Identification of UDP-*N*-acetylglucosamine-phosphotransferase-Binding Sites on the Lysosomal Proteases, Cathepsins A, B, and D[†]

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ABSTRACT: A key step in the targeting of soluble lysosomal enzymes is their recognition and phosphorylation by a 540 kDa multisubunit enzyme, UDP-*N*-acetylglucosamine-phosphotransferase (phosphotransferase). The molecular mechanism of recognition is still unknown, but previous experiments suggested that the phosphotransferase-binding sites on lysosomal proteins are represented by structurally conserved surface patches of amino acids. We identified four such regions on nonhomologous lysosomal enzymes, cathepsins A, B, and D, which were superimposed by rotating their structures around the Cα atom of the glycosylated Asn residue. We proposed that these regions represent putative phosphotransferase-binding sites and tested synthetic peptides, derived from these regions on the basis of surface accessibility, for their ability to inhibit in vitro phosphorylation of purified cathepsins A, B, and D. Our results indicate that cathepsin A and cathepsin D have one closely related phosphotransferase recognition site represented by a structurally and topologically conserved β-hairpin loop, similar to that previously identified in lysosomal β-glucuronidase. The most potent inhibition of phosphorylation was demonstrated by homologous peptides derived from the regions located on cathepsin molecules opposite the oligosaccharide chains which are phosphorylated by the phosphotransferase. We propose that recognition and catalytic sites of the phosphotransferase are located on different subunits, therefore, providing an effective mechanism for binding and phosphorylation of lysosomal proteins of different molecular size.

The biogenesis of soluble lysosomal proteins involves their transport from the ER to the pre-Golgi and Golgi compartments, where they obtain a mannose-6-phosphate lysosomal targeting marker (reviewed in refs *1* and *2*). Synthesis of the mannose-6-phosphate signal on the lysosomal enzymes is a two step process. The initial step occurs in the pre- and cis-Golgi, where UDP-GlcNAc:lysosomal enzyme *N*-acetylglucosamine-1-phosphotransferase (phosphotransferase) transfers the *N*-acetylglycosaminyl phosphate (GlcNAc) from UDPGlcNAc to α1,6 and α1,3 branches of a high mannose oligosaccharide chain on lysosomal enzymes to produce a phosphodiester. Then, in the medial Golgi, a specific diesterase removes the terminal GlcNAc from the mannose-6-phosphate (*3*−*6*).

The determinant step in the sorting of lysosomal enzymes is their specific recognition by the phosphotransferase among the other glycoproteins which pass through the endoplasmic reticulum. A genetic deficiency of the phosphotransferase causes the inborn diseases, I-cell disease and pseudo-Hurler polydystrophy, which are characterized by the massive secretion of lysosomal enzymes. Reitman and Kornfeld (*7*) demonstrated that lysosomal enzymes are phosphorylated at least a 100-fold more efficiently than nonlysosomal glycoproteins or short glycopeptides, containing the identical high-mannose oligosaccharides, which suggests that the high affinity of phosphotransferase for lysosomal enzymes is mediated by specific protein−protein interactions. Thus, phosphotransferase should bind surface determinants common to all lysosomal proteins but absent in other glycoproteins. Since more than 50 soluble lysosomal proteins cloned to date do not exhibit any obvious common amino acid sequence motif, it is probable that the phosphotransferase recognition site is determined by the tertiary structure of lysosomal enzymes. Indeed, the disruption of the native conformation of lysosomal enzymes significantly or completely inhibits phosphorylation (*7*−*9*).

Recent studies using the lysosomal protease, cathepsin D, as a model showed that several noncontinuous amino acid segments and single Lys residues from either the carboxyl terminal lobe (Ser188−Val230, Cys265−Leu292, and Lys203) or the amino terminal lobe (propeptide region, Leu1−Glu44 and His77) were important for phosphorylation (*10*−*12*). However, these data were insufficient to define the elements of the phosphotransferase-binding site common to all lysosomal enzymes. Moreover, these results contradict those of Schorey et al. (*13*), who showed that cathepsin D mutants,

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where the C-terminal lobe Lys residues had been converted to Glu were normally targeted to the lysosome and processed to the mature form.

We presumed that if the recognition site is structurally conserved among different lysosomal enzymes, it could be identified by superimposing their tertiary structures. Following this strategy, we found four structurally conserved surface patches in the nonrelated lysosomal proteases, cathepsins A, B, and D. These sites were examined as potential phosphotransferase-binding domains and analogous synthetic peptides were tested for their capacity to mimic the binding to phosphotransferase and thus inhibit in vitro phosphorylation of the corresponding cathepsin.

