Protein Matrix Effects on Glycan Processing by Mannosidase II and Sialyl Transferase from Rat Liver†

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ABSTRACT: The effect of the protein environment on the reaction sequence and the relative rates of two two-step reactions involved in the biosynthesis of complex glycans in glycoproteins has been explored by comparing the processing of biotinylated substrates either free or bound to avidin. By use of biotinyl and biotinamidohexanoyl derivatives, the display of the glycan in a proximal and distal association with the avidin surface could also be assessed. Mannosidase II removes two Man residues from the substrate $GlcNAcMan_5GlcNAc_2$-R to yield $GlcNAcMan_3GlcNAc_2$-R. The NMR spectra of the substrate, intermediate, and product showed that the first Man is removed from the 6-arm of the substrate. The rate constants for the first and second step (estimated by direct analysis of the reactants by anion-exchange chromatography with a pulsed amperometric detector) were determined to be about 0.05 and 0.08 min^{-1}, respectively, for the free substrates. In the proximal complex k_1 was reduced 80-fold, and the k_2 step could not be observed under the same conditions. In the distal complex both k_1 and k_2 were reduced about 8-fold. Sialyl transferases transfer Sia from CMP-Sia to the biantennary substrate $Gal_2GlcNAc_2$-Man_3GlcNA_2-R to yield the product Sia_2Gal_2-$GlcNAc_2Man_3GlcNAc_2$-R with the Sia linked either 2–3 or 2–6 to the Gal residues. The NMR spectra showed that the first step involved the Gal on the 3-arm of the substrate and that both Sia residues were added 2–6. The rate constants for the two steps in the proximal complex showed an 8- and 5-fold decrease for k_1 and k_2, respectively, compared to those of the free substrate; in the distal complex both constants were decreased about 2-fold. The results confirm that the protein environment exerts a significant effect on the substrate quality of glycans in glycoproteins. Although the specific effects studied to date have not yielded any evidence that the protein matrix causes major changes in enzyme specificity, the observed effects are of sufficient magnitude to explain how one glycan may remain as an oligomannose-type structure while its neighbor is converted to a typical complex structure or how heterogeneity can arise during the different biosynthetic steps.

It is now well established that the protein matrix on which a given N-linked glycan is displayed in a glycoprotein exerts a significant regulatory effect on the enzymes involved in glycoprotein processing (Kornfeld & Kornfeld, 1985; Schachter, 1984; Tanner & Lehle, 1987). Although the nature, specificity, and relative amounts of the processing enzymes in a given compartment unquestionably represent key factors determining the glycan end products of glycoprotein processing in that compartment (Beyer et al., 1981; Schachter, 1984; Gopal & Ballou, 1987), the fact that so many different glycan end products are encountered in different glycoproteins and indeed in a single protein from the same compartment must mean that each glycan structure in its specific protein environment is a unique substrate for the processing enzymes. This phenomenon of differential processing specificity within a given glycoprotein has been observed in animals (Hsieh et al., 1983; Sheares & Robbins, 1987; Parekh et al., 1987; Yet et al., 1988a), in yeast (Trimble et al., 1983), and in plants (Sturm et al., 1987), and the nature of these protein matrix effects appears to be a major unknown in our understanding of the specificity of glycan processing in vivo. Is the protein simply a nonspecific mechanical barrier to the approach of processing enzymes, or do specific glycan–protein interactions induce unique glycan conformations at each individual glycosylation site (Yet et al., 1988b)? We have compared the processing rate of free and protein-bound glycans using an artificial system in which the glycoprotein model is prepared through the interaction of biotinylated glycans with avidin (Chen & Wold, 1986; Shao & Wold, 1987). Marked decreases in processing rates were observed for most of the protein-bound substrates when compared to those of the free ones; if an extension arm was inserted between the glycan and biotin, to move the glycan away from the protein surface, most of the inhibition was removed in many, but not all, of the reactions (Shao et al., 1987). Two of the reactions studied, those catalyzed by mannosidase II and sialyl transferase, are consecutive, two-step reactions of the type A → B → C, and from the fact that intermediate B appeared to accumulate in the presence of avidin, it was concluded that the two steps of each reaction were affected by the protein matrix. The two reactions have been extensively studied with a number of substrates and enzymes from different sources. Harpaz and Schachter (1980) have demonstrated that the first step of the rat liver mannosidase II catalyzed reaction is slower than the second step and suggested, on the basis of their concanavalin A binding studies that the removal of the 1–6-linked Man is the first step in the reaction. Two sialyl transferases have been isolated from liver and shown to catalyze the incorporation of either α2–6- or α2–3-linked sialyl residues into biantennary structures like the one used here (Weinstein et al., 1982), and an α2–6 sialyl transferase from bovine colostrum has been shown to follow a specific sequence of addition of the two sialyl residues to biantennary glycans: the first, and fastest, addition to the 1–3 arm; the second, and slower, to the 1–6 arm (Joziasse et al., 1985, 1987). On the basis of this information,

