Pure & Appl. Chem., Vol. 59, No. 11, pp. 1457–1464, 1987.
Printed in Great Britain.

Basic monosaccharide derivatives: tools for exploring the active site of glycohydrolases and for studies in glycoprotein biosynthesis

Günter Legler

Institut für Biochemie, Universität Köln, D-5000 Köln 41, FRG

Abstract - The majority of glycoside hydrolases is strongly inhibited by monosaccaride derivatives bearing a basic NH-group adjacent to C-1, i. e. by glycosylamines, 5-amino-5-deoxyhexopyranoses, and 1,5-iminoalditols. This inhibition with K_i-values up to 10^4-times smaller than those of neutral analogs is explained by a catalytic site which features an acid and a carboxylated in iuxtaposition close to the anomeric carbon of the bound inhibitor or substrate. The inhibitor protonated by the acid forms an ion pair with the carboxylate. The stability of this ion pair and the lack of influence of added salt on the inhibition calls for an active site strongly shielded from the aqueous environment. The acid/carboxylate arrangement of functional groups is supported by inhibition studies with substrate related epoxides that are activated by proton transfer from the acid and form an ester with carboxylate. N-Alkylation of the basic inhibitors leads, in many cases, to an enhanced inhibition. Structural variation of the alkyl substituents permits a detailed characterisation of the aglycon site as exemplified by studies on lysosomal ß-glucosidase.

Inhibition by 1,5-dideoxy-1,5-imino-D-glucitol and -mannitol of α-glucosidases and -mannosidases participating in the biosynthesis of carbohydrate chains linked to asparagine residues of glycoproteins results in altered glycan structures of newly synthesized proteins. The effects of these alteration on surrace expression and secretion of the proteins, on intracellular compartmentation and on the formation and infectivity of enveloped viruses is discussed.

INTRODUCTION

The first detailed picture of the active site of an enzyme catalysing the hydrolysis of a glycosidic bond was published almost 20 years ago by D. C. Phillips and his colleagues (ref. 1). X-Ray structure analysis of the lysozyme chitotriose complex gave strong evidence that a carboxylic acid and a carboxylate group (Glu-35 and Asp-52) in close proximity to the cleavage site could effect bifunctional catalysis by proton transfer to the glycosidic oxygen and electrostatic stabilisation of a putatiive oxocarbonium ion intermediate (Fig. 1)

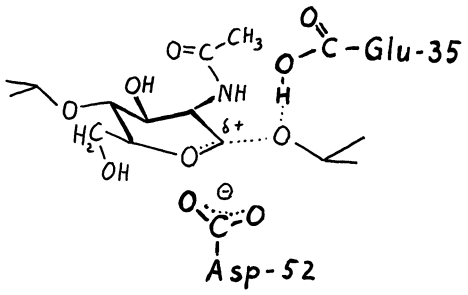


Fig. 1. Hypothetical transition state for cleavage of the glycosidic bond at subsite D of the substrate binding cleft of lysozyme

In addition, it was assumed that the ground state energy of the bound substrate was raised by deformation of the pyranose chair conformation towards a planar geometry at the anomeric carbon, thereby lowering the energy of activation. This latter factor seemed to be supported by the strong inhibition of lysozyme (ref. 2) and other glycosidases (ref. 3) by glycon derived hexonolactones. The importance of deformation was considered small, however, when it was realized that proteins are too flexible to put sufficient strain on the bound substrate (ref. 4).

GENERAL INFORMATION FROM INHIBITION STUDIES

As X-ray data comparable to those for lysozyme are not available for other glycoside hydrolases we have to rely on indirect evidence for a characterisation of their active sites. Experiments with glycon related epoxides (ref. 5 and Table 1) have shown that a carboxylate group can be specifically labeled with these active site directed inhibitors in most of the ß-specific enzymes and in ~60% of the α-specific ones. An even higher proportion of α- and ß-specific glycosidases is reversibly inhibited up to 10^4-times better by basic monosaccharide derivatives in comparison with their neutral counterparts (Table 1). The results obtained with both types of inhibitors support a model for the catalytic site of most glycosidases that features a carboxylate and an acidic group acting synergistically on the glycosisic bond as shown in Fig. 1.