EXPERIMENTAL PROCEDURES

Peptides. The 9−12 amino acid peptides derived from sequences of cathepsin A or cathepsin D were purchased from Sheldon Biotechnology Center (McGill University, Montréal), and those derived from the sequence of cathepsin B were synthesized in the laboratory of J.S.M. All peptides were of 90% or higher purity as analyzed by reversed-phase HPLC and had correct monoisotopic molecular masses determined by FAB-mass spectrometric analysis.

Synthesis of [β-^{32}P]UDP-GlcNAc. [β-^{32}P]UDP-GlcNAc was synthesized by the method of Lang et al. (*8*) and purified on a QAE-Sephadex column according to Cuozzo et al., (*14*). The final preparation of [β-^{32}P]UDP was homogeneous as determined by TLC (*8*).

Cathepsins A, B, and D. Cathepsin A and cathepsin D from human placenta were purified using the affinity chromatography on concanavalin A-Sepharose and Phe-Leu-agarose as described (*15*). After affinity chromatography, the preparation was dialyzed against 10 mM Tris-HCl buffer, pH 7.5, centrifuged at 10000*g* for 10 min, applied to a FPLC Mono Q column (Parmacia) equilibrated with the same buffer and eluted with a linear NaCl gradient from 0 to 0.4 M. Under these conditions, cathepsin A and cathepsin D were eluted as two separate peaks. Fractions containing cathepsin A activity were pooled and concentrated. The cathepsin D preparation was additionally purified by FPLC gel filtration on a Superose 12 column.

Recombinant rat procathepsin B was expressed in *Pichia pastoris* yeast and purified as described (*16*). All enzymes were more than 95% pure by Laemmli SDS−PAGE (*17*) and stable for several months when stored at −20 °C.

Purification of Phosphotransferase. Phosphotransferase was partially purified from rat liver by a modification of the method of Reitman et al. (*18*). A crude preparation of rat liver membranes was washed 3 times with a 10 mM Tris-HCl buffer, pH 7.4, 0.1% (w/v) Lubrol PX (ICN Biochemicals, Mississauga), 5 mM $MgCl_2$, 0.5 mM DTT, and 10 mM mannose-6-phosphate. Phosphotransferase was solubilized by a sonication of membranes (2 times by 15 s at 60 W) in 25 mM Tris-HCl buffer, pH 7.4, 2% (w/v) Lubrol PX, 0.5% (w/v) deoxycholate, 20% (v/v) glycerol, 5 mM $MgCl_2$, and 3 mM mercaptoethanol. The enzyme was typically purified about 160-fold with a recovery of 26% of total initial activity.

Enzyme Assays. Cathepsin A activity was measured by the method of Tranchemontagne et al., (*19*) with CBZ-Phe-Leu as a substrate and cathepsin D activity − according to the method of Barret (*20*) with bovine hemoglobin (Sigma) as a substrate. One unit of enzyme activity (U) is defined as the conversion of 1 μmol of substrate per min. Proteins were assayed according to Bradford (*21*) with BSA (Sigma) as a standard. Phosphotransferase activity was assayed by the method of Reitman et al. (*18*) with [β-^{32}P]UDP-GlcNAc as a phosphate donor and α-D-methyl-mannopyranoside (Sigma) as an acceptor. The enzyme activity was expressed as pmoles of GlcNAc-1−[^{32}P]phosphate transferred per h.

In Vitro Phosphorylation of Lysosomal Proteins. In vitro phosphorylation of human placental cathepsins A, D, and recombinant rat procathepsin B was performed as described by Cuozzo and Sahagian (*14*) using the partially purified rat liver phosphotransferase and [β-^{32}P]UDP-GlcNAc as a phosphate donor. The reaction mixture (50 μL) contained 0.5 μCi of [β-^{32}P]UDP-GlcNAc, 1−5 μg of cathepsin, 12 units of phosphotransferase, 50 mM Tris-HCl, pH 7.5, 50 mM GlcNAc, 10 mM $MgCl_2$, 6 mM ATP, 0.25 mM DTT, 1 μM UDP-GlcNAc, and 1% (v/v) Triton X-100. Phosphorylated proteins were analyzed by SDS−PAGE according to Laemmli (*17*). After electrophoresis, gels were stained for protein with Coomasie blue R250 and vacuum-dried. Protein phosphorylation was analyzed by autoradiography and quantitated with a 425 PhosphorImager (Molecular Dynamics Inc.) using a ImageQuaNT software. Preliminary experiments with [β-^{32}P]-phosphorylated cathepsin A preparation showed that the PhosphorImager response was directly proportional to the amount of radioactivity applied on gel in the range of 0.10−1.00 μCi.