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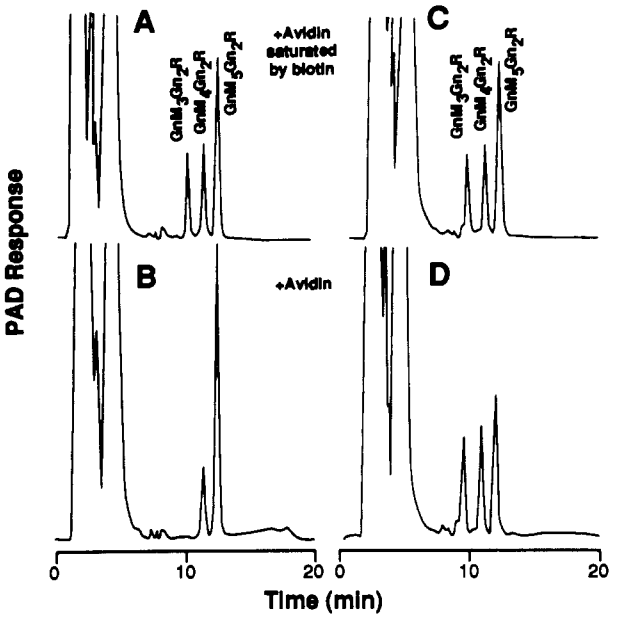


FIGURE 1: Typical analytical chromatograms for the mannosidase II reaction with $GlcNAcMan_5GlcNAc_2$-(biotinyl)Asn (A and B) and $GlcNAc\text{-}Man_5GlcNAc_2$-(6-biotinamidohexanoyl)Asn (C and D) as substrates either free (A and C) or bound to avidin (B and D). Spectra A and C represent reaction mixtures after 15-min incubation; spectrum B represents the reaction mixture after 4 and panel D that after 2 h. The elution was carried out with a 12-min gradient from 30 to 100 mM NaOAc in 100 mM NaOH, followed by a 5-min reverse gradient back to the starting conditions and a 5-min equilibration at this condition (30 mM NaOAc in 100 mM NaOH) before a new sample was applied. The flow rate was 1 mL/min.

we are here examining whether the protein matrix provided by the artificial avidin–glycan complex will significantly affect these established reaction sequences and relative rates in a manner that may reflect specific glycan–protein interactions.

Experimental Procedures

Preparation of Substrates. In all the glycan structures designated, the R group is either $GlcNAc_2$-(biotinyl)Asn or $GlcNAc_2$-(biotinamidohexanoyl)Asn. It should be noted that the preparation of individual substrates from the starting material (Man_5-$GlcNAc_2$-Asn isolated from Pronase-digested ovalbumin) by enzymatic means required that the α-amino group of Asn be blocked. Golgi enzyme preparations contain sufficient levels of glycosyl-Asn amidase activity to cause extensive hydrolysis of the starting material (Kohno & Yamashita, 1972; Shao et al., 1989). Since the amidase requires a free amino group for activity, the two biotinylated derivatives were resistant to the amidase, but the two derivatives had to be prepared and converted separately to the desired products. The general procedures used for the preparation of substrates have been described (Shao & Wold, 1987; Shao et al., 1987); the experimental conditions were simply scaled up to provide the larger quantities of glycans needed for the NMR experiments. All the reactions were carried out in the presence of 30 mg/mL bovine serum albumin.

The substrate $GlcNAc\text{-}Man_5$-R was produced by incubating the ovalbumin–glycopeptide Man_5-R with UDP-GlcNAc and Golgi enzymes in the presence of the mannosidase II inhibitor swainsonine. The same reaction, but without swainsonine, was used to prepare $GlcNAc_2$-Man_3-R. The substrate Gal_2-$GlcNAc_2$-Man_3-R was in turn prepared from this latter derivative by incubation with Golgi enzymes in the presence of UDP-Gal (Shao et al., 1989). The product of each incubation was purified by gel filtration and characterized by mass spectrometry (Shao et al., 1987).

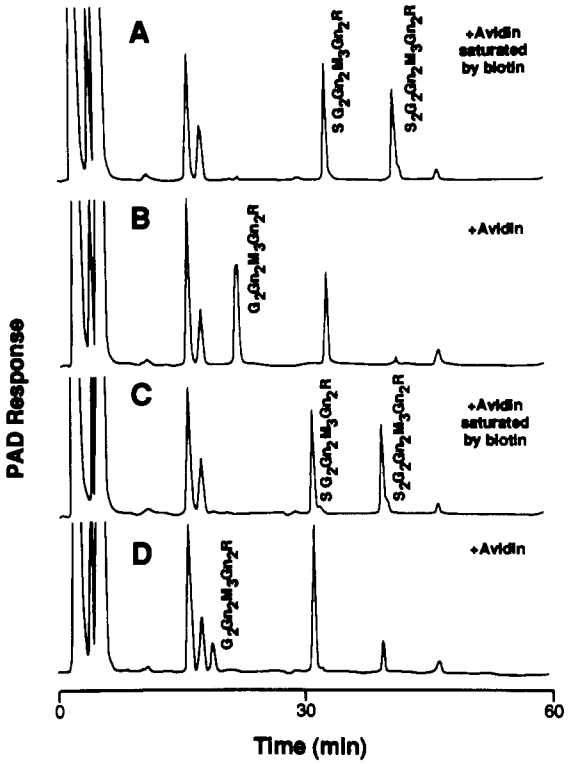


FIGURE 2: Typical analytical chromatograms for the sialyl transferase reaction with $Gal_2GlcNAc_2Man_3GlcNAc_2$-(biotinyl)Asn (A and B) and $Gal_2GlcNAc_2Man_3GlcNAc_2$-(6-biotinamidohexanoyl)Asn (C and D) as substrates either free (A and C) or bound to avidin (B and D). All samples represent reaction mixtures after 2-h incubation. The elution was carried out with a 20-min gradient from 15 mM NaOAc in 78 mM NaOH to 30 mM NaOAc in 91 mM NaOH, followed by a 35-min gradient from this eluent to 125 mM NaOAc in 100 mM NaOH. At 55 min a 5-min reverse gradient returned the eluent the starting point (15 mM NaOAc in 78 mM NaOH), and a 10-min equilibration at this level was carried out before a new sample was applied. The flow rate was 1 mL/min. The two peaks at 16 and 18 min are derived from CMP-Sia (control not shown).

