

**In Vivo N-Glycosylation and Fate of Asn-X-Ser/Thr Tripeptides**

M. Geetha-Habib, Helen R. Park, and William J. Lennarz

From the Department of Biochemistry and Molecular Biology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

The minimum primary structural requirement for a tripeptide to serve as a substrate for oligosaccharyl transferase is the sequence -Asn-X-Ser/Thr-. In the present study the activities of three structurally different tripeptides containing acceptor sequences for oligosaccharyl transferase were compared in three systems: Xenopus oocytes, in which they were introduced into the cytoplasm by microinjection, cultured mammalian cells, and isolated rat liver microsomes. In the last two systems, the peptides were added exogenously to the culture or to the incubation medium, respectively. On the basis of lectin column and paper chromatographic analysis it was established that the microinjected acceptor tripeptides were glycosylated in Xenopus oocytes. However, lectin column analysis and retention of sensitivity to endoglycosidase H revealed that none of the three glycopeptides was processed to complex oligosaccharide chains and none was subsequently secreted. Rather, over a 24-h period the glycopeptides were degraded. Chloroquine was found to block this degradation process, but even under these conditions, the glycopeptides were not secreted into the medium. In the isolated microsomes the glycosylation of the acceptor tripeptides was time-dependent and the tripeptide with an iodotyrosine residue in the X position was found to be a poor substrate. When added to cultured mammalian cells, all three of the tripeptides were taken up, glycosylated, and subsequently secreted. These results are discussed in the context of the wide differences in glycosylation of the three peptides and their lack of secretion after glycosylation in Xenopus oocytes.

Xenopus oocytes have been shown to not only efficiently carry out in vivo translation of microinjected mRNAs, but also to correctly co- or posttranslationally modify many heterologous proteins (1-6). Because of their large size, microinjection is a direct and quantitative method for introducing macromolecules into oocytes, thus obviating many difficulties that are inherent to other delivery techniques. Moreover, both the large size and metabolic activity of the oocytes make it possible to carry out product analysis of even a single cell after microinjection.

One important cotranslational event that occurs in the endoplasmic reticulum is the N-glycosylation of proteins, which involves the transfer of the oligosaccharide from oligosaccharylpyrophosphoryldolichol to nascent polypeptide acceptors (7, 8). Oligosaccharyl transferase, the enzyme catalyzing this reaction, was studied earlier with respect to specificity of the Asn sites that become glycosylated (9, 10). These studies established that simple Asn-X-Ser/Thr tripeptides could serve as substrates for this enzyme provided that both the N and C termini were blocked. Subsequently it was established that such peptides readily enter microsomes, become glycosylated, and remain associated with the microsomes, presumably entrapped in the lumen. In contrast, the unglycosylated peptide readily could be removed from the membranes by washing (11).

These findings, coupled with the observation that acceptor peptides inhibited cotranslational glycosylation in vitro and that their inhibitory activity positively correlated with their hydrophobicity (12), raised the possibility that such acceptor peptides might be useful in in vivo studies on glycosylation. In fact, while this work was in progress, it was reported that derivatives of tripeptides presented to human hepatoma or Chinese hamster ovary cells were taken up, glycosylated, and subsequently secreted with half-times of about 5 and 10 min, respectively (13). In the present paper we have investigated the fate of three 125I-labeled acceptor tripeptides: N\(^{\alpha}\)-3-(4-hydroxyphenylpropionyl)-Asn-Lys(N\(^{\alpha}\)-p-azidobenzozy)-Thr-NH\(_2\), N\(^{\alpha}\)-3-(4-hydroxyphenylpropionyl)-Asn-Tyr-Thr-NH\(_2\), and N\(^{\alpha}\)-(acetyl)-Asn-Tyr-Thr-NH\(_2\) in Xenopus oocytes and in isolated microsomes incubated in vitro. It was found that all three peptides were glycosylated in the oocytes, although their efficacy as substrates differed by 100-fold. Based on their behavior upon lectin chromatography and sensitivity to endoglycosidase H, it was concluded that none of the glycopeptides was processed. Moreover, they were not secreted and, over a 24-h period, were degraded by a chloroquine-inhibitable process. When incubated in vitro with isolated microsomes, the three peptides exhibited the same relative efficacy as substrates that was observed when the peptides were microinjected into oocytes. These results were compared with those of experiments using mammalian cells in which these exogenous acceptor peptides were taken up, glycosylated, and then secreted.

