Lysosomal Enzyme Phosphorylation

I. PROTEIN RECOGNITION DETERMINANTS IN BOTH LOBES OF PROCATHEPSIN D MEDIATE ITS INTERACTION WITH UDP-GlcNAc:LYSOSOMAL ENZYME N-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE*

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We have investigated the nature of a protein domain that is shared among lysosomal hydrolases and is recognized by UDP-GlcNAc:lysosomal enzyme N-acetylglucosamine-1-phosphotransferase, the initial enzyme in the biosynthesis of mannose 6-phosphate residues. Previously, elements of this recognition domain were identified using a chimeric protein approach. The combined substitution of two regions (amino acids 188-230, particularly lysine 203, and 265-292) from the carboxyl lobe of the lysosomal hydrolase cathepsin D into the homologous positions of the related secretory protein glycopepsinogen was sufficient to confer recognition by phosphotransferase and subsequent phosphorylation of the oligosaccharides when this chimeric protein was expressed in Xenopus oocytes. (Baranski, T. J., Faust, P. L., and Kornfeld, S. (1990) Cell 63, 281-291). The current study demonstrates that when these two regions are replaced in cathepsin D by the homologous glycopepsinogen amino acids, the resultant chimeric molecule is poorly phosphorylated. However, when either of these regions is substituted individually, the chimeric molecules are well phosphorylated. The phosphorylation of these latter chimeric proteins is dependent on the presence of procathepsin D amino lobe elements. By analyzing a series of chimeric proteins that contain all eight combinations of three consecutive segments of the entire amino lobe of procathepsin D, it was found that multiple regions of the amino lobe of cathepsin D enhance phosphorylation of the chimeric proteins. These elements may be part of an extended carboxyl lobe recognition domain or comprise a second independent recognition domain.

In many cell types, lysosomal hydrolases acquire phosphomannosyl residues that mediate their binding to mannose 6-phosphate (Man-6-P) receptors and targeting to an endosomal compartment where the hydrolases are subsequently packaged into lysosomes. Phosphomannosyl residues are synthesized by the concerted action of two enzymes (1, 2). First, UDP-GlcNAc:lysosomal enzyme N-acetylglucosaminylphosphotransferase (phosphotransferase) selectively transfers N-acetylglucosamine 1-phosphate to mannose residues on lysosomal enzymes to form phosphodiester intermediates. Then, the N-acetylglucosamine residues are removed by N-acetylglucosamine 1-phosphodiesterase-α, N-acetylgalactosaminidase to produce Man-6-P monoesters. The efficient targeting of acidic hydrolases to lysosomes is dependent on the ability of the first enzyme, phosphotransferase, to recognize and bind with high affinity to a protein determinant that is common to lysosomal enzymes but absent from non-lysosomal glycoproteins. The catalytic site of phosphotransferase then specifically phosphorylates the high mannose oligosaccharides of the lysosomal enzymes (3-5).

Cathepsin D is an aspartyl protease that is phosphorylated and targeted to lysosomes in several systems (2, 6, 7), including Xenopus oocytes (8). Pepsinogen is a well-studied secretory aspartyl protease that shares 45% identity in amino acid sequence with cathepsin D. A glycosylated form of human pepsinogen that contains two N-linked glycosylation sites mutated into positions homologous to the two sites in cathepsin D was secreted and not detectably phosphorylated when expressed in Xenopus oocytes (9). Cathepsin D and pepsinogen are predicted to have very similar structures since the overall secondary and tertiary structures of the bilobed aspartyl proteases are remarkably conserved in the fungal and mammalian enzymes that have been crystallized (10).

To identify the protein determinants of cathepsin D that comprise the phosphotransferase recognition domain, we previously generated cDNA constructs that encode a number of chimeric proteins in which regions of human cathepsin D are substituted into the backbone of human glycopepsinogen. When these chimeric molecules were expressed in Xenopus oocytes and analyzed for phosphorylation, we found that two noncontinuous primary sequences from the carboxyl lobe of cathepsin D (amino acids 188-230, particularly lysine 203, and 265-292) were sufficient to generate an efficient phosphotransferase recognition domain in glycopepsinogen (9). When localized to the homologous position in the crystal structure of porcine pepsinogen, these two sequences were found to be in direct apposition on the surface of the molecule. Moreover, this recognition domain on the carboxyl lobe of cathepsin D is sufficient to direct not only the phosphorylation of the oligosaccharide on the carboxyl lobe, but also the phosphorylation of the oligosaccharide on the amino lobe of the molecule, although to a lesser extent (11). However, the

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§The abbreviations used are: Man-6-P, mannose 6-phosphate; CP, chimeric protein; CI-MPR, cation-independent mannose 6-phosphate/IGF-I1 receptor; phosphotransferase, UDP-GlcNAc:lysosomal enzyme N-acetylglucosaminylphosphotransferase; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; endo H; endo-β-N-acetylgalactosaminidase H.