Kinetic Analysis. The 200-μL reaction mixtures contained 250 nmol of glycan, 2 μmol of CMP-Sia (in the case of the transferase reaction), and 240 μg of Golgi protein in 10 mM MES (Sigma) buffer, pH 6.7, containing 10 mM $MnCl_2$, 5 μL/mL Nonidet, and either 7 mg of avidin–biotin (free glycan) or 7 mg of avidin (glycan–avidin complex). The reaction was initiated by the addition of the enzyme and incubated at 37 °C. Aliquots (40 μL) were removed at different times, mixed with 4 μL of biotin (4 mg/mL), and heated at 95 °C for 10 min. The reaction mixture was transferred, along with a total of 130 μL of water to wash the reaction vessel, to Amicon Centrifree filter tubes, and the filtrate was collected after centrifugation. Aliquots of 24 mL (containing approximately 5 nmol of total glycan) were diluted with 25 μL of water, and a 5-μL sample was analyzed on the Dionex BioLC carbohydrate analyzer (Chen et al., 1988). The analysis was carried out on an AS-6 anion-exchange column with an AG-6 guard column by use of a three-solvent elution gradient at high pH. The specific elution conditions are given in Figures 1 and 2. The detection system was the Dionex pulsed amperometric detector (PAD) consisting of a gold working electrode and an Ag/AgCl reference electrode and operating at an applied potential of 0.05 V. The eluted peaks were integrated, and the first-order rate constants were estimated by assuming that the PAD response is the same for substrate, intermediate, and product. The rate equations expressing the change in substrate, intermediate, and product as functions of time and the rate constants k_1 and k_2 were solved with the Microsoft EXCEL