To study the inhibition of phosphorylation, cathepsin-derived synthetic peptides were added to the incubation mixture in concentrations of 0, 0.25, 0.50, 0.75, and 1.00 mM. The phosphorylation of cathepsins was quantitated as above, and the dependence of the phosphorylation on concentration of cathepsin-derived peptides was analyzed by nonlinear regression.

Structural Analysis. Structural analysis was performed with the QUANTA-CHARMm software package (Molecular Simulations Inc.). The atomic X-ray structures of rat procathepsin B, human cathepsin D, and β-glucuronidase were obtained from the Brookhaven Protein Databank (1cte, 1lya and 1bhg). Due to the hold placed on the cathepsin A coordinates (1ivy; 22) initial analysis and peptide selection were made with the homology modeled structure (*23*). Final analyses were subsequently performed with the crystallographic coordinates.

RESULTS AND DISCUSSION

Selection of Putative Phosphotransferase-Binding Regions on Lysosomal Cathepsins A, B, and D. Assuming that phosphotransferase-binding domains share the same structure in all soluble lysosomal enzymes, we searched for structurally conserved regions in cathepsins A, B, and D. Although all three enzymes have a proteolytic function, they differ in their overall tertiary structure and catalytic properties. The serine carboxypeptidase, cathepsin A, is a member of the α/β hydrolase fold family, the cysteine endo/dipeptidyl peptidase, cathepsin B, belongs to the papain superfamily, and the aspartic endopeptidase, cathepsin D, has structural similarities to pepsin. Therefore, it can be assumed that their phosphotransferase recognition sites have been acquired by convergent evolution and are probably the only structurally

FIGURE 1: Ribbon representations of lysosomal cathepsin A (A), B (B), and D (C) tertiary structure. Putative phosphotransferase recognition sites are shown: green, the "Lys region" comprised by Gly24−His33 for cathepsin A, Phe177−Val187 for cathepsin B, and Leu276−Leu285 for cathepsin D; blue, the "His region" (Lys414−Leu422 for cathepsin A, Gly92-Gly99 for cathepsin B, and Ser73-Gly81 for cathepsin D); orange, the "Pro region" (Phe443−Pro451 for cathepsin A, Glu133−Lys141 for cathepsin B and Thr119−Ala128 for cathepsin D); and pink, a loop opposite to the Asn glycosylation site (Gln76−Tyr87 for cathepsin A, Ile44−Glu53 for cathepsin B, and Ser170−Gly179 for cathepsin D). Asn-glycosylation sites are shown in red.

conserved regions between these enzymes. Although cathepsins are phosphorylated and transported to the lysosome as precursors, we have examined in vitro phosphorylation, and the structures of the mature forms of cathepsins A and D. We assumed from studying the modeled structures of cathepsin A and D precursors (*22, 24*) and crystal structure of cathepsin B precursor (*25*) that these regions are retained in the mature forms and are positioned similarly as in the precursors.

By rotating the cathepsin structures around the Cα atom of the glycosylated Asn residues, we identified spatially conserved surface amino acid residues: Lys32, His418, and Pro451 of cathepsin A; Lys184, His97, and Thr138 (Pro138 in human enzyme) of cathepsin B; and Lys281, His77, and Pro122 of cathepsin D. In all three cathepsins these residues occupy spaces centered at the apexes of triangles which can be superimposed within an average rms deviation of 1.8 Å. Lys32 of cathepsin A is located in a loop region spanning residues Gly24−Phe38 (shown in green in Figure 1). This loop superimposes (rms deviation of 0.95 Å) with the cathepsin D Pro272−Pro287 loop earlier proposed (*10, 11*) as an element of the phosphotransferase recognition site. In both proteins these loops contain several Lys residues which, as previously shown by a chemical modification (*14*) or site