TABLE 1. Number of glycosidases of microbial, plant, and animal origin susceptible to an enhanced inhibition by basic sugar analogs (a) and inactivation by conduritol epoxides (anhydro-inositols) (b).
(a) +: K_i(hexose)/K_i(basic analog) > 300, -: K_i(hexose)/K_i(basic analog) < 50
(b) +: rate constant for inactivation $k_i > 0.02\ M^{-1}min^{-1}$, -: no inactivation

	basic analogs		epoxides	
	+	-	+	-
α-D-Glucosidases	5	0	5	3
ß-D- "	4	1	6	2
α-D-Galactosidases	2	0	2	2
ß-D- "	2	1	4	0
α-D-Mannosidases	4	1	1	2
ß-D- "	1	1	1	2
ß-N-Acetyl-D-glucosaminidases	3	1	no data	

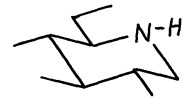
EXPLORING THE ACTIVE SITE WITH BASIC INHIBITORS

Structural requirements

Basic sugar derivatives with strong affinity for glycosidases have their basic group adjacent to the anomeric carbon, i. e. they are glycosylamines (1), 5-amino-5-deoxyhexoses (nojirimycins) (2), or 1,5-dideoxy-1,5-iminoalditols (deoxynojirimycins) (3).

1

2



3

N-Arylation or N-acylation decreases basicity, resulting in a greatly reduced affinity (Table 2). As hydrogen bonding is only moderately impaired by these structural modifications we assume that the main cause for the strong binding of the above hexose derivatives is the formation of an ion pair consisting ot the protonated inhibitor and a carboxylate group at the catalytic site. An amino group at C-2 enhances binding only moderately or not at all over that of the parent hexose (Table 2). This shows that thre is little flexibility with respect to the mutual orientation of the carboxylate relative to the bound inhibitor.

TABLE 2. Effects of basicity and position of the amino group on the inhibition of ß-glucosidase A_3 from *Aspergillus wentii* and ß-galactosidase from *Escherichia coli* by derivatives of D-glucose and D-galactose, respectively.

Inhibitor	pK_a	ß-Glucosidase K_i (µM)	ß-Galactosidase K_i (µM)
Glycose	-	2,800	21,000
Glycosylamine	5.6	1.6	7
2-Amino-2-deoxyglycose	7.8	18,000	1,300
N-Bromonacetylglycosylamine	-	250	1,100
N-Glycosylbenzylamine	5.3	n. d.	0.009
N-Glycosyl-p-toluidine	1.5	n. d.	760
Nojirimycin	5.3	0.36	0.045
1-Deoxynojirimycin	6.4	2.7	12.5

Access of water to the catalytic site

From a comparison of K_i-values for basic inhibitors with those of their neutral counterparts we can calculate that an additional binding energy of up to 31 kJ/mol (7.4 kcal/mol) is provided by the basic group (ref. 6). If we ascribe this energy contribution to the electrostatic interaction discussed above we have to assume that water with its high dielectric constat has little access to this part of the enzyme inhibitor complex. Support for a catalytic site strongly shielded from the aqueous environment comes from inhibition studies in the presence of salt concentrations from 5 to 300 mM: the K_i-values of 1-deoxynojirimycin with α-glucosidase from yeast, ß-glucosidase from Asp. wentii, almonds and mammalian lysosomes were either independent of the ionic strength or they varied to the same extent as the K_m-values of the neutral substrates (Legler and Petzold, unpublished data). The restricted solvation of the catalytic site may well be of importance for a proper alignment of the functional groups with respect to the bond to be cleaved and for maximal efficiency of electrostatic effects during catalysis.

pH-Dependence

In order to understand the pH-dependence of the inhibition we have to consider the ionisation state of the inhibitor in solution and that of the ionizing and charged groups at the active site. The former is known from pK_a-data, that latter can, at least in part, be deduced from the pH-dependence of V_{max} and K_m. Ambiguities arising from overlapping ionisation processes, e. g. when an observed increase of the inhibition with pH could either be due to an increased ionisation of a carboxylate binding the inhibitor cation or to an increasing proportion of the unprotonated form of the inhibitor which is protonated at the active site, may sometimes be resolved by studies with permanently cationic inhibitors. The identical pH-dependence of pK_i for ß-glucosyl pyridinium ion and the protonated forms of ß-glucosylamine and nojirimycin (Fig.2) shows that the inhibitor cation is the species which is bound by the enzyme (ref. 7). From studies like this and from a comparison of K_i-values for permanently cationic inhibitors with their neutral analogs (e. g. ß-glycosyl pyridinium ion vs. ß-glycosyl benzene) two classes of glycosidases can be distinguished: a) the enzyme is inhibited by the inhibitor cation; b) the inhibiting species is the unprotonated form of the inhibitor; permanently cationic inhibitors are bound only to the same extent as their neutral counterparts. Examples for class (a) enzmyes are ß-glucosidase A_3 from Asp. wentii (ref. 7) and 'trimming' glucosidase I from mammalian microsomes (ref. 8). Most other enzymes belong to class (b), e. g. ß-glucosidase from almonds (ref. 6 and 8) and from mammalian lysosomes (ref. 10), ß-galactsidase from E. coli (ref. 11), and microsomal 'trimming' glucosidase II (ref. 9).