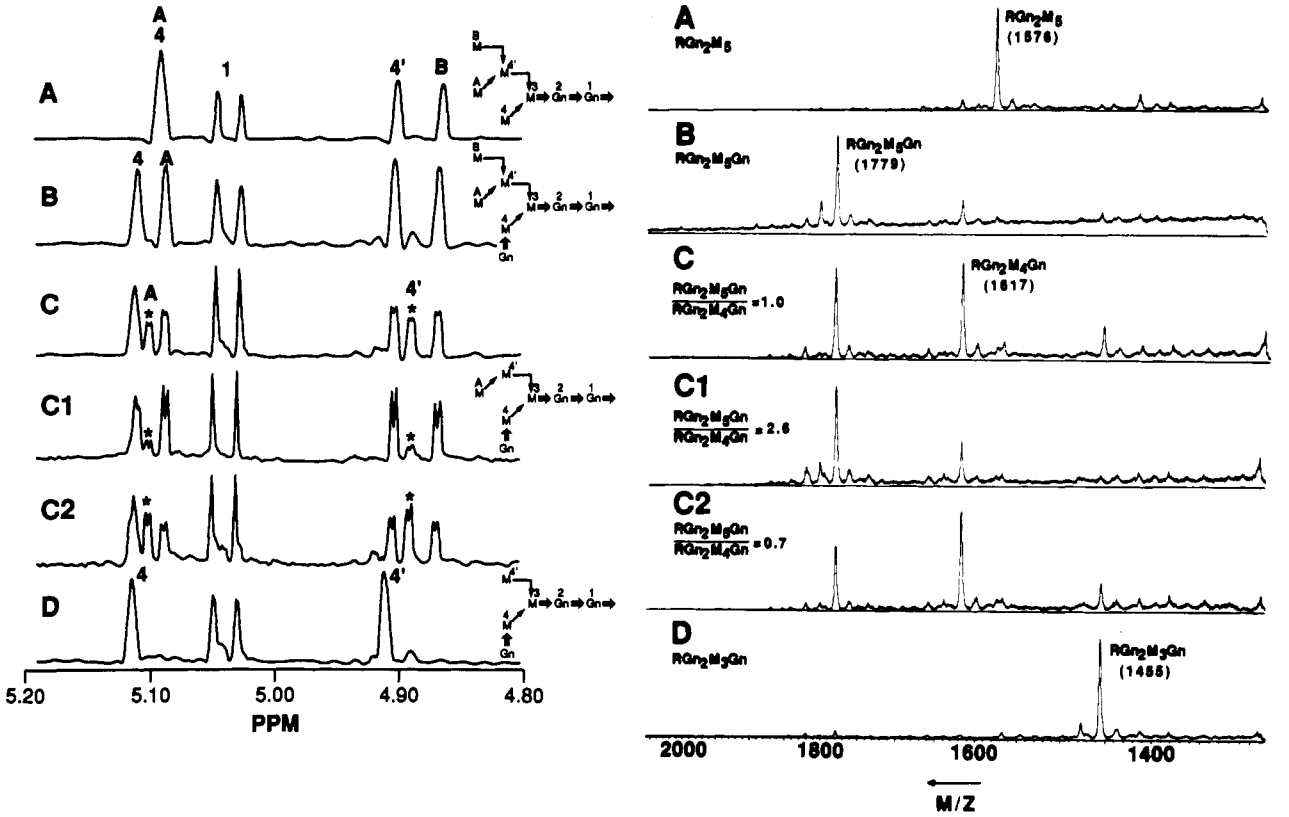


FIGURE 3: Characterization of the components of the mannosidase II catalyzed reaction: (right) mass spectra; (left) anomeric proton NMR spectra. (A) Reference compound; (B) substrate; (C) reaction mixture containing about equal amounts of substrate and intermediate and a small amount of product; (C_1) reaction mixture enriched for substrate; (C_2) reaction mixture enriched for intermediate (and product); (D) product. The integration of the NMR signals in spectra C, C_1, and C_2 correspond well to the corresponding molecular ion peak intensities from the mass spectra. The signal at 5.10–5.11 marked with an asterisk is due to the A residue after B has been removed; the signal at 4.88–4.89 also marked with an asterisk, to the 4′ residue after B has been removed. When A is also removed in the k_2 step, the unsubstituted 4′ chemical shift settles at 4.91 ppm. The structure code is the same as that used in Scheme I.

program, yielding the values for k_1 and k_2 from the best fit of the data. In the long-term incubations there was evidence of loss of enzyme activity with time (a decreasing trend in the values of the rate constants). In these cases, the early data points were used for the estimation of the rate constants.

Preparation of the Reaction Intermediates and Products for NMR. (*A*) *Mannosidase II.* The substrate GlcNAc-Man_5-R (8.9 mg; 5 μmol) was incubated with the Golgi membrane preparation (total of 4.8 mg of protein) in a total volume of 4.0 mL of 10 mM MES buffer, pH 6.8, containing 200 μL of 10% Nonidet P-40 and 10 mM $MnCl_2$. In addition, one reaction mixture contained 35 mg/mL BSA (free glycan) and another 35 mg/mL avidin (protein-bound glycan). After incubation at 37 °C (12 h for the free glycan and 8 h for the avidin-containing sample), the reactions were stopped by heating at 95 °C for 10 min in the presence of biotin, precipitated protein was removed by centrifugation, and the supernatant solution was lyophilized. The products were redissolved in water and subjected to gel filtration on a 1.6 × 35 cm column of Bio-Gel P-6 (200–400 mesh) eluted with 0.1 M ammonium acetate. The fractions containing biotinylglycan were identified (Shao et al., 1987), pooled, lyophilized, and redissolved in 0.15 mL of water. A small sample was removed for MS analysis, and the remainder was used for the NMR experiment. When it was found that the collected glycan fraction from the sample containing avidin consisted of an approximately equal mixture of starting substrate and intermediate, an attempt was made to separate the two glycans on a large (1 × 240 cm) column of Bio-Gel P-4. The mixture eluted as a broad partially resolved peak; the central part of which was collected, lyophilized, and rerun on the same P-4 column. The fractions constituting the front half of the resulting peak and those constituting the trailing half were pooled separately and prepared for MS and NMR analysis. The mannosidase II reaction was run only with the biotinylated substrate, free and bound to avidin.

(*B*) *Sialyl Transferase.* The substrate Gal_2-$GlcNAc_2$-Man_3-R (20 mg; ~10 μM) was incubated as above but the CMP-Sia as donor substrate. The workup was also the same as above, but in this case the gel filtration on P-6 partially resolved the mono- and disialylated derivatives, permitting the isolation of ~90% pure ntermediate and 80–85% pure product for the MS and NMR analyses. In the original work (Shao et al., 1987) it was found that both the biotinylated and the biotinamidohexanoylated derivatives reflected a significant effect of avidin on the rate of incorporation of the second Sia resdue, and the reaction was consequently carried out with both substrates bound to avidin; only the derivative with the extension arm was studied in the absence of avidin.

Fast Atom Bombardment Mass Spectrometry. A concentrated aqueous solution of the lyophilized samples from the gel filtration columns was used for the MS analyses. A 1-μL aliquot, containing 1–5 nmol of glycan, was mixed well with 1 μL of 90:10 mixture of glycerol and a saturated aqueous oxalic acid solution, and the entire sample was applied to the probe of a Kratos MS50RF high-resolution mass spectrometer. Mass spectra were obtained with a wide-range multichannel analyzer program on the DS90 data system and were calibrated with cesium iodide.

^1H NMR Spectroscopy. The remainder of the lyophilized samples from the gel filtration columns (1–4 μmol) was dissolved in D_2O and lyophilized three times. The sample was