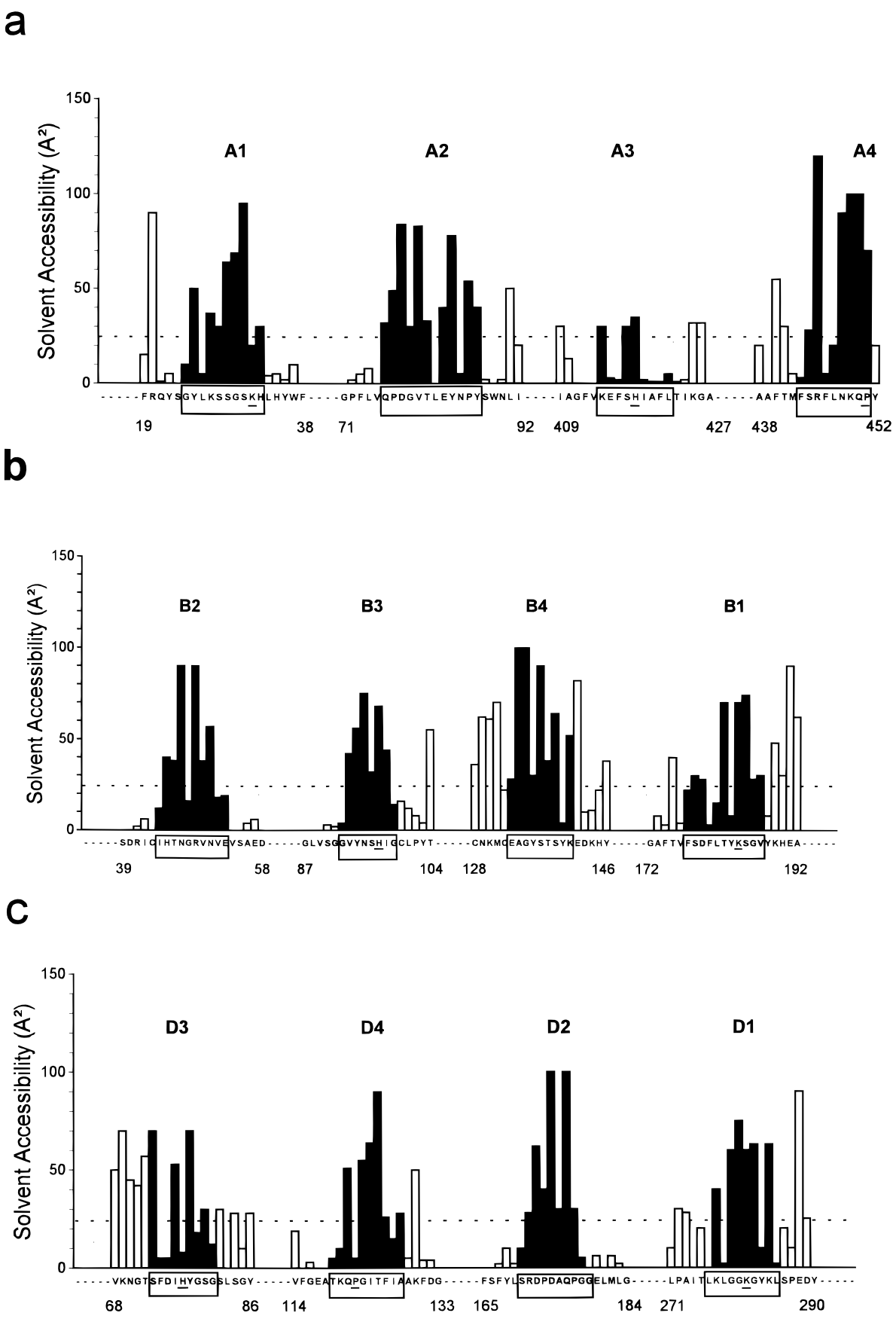

FIGURE 2: Solvent accessibility (Å^2) of amino acid residues of cathepsin A (a), procathepsin B (b), and cathepsin D (c) putative phosphotransferase recognition sites. Candidate segments which corresponded to our criteria for possible interaction with phosphotransferase (25 Å^2 cutoff, dotted line) and were selected for the synthesis of peptides (solid black) are labeled and their sequence is boxed. The topologically conserved amino acid residues are underlined.

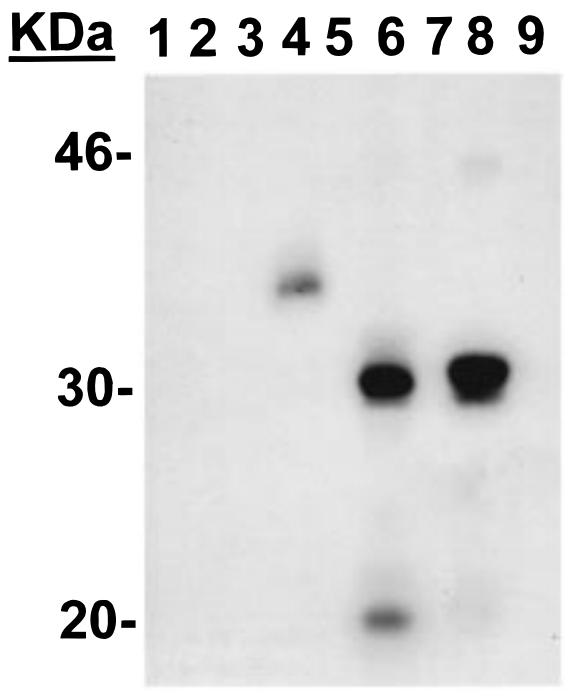

FIGURE 3: Autoradiogram showing in vitro phosphorylation of ovalbumin (lanes 2 and 3), recombinant rat procathepsin B (lanes 4 and 5) human placental cathepsin A (lanes 6 and 7) and human placental cathepsin D (lanes 8,9) by rat liver phosphotransferase. Lane 1, phosphotransferase control. Each sample contained 0.5 μCi of [β-^{32}P]UDP-GlcNAc and 5 μg of the acceptor protein. Samples applied on lanes 1, 2, 4, 6, and 8 contained 12 units of phosphotransferase activity. The positions of ^{14}C-labeled M_r standards are shown.