2 Human cathepsin D numbering is used throughout (10).
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chimeric protein containing these two regions was not phosphorylated as well as cathepsin D, indicating that cathepsin D contains yet other elements that contribute to the optimal interaction with phosphotransferase.

In the current study, we have replaced the lysine 203 region and amino acids 265–292 (loop region) of cathepsin D with the homologous glycopepsinogen sequences to determine if these regions are necessary for recognition by phosphotransferase. Our experiments have revealed that both regions are required for maximal phosphorylation of procathepsin D.

However, replacement of either region individually results in only a partial loss of phosphorylation, indicating that additional regions of procathepsin D contribute to the phosphotransferase recognition domain. These additional elements have been localized to the amino lobe of procathepsin D.

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**EXPERIMENTAL PROCEDURES**

**Materials**—The preparation of rabbit antisera to human cathepsin D was described previously (12). Rabbit antiserum to human pepsinogen was a generous gift of Dr. I. M. Samloff (UCLA School of Medicine, Los Angeles, CA). Immobilized bovine mannos 6-phosphate/IGF-II receptor (MPR) was prepared as described previously (13). Mannose 6-P was prepared as described (14). All oligonucleotides were synthesized on an Applied Biosystems 380A Gold phase synthesizer. Adult female Xenopus frogs were obtained from Nilbo (Fort Atkinson, WI). The pSP64 plasmid vector was purchased from Promega, Inc., and pGBT plasmid vector was from Gold Biotechnologies, Inc. Escherichia coli strain (CJ236) was obtained from American Type Culture Collection (Baltimore, MD). Immobilized bovine mannose 6-phosphate/IGF-II receptor affinity columns were used for the initial experiments and were later replaced with anti-IGF-II receptor affinity columns. Column buffer was 50 mM imidazole/HCl, pH 6.5, 150 mM NaCl, 0.05% Triton X-100, 5 mM sodium β-glycophosphate, and 5 mM EDTA. Fractons containing 20% of column run-through and Man-6-P eluted material were immunoprecipitated with either anti-pepsinogen antiserum alone or in combination with anti-cathepsin D antisera and subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (18) as described (8). The gels were fluorographed and the appropriate regions of the dried gel were excised, solubilized with 90% PROTOSOL, and radioactivity was measured by liquid scintillation counting according to the manufacturer’s instructions. The total radioactivity recovered for an individual determination ranged from 2,000 to 360,000 cpm with a median value of 13,000 cpm. The “high density” MPR-affinity columns bound up to 25% more of the expressed proteins compared with the binding to the “low density” columns.

**RESULTS AND DISCUSSION**

**Replacement of the Lysine 203 and Loop Regions in Cathepsin D Decreases Phosphorylation**—A series of constructs encoding CPs in which human cathepsin D sequences have been substituted with the analogous glycopepsinogen sequences were prepared to determine if the lysine 203 region (amino acids 188–230) and the loop region (265–292) of cathepsin D are necessary for the efficient phosphorylation of cathepsin D. These cDNAs were used to generate mRNAs by in vitro transcription, and the mRNAs were injected into Xenopus oocytes which were then incubated with [35S]methionine/cysteine for 72 h. The extent of phosphorylation of the expressed proteins was determined by their ability to bind to a MPR affinity column. This affinity procedure detects most of the phosphorylated molecules as confirmed by direct analysis of [3H]mannose-labeled oligosaccharides of expressed glycoproteins (9). The expressed proteins in the receptor column run-through and the Mann-6-P eluate fractions were immunoprecipitated with anti-cathepsin D and/or anti-pepsinogen antiseras and analyzed by SDS-PAGE.

The chimeric proteins CP51 and CP52 (amino acids 188–230 and either 265–319 or 265–292 of cathepsin D substituted with glycopepsinogen sequences, respectively) were phosphorylated poorly (0.8 and 2.2% receptor binding, respectively, Fig. 1, lanes 5–12). This is to be compared to 94% MPR binding for cathepsin D (Fig. 1, lanes 1–4). In fact, CP51 and CP52 were phosphorylated at levels comparable to CP 1, which contains the amino lobe of cathepsin D and the entire carboxyl lobe of glycopepsinogen (0.8 and 2.2% receptor binding versus 1.6% receptor binding, respectively, Fig. 1). These results indicate that when both the lysine 203 region and the loop region of cathepsin D are substituted with
glycopepsinogen sequence, no other regions of cathepsin D are able to generate an efficient recognition domain.