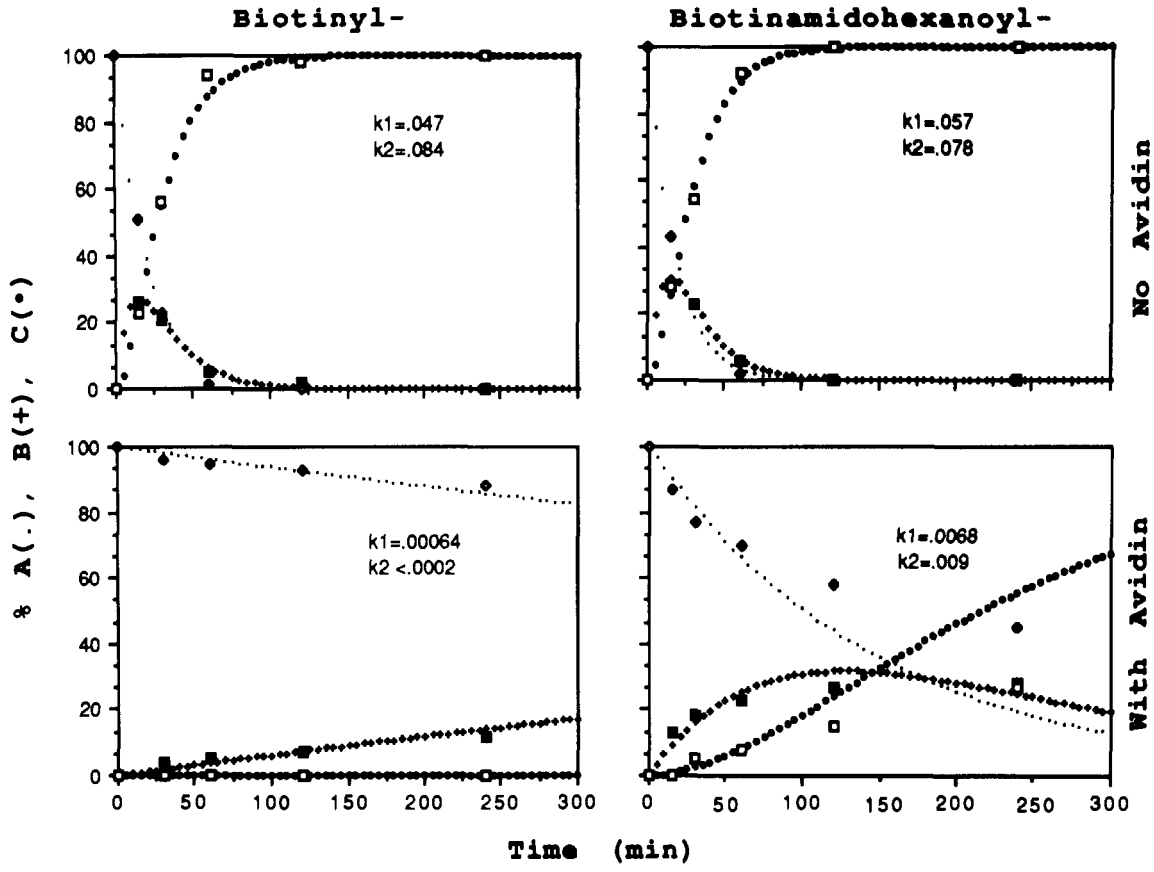


FIGURE 4: Reaction progress curves for the mannosidase II catalyzed reactions. The theoretical curves for substrate (A), intermediate (B), and product (C) at the estimated k_1 and k_2 values are indicated with the symbols on the ordinate; the corresponding experimental points are (○) A, (■) B, and (□) C.

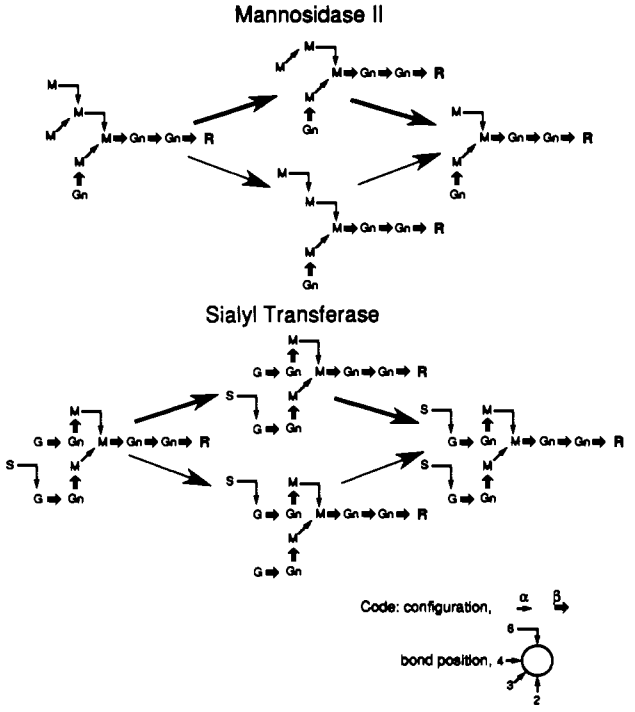
finally dissolved in 400 μL of D_2O (99.996 atom % D, Aldrich Chemical Co.). ^1H NMR spectra were obtained at 500 MHz with a GE GN500 spectrometer, using 32K data points, 16 bit A/D conversion, a sweep width of 7000 Hz, a 90° pulse, a recycle time of 7.5 s, and presaturation of the residual HDO at room temperature. Resolution was enhanced by apodization of the FID by Gaussian multiplication and an exponential multiplication with a negative time constant. Proton chemical shifts are reported relative to internal acetone at 2.217 ppm.

Results and Discussion

Scheme I illustrates the possible paths for the two steps catalyzed by the two processing enzymes; the question addressed in this study is whether the protein matrix may alter the established path (heavy lines in the scheme) and affect the two rate constants to a different extent.