directed mutagenesis (*26*), are also important for phosphotransferase recognition of cathepsin L. This loop is not conserved in cathepsin B, although one of the Lys residues (Lys184) is found at a topologically equivalent position (Figure 1). The second structurally conserved element is a His-containing loop, spanning residues Lys414−Leu422 in cathepsin A, Gly92−Gly99 in cathepsin B, and Ser73−Gly81 in cathepsin D (shown in blue in Figure 1). Interestingly, in cathepsin D, this segment contains a His77 residue, which was also shown to be essential for the phosphorylation (*12*). Two other putative phosphotransferase-binding sites included a structurally conserved loop containing Pro residues (shown in orange in Figure 1) and a loop opposite the Asn glycosylation site (Gln76−Tyr87 in cathepsin A, Ile44−Glu53 in cathepsin B, and Ser170−Gly179 in cathepsin D, all shown in pink in Figure 1).

Inhibition of in Vitro Phosphorylation by Synthetic Peptides. To verify if the selected sites are involved in phosphotransferase binding, we used a peptide-mapping approach which has been successfully applied in our (*27*) and other laboratories (*28*−*32*) to define regions involved in protein−protein interactions. The peptide-mapping approach is based on the ability of peptides derived from protein recognition interface to mimic the binding with sufficient affinity to displace or hamper protein−protein interactions resulting in the inhibition of a certain biological activity (in our case, the phosphorylation of acceptor proteins). Using the criteria of surface accessibility (solvent accessibility of residue side chains greater than 25 Å^2), we selected peptides from each of the topologically conserved regions to test for their ability to mimic the interaction of cathepsins with phosphotransferase. Each of the selected 10−12 amino acid peptides contained a stretch of at least six solvent-accessible residues (Figure 2).

The phosphorylation of lysosomal cathepsins A, B, and D was studied in vitro using a partially purified phosphotransferase and a phosphate donor, [β-^{32}P]UDP-GlcNAc. Phosphorylated proteins were not detected when phosphotransferase alone was incubated with [β-^{32}P]UDP-GlcNAc (Figure 3, lane 1), confirming the absence of endogenous lysosomal proteins in the preparation. Unspecific phosphorylation of cathepsins did not occur in the absence of phosphotransferase (Figure 3, lanes 3, 5, 7, and 9). In the presence of phosphotransferase, cathepsin B (lane 4), cathepsin A (lane 6), and cathepsin D (lane 8) were intensely phosphorylated. Neither ovalbumin, a nonlysosomal glycoprotein (lane 2), nor heat-denatured lysosomal cathepsins A and D (not shown) were phosphorylated under similar conditions, indicating that the observed phosphorylation is structure-specific.

All peptides derived from the putative phosphotransferase-binding sites showed a dose-dependent inhibition of phosphorylation although the effect varied from peptide to peptide (Figure 4). Control peptides (randomly synthesized 10 amino acid peptides, peptides depleted of spatially conserved

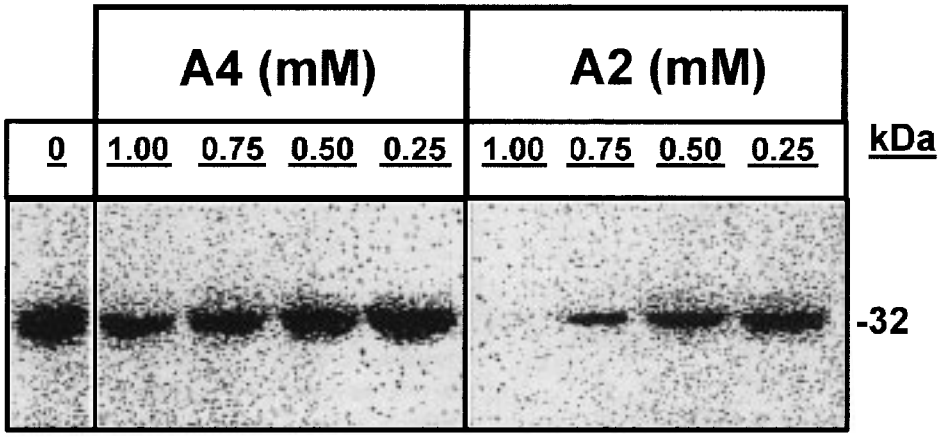

FIGURE 4: Autoradiogram showing in vitro phosphorylation of 32 kDa subunit of cathepsin A in the presence of synthetic peptides A2 and A4. Each sample contained 0.5 μCi of [β-^{32}P]UDP-GlcNAc, 5 μg of cathepsin A, 12 units of phosphotransferase activity, and A2 or A4 peptide in indicated molar concentration. The molecular mass of the phosphorylated protein is shown.