The poor phosphorylation of CP51 and CP52 is unlikely to be a consequence of improper folding of the chimeric molecules since 90% of CP51 and 77% of CP52 were secreted into the medium, in contrast to misfolded proteins that are usually retained in the endoplasmic reticulum (19). The decreased mobility of the secreted glycoproteins on SDS-PAGE (Fig. 1, lanes 7 and 11) is consistent with the oligosaccharides having been processed to complex-type forms.

Since the substitution of lysine 203, in combination with amino acids 265-348 of cathepsin D, into the glycopepsinogen backbone results in a chimeric protein (CP17, Fig. 1) that is phosphorylated as well as a chimeric molecule containing the entire cathepsin D sequence 188-230 (9), we next analyzed the consequence of replacing lysine 203 of cathepsin D with an alanine residue. When this was done, in combination with the replacement of amino acids 265-319, the resultant chimeric protein (CP54) was phosphorylated considerably better than CP51 and CP52, but less well than cathepsin D (22% receptor binding, Fig. 1, lanes 13-16). The finding that CP53 is phosphorylated better than CP51 and CP52 indicates that other residues in the lysine 203 region contribute to the recognition domain. One candidate residue is the positively charged arginine at position 202 in cathepsin D. To test this possibility, we constructed CP54 which contains alanine residues at positions 202 and 203 and amino acids 265-319 of cathepsin D substituted with glycopepsinogen residues. When expressed, CP54 was phosphorylated to the same extent as CP53 (22% receptor binding, Fig. 1) indicating that residues in the lysine 203 region (amino acids 188-230 of cathepsin D) other than the positively charged arginine 202 and lysine 203 contribute to the phosphotransferase recognition domain.

We next tested the consequence of separately replacing the cathepsin D lysine 203 region or the region encompassed by amino acids 265-348 with pepsinogen sequence. When amino acids 188-230 of procathepsin D were substituted with glycopepsinogen sequence, the resultant chimeric protein (CP55) was phosphorylated 39%, which is less well than cathepsin D, but considerably better than CP51 which lacks both of the critical regions (Fig. 2, lanes 1-4). CP56, which has amino acids 188-265 of procathepsin D substituted with glycopepsinogen residues, was only phosphorylated 12% (Fig. 2, lanes 5-8), indicating that amino acids 230-265 of cathepsin D may play a role in the recognition domain. However, this region is not sufficient for phosphorylation even in combination with the amino lobe of procathepsin D as illustrated by the poor phosphorylation of CP51 and CP52 (Fig. 1). On the other hand, CP55 and CP56, which contain the entire amino lobe of procathepsin D, were phosphorylated about 19-fold better than the respective chimeric proteins that contain the amino lobe of glycopepsinogen (CP53 and CP55, summarized in Fig. 3; 39% versus 41% receptor binding and 12% versus 1.4% receptor binding, respectively). Therefore, the amino lobe of procathepsin D appears to contribute to the efficient phosphorylation of CP55 and CP56.

CP57 was tested to determine the effect of replacing amino acids 265-319 of procathepsin D with glycopepsinogen sequence. When expressed, CP57 was also phosphorylated less well than cathepsin D but much better than CP51 (30% receptor binding, Fig. 2, lanes 9-12). The results obtained with CP57 and CP55 demonstrate that either the lysine 203 region or the loop region of cathepsin D can be separately replaced with glycopepsinogen sequence without severely impairing the ability of procathepsin D to interact with phosphotransferase. However, these chimeric proteins are phosphorylated 2-3-fold less well than procathepsin D (30 and 39% receptor binding versus 94% receptor binding, Fig. 2).