Mannosidase II Reaction. The NMR and FAB-MS spectra of the substrate, intermediate, and product of the mannosidase II catalyzed reaction are shown in Figure Figure 3. Panels A show the data for the reference compound Man_5-R, panels B the substrate GlcNAc-Man_5R, and panels D the product GlcNAc-Man_3-R. This was the reaction product from the long-time incubation in the absence of avidin (free glycan). Under similar conditions (shorter time to favor intermediate formation), the incubation in the presence of avidin gave the product shown in panels C, containing about 50% substrate, 8% product, and 42% of the intermediate GlcNAc-Man_4-R according to the MS data. The mixture in panels C was fractionated by gel filtration to eliminate the small product peak and to enrich for starting material in fraction C_1 and for intermediate in C_2. According to the numbering system and the chemical shifts established by Vliegenthart et al. (1983), the anomeric proton resonances for all these samples show that the missing Man in the intermediate must be the outer 1–6-linked one (B). The rate constants for the two steps catalyzed by the rat liver Golgi enzyme have been determined by Harpaz and Schachter (1980) to be 0.065 and 0.14 min^{-1}, respectively, but which Man residue is removed first has not been unequivocally determined. Using the carbohydrate analyzer, we estimated the rate constants for the two substrates free and bound to avidin. The results are summarized in Figure 4 and show the effects of the protein matrix on the individual rate constants for the two steps of the mannosidase II reaction. With the k_2 steps being faster than the k_1 step for the free substrate, it has been difficult to accumulate enough intermediate to establish its structure; in the following discussion we shall assume that it is the same as that established for the avidin-bound derivative (Figure 3). With this assumption, the data in Figure 4 show that the protein matrix caused a 70-fold decrease in the rate of the removal of Man on the 6-arm and a greater than 400-fold decrease in the removal of the Man on the 3-arm. Since no product was observed in the kinetic analysis, the value of $k_2 < 0.0002$ is an estimate based on the calculation that a value of 0.0002 should have yielded observable amounts of product at the longest incubation times. The data in Figure 4 suggest that the reaction is slowing down in some of the longer incubations, indicating that the enzyme loses activity, and the value for k_2 may consequently be underestimated. Nevertheless, the ready accumulation of intermediate even in cases where product was produced in experiments involving the avidin complex (Figure 3) is consistent with the general conclusion made earlier (Shao & Wold, 1987) that the second step of the reaction is more strongly retarded than is the first. The effect of the extension arm is to reduce the effect of the avidin to a 8–9-fold decrease in both the k_1 and k_2 steps, leaving the k_2 step as the fastest for both free

Scheme I



and avidin-bound substrate. Glycoproteins with a glycan exposure similar to that in the proximal avidin complex should therefore be expected to yield primarily products with five and four Man residues. A minor fraction of ovalbumin glycans does indeed show a GlcNAc-Man_4 structure, with the structure identical with that of the intermediate characterized here.

Sialyl Transferase Reaction. For the second enzyme, sialyl transferase, both the substrate without and the one with the extension arm were used. The original studies had suggested that the effect of the protein matrix was expressed approximately equally for the two differently displayed substrates, and it was considered of interest to explore whether the apparently equal processing of the two different substrates might still be different in terms of the specificity of either the position or the linkage of the first and second sialyl incorporation. Two major sialyl transferases responsible for the formation of the α2–6 or α2–3 linkage to the terminal Gal residues have been isolated from liver (Weinstein et al., 1982), and the two distinct sialyl transferases could well be affected differently by the protein matrix. The results in Figure 5 (lower panels) show that both in the absence (panels B and C) and in the presence (panels E and F) of avidin only the 2–6 transferase is involved in the reaction (a 2–3 sialyl residue would cause a slight upfield shift rather than the downfield shift observed) and also that the first, fast step in the reaction is the addition of Sia to the 1–3 arm of the biantennary glycan. The interpretation of the data is based on the chemical shift assignments of Joziasse et al. (1985). Thus, the protein matrix in this system does not appear to affect the linkage position of the Sia incorporation. The same type of branch specificity for the 1–3 arm of biantennary glycans has been clearly documented for the bovine colostrum α2–6 sialyl transferase (Joziasse et al., 1985, 1987); the results reported here thus extend this phenomenon to the rat liver enzyme and perhaps suggest that it may be a general feature of α2–6 sialyl transferases. Only the data for the biotinamidohexanoyl derivatives are shown. The biotinyl derivatives gave essentially identical spectra and are not shown.

The reason the chemical shift of the methyl protons of GlcNAc was used for this identification is the unfortunate fact

