causes an Apparent Block in Translation of Immunoglobulin Heavy Chain*

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Analysis of nascent heavy chains isolated from MPC11 (α2h heavy chains) and MOPC 21 (γ1 heavy chains) mouse myeloma cells demonstrates an accumulation of nascent heavy chains which are slightly smaller in mass (~35,000 daltons) than nascent heavy chains which have just been glycosylated (~38,000 daltons). The accumulation of 35,000-dalton nascent heavy chain appears to be a consequence of the glycosylation process since tunicamycin, an inhibitor of glycosylation, abolishes the apparent translational block manifested by the accumulation of 35,000-dalton nascent chains. Tunicamycin also causes a 15 to 25% increase in the relative rate of synthesis of heavy chain compared to the corresponding rate of synthesis of the nonglycosylated light chain synthesized by the same cell. These results suggest that the translation block, caused by the glycosylation process, of heavy chain synthesis contributes to the imbalance of heavy chain and light chain biosynthesis observed in malignant and normal lymphoid cells.

Recently, there has been substantial progress toward clarifying the pathways by which glycoproteins containing asparagine-linked oligosaccharides are synthesized in eukaryotic cells. The initial glycosylation event involves the transfer of a large molecular weight oligosaccharide, containing N-acetylglucosamine, mannose, and glucose, as a lipid intermediate to an asparagine acceptor residue on the polypeptide (1-4). We and others have shown that the transfer of the oligosaccharide occurs while the nascent polypeptide is still bound to the ribosome, apparently very soon after the acceptor residue is synthesized and inserted into the lumen of the endoplasmic reticulum (5-9). However, very little is known about the molecular mechanism facilitating this transfer event and about the interactions between the nascent polypeptide, the lipid intermediate, and the enzyme(s) involved in this glycosylation event.

Tunicamycin is a glucosamine-containing compound (10) which selectively prevents the initial glycosylation of newly synthesized peptidoglycan and proteins in bacteria (11), yeast (12, 13), viruses (14, 15), and mammalian cells (16, 17). Its mechanism of action is to inhibit the formation of N-acetylglucosamine-lipid intermediates (13, 18) which serve as the donors for the core regions of the oligosaccharides units found on glycoproteins (1-4).

Previously, in our studies of the initial glycosylation of nascent immunoglobulin heavy chains (5-6), we have noticed the accumulation of nascent heavy chains which are slightly smaller in mass (~35,000 daltons) than nascent heavy chains which have just been glycosylated (~38,000 daltons). Results presented in this paper indicate that accumulation of the 35,000-dalton nascent heavy chains is directly related to the glycosylation of the nascent heavy chains. Inhibition of glycosylation with tunicamycin specifically relieves this apparent translational block, and causes an increase in the relative rate of synthesis of the heavy chain when compared to the rate of synthesis of the nonglycosylated immunoglobulin light chain produced by the same cell.

EXPERIMENTAL PROCEDURES

Materials—Tunicamycin was a generous gift of the Eli Lilly Co. and was stored as a 1.0 mg/ml stock in methanol. Cycloheximide was purchased from Sigma Chemical Co. and was stored as a 5.0 mg/ml stock in H2O.

Cells—Clone 45.6, a clone derived from the MPC 11 plasmacytoma cell line (19), synthesizes glycosylated yH, heavy (H) chains, κ light (L) chains, and a α light chain constant region fragment (Fv$^\alpha$). The products represent 12, 8, and 1%, respectively, of proteins synthesized by this clone (20). Clone 66.2 has lost the ability to synthesize intact MPC 11 H chains but continues to synthesize MFC 11 L chains and Fv$^\alpha$ (21). PB00.1, a clone derived from the MOPC 21 plasmacytoma cell line, synthesizes glycosylated γH, heavy chains and κ light chains (22). Clone NS-1 has lost the ability to synthesize intact γH chains but continues to synthesize the MOPC 21 L chain (23). The cells were maintained in suspension in Dulbecco’s modified Eagle’s medium supplemented with 16% heat-inactivated horse serum, 2 mM glutamine, and nonessential amino acids.