CP58 was constructed to examine the role of the COOH-
Chimera. The schematic representation of the various chimeric proteins that occur at position 339 in the terminal loop of cathepsin D could function in the recognition domain when that is accessible on the surface and in close proximity to the lysine 203 is absent. In CP58, the glycopepsinogen COOH terminal 28 amino acids of cathepsin D. In the crystallo-proteins and the details of the sample analysis are described in the legend to Fig. 1. The results for each chimeric protein are summarized and represent the average of two to four separate experiments with the exception of CP5 and CP63 which are single determinations. The data for CP3 and CP5 include determinations from Ref. 9.

terminal 28 amino acids of cathepsin D. In the crystallographic structure of porcine pepsinogen, this region is buried internally in the molecule, except for a turn of \( \beta \)-loop structure that is accessible on the surface and in close proximity to lysine 203. It was possible, therefore, that in CP55 an arginine that occurs at position 339 in the turn of the \( \beta \)-loop of cathepsin D could function in the recognition domain when the lysine 203 is absent. In CP58, the glycopepsinogen COOH terminus, which contains a glutamine at position 339, was substituted for the COOH terminus of cathepsin D. When expressed, CP58 was phosphorylated to a similar extent as CP55 (Fig. 2, 46 versus 39% receptor binding, respectively), excluding a critical role for arginine 339.

CP59 and CP60 were prepared to determine whether the single replacement of lysine 203 or the replacement of arginine 202 and lysine 203 with alanine residues would have any effect on the phosphorylation of procathepsin D. When expressed, both chimeric proteins were well phosphorylated, although at somewhat diminished levels compared with procathepsin D (80 and 74% versus 93% receptor binding, respectively, Fig. 2). These results demonstrate that, despite its critical role in the generation of a minimal carboxyl lobe recognition domain, lysine 203 can be replaced by alanine in procathepsin D without a major deleterious effect on the phosphorylation of the resultant molecule.

To examine the roles of NH\(^{-}\) and COOH-terminal residues in procathepsin D, CP61 and CP62 were prepared. When expressed, these chimeric proteins were well phosphorylated (84 and 76% receptor binding, Fig. 2). Thus, amino acids -44 to 15 and 319-348 of procathepsin D are not necessary to generate an efficient phosphotransferase recognition domain. However, shared residues between procathepsin D and glycopepsinogen in these regions may contribute to the recognition domain, since they would fail to be identified by this approach.

It should be noted that CP62 was secreted to a much greater extent than the other chimeric proteins with a similar degree of phosphorylation (compare CP62 to CP61 for example). The reason for this is not clear. Overall, there was a correlation between the extent of phosphorylation of a given chimeric protein and its retention in the cell, although a number of other exceptions were noted. Most of these were chimeric proteins that were poorly phosphorylated but only secreted to a limited extent (such as CP64 and CP63, Fig. 3). The most likely explanation for this phenotype is that the protein is impaired in folding and consequently is retained in the endoplasmic reticulum.

**Analysis of Amino Lobe Elements of Procathepsin D That Contribute to the Recognition Domain**—The finding that CP55 is phosphorylated 10-fold better than CP3 indicates that elements of the amino lobe of procathepsin D contribute to the efficient phosphorylation of the protein. To begin to identify these elements, constructs were prepared that systematically substituted three consecutive segments of the amino lobe of procathepsin D, in combination with amino acids 230–348 of cathepsin D, into glycopepsinogen. CPs 63–65 contain individual regions of procathepsin D (amino acids -44 to 14, 15–131, or 132–187) substituted into the amino lobe of CP3 or CP5 (see Fig. 3). When expressed, the chimeric proteins were all poorly phosphorylated (4.4, 2.2, and 4.4% receptor binding, respectively, Fig. 3). Therefore, no individual segment of the amino lobe functions as well as the entire amino lobe of procathepsin D in CP55.

CPs 66–68 contain combinations of the individual segments of procathepsin D. When expressed, CPs 66 and 68 were phosphorylated at intermediate levels relative to CP3 and CP55 (12 and 14% versus 4.1 and 39%, receptor binding, respectively, Fig. 3). Therefore, the amino acids 15–131 of cathepsin D in combination with either amino acids -44 to 14 or amino acids 132–187 of procathepsin D are sufficient to mediate increased levels of phosphorylation. However, CP67, which contains amino acids -44 to 14 and 132–187 in combination, was poorly phosphorylated (1.7% receptor binding, Fig. 3). These results are consistent with two interpretations. First, multiple regions of the amino lobe of procathepsin D may contribute specifically to the recognition domain. Second, the critical determinants for the recognition domain may be located within the segment that contains amino acids 15–131, but the other amino lobe segments of procathepsin D are required for this segment to achieve a proper conformation.