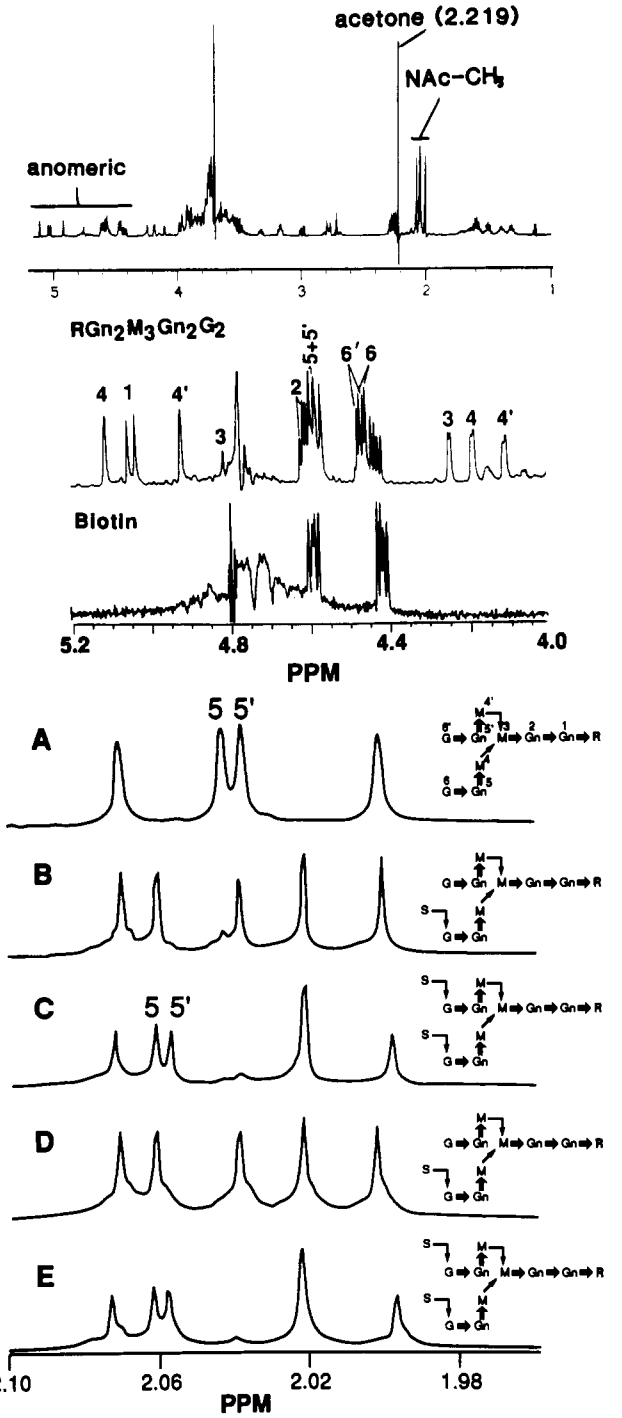


FIGURE 5: Characterization of the components of the sialyl transferase reaction. (Upper panel) Top, Overall ^{1}H NMR spectrum of the biotinylated substrate for the sialyl transferase reaction; middle, expanded spectrum for the anomeric protons; bottom, expanded spectrum for free biotin. (Lower panel) Expanded NMR spectra for the acetyl methyl proton region of the structures indicated in the figure (the signal at 1.995–2.005 represents GlcNAc-1; the one at 2.070–2.075, GlcNAc-2; the one at 2.020–2.025, Sia at both the 6 and 6′ positions). The mass spectra (not shown) indicated the presence of about 10% of the substrate in the samples of intermediate and 15% of the intermediate in the samples of product. Samples B and C were obtained from the reaction in the absence of avidin and samples D and E from the reaction in the presence of avidin. The structure code is the same as that used in Scheme I.

that the biotin signals interfere with the proper interpretation of the chemical shifts of the anomeric protons of Gal (Figure 5, upper panels). The disappearance of the signals at 4.44–4.49 ppm was consistent with the 3-arm reacting first and the 6-arm last, but the biotin resonances (at 4.40–4.43 and 4.57–4.6 ppm)

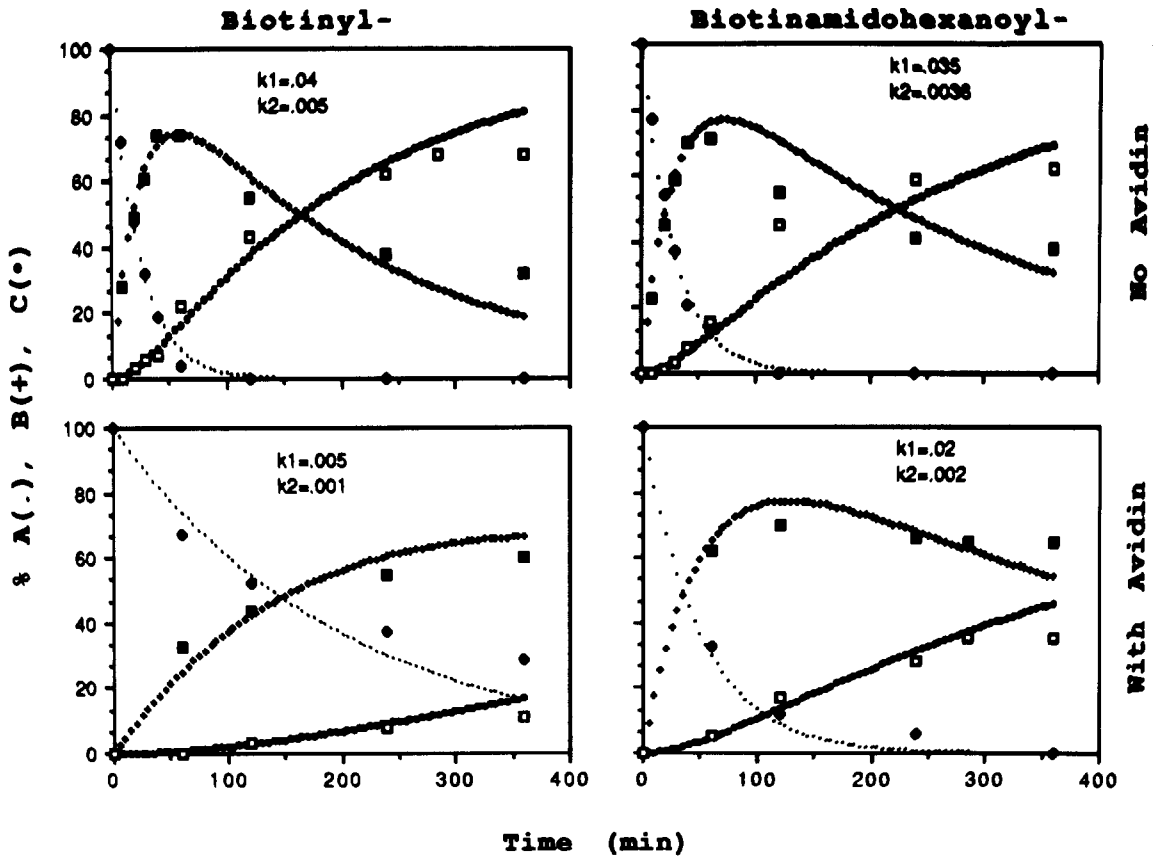


FIGURE 6: Reaction progress curves for the sialyl transferase reactions. The symbols and representations are the same as in Figure 3.

made the assignment of an upfield or downfield shift ambiguous.