**EXPERIMENTAL PROCEDURES**

**Synthesis of Peptides**—The trifluoroacetate salt of Asn-Lys(N\(^{\alpha}\)-p-azidobenzozy)-Thr-NH\(_2\), was synthesized as described previously (11). Synthesis of Asn-Tyr-Thr-NH\(_2\), was carried out according to the solid phase synthesis method (14) by the phosphate synthesizing facility of The University of Texas M. D. Anderson Cancer Center. Conversion of both tripeptides to the corresponding 125I-labeled N\(^{\alpha}\)-3-(4-hydroxyphenylpropionyl) derivatives was performed according to the procedure described by Welply et al. (15). 125I-Labeled Bolton-Hunter reagent (2562 Ci/mmol) was purchased from ICN Radiochemicals (Irvine, CA). Acetylation of Asn-Tyr-Thr-NH\(_2\) was performed ac-
Microinjection of Xenopus Oocytes—Individual stage V oocytes were manually dissected from surgically removed ovaries and were maintained at 19 °C for up to 48 h prior to injection in modified Barth’s saline solution. Five oocytes were each injected with approximately 35 pmol (20-50 nl) of the 125I-labeled tripeptide in 20% dimethyl sulfoxide and then were allowed to incubate in modified Barth’s saline solution at 19 °C for the indicated times. After microinjection the final concentration of dimethyl sulfoxide in the oocytes was less than 1%, higher concentrations of dimethyl sulfoxide resulted in leakage of the unglycosylated and glycosylated peptides from the oocytes. At the end of each incubation time, each group of oocytes was separated from the media. The oocytes were homogenized and the resulting homogenate and the culture media were separately treated with 10% trichloroacetic acid to precipitate the macromolecules. After 2 h on ice, treated samples were centrifuged in a Beckman Microfuge 11 at 10,000 × g for 10 min. The supernatant was saved and the pellet was washed twice with 20 mM Hepes, pH 7.5, containing 1 mM CaCl2, 1 mM MnCl2, and 0.2% Nonident P-40. The original supernatant and the supernatant from the washes were pooled, back-washed three times with 1 ml of ether, and passed with N2 to remove residual ether. The supernatant was then lyophilized and analyzed for glycopeptides by lectin chromatography followed by paper chromatography. In some cases the results were then checked by endoglycosidase H digestion.

Lectin Chromatography—Concanavalin affinity chromatography was done by a batchwise procedure: Samples were added to 100 μl of concanavalin A-agarose beads preswashed in 20 mM Hepes, pH 7.5, containing 1 mM CaCl2 and 1 mM MnCl2. After 1 h the beads were washed 5 times with buffer and glycosylated tripeptide was eluted by overnight incubation with 0.5 ml of the same buffer containing 0.5 M α-methylmannoside. In some cases (data not shown) labeled peptide that did not bind to concanavalin A-agarose was tested for binding to wheat germ agglutinin-Sepharose.

Paper Chromatography—Descending paper chromatography of peptide and glycopeptide was performed in 1-butanol/acetate/acidwater (12:3:5, v/v) on Whatman 3-mm paper for 16 h. Strips of 1 cm were cut from the chromatograph and counted in a LKB 1275 Minigamma counter.

Endoglycosidase H Digestion—The glycopeptides that remained at the origin after paper chromatography were eluted off the paper in 0.15 M citrate buffer (pH 5.8). Endoglycosidase H was added (10 units/ml) and incubated overnight at 37 °C. Controls consisted of mock incubation mixtures lacking endoglycosidase H. The digested samples were subsequently analyzed by descending paper chromatography.