Cell Labeling, Fractionation, and Nascent Chain Isolation—For amino acid labeling of nascent chains, cells were collected by centrifugation at 500 × g for 5 min, resuspended in 200 ml of Dulbecco’s modified Eagle’s medium containing 2% horse serum. After preincubation (see below), the cells were collected by centrifugation and resuspended in 10 ml of Dulbecco’s modified Eagle’s medium minus methionine containing 1% horse serum in the presence or absence of the appropriate inhibitor, and labeled for 20 min with [35S]methionine (Amersham/Searle, 800 to 1200 Ci/mmol) at an isotopic concentration of 50 to 200 μCi/ml. The labeling was terminated by chilling in an ice-cold H2O bath and subsequent centrifugation at 1000 × g for 5 min at 4°C.

To examine the effect of inhibitors on the nascent H chain profiles, the cells were preincubated for 120 min at 37°C in air containing 5% CO2 with cycloheximide, NaCl, or tunicamycin (concentrations are indicated in text) being added to individual aliquots at 100 min, 105 min, or 0 min, respectively, after initiation of the preincubation (no inhibitor was added to the fourth aliquot which served as the control). Preliminary experiments were done to determine the extent of inhibition of protein synthesis (CLCCOBI-insoluble [35S]methionine incorp-
corporation in 30 min) when different levels of NaCl (30, 31), cycloheximide (34), and tunicamycin (14–17) were present. For the experiment shown in Fig. 1, levels of NaCl and cycloheximide were chosen so that the extent of inhibition of protein synthesis was comparable to that seen with tunicamycin. Similar preliminary experiments established the level of tunicamycin necessary to completely block glycosylation of H chain (6).

The isolation of membrane-bound ribosomes and nascent polypeptides was essentially as previously described (5, 6). To specifically isolate nascent heavy chains, the dialyzed nascent chain fraction was subjected to indirect immunoprecipitation using the antisera described below in the presence of a vast excess of unlabeled post-nuclear supernatant from the appropriate variant cell line which synthesizes L chain but not H chain. Control experiments demonstrated that the presence of excess unlabeled post-nuclear supernatant from the appropriate variant fully blocks immunoprecipitation of completed L chains under these conditions. Nonspecific immunoprecipitation of nascent chains was less than 1% of the total [35S]-methionine in the nascent chains. Attempts to specifically immunoprecipitate intact polysomes containing nascent H chains were unsuccessful because of high levels (~5 to 10% of total [35S]-methionine present in nascent chains) of nonspecific immunoprecipitation.

For analysis of the completed immunoglobulin polypeptides, cells were labeled with [35S]-methionine as described above. After labeling, the cells were resuspended in 5 ml of 0.15 M NaCl, 0.0015 M MgCl₂, 0.01 M Tris/HCl, pH 7.4, containing 1.0% Nonidet P-40 (v/v) to lyse the cells. A post-nuclear supernatant was prepared by centrifugation of the lysate at 1000 x g for 5 min. NaDodSO₄ was added to the post-nuclear supernatant to a final concentration of 0.1% and the sample was reduced with 10 mM dithiothreitol for 2 to 4 h at 37°C. After reduction, the sample was alkylated with 50 mM iodoacetamide for 15 min at 37°C in the dark, and the sample was subsequently dialyzed extensively against 10 mM Tris/HCl, pH 7.8, 0.05 M NaDodSO₄. After dialysis, the pH of the sample was lowered by addition of one-tenth volume of 1.0 M sodium phosphate, pH 6.8. Subsequently, the sample was mixed with reduced and alkylated [3H]leucine-labeled post-nuclear supernatant from the appropriate cell line (prepared as just described), and then subjected to indirect immunoprecipitation and NaDodSO₄-gel electrophoresis as described below.