The kinetic data for the two steps of Sia incorporation are given in Figure 6. The effect of the protein matrix is definitely more significant for the proximal complex than for the more distal one, although both complexes show a decreased rate compared to that of the free substrate. The deviation of the experimental points from the theoretical curves at the later stages of the reaction strongly suggests that the enzyme loses activity during the long incubations; this feature must be considered in interpreting the original observations (Shao & Wold, 1987; Shao et al., 1987). It also emphasizes the need for caution in the interpretation of this type of results. We did not observe any evidence for the 2–3 transferase in this work, if this enzyme should be even more labile than the 2–6 transferase, our reaction conditions could have precluded the possibility of observing the 2–3 activity.

The results presented here confirm that the protein matrix has a significant effect on the processing efficiency of Golgi enzymes. However, the concept of short-range and long-range effects introduced to describe respectively the strong inhibition for the biotinyl derivatives only and the same extent of inhibition for both the biotinyl and the biotinamidohexanoyl derivatives appears to represent an oversimplification. As more detailed kinetic analyses are performed (Shao & Wold, 1988), the protein matrix effects emerge as rather complex processes convering a wide range of altered processing rates. The most dramatic effects are expressed in the proximal complexes of the biotinylated glycans with avidin; for some early processing steps the net result of this effect is to virtually block these steps, leaving "unprocessed" oligomannose structures as the major products. In the distal complexes of biotinamidohexanoylated glycans with avidin, the strong inhibition is reduced, but significant rate effects are still observed for the distal complexes. Similarly, with proper analysis, both types of complexes show some degree of rate inhibition in comparison to the free complexes even for processing steps that were concluded not to be affected by the protein matrix in the initial survey (Shao & Wold, 1987, 1988). The picture that emerges is that some early steps are very sensitive to the proximity of the protein matrix; these effects are most readily explained as a simple steric hindrance by the protein surface, leading to the kind of inhibition as that observed for mannosidase II in this work. In the absence of the direct steric hindrance, more subtle rate effects appear to come into play, such as those observed for the botinamidohexanoyl derivative in the mannosidase reaction and for both derivatives in the sialyl transferase reaction. The 2–10-fold decrease in rate observed in these cases are not readily explained on the basis of direct steric hindrance and are proposed to reflect conformational modification of the substrates induced by the protein surface. It may well be that these less dramatic effects will be the most significant ones in explaining the multitude of covalent glycan structures that are produced from a single precursor in different eukaryotic systems.

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Conformational States of *Xenopus* Transcription Factor IIIA†

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ABSTRACT: The conformation of *Xenopus* transcription factor IIIA (TFIIIA) free in solution, bound to 5S RNA in the 7S particle, depleted of zinc, or bound to plasmid DNA was analyzed by (1) trypsin digestion and electrophoretic analysis of proteolytic fragments or (2) measurement of the fluorescence of TFIIIA mildly derivatized with *N*-[[(iodoacetyl)amino]ethyl]-5-naphthylamine-1-sulfonic acid (IAEDANS). TFIIIA free or complexed with 5S RNA has a similar conformation as judged (a) by trypsin-dependent generation of similar metastable 20-kDa domains (corresponding to the N-terminal half of the protein) or (b) by the negligible change in AEDANS–TFIIIA fluorescence when free or bound to 5S RNA. When TFIIIA binds plasmid DNA, its N-terminal half becomes hypersensitive to trypsin digestion, indicating a structural change in this region of the protein upon interaction with DNA. Quenching of AEDANS–TFIIIA fluorescence is observed upon interaction of the protein with plasmid DNA, a result also indicative of a conformational change upon protein–DNA interaction. Removal of zinc from TFIIIA by EDTA chelation results in (a) increased proteolysis of this 20-kDa domain, indicating a structural change in the N-terminal half of the protein upon zinc removal, and (b) large enhancement of AEDANS–TFIIIA fluorescence. EDTA chelation of TFIIIA bound to 5S RNA in the 7S particle, a procedure which does not deplete all zinc from the protein, neither increases the trypsin sensitivity of the 20-kDa domain nor alters appreciably the fluorescence of AEDANS–TFIIIA. These results indicate that zinc is involved in maintaining the native conformation of at least the N-terminal half of the protein.

Elucidating the structure and function of eukaryotic gene regulatory proteins is necessary for understanding gene expression and cell differentiation. *Xenopus* transcription factor IIIA (TFIIIA)[1] regulates 5S RNA synthesis during oogenesis in *Xenopus laevis* by binding to an intragenic control region (ICR) of the 5S RNA gene (Engelke et al., 1980; Sakonju et al., 1980; Bogenhagen et al., 1980). Specific DNA binding by TFIIIA is initiated by the interaction of the N-terminal portion of the protein with the 3′ region of the ICR (Miller et al., 1985; Smith et al., 1984; Fiser-Littell et al., 1988; Vrana

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[1] Abbreviations: TFIIIA, transcription factor IIIA from *Xenopus laevis* immature oocytes; ICR, internal control region of the *Xenopus laevis* oocyte 5S RNA gene; DTT, dithiothreitol; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; IAEDANS, *N*-[[(iodoacetyl)amino]ethyl]-5-naphthylamine-1-sulfonic acid.

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