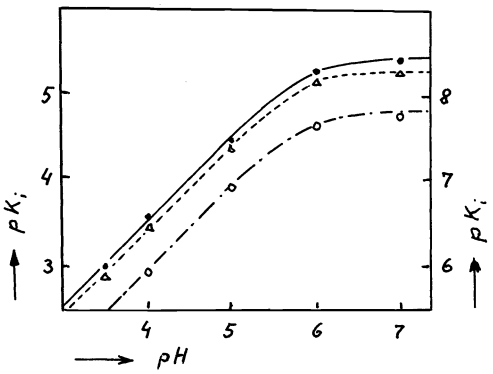


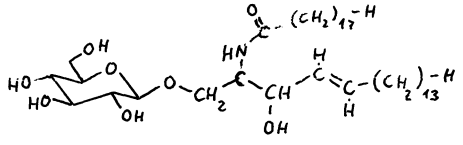
Fig. 2. pH-Depndence of the inhibition of ß-glucosidase A_3 from Asp. wentii by ß-D-glucosylpyridinium ion (—●—, left) and the protonated forms of D-glucosylamine (—·—o—·—, right) and nojirimycin (---▲--, right).

A possible explanation for the inhibtion characteristics of the class (b) enzmyes could be the presence of a cationic acid (e. g. protonated histidine) at the catalytic site. A permanently cationic inhibitor would be attracted by the carboxylate group but it would at the same time be repelled by the cationic acid group. A basic inhibitor, on the other hand, would be protonated by the cationic acid and could then form an ion pair with the carboxylate and a hydrogen bond to the unprotonated acid.

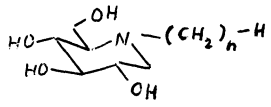
The aglycon site

A further exploration of the active site is possible by the introduction of substituents at the nitrogen of compounds 1 or 3. Experiments with N-alkyl glycosylamines (ref. 6 and 11) and N-alkyl-1-deoxynojirimycins (ref. 10 and 12) have often indicated a hydrophobic domain at the aglycon site, even in cases where one would not expect it from the structure of the natural substrate. ß-Galactosidase from E. coli has probably evolved for the efficient hydrolysis of lactose and for its transformation into allo-lactose which is the natural inducer for the synthesis of ß-galactosidase in this bacterium. The latter function is facilitated by the retention and reorientation of glucose at the aglycon site after bond cleavage: the formation of allo-lactose by the reaction of the C-6 hydroxyl group of glucose with the galactosyl enzyme intermediate is favored 1:2 over hydrolysis (ref. 13). In spite of an expected polar aglycon site we find that ß-galactosidase from E. coli is inhibited 740- and 1200-times better by N-benzyl- and N-heptyl-D-galactosylamine than by D-galactosylamine itself (ref. 11).

An aglycon site which appears to be closely adapted to the hydrophilic/hydrophobic features of its natural substrate is that of ß-glucosidase from mammalian lysosomes. Patients who carry a hereditary defect of this enzyme suffer from the different types of Gaucher's disease in which various symptoms are due the intracellular accumulation of the natural substrate, ß-glucosylceramide 4 (ref. 14). Inhibition of the enzmye from calf spleen (ref. 12) and human placenta (ref. 10) by N-alkyl derivatives of 1-deoxynojirimycin 5 gave K_i-values from which the free energy plot shown in Fig. 3 was calculated. It is seen that hydrophobic interactions become noticeable only with alkylchains larger than butyl. We interpret this as the result of an adaption to the natural substrate: near the cleavage site there are polar groups in the aglycon site for the interaction with the acylamido and hydroxyl groups of ceramide, further removed is a hydrophobic domain for its alkyl and/or acyl chains.