Antisera—Rabbit antisera directed against MPC 11 γ, H chain and κ L chain, and goat antisera directed against rabbit γ-globulin were prepared as described previously (25). Rabbit antisera directed against MOPC 21 γ, H chain and κ L chain was a gift from Dr. S. Morrison, Columbia University. The direct or indirect immunoprecipitations were performed in antibody excess and incubated overnight at 4°C (26). The immunoprecipitates were collected by centrifugation through 1.0 M sucrose in phosphate-buffered saline (138 mM NaCl, 3 mM KCl, 8 mM Na₂HPO₄, 12.0H₂O, 1 mM KH₂PO₄), and washed once with the same buffer (27).

NaDodSO₄-Polyacrylamide Gel Electrophoresis—The immunoprecipitates were boiled in NaDodSO₄ sample buffer, and then reduced with 0.15 M β-mercaptoethanol at 37°C. They were then electrophoresed in a 10% polyacrylamide—12-cm cylindrical gel using a discontinuous NaDodSO₄/Tris/glycine buffer system essentially as described by Laemmli (28) and Mazel (29). The gels were sliced into 2-mm fractions with a Gilson Aliquogel fractionator and counted in a Triton X-100/toluene (12) scintillation fluid containing 0.4% 2,5-diphenyloxazole (w/v) and 10% H₂O (v/v) in a Beckman LS 230 scintillation counter.

RESULTS

Previously, we have noticed the accumulation of nascent heavy chains of approximately 35,000 daltons in MPC 11 mouse myeloma cells (5, 6). To investigate the nature of this apparent translational block, we have analyzed the effect of inhibitors of protein synthesis (cycloheximide and high salt concentrations) and glycosylation (tunicamycin) on the size distribution of nascent heavy chains. Approximately 8 to 10 x 10⁸ MPC 11 cells were collected, divided into four equal aliquots, and individual aliquots preincubated (see "Experimental Procedures") in the following levels of inhibitor to establish inhibition of either translation or glycosylation: cycloheximide, 0.4 µg/ml; NaCl, 35 mM; and tunicamycin, 1.0 µg/ml. After preincubation, the cells were then labeled for 20 min with [35S]-methionine in the presence of the inhibitor, and aliquots were removed to assay the extent of inhibition of protein synthesis in each case. As determined by Cl/CCO₄-precipitable radioisotope, the percentage of control (no inhibitor) protein synthesis was 73% for tunicamycin-treated cells, 58% for cycloheximide-treated cells, and 66% for NaCl-treated cells. In the tunicamycin-treated cells, all of the radiolabeled completed heavy chain was nonglycosylated as determined by NaDodSO₄-gel electrophoretic analysis (data not shown). In each case, microsomal nascent chains were isolated by two cycles of QAE-Sephadex chromatography, and subsequently nascent heavy chains were isolated by specific immunoprecipitation in the presence of an excess of unlabeled MPC 11 light chains as described under "Experimental Procedures." Fig. 1 shows the NaDodSO₄-gel electrophoretic profiles of the nascent heavy chains. The control sample (Panel A) shows a size distribution of 55,000 daltons (full size) to approximately 15,000 daltons, with an accumulation of nascent heavy chains of approximately 35,000 daltons (Fractions 26 to 30). This