Oligosaccharyl Transferase Assay—Oligosaccharyl transferase activity in microsome prepared from liver as described earlier (17) was estimated by using saturating amounts of the synthetic peptide acceptors [125I]labeled N-3-[4-hydroxyphenylpropionyl]-Asn-Lys-[125I]Tyr-Thr peptide. In some cases (data not shown) labeled peptide containing 125I was used as a control. The labeled material that did not bind to concanavalin A-agarose was tested for binding to wheat germ agglutinin-Sepharose. The labeled peptide was analyzed by descending paper chromatography. The results of this analysis, shown in Fig. 2, reveal that endoglycosidase H digestion completely converts all of the glycopeptide to a high mobility form. This finding establishes that all of the chains are susceptible to endoglycosidase H, e.g. only high mannose chains were present on the peptide.

The time course of glycosylation of the three tripeptides was followed after their microinjection into the oocytes. As shown in Fig. 3, the efficacy of the three peptides as acceptors varied by 100-fold. However, in all three cases maximal glycosylation was observed after 1–2 h; at later times, the amount of glycosylated peptide actually decreased. This decline in occurs after their glycosylation in vivo. A basic technical problem to be resolved before tripeptide acceptors can readily be utilized in glycosylation studies involving intact cells is how to deliver them to the cytoplasm and ultimately to the endoplasmic reticulum. Since earlier studies indicate that hydrophobic peptides readily enter the endoplasmic reticulum, in theory the problem is simplified to one of delivery of the peptide into the cellular cytoplasm. One system in which potential delivery problems can be minimized is the Xenopus oocyte, which can readily be microinjected. Therefore, we initially utilized this system to study the glycosylation and processing of tripeptide substrates in vivo. The structures of the three tripeptides that were tested as substrates are shown in Fig. 1.

To measure the possible glycosylation of microinjected 125I-labeled tripeptides we utilized the techniques previously developed for assessing in vitro peptide glycosylation in isolated hen oviduct microsomes (15). Thus, the formation of glycosylated tripeptides was initially quantitated by binding to concanavalin A-agarose. To document that all of the peptide that bound to concanavalin A-agarose was in fact a glycopeptide, after elution with α-methylmannoside it was subjected to paper chromatography according to the procedure described by Welgyl et al. (16). In the chromatographic system used, authentic standards of peptide substrate migrated near the solvent front, whereas the glycosylated peptide remained at the origin. Evidence that the tripeptide was, in fact, converted to a high mannose glycopeptide was obtained by digesting the recovered glycotripeptide from the paper with endoglycosidase H and analyzing the digestion product by paper chromatography. The results of this analysis, shown in Fig. 2, reveal that endoglycosidase H digestion completely converts all of the glycopeptide to a high mobility form. This finding establishes that all of the chains are susceptible to endoglycosidase H, e.g. only high mannose chains were present on the peptide.

The time course of glycosylation of the three tripeptides was followed after their microinjection into the oocytes. As shown in Fig. 3, the efficacy of the three peptides as acceptors varied by 100-fold. However, in all three cases maximal glycosylation was observed after 1–2 h; at later times, the amount of glycosylated peptide actually decreased. This decline in

RESULTS

Earlier studies from this laboratory have utilized a wide variety of Asn-X-Ser/Thr-containing peptides (see Introduction) as in vitro substrates for oligosaccharyl transferase. As an extension of these studies, we sought to determine if, like intact proteins, processing and secretion of simple tripeptides

\[ \text{Peptide I} \]

\[
\begin{array}{c}
\text{HOOC-} \text{CH}_2- \text{CH}_2-O-C \cdot \{N^+\} \text{Asn-Lys-Thr-NH}_2 \\
\end{array}
\]

\[
\begin{array}{c}
\text{NH}_2-C-\text{C}^\ominus \text{-} N_3
\end{array}
\]

\[ \text{Peptide II} \]

\[
\begin{array}{c}
\text{HOOC-} \text{CH}_2- \text{CH}_2-O-C \cdot \{N^+\} \text{Asn-Tyr-Thr-NH}_2 \\
\end{array}
\]

\[ \text{Peptide III} \]

\[
\begin{array}{c}
\text{CH}_2-C \cdot \{N^+\} \text{Asn-Tyr-Thr-NH}_2
\end{array}
\]

FIG. 1. Structures of the acceptor tripeptides.