4



5

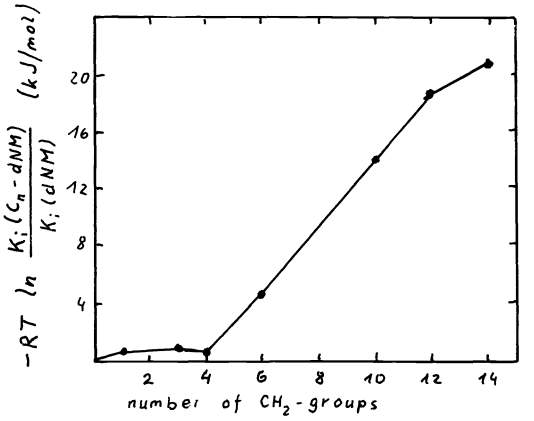


Fig. 3. Apparent free energy increments for binding alkyl chains to the active site of lysosomal ß-glucosidase calculated from the inhibition constants of N-alkylderivatives of 1-deoxynojirimycin (C_n-dNM) relavtive to the parent compound (dNM)

Glycosylamines vs. nojirimycin derivatives

If we compare the inhibition of glycosidases by these two types of basic hexose derivatives we note that the compounds with an endocyclic nitrogen are, in many examples, better inhibitors than those with an exocyclic basic group. As good inhibitors resemble the enzymic transition state or a reactive intermediate rather than the substrate (ref. 15) the strong inhibition by 1-deoxynojirimycin and related compounds was taken as an indication that the enzyme would protonate the substrate on the pyranose oxygen (ref. 16) instead of the glycosidic one as shown in Fig. 1. While this may hold for some enzymes we find that with most examples of strong inhibition by type 2 and 3 compounds the final state is reached slowly, i. e. on the time scale of minutes (Fig. 4). With the glycosylamines, on the other hand,

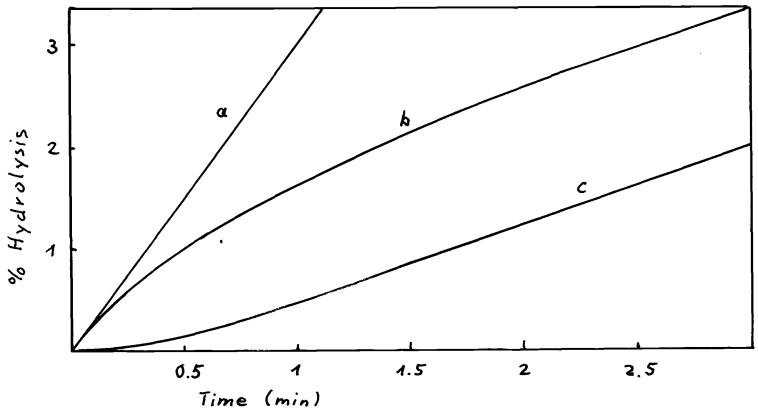


Fig. 4. Hydrolysis of 4-methylumbelliferyl α-D-mannopyranoside by jack bean α-D-mannosidase at pH 5.5 in the absence (a) and presence (b) of 10 µM 5-amino-5-deoxy-mannopyranose. For trace c, the enzyme was preincubated in 200 µM inhibitor and then added to 18 vol. of substrate.

a slow complex formation is noticed only in cases where diffusion becomes rate limiting (ref. 11). As the inhibition is reversible (Fig. 4, trace c) and does not involve a chemical reaction it has been proposed that the slow approach to the inhibitor complex equilibrium with type 2 and 3 compounds is due to a slow conformational change that alignes the active site groups for optimal binding (ref. 17). From the concentration dependence of the rate constant for the approach to the final inhibition we can calculate that the enzyme in its normal form has an at least 50-fold smaller affinity than in its complexed state with the inhibitor. We conclude, therefore, that nojirimycins and 1-deoxynojirimycins are not better models for the protonated substrate or cationic intermediate than the glycosylamines.

A distinct advantage of nojiirmycin and especially 1-deoxynojirimycin type inhibitors over glycosylamines is their stability in solution. Glycosylamines show a rapid mutarotation due to the equilibration of α- and ß-anomers and are subject to hydrolysis and other reactions. Their use is limited to kinetic experiments on the time-scale of 5 to 10 min. Experiments concerned with the physiological role of glycosidases usually are on the time-scale of hours even days and can only be made with nojirimycins and their 1-deoxy derivatives. Some of them will be described in the next section.