![Figure 1](image-url)
accumulated peak of nascent heavy chains is slightly more heterogeneous in size than the co-electrophoresed marker MPC 11 completed heavy chains, thereby suggesting that the peak is not due to nascent heavy chains having a homogeneous length. The accumulation of a peak of nascent heavy chains having an apparent mass of approximately 35,000 daltons was also observed when the labeled nascent chains were analyzed by gel filtration on Sephadex G-200 in an NaDodSO4 buffer system (data not shown). However, in the presence of tunicamycin (Panel B), there is no accumulation of 35,000-dalton nascent heavy chains, and there is an increase in the amount of nascent heavy chains in the 38,000- to 55,000-dalton size range compared to the control (Panel A). To ensure that the effect of tunicamycin on the nascent heavy chains distribution was related to the inhibition of glycosylation and not due to the partial inhibition of protein synthesis seen in tunicamycin-treated cells, we have analyzed the effect of cycloheximide (an inhibitor of elongation) (34) and high NaCl concentration (an inhibitor of initiation) (30, 31) on the size distribution of nascent heavy chains. In the presence of 0.4 μg/ml of cycloheximide (Panel C) or 35 mM NaCl (Panel D), the accumulation of the 55,000-dalton nascent heavy chains is similar to the control (Panel A). In the aliquot inhibited by high NaCl concentrations, the nascent heavy chain size profile is essentially identical with the control aliquot. As expected, however, in the cycloheximide-treated sample there is a small increase in the amount of 38,000-dalton to full size nascent heavy chains compared to the control, even though the intrinsic translational block is still apparent. There is an increased yield of nascent chains when cycloheximide is present and a decreased yield of nascent chains when NaCl is present (data not shown); consistent with inhibition of elongation and initiation, respectively, in these experiments with myeloma cells.

We have analyzed nascent H chains from MOPC 21 cells to determine whether the translational block due to glycosylation was unique to MPC 11 cells or was present in a second cell line synthesizing a different subclass of heavy chain. MOPC 21 cells were labeled with [35S]methionine in the presence of the various inhibitors, and nascent heavy chains isolated and analyzed as described above. The percentage of control protein synthesis was 69%, 50%, and 71% for tunicamycin-, cycloheximide-, and high salt-treated cells, respectively. Glycosylation of completed heavy chains was completely blocked in the tunicamycin-treated cells (data not shown). The results, seen in Fig. 2, indicate that the control sample from untreated cells (Panel A) has an accumulation of nascent MOPC 21 heavy chains of approximately 35,000 daltons. In the cells inhibited with tunicamycin (Panel B), there was no accumulation of nascent heavy chains of 35,000 daltons. Compared to the control MOPC 21 cells, inhibition with cycloheximide or 35 mM NaCl had no significant effect on the nascent heavy chain profile (data not shown).

These results indicate that there is a block in translation of heavy chain mRNA in MOPC 11 and MOPC 21 cells, causing the accumulation of nascent heavy chains of 35,000 daltons. The apparent translational block is relieved by tunicamycin, an inhibitor of glycosylation. The apparent translational block is not affected by inhibitors of either elongation (cycloheximide) (34) or initiation (high NaCl concentration) (30, 31) of polypeptide translation, i.e. at levels of inhibition of protein synthesis comparable to the levels of inhibition of protein synthesis caused by tunicamycin.

We have further examined the effect of various levels of cycloheximide on the size distribution of nascent heavy chains to determine whether the effect of the apparent translational block on the nascent H chain profile can be eliminated by decreasing the rate of polypeptide chain elongation (34). Aliquots of MPC 11 cells were preincubated at 0, 0.4, 1.0, and 4.0 μg/ml of cycloheximide. The results in Fig. 3 indicate that with increasing levels of inhibition of protein synthesis, there is a corresponding increase in the number of nascent heavy chains between 38,000 and 55,000 daltons. At 0.4 μg/ml of cycloheximide (68.1% of control protein synthesis), the accumulation of 35,000-dalton nascent heavy chains (Panel B) is markedly diminished compared to the control (Panel A). At 4.0 μg/ml of cycloheximide (9.2% of control protein synthesis), there is no discernible accumulation of 35,000-dalton nascent heavy chain (Panel D). Thus, these data suggest that the translational block resulting in accumulation of 35,000-dalton nascent heavy chains essentially is abolished by substantial inhibition (>60%) of polypeptide elongation with cycloheximide.