**Oligosaccharide Analysis on CP55**—There are several possible mechanisms by which the amino lobe of procathepsin D could enhance the phosphorylation of chimeric proteins. First, the amino lobe could directly contribute to the increased phosphorylation of the molecule by specific regions serving as direct contact sites for the binding of phosphotransferase. Alternatively these elements could act to position the amino lobe oligosaccharide in such a manner that it becomes a better substrate for phosphotransferase. Another possible role for the amino lobe is to improve the overall folding of the carboxyl lobe and thereby generate a more potent carboxyl lobe recognition domain. In this case, the effect of the amino lobe would be an indirect one. One approach to distinguish between these mechanisms is to compare the oligosaccharide site-specific phosphorylation of CP55 with that obtained with cathepsin D and a number of the other chimeric proteins (11).

In procathepsin D, the amino lobe oligosaccharide is 76% phosphorylated whereas the carboxyl lobe oligosaccharide is 96% phosphorylated (11). On the other hand, in CP2 which contains the amino lobe of glycopepsinogen joined to the carboxyl lobe of cathepsin D, the amino lobe oligosaccharide is only 37% phosphorylated while the carboxyl lobe is 93% phosphorylated (11). Therefore, in CP2, the pattern of phosphorylation at the two glycosylation sites appears to reflect the position of the phosphotransferase recognition domain in the molecule. If the role of the amino lobe of procathepsin D in
CP55 is to directly interact with phosphotransferase, then a greater percentage of the amino lobe oligosaccharides might be phosphorylated relative to the carboxyl lobe oligosaccharides. Conversely, if the role of the amino lobe of procathepsin

D in CP55 is to promote a more properly folded carboxyl lobe recognition domain, then the carboxyl lobe oligosaccharides would be phosphorylated to a greater extent than the amino lobe oligosaccharides, as is the case for CP2. In an attempt to differentiate between these two possibilities, an analysis of the extent of phosphorylation of the N-linked oligosaccharides at the two glycosylation sites of CP55 was performed.

Xenopus oocytes were coinjected with GDP-[3,4-3H]mannose and CP55 RNA transcripts, and then incubated for 72 h. The cellular and secreted CP55 molecules were immunoprecipitated with anti-cathepsin D serum and digested with chymotrypsin to generate glycopeptides. These were fractionated by reversed-phase liquid chromatography under conditions where the amino lobe glycopeptide is separated from the carboxyl lobe glycopeptide (11). The elution profiles of the glycopeptides are shown in Fig. 4. As expected, the amino lobe glycopeptide of CP55 fractionated in the position of the amino lobe glycopeptide of procathepsin D whereas the carboxyl lobe glycopeptides of CP55 fractionated in the position corresponding to the carboxyl lobe of glycopepsinogen.

Each glycopeptide was incubated with endo H to release the high mannose oligosaccharides which were then analyzed for the presence of phosphomannosyl residues by QAE-Sephadex chromatography (20). As shown in Fig. 5, the oligosaccharides of CP55 contained species that bound to QAE-Sephadex and eluted at positions characteristic for oligosaccharides with one phosphomonoester (70 mM NaCl) or two phosphonomonoesters (140 mM NaCl). The endo H-resistant material was subjected to concanavalin A-Sepharose chromatography to determine the amount of complex-type units for each glycopeptide (21).

The results of these oligosaccharide analyses are summarized in Table I. The amino lobe and the carboxyl lobe oligosaccharides of CP55 were equally phosphorylated (48 and 53%, respectively). This pattern of phosphorylation is similar to that observed with procathepsin D and is significantly different from that observed with CP2. These results are consistent with the proposal that the procathepsin D amino lobe elements in CP55 contribute directly to the interaction with phosphotransferase rather than just serving to enhance the folding of the carboxyl lobe elements.

CONCLUSIONS

Previously we identified two noncontinuous primary sequences (amino acids 188-230 and 265-292) in the carboxyl

![Image](https://example.com/image.png)

**Fig. 4.** Fractionation of glycopeptides by reversed-phase liquid chromatography. Xenopus oocytes were co-injected with GDP-[3,4-3H]mannose and the CP55 mRNA transcript and the oocytes were incubated for 72 h. The cellular homogenates and media samples of CP55 were immunoprecipitated, and glycopeptides were generated by chymotryptic digestion and then fractionated by reversed-phase liquid chromatography, as described (11). The glycopeptides of cathepsin D and glycopepsinogen expressed in the same experiment served as controls for the identification of each glycopeptide, and the positions of their elution are shown by the arrows at the top of the figure. C-NH₂, cathepsin D amino lobe glycopeptide; C-COOH, cathepsin D carboxyl lobe glycopeptide; P-NH₂, glycopepsinogen amino lobe glycopeptide; P-COOH, glycopepsinogen carboxyl lobe glycopeptide.