The abbreviations used are: Hepes, 4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid; Ac, acetyl.

\[ ^{1} \text{The abbreviations used are: Hepes, 4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid; Ac, acetyl.} \]
In Vivo N-Glycosylation of Triptides

13657

which is known to raise the pH of lysosomes, would be expected to retard catabolism (18). As shown in Fig. 4, in the presence of injected chloroquine the disappearance of glycopeptide was essentially abolished. The same results were obtained in two other experiments using chloroquine and measuring glycopeptide formation at 10 and 19 h. However, in no case was secretion of the glycosylated peptide into the medium observed. Thus, apparently the lack of secretion in control oocytes was not due to their degradation, because secretion of the glycopeptides did not occur even in oocytes blocked in degradation with chloroquine.

Fractionation of the oocytes 2 h after microinjection of peptide I into crude membrane and soluble fractions (100,000 x g pellet and supernatant) showed that all of the glycopeptide was associated with the membrane fraction (data not shown). This indicated that, as expected, the glycosylation is indeed taking place in a membrane-associated organelle.

Earlier studies from this laboratory with a variety of -Asn-X-Ser/Thr- derivatives delineated the structural modifications that allow optimal recognition of the tripeptides by oligosaccharyl transferase (16). In vitro studies using these peptides have shown that tripeptides blocked at both the amino and carboxyl termini permeate the microsomal membrane, but once they are glycosylated they are trapped in the lumen (11). Our findings on the marked differences in the glycosylatability of the three tripeptides utilized in the in vivo Xenopus microinjection experiments prompted us to compare their acceptor activities in isolated microsomes in vitro. The results of this experiment, shown in Fig. 5, indicate that glycotripeptide formation was approximately linear with time for the first 8 min. Under these conditions in microsomes, the relative efficacy of the three tripeptides as acceptors paralleled that found in the in vivo oocyte system, with the acceptor activity of peptide I being highest and that of peptide III lowest.

Relatively little is known about the mechanisms of peptide uptake in eukaryotic cells, but apparently small peptides can enter cells. Thus, chymoestatin, a hydrophobic tetrapeptide that inhibits chymotrypsin, was found to freely enter hepatocytes when added directly to the culture medium and inhibit both cytoplasmic and lysosomal proteolysis (19). More relevant to the current study was the demonstration that acceptor tripeptides such as N-acyl-Asn-Tyr-Thr-NH₂, are taken up by the cellular glycopeptide was not due to its secretion since, as shown, at all time points little or no glycopeptide was found in the culture medium. (The values for putative secreted glycopeptide III are not significant since the signal was 100-fold lower than that of glycopeptide I and only 2–3-fold above background values.)

These findings led us to consider the possibility that disappearance of glycosylated peptides is due to their catabolism. The fact that the site of the ¹²⁵I label was different in peptide III, as compared to peptides I and II, yet all three disappeared, suggested that the glycopeptide was degraded, rather than merely deiodinated. If the observed degradation occurred in the lysosomes, coinjection of labeled peptide and chloroquine.

Fig. 4. The effect of chloroquine on the disappearance of ¹²⁵I-glycotripeptide in Xenopus oocytes. Chloroquine (50 μM) was coinjected into Xenopus oocytes with peptide I and the formation of ¹²⁵I-glycopeptide in the oocytes and in the media was determined.

FIG. 2. Paper chromatographic analysis of ¹²⁵I-labeled glycopeptide incubated without ([]) or with (○) endoglycosidase H. Peptide I was used in this experiment.

FIG. 3. Rate of appearance of ¹²⁵I-glycotripeptides in Xenopus oocytes and the culture medium. The indicated ¹²⁵I-tripeptide was microinjected into five oocytes per time point. Glycopeptides in the oocytes ([]) and in the medium (○) were analyzed as described under "Experimental Procedures." From the specific activity of peptide III indicated in the footnote to Table I it can be calculated that less than 0.032 pmol, i.e. 160 cpm (2–3 times over background) of labeled peptide was found in the oocyte medium.