Finally, we have attempted to determine whether the block in translation of the heavy chains causes a relative decrease in the rate of heavy chain synthesis compared to the rate of synthesis of light chain, a nonglycosylated polypeptide. Cells were labeled for 20 min with [35S]methionine in the presence of various concentrations of inhibitors as described under "Experimental Procedures." Each sample was reduced, alkylated, mixed with a post-nuclear supernatant from [3H]leucine-labeled control cells (prepared in the same manner but in the absence of inhibitors), and subjected to indirect immunoprecipitation as described under "Experimental Procedures." The immunoprecipitates were then analyzed by NaDodSO4-poly-
The proportion of radiolabel in completed heavy chain and completed light chain in each sample was determined from these results. The data in Table I indicate that inhibition of glycosylation with tunicamycin causes an increase in the ratio of heavy chain to light chain (H/L) from 1.19 for the control sample to 1.47 for the tunicamycin-treated cells. This is a 23.5% increase in the relative rate of heavy chain synthesis compared to light chain synthesis. This effect, however, does not appear to be due to a direct effect of tunicamycin on protein synthesis in that there is no effect on the ratio of heavy chain to light chain at two levels of cycloheximide (H/L ratios for the cycloheximide-treated cells are 1.20 and 1.24 for 0.4 µg/ml and 5.0 µg/ml of cycloheximide). Inhibition of initiation of protein synthesis with high concentrations of NaCl, in fact, has the opposite effect in that there is a decrease in the relative rate of heavy chain synthesis compared to light chain synthesis, as seen by the decrease in the ratio of heavy chain to light chain from 1.19 for the control to 0.98 and 0.62 for cells incubated with 35 mM and 100 mM NaCl, respectively (30, 31).

The effect of tunicamycin on the relative synthesis of heavy chain is also seen in MOPC 21. Table II reveals that the ratio of heavy chain to light chain for MOPC 21 increases from 1.32 for the control to 1.52 for the tunicamycin-treated cells. This is an approximately 15% increase in the relative rate of synthesis at normal NaCl concentrations. This is in contrast to the effect of tunicamycin on the ratio of heavy chain to light chain for MPC 11, where the ratio decreases from 1.19 for the control to 0.98 and 0.62 for cells incubated with 35 mM and 100 mM NaCl, respectively. The effect of tunicamycin on the ratio of heavy chain to light chain for MOPC 21 increases from 1.32 for the control to 1.52 for the tunicamycin-treated cells.

![Fig. 4. NaDodSO₄-gel electrophoresis of immunoprecipitated MPC 11 completed H and L chains. Two aliquots of 2.2 × 10⁶ MPC 11 cells were collected and labeled for 20 min with [³⁵S]methionine in the absence (Panel A) or presence of 0.4 µg/ml of tunicamycin (Panel B), as described under "Experimental Procedures." The post-nuclear supernatant was prepared and then treated with 0.1% NaDodSO₄. It was then reduced with 10 mM dithiothreitol, alkylated with 50 mM iodoacetamide, and subsequently dialyzed extensively against 10 mM Tris/HCl, pH 7.4, 0.05% NaDodSO₄, as described under "Experimental Procedures." After dialysis, the sample was mixed with reduced and alkylated [³⁵S]leucine-labeled MPC 11 post-nuclear supernatant (prepared as just described but from control cells), and subjected to indirect immunoprecipitation and NaDodSO₄-gel electrophoresis. Electrophoretic migration is from left to right. Solid line (—) [³⁵S]methionine-labeled MPC 11 completed H and L chains; dashed line (— —) [³⁵S]leucine-labeled MPC 11 completed H and L chains.](image)