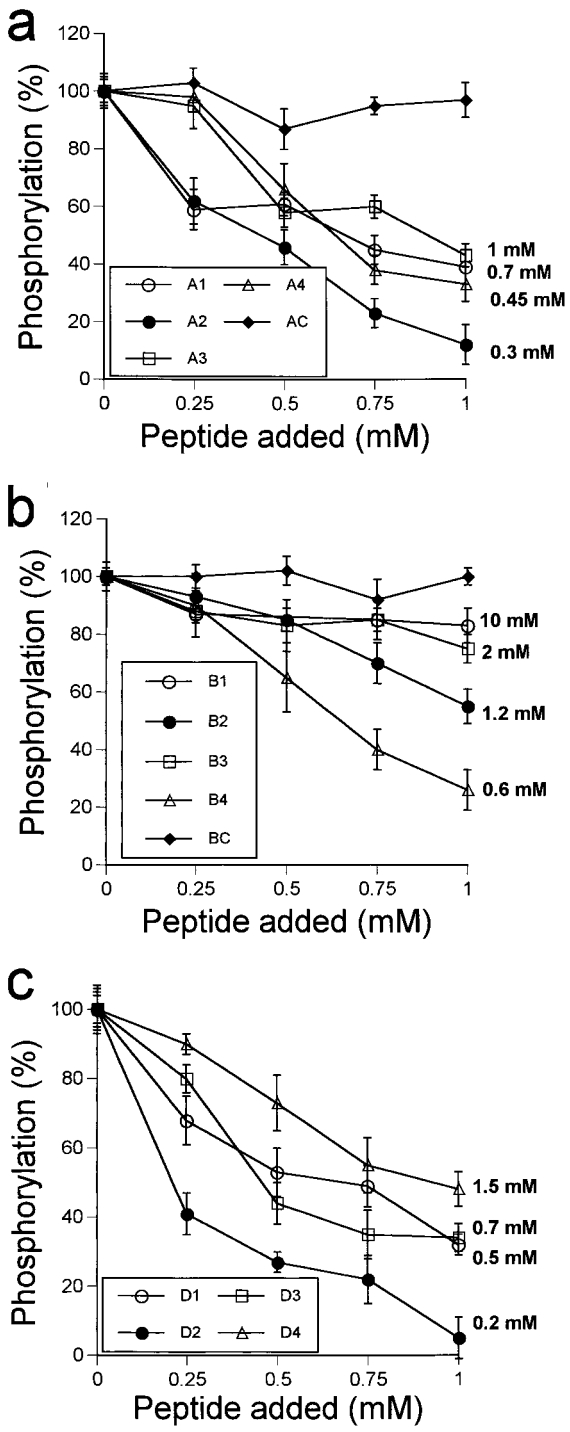

FIGURE 5: Inhibition of in vitro phosphorylation of cathepsins A (a), B (b), and D (c) in the presence of synthetic peptides derived from putative phosphotransferase recognition sites on cathepsin A (A1, G24YLKSSGSKH33; A2, Q76PDGVTLEYNPY87; A3, K414EFSHIAFL422; A4, F433SRFLNKQP451), cathepsin B (B1, F177SDFLTYKSGV187; B2, I44HTNGRVNVE53; B3, G92VY-NSHIG99; B4, E133AGYSTSYK141) and cathepsin D (D1, L276-KLGGKGYKL285; D2, S170RDPDAQPGG179; D3, S73FDIHY-GSG81; D4, T119KQPGITFIA128). Two of the control peptides are also shown: AC (Y400KYGDSGE407) which is selected from cathepsin A loop presumably not involved in phosphotransferase binding; BC (G92VNSMIG99) which is the B3 peptide where His97 was replaced by Met residue. Estimated K_i values for the peptides are shown. The phosphorylation of cathepsins was performed as described in the Experimental Procedures. The reaction mixtures were subjected to SDS−PAGE and protein phosphorylation was analyzed and quantitated with the 425 PhosphorImager using a ImageQuaNT software.