FIG. 4. The effect of chloroquine on the disappearance of ¹²⁵I-glycotripeptide in Xenopus oocytes. Chloroquine (50 μM) was coinjected into Xenopus oocytes with peptide I and the formation of ¹²⁵I-glycopeptide in the oocytes and in the media was determined.

0 6 12 24
Time (Hours)

100
75
50
25
0
[pg ¹²⁵I/μg] Glycopeptide (relative maximum activity)

Fractionation of the oocytes 2 h after microinjection of peptide I into crude membrane and soluble fractions (100,000 x g pellet and supernatant) showed that all of the glycopeptide was associated with the membrane fraction (date not shown). This indicated that, as expected, the glycosylation is indeed taking place in a membrane-associated organelle.

These findings led us to consider the possibility that disappearance of glycosylated peptides is due to their catabolism. The fact that the site of the ¹²⁵I label was different in peptide III, as compared to peptides I and II, yet all three disappeared, suggested that the glycopeptide was degraded, rather than merely deiodinated. If the observed degradation occurred in the lysosomes, coinjection of labeled peptide and chloroquine.
ever, in this case peptides II and III were comparable to glycosylated peptide I, whereas in the case of glycopeptides II and III no lag could be detected between formation of intracellular and extracellular forms. Experiments with Chinese hamster ovary cells in isolation revealed that the glycosylation of tripeptide and appearance of extracellular glycopeptide and cellular glycopeptide followed a similar process as described in Table I. The specific activities of peptides I, II, and III were significantly less active as substrates than peptide I. Further comparison of the three biological systems is shown in Table II. It is apparent that both the specific activity of the glycosylation process and the percentage conversion of microinjected or added peptide I to glycopeptide are greatest in microsomes, and that lower and similar values for each parameter are obtained in both oocytes and somatic cells.

### DISCUSSION

The earlier findings on the ability of simple tripeptides to serve as substrates for the endoplasmic reticulum (10), coupled with the subsequent demonstration that such peptides would block the in vitro cotranslational glycosylation of nascent polypeptide chains (12), led us to consider the possible utility of such peptides in vivo. Specifically, if such peptides could be delivered to the cytoplasm, and if they subsequently entered the endoplasmic reticulum, they could be used to study the fate of glycosylated peptides vis à vis glycosylated proteins in the endomembrane system. In addition, introduction of such peptides at sufficiently high concentration might result in inhibition of protein glycosylation in vivo, as was earlier demonstrated in vitro (11).

With respect to delivery of acceptor peptides to cells, two obvious alternatives are (a) addition to the culture medium on the assumption that, because of their hydrophobicity, some of the peptide would enter the cells and (b) introduction by microinjection. While our studies utilizing the latter approach were in progress, Wieland et al. (13) reported the results of studies using the former approach. These studies show that exogenous peptides were glycosylated by a variety of cultured mammalian cells and that they were subsequently secreted.
In Vivo N-Glycosylation of Tripeptides

We chose the microinjection approach because earlier studies have established that Xenopus oocytes are capable of translating a wide range of secretory protein mRNAs and carrying out a variety of posttranslational modifications (1). Indeed, oocytes have been shown to provide a surrogate system of high fidelity for the purpose of studying protein secretion (20). Moreover, recently it has been shown that the mammalian mannose-6-phosphate-dependent pathway for targeting lysosomal enzymes to lysosomes is functional in oocytes (21), and Xenopus oocytes injected with mRNA for human low density lipoprotein receptor synthesized the receptor, added O-linked sugars, and transported it to the cell surface as in the case of mammalian cells (22). Thus the frog oocyte seems to be fully equipped to carry out the diversity of co- and posttranslation processes involved in the assembly and disposition of secretory and cell surface glycoproteins.