![Fig. 3. NaDodSO₄-gel electrophoresis of immunoprecipitated MPC 11 nascent heavy chains. Four aliquots of 2.5 × 10⁶ MPC 11 cells were collected and labeled for 15 min with [³⁵S]methionine in the absence (Panel A) or presence of 0.4 µg/ml of cycloheximide (Panel B), 1.0 µg/ml of cycloheximide (Panel C) or 4.0 µg/ml of cycloheximide (Panel D), as described under "Experimental Procedures." Microsomal nascent heavy chains were isolated and analyzed as described in Fig. 1. Electrophoretic migration is from left to right. Solid line (—) [³⁵S]methionine-labeled nascent heavy chains; dashed line (— —) [³⁵S]leucine-labeled marker MPC 11 H and L.](image)

**Table I**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control synthesis</th>
<th>[³⁵S]Met H/L</th>
<th>[³⁵S]Leu control H/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>1.19</td>
<td>1.56</td>
</tr>
<tr>
<td>1.0 µg/ml tunicamycin</td>
<td>65</td>
<td>1.47</td>
<td>1.51</td>
</tr>
<tr>
<td>0.4 µg/ml cycloheximide</td>
<td>53</td>
<td>1.20</td>
<td>1.61</td>
</tr>
<tr>
<td>5.0 µg/ml cycloheximide</td>
<td>8</td>
<td>1.24</td>
<td>1.61</td>
</tr>
<tr>
<td>35 mM NaCl</td>
<td>47</td>
<td>0.98</td>
<td>1.54</td>
</tr>
<tr>
<td>100 mM NaCl</td>
<td>7</td>
<td>0.62</td>
<td>1.51</td>
</tr>
</tbody>
</table>

*The percentage control synthesis was determined by CI/COOH-precipitation of aliquots removed from the post-nuclear supernatant of each sample. The amount of [³⁵S]methionine incorporated in the control sample was set as 100%.

*The [³⁵S]leucine was included for each sample to determine the variability in the H/L ratio of the same control lysate in different experiments.
mass of approximately 35,000 daltons. Previous studies (5, 6) have demonstrated that when nascent heavy chains from MOPC 21 (Fig. 1) have reached a mass of approximately 38,000 daltons, it may be a block in translation of heavy chains which is related to the glycosylation process. Therefore, the accumulation of nascent heavy chains having a mass of approximately 35,000 daltons suggests that there may be a block in translation of heavy chains which is related to the glycosylation process.

Experiments were performed to determine the nature of the apparent block in translation of heavy chain mRNA in MPC 11 and MOPC 21 cells which causes the accumulation of nascent heavy chains of 35,000 daltons. We have shown that inhibition of glycosylation with tunicamycin abolishes the presumptive translational block and results in the size distribution expected for both MPC 11 and MOPC 21 nascent heavy chains (see Fig. 1) and MOPC 21 nascent heavy chains (see Fig. 2). This result strongly suggests that the apparent translational block is not inherent in the mRNA translation, but is rather a secondary effect of the glycosylation process. (Although proteolysis during nascent chain isolation cannot be excluded, it is difficult to account for the accumulation of a 35,000 dalton peak only in the absence of tunicamycin (Figs. 1 and 2) or high levels of cycloheximide (Fig. 3) as a result of proteolysis.) Tunicamycin, by an unknown mechanism, also slightly inhibits protein synthesis but comparable inhibition of either elongation with cycloheximide or initiation with high salt had no effect on the specific translational block noted above (see Fig. 1 and corresponding text). Tunicamycin inhibits the formation of N-acetylgalactosamine-dolichol (13, 18), a precursor of the oligosaccharide-dolichol which mediates the transfer of the oligosaccharide to the nascent heavy chain molecule (2-6, 17). It is possible that the binding of the lipid intermediate and the enzyme(s) involved in glycosylation to the asparaginyl acceptor site results in a slowing of polypeptide elongation causing an accumulation of nascent heavy chains of 35,000 daltons in mass; association of the asparaginyl acceptor site with the enzyme(s) in the absence of the lipid oligosaccharide intermediate may be much weaker and therefore not affect peptide elongation.