![Image](https://example.com/image.png)

**Fig. 5.** QAE-Sephadex analysis of CP55 oligosaccharides. The fractionated glycopeptides (Fig. 4) were treated with endo H to release the high mannose type-oligosaccharides which were then separated from endo H-resistant glycopeptides by reversed-phase chromatography, as described (11). Cell-associated (A and B) and medium (C and D) high mannose type-oligosaccharides derived from the amino lobe (A and C) or the carboxyl lobe (B and D) of CP55 were applied to analytical QAE-Sephadex columns and eluted with either 20, 70, 100, or 140, mM NaCl.
lobe of cathepsin D that, when substituted together into the homologous regions of glycopepsinogen, were sufficient to generate a phosphotransferase recognition domain (9). The data presented in the current study demonstrate that replacement of these two regions in procathepsin D with the homologous glycopepsinogen sequences results in a chimeric protein that is very poorly phosphorylated, thereby establishing that these two carboxyl lobe elements are also necessary for the expression of the phosphotransferase recognition domain in procathepsin D. However, when these regions were replaced individually, the resultant chimeric proteins were well phosphorylated (although not to the same extent as procathepsin D). The ability of these latter chimeric proteins to serve as substrates for phosphotransferase was found to be dependent on the presence of amino lobe elements of cathepsin D.

These results can be explained in two ways. The first possibility is that procathepsin D contains two independent phosphotransferase recognition sites, one on each lobe of the molecule, with the carboxyl lobe elements being more potent than the amino lobe elements. Secondly, there could be a single extended recognition site that includes the carboxyl lobe elements as well as regions of the amino lobe. In addition, the amino lobe elements of cathepsin D may improve the overall folding of the carboxyl lobe and thereby enhance the expression of the carboxyl lobe recognition domain. The independent site model is an interesting possibility since the aspartyl proteases are thought to have arisen by a gene duplication event (22). However, when the homologous lysine 203 and loop regions in the amino lobe of procathepsin D are localized on the crystal structure of porcine pepsinogen, they are predicted to be buried at the interface of the two lobes of procathepsin D. Therefore, a second independent phosphotransferase recognition site would have to be located elsewhere on the amino lobe. The single extended site model would necessitate that phosphotransferase bind a very large area on the surface of procathepsin D, since molecular modeling predicts the surface area of the carboxyl lobe recognition domain to be about 1,600 square angstroms (23). This would appear to be possible since protein-protein surface interactions up to 5,000 square angstroms have been reported, primarily in the association of subunits in multimeric proteins (24-26). To differentiate between these various models, the elements in the amino lobe that lead to enhanced phosphorylation will have to be more precisely defined.

Any model of phosphotransferase action must explain how this enzyme can interact with 40-50 different lysosomal hydrolases while displaying low affinity for hundreds of other glycoproteins with identical high mannose-type oligosaccharides. One possible mechanism that we have previously suggested is that phosphotransferase binding may involve multiple contacts extending over a broad surface of the lysosomal hydrolase, with only a portion of these required to generate a productive interaction (9). In this way the different lysosomal hydrolases need only express some components of the entire recognition marker to be able to bind to phosphotransferase. The single extended site model is compatible with this view. An alternative possibility is that phosphotransferase contains binding sites for a variety of protein motifs. This would be analogous to the interaction of human growth hormone with its receptor (27). In this system a single molecule of growth hormone binds and brings together two molecules of the receptor. DeVos et al. (27) have found that both receptor molecules are involved in the growth hormone binding and utilize essentially the same residues to interact with the ligand. However, the two binding sites on growth hormone have no structural similarity. These data show that the single binding site on the growth hormone receptor is able to interact with two different structural elements. In a similar manner, phosphotransferase could potentially bind to a number of different protein motifs that are present on the various lysosomal hydrolases. Consequently, there would be no need for all lysosomal hydrolases to express the same determinants. We are attempting to distinguish between these possibilities by examining the phosphotransferase binding determinant on a second lysosomal hydrolase.

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REFERENCES

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* The data are reported as molar percents by assuming that the high mannose oligosaccharides contain 5.1 mannoses (11) and the complex-type oligosaccharides contain three mannose residues.

As described (11), the recoveries of the carboxyl lobe hydrophobic glycopeptides were consistently lower than the recoveries of the amino lobe glycopeptides, thus less material was available for subsequent analysis.
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