Our initial findings that all three of the microinjected peptides were glycosylated support this generalization. The efficacy of the three peptides as acceptors in vivo varied over a 100-fold range, with peptide I being the best acceptor and peptide III the worst; the difference may be due to a progressive decrease in hydrophobicity between peptides I and III, although this has not been directly determined. Interestingly, the same order of acceptor activity was observed when the three peptides were compared in the in vitro microsomal glycosylation system. Although the concentrations of the injected or added peptide were within the same order of magnitude in oocytes, somatic cells, and microsomes (98, 160, and 35 nM, respectively), the specific activity of the glycosylation process was much higher (100-300 fold) in microsomes. One explanation for this, of course, is that oligosaccharyl transferase is localized to the endoplasmic reticulum, and this organelle would only represent a fraction of the total protein mass of the oocyte or the somatic cell. In addition, at least in the case of the oocyte which contains a large amount of lipid-rich yolk, it is very likely that the effective concentration of the hydrophobic peptides available to oligosaccharyl transferase in the endoplasmic reticulum could be much lower than 98 nM. Perhaps under these conditions substrate rather than enzyme has become limiting. Also of interest, with respect to the comparison of isolated microsomes and the cells, is the percentage conversion of peptide to glycopeptide. Not unexpectedly the highest conversion was observed with microsomes. The conversion of peptide was 10-fold lower in somatic cells, an intermediate value was observed for conversion in the oocytes.

Despite the fact that in oocytes all three peptides were glycosylated and therefore are presumed to have entered the endoplasmic reticulum, in no case was secretion of the resultant glycopeptides observed. Initially we thought that this lack of secretion might be caused by catabolism of the glycosylated peptides, which was found to occur within 2 h of their formation. However, coinjection of chloroquine (which had no inhibitory effect on glycosylation) resulted in a block in this degradatory process without facilitating secretion of the glycosylated peptide. These findings suggest that following its glycosylation the peptide had been routed out of the normal secretory pathway to acidic, lysosomal compartments. Consistent with the idea that the glycopeptide did not move through the normal pathway that culminates in secretion is the observation that there was no processing of the high mannose oligosaccharide chains on the glycopeptide to complex forms that would bind to wheat germ agglutinin. Given the demonstrated ability of the oocyte to correctly route glycosylated proteins, these findings on the absence of secretion of glycosylated peptides are interesting and warrant further study to define the reason for the apparent misrouting. In this context, it is of interest that one exception to the generalization that secretion of de novo synthesized secretory proteins is observed in oocytes (1, 20) is the case of the hydrophobic protein melittin, which is translated and inserted into the endomembrane system, but is neither secreted nor degraded (23). Perhaps in the oocyte system hydrophobic polypeptides and glycopeptides partition into the membranes and therefore cannot exit from the cell.

Finally, it is of interest that the same three peptides, when added to mammalian somatic cells in culture, were taken up and both glycosylated and secreted. Because the three peptides exhibited the same relative efficacy as acceptors in both oocytes and somatic cells, it is clear that the lack of secretion in the oocytes is due to differences in routing, rather than in the structures of the peptides per se. Additionally, it should be noted that based on our limited studies with mammalian cells, the kinetics of secretion appeared to depend on the structure of the peptide. Thus, upon glycosylation peptide I exhibited a discernible lag prior to secretion, whereas no lag could be detected with the glycosylated forms of either peptides II or III. Wieland et al. (13) have proposed that the rapid exit of glycosylated peptides from cells is a measure of bulk flow through the endomembrane system. Our observation on the differences between secretion of various glycosylated peptides in somatic cells, as well as the finding that glycopeptide secretion does not occur in oocytes that are known to be capable of secreting both glycoproteins and proteins, suggest that the fate of these simple glycosylated peptides in the endomembrane system may be more complex than expected.

Acknowledgments — W. J. L., formerly a Robert A. Welch Professor of Chemistry, acknowledges the Robert A. Welch Foundation. We are indebted to Dr. David Wright and Michael L. Harless for the ooey microinjections, which were supported by Core Grant C41667. Dr. Robert Nisson is thanked for his help in the iodination of Chemistry, acknowledges the Robert A. Welch Foundation. We are indebted to Dr. David Wright and Michael L. Harless for the ooey microinjections, which were supported by Core Grant C41667.