It is difficult to estimate the extent of blockage of translation which causes the accumulation of nascent heavy chains having a mass of approximately 35,000 daltons. Estimates based on the amount of radioactivity present in nascent heavy chain fractions in the accumulated peak and in comparable fractions which contain slightly smaller or larger nascent heavy chains suggest that the block in translation is roughly 2- to 3-fold, i.e., it takes approximately two to three times longer to translate the region of mRNA corresponding to the site of the block than to translate other comparable regions of the heavy chain mRNA. Experiments with cycloheximide are consistent with this estimate since the apparent specific translational block is largely abolished when overall translation (elongation) rates are decreased 2- to 3-fold (see Fig. 3 and corresponding text). The net effect of the marked slowdown of elongation in this region of the mRNA is minimized due to the localized accumulation of nascent heavy chains, so that the overall transit time for synthesis of a heavy chain is increased by only 15 to 25% (see Tables I and II and corresponding text).

The nascent chain profiles obtained in the experiments described can provide information about the rate-limiting processes involved in heavy chain translation. The accumulation of nascent heavy chains of approximately 35,000 daltons indicates the nascent heavy chains (and therefore ribosomes) are more highly packed on the mRNA at the site of the block compared to regions of mRNA preceding or following the block. Based on the polysome model, one would expect that a slowing of translation (elongation) at a specific site on the mRNA should generate a sharp peak corresponding to the site of the block (Figs. 3A, B, and C) only if initiation of translation is rate-limiting; this result is obtained under conditions whether initiation, and not elongation, is known to be rate-limiting (Fig. 1D). If elongation is rate-limiting, one would expect an accumulation of all nascent chains behind the site of the specific block, i.e., a radiolabeled peak of accumulated nascent chains which should show a gradual rather than a sharp decline for small nascent chains. This result is observed when cycloheximide is used to inhibit elongation so that elongation rather than initiation is rate-limiting (see Figs. 1C, 3B, and 3D). Thus, the presence of a sharp peak in control cells (Figs. 1A, 2A, and 3A) suggests that in the absence of inhibitors, initiation, and not elongation, is the rate-limiting step in translation of heavy chain mRNA in the two myeloma cell lines examined.

If there is a block in heavy chain mRNA translation related to the glycosylation process, one would expect a relative increase in heavy chain synthesis when this block is abolished. Analysis of the relative rates of synthesis of heavy chains and light chains in control and tunicamycin-treated MPC 11 (Table I) and MOPC 21 (Table II) mouse myeloma cells indicate that there is a relative increase of heavy chain (relative to light chain) synthesis when glycosylation is inhibited with tunicamycin. The lack of a similar relative increase of heavy chain synthesis in the presence of high levels of cycloheximide (i.e., conditions where the apparent specific translational block is largely abolished) is not readily explained unless cycloheximide has a secondary effect, such as some inhibition of initiation. It is known, for instance, that light chain synthesis is less sensitive than heavy chain synthesis to some inhibitors of initiation, such as high salt (Tables I and II and Ref. 31).

Finally, it is of interest that biosynthetic studies with myeloma tumors and cell lines, as well as with normal spleen cells, have demonstrated that there is generally synthesis of a significant molar excess of light chains compared to heavy chains (32, 33). This is physiologically significant since synthesis of excess heavy chains, but not excess light chains, appears to be detrimental to the cell. The present studies suggest that the apparent translational block of heavy chain synthesis caused by the glycosylation process contributes significantly to the imbalance of heavy chain and light chain biosynthesis observed in normal and malignant lymphoid cells.

### Table II

**Effect of tunicamycin on the relative rate of synthesis of MOPC 21 heavy chain**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control synthesis [%]</th>
<th>[35S]Methionine</th>
<th>[3H]Leucine control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>1.32</td>
<td>0.94</td>
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<tr>
<td>1.0 µg/ml tunicamycin</td>
<td>71</td>
<td>1.52</td>
<td>0.97</td>
</tr>
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REFERENCES

706 Glycosylation of Ig Heavy Chain