FIGURE 6: Superimposition of the β-hairpin loop regions found in cathepsin A (black), cathepsin D (gray) and β-glucuronidase (white).

residues, or peptides selected from loops presumably not involved in the phosphotransferase binding) did not show this effect. The quantitative results are shown for all cathepsins studied on Figure 5. For both cathepsin A and cathepsin D, the peptides A1 and D1 derived from a Lys-containing loop with a conserved β-hairpin structure had a significant inhibitory effect on phosphorylation (~60% at 1 mM peptide concentration), confirming that these regions are involved in phosphotransferase recognition. These results corroborate previous findings for cathepsin D (*10, 11*) and indicate that cathepsin A has a closely related recognition site.

A similar β-hairpin loop was also identified as the phosphotransferase recognition site in another lysosomal enzyme, β-glucuronidase (*33*). The structures of all three loops can be superimposed with a rms deviation of 0.95 Å (cathepsin A versus cathepsin D) or of 1.1 Å (cathepsin A versus β-glucuronidase) (Figure 6). Moreover, all three regions are enriched in Lys, Tyr, and Gly residues which are suggested to form a short consensus sequence for phosphotransferase recognition (*33*). To date, cathepsin A, cathepsin D, and β-glucuronidase are the only three lysosomal proteins that seem to possess this structurally conserved β-hairpin loop in a total of seven available crystallographic structures (cathepsins A, B, D, L, β-glucuronidase, arylsulfatase B, and aspartyl glucosaminidase). In addition,

a surface-exposed loop on aspartyl glucosaminidase involved in phosphotransferase recognition, although it does not form a β-hairpin, contains consensus amino acid residues Lys177 Tyr Cys Gly Pro Tyr Lys183 (*34*).

Neither a β-hairpin structure nor the consensus amino acid residues are conserved in a topologically similar region of cathepsin B. Therefore, it is not surprising that the peptide B1 selected from this region did not show any significant inhibition of phosphorylation (Figure 5b).

Peptides derived from Pro-containing regions (A4 and D4) generally demonstrated relatively low inhibitory effect with an exception of B4 peptide which contained an amino acid sequence, Gly135 Tyr Ser Thr Ser Tyr Lys141, partially resembling that of structurally conserved β-hairpin loop region of cathepsins A and D. Low inhibitory effect was also observed for peptides derived from His-containing regions (A3, B3, and D3). However, peptides A3, B3, and D3 in which the conserved His was replaced by Met residue did not inhibit phosphorylation even at 1 mM concentration, suggesting that conserved His residues are important for phosphotransferase binding and recognition (Figure 5).

Surprisingly peptides A2, B2, and D2 derived from the regions located opposite to the oligosaccharide chain phosphorylated by phosphotransferase demonstrated very potent inhibition of phosphorylation ($\sim$90% for cathepsins A and D and $\sim$50% for cathepsin B at 1 mM peptide concentration) (Figure 5), suggesting that these regions are involved in phosphotransferase binding. Peptides A2 and D2 also share a Gln/Asp Pro Asp/Gly Gly amino acid sequence which may represent a new consensus for phosphotransferase recognition.

The fact that the phosphotransferase recognition domains on cathepsins were found located on the side opposite to the phosphorylated oligosaccharide raised the question of how the phosphotransferase can maintain contact with these regions and the oligosaccharide chain. This can be explained partially by recent data (*35*) showing that phosphotransferase is a 540 kDa complex composed of disulfide-linked homodimers of 166 and 51 kDa subunits and two 56 kDa subunits. These data together with our present results suggest that catalytic and recognition sites of phosphotransferase are probably located on two different protomers which simultaneously interact with the opposite sides of cathepsins. Such a structure would provide effective recognition and phosphorylation of lysosomal enzymes independently of their molecular size and also would facilitate the phosphorylation of several oligosaccharide chains located at some distance from each other on one protein. While this paper was in preparation, Cuozzo et al. (*24*) have identified structurally conserved Lys residues in procathepsins D and L by superimposing modeled structures of these enzymes and showed that these residues are important for phosphotransferase recognition. One of these residues in cathepsin D (Lys293) is proximal to the Leu276−Leu285 region mapped in this paper, whereas the other (Lys203) maps to the opposite side of the enzyme and can be considered as a complementary region to those described here. The identification of two spatially distant residues important for phosphotransferase recognition on cathepsins D and L is consistent with our hypothesis that multiple phosphotransferase-binding sites are present on lysosomal enzymes.

Although the determination of the tertiary structure of phosphotransferase crystallized bound to its lysosomal protein substrates will most likely be necessary to clarify the recognition mechanism of phosphotransferase, we believe that our results on the identification of new phosphotransferase binding sites on cathepsins A, B, and D add new dimension to the understanding of the biogenesis and sorting of lysosomal proteins.