Another aspect to be considered is ease of preparation. Glycosylamines are readily prepared from unprotected monosaccharides and ammonia or an amine (ref. 18). 5-Amino-5-deoxyhexopyranoses and their derivatives, however, have to be synthesized by routes involving up to 12 steps from the common hexoses unless they happen to be microbial products like nojirimycin (ref. 19) or 1-deoxynojirimycin (ref. 20). Following the pioneering work of Paulsen (ref. 21) procedures have been described for the synthesis of the 5-amino-5-deoxyanalogs of D-glucose (ref. 22), D-mannose (ref. 23), and D-galactose (ref. 24). The routes involve the preparation of a protected furanose, its oxidation to the 5-ketone, and conversion of the latter to the 5-amine. Deprotection in the presence of sulfurous acid gives the 5-amino-5-deoxyhexofuranose as hydrogensulfite adduct. Formation of this adduct ensures stability against acids that otherwise cause dehydration to pyridine derivates and other reactions (ref. 21 and 22). Dissociation of the adduct is slow process (ref. 25) but the free base is easily prepared by treating the adduct with barium hydroxide or a strongly basic ion exchange resin.

Less involved procedures have been described for the synthesis of 1-deoxynojirmycin and its D-manno analog (ref. 26 and 27) and for the analogs of L-fucose (ref. 28) and N-acetyl-D-glucosamine (ref. 29).

STUDIES ON GLYCOPROTEIN BIOSYNTHESIS

The pathway of N-glycosylation and glycoprotein processing

Biosynthesis and attachment of carbohydrate side chains to specific asparagine residues of glycoproteins imvolves a common pathway that terminates with transfer of the oligosaccharide unit $Glc_3Man_9GlcNAc_2$ (Fig. 5) to the protein acceptor (ref. 30).

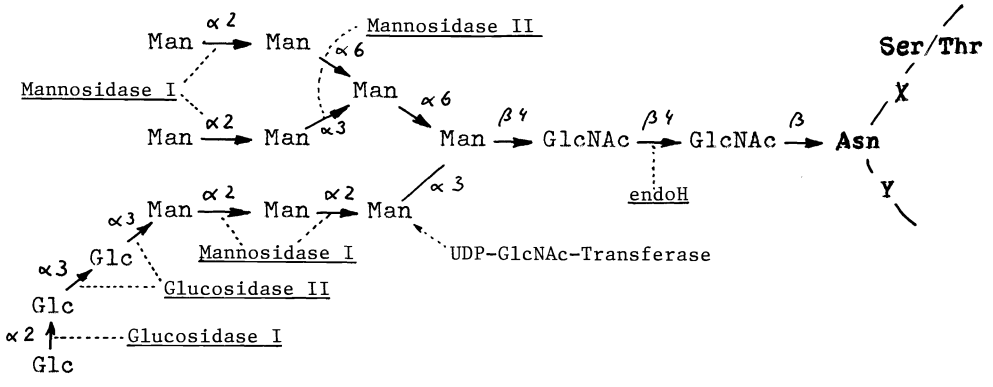


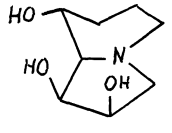
Fig. 5. Structure of oligosaccharide precursor as transferred from dolichylpyrophosphate to specific asparagine residues of glycoproteins. Cleavage sites for trimming glycosidases and endoglucosaminidase H (endoH) are indicated (·····).

The oligosaccharide structure shown in Fig. 5 is, however, never found in mature glycoproteins. Further modifications occur in the endoplasmatic reticulum and Golgi vesicles that convert the original glycan to either 'complex' or 'high mannose' side chains. The first step of this 'processing' are catalyzed by 'trimming' glucosidases I and II and mannosidases I and II which are localized in the endoplasmatic reticulum and (in part) in the Golgi vesicles.

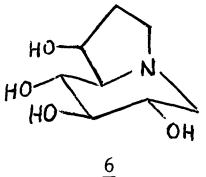
Recognition signal for mannosidase II is an N-acetylglucosamine transferred to a mannose residue that became terminal after the removal of the appropriate glucose and mannose residues as shown in Fig. 5. Conversion of the fully trimmed $Man_3GlcNAc_2$-intermediate to 'complex' type glycans involves the sequential addition of N-acetylglucosamine, galactose and sialic acid by specific glycosyl transferases of the