REFERENCES

1. Lane, C. D., Colman, A., Mohun, T., Morser, J., Champion, J., Kaurides, J. Craig, and R (1980) Eur J Biochem 111, 225-235

2. Richter, J. D., and Smith, L. D. (1981) Cell 27, 183-191

3. Richter, J. D., Jones, N. C., and Smith, L. D. (1982) Proc. Natl. Acad. Sci. U. S. A. 78, 3739-3743

4. Richter, J. D., Evers, D. L., and Smith, L. D. (1983) J Biol. Chem 258, 2614-2620

5. Richter, J. D., Lorenz, L. J., and Audet, R. G. (1986) J Biol. Chem 260, 4448-4454

6. Krog, P., Strachan, K., Walia, E., Tabe, L., and Colman, A. (1984) J Mol. Biol. 180, 615-643

7. Kiyel, M. L., McNight, G. S., and Schimke, R. T. (1976) J Biol. Chem. 251, 5490-5495

8. Giabe, C. G., Hanover, J. A., and Lennarz, W. J. (1980) J Biol. Chem. 255, 9236-9242

9. Stuck, D. K., and Lennarz, W. J. (1980) in The Biochemistry of Glycopeptides and Proteoglycans (Lennarz, W. J., ed) pp. 35-84, Plenum Publishing Corp., New York

10. Hart, G. W., Brew, K., Grant, G. A., Bradshaw, R. A., and Lennarz, W. J. (1979) J Biol. Chem. 254, 9747-9753

11. Welply, J. K., Shenbagamurthi, P., Naider, F., Park, H. R., and Lennarz, W. J. (1986) Arch. Biochem. Biophys. 246, 808-819

12. Welply, J. K., Shenbagamurthi, P., Lennarz, W. J., and Naider, F. (1983) J. Biol. Chem. 258, 11850-11863

13. Wieland, F. T., Gleason, M. L., Serafini, T. A., and Rothman, J. E. (1987) Cell 50, 289-300

14. Berney, H. R., Wieland, F. T., and Boman, H. G. (1982) Biochemistry 21, 5020-5031

15. Wieland, J. K., Kaplan, H. A., Shenbagamurthi, P., Naider, F., and Lennarz, W. J. (1986) Arch. Biochem. Biophys. 246, 808-819
In Vivo N-Glycosylation of Tripeptides

F. (1983) J. Biol. Chem. 258, 11856–11863
Chowdhury, J. R., and Arias, I. M. (1981) Methods Enzymol. 77, 192–197
Morser, J. (1981) Eur. J. Biochem. 113, 339–348
17. Chowdhury, J. R., and Arias, I. M. (1981) Methods Enzymol. 77, 192–197
18. Mellman, I., Fuchs, R., and Helenius, A. (1986) Annu. Rev. Biochem. 55, 663–700
Grinde, B., Galpin, I. J., Wilby, A. H., and Beynon, R. J. (1983) J. Biol. Chem. 258, 10821–10823
19. Grinde, B., Galpin, I. J., Wilby, A. H., and Beynon, R. J. (1983) J. Biol. Chem. 258, 10821–10823
Colman, A., Lane, C. D., Craig, R., Bolton, A., Mohun, T., and Morser, J. (1981) Eur. J. Biochem. 113, 339–348
20. Colman, A., Lane, C. D., Craig, R., Bolton, A., Mohun, T., and Morser, J. (1981) Eur. J. Biochem. 113, 339–348
21. Faust, P. L., Wall, D. A., Perara, E., Lingappa, V. R., and Kornfeld, S. (1987) J. Cell Biol. 105, 1937–1945
22. Peacock, S. L., Bates, M. P., Russell, D. W., Brown, M. S., and Goldstein, J. L. (1988) J. Biol. Chem. 263, 7838–7845
23. Lane, C. D., Champion, J., Haine, L., and Kreil, G. (1981) Eur. J. Biochem. 113, 273–281