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REFERENCES

1. Kornfeld, S., and Mellman, I. (1989) *Annu. Rev. Cell Biol. 5*, 483−525.
2. Neufeld, E. F. (1991) *Annu. Rev. Biochem. 60*, 257−280.
3. Gonzalez-Noriega, A., Grubb, J., Talkad, V., and Sly, W. (1980) *J. Cell Biol. 85*, 839−852.
4. Hasilik, A., Waheed, A., and von Figura, K. (1981) *Biochem. Biophys. Res. Commun. 98*, 761−767.
5. Varki, A., and Kornfeld, S. (1981) *J. Biol. Chem. 255*, 8398−8401.
6. Reitman, M., and Kornfeld, S. (1981) *J. Biol. Chem. 256*, 4275−4281.
7. Reitman, M., and Kornfeld, S. (1981) *J. Biol. Chem. 256*, 11977−11980.
8. Lang, L., and Kornfeld, A. (1984) *Anal. Biochem. 140*, 264−269.
9. Tao, K., Stearsn, N., Dong, J., Wu, Q., and Sahagian, G. (1994) *Arch. Biochem. Biophys. 311*, 19−27.
10. Baranski, T., Faust, P., and Kornfeld, A. (1990) *Cell 63*, 281−291.
11. Baranski, T., Koelsch, G., Hartsuck, J., and Kornfeld, A. (1991) *J. Biol. Chem. 266*, 23365−23372.
12. Dustin, M., Baranski, T., Sampath, D. and Kornfeld, A. (1995) *J. Biol. Chem. 270*, 170−179.
13. Schorey, J. S., Fortenberry, S. C., and Chirgwin, J. M. (1995) *J. Cell Sci. 108*, 2007−2015.
14. Cuozzo, J., and Sahagian, G. (1994) *J. Biol. Chem. 269*, 14490−14496.
15. Pshezhetsky, A. V., and Potier, M. (1993) *Biochem. Biophys. Res. Commun. 4*, 362.
16. Sivaraman, J., Coulombe, R., Magny, M.-C., Mason, P., Mort, J. S., and Cygler M. (1996) *Acta Crystallogr., Sect. D52*, 874−875.
17. Laemmli, U. (1970) *Nature 227*, 680−685.
18. Reitman, M., Lang, L., and Kornfeld, A. (1984) *Methods Enzymol. 107*, 163−172.
19. Tranchemontagne, J., Michaud, L., and Potier, M. (1990) *Biochem. Biophys. Res. Commun. 168*, 22−29.
20. Barrett, A. J. (1970) *Biochem. J. 117*, 601−607.
21. Bradford, M. (1976) *Anal. Biochem. 72*, 248−254.
22. Rudenko, G., Bonten, E., d'Azzo, A., and Hol, W. (1995) *Structure 3*, 1249−1259.
23. Elsliger, M.-A., and Potier, M. (1994) *Proteins 18*, 81−93.
24. Cuozzo, J., Tao, K., Cygler, M., Mort, J. S., and Sahagian, G. (1998) *J. Biol. Chem. 273*, 21067−21076.
25. Cygler, M., Sivaraman, J., Grochulski, P., Coulombe, R., Storer, A. C., and Mort, J. S. (1996) *Structure 4*, 405−416.
26. Cuozzo, J. Tao, K., Wu, Q., Young, W., and Sahagian, G. (1995) *J. Biol. Chem. 270*, 15611−15619.
27. Pshezhetsky, A. V., Elsliger, M.-A., Vinogradova, M. V., and Potier, M. (1995) *Biochemistry 34*, 2431−2440.
28. Cohen, E. A., Gaudreau, P., Brazeau, P., and Langelier, Y. (1986) *Nature 321*, 441−443.
29. Dutia, B., Frame, M., Subak-Sharpe, J., Clark, W., and Marsden, H. (1986) *Nature 321*, 439−441.

30. Paradis, H., Gaudreau, P., Brazeau, P., and Langelier, Y. (1988) *J. Biol. Chem. 263*, 16045−16050.
31. Joseph, G., Gorzalczany, Y., Koshkin, V., and Pick, E. (1994) *J. Biol. Chem. 269*, 29024−29031.
32. Divita, G., Restle, T., Goody, R., Chermann, J., and Baillon, J. (1994) *J. Biol. Chem. 269*, 13080−13083.
33. Jain, S., Drendel, W. B., Chen, Z. W., Mathews, F. S., Sly, W. S., and Grubb, J. H. (1996) *Nat. Struct. Biol. 3*, 375−381.
34. Tikkanen, R., Peltola, M., Oinonen, C., Rouvinen, J., and Peltonen, L. (1997) *EMBO J. 22*, 6684−6693.
35. Bao, M., Booth, L., Elmendord, B., and Canfield, W. (1996) *J. Biol. Chem. 271*, 31437−31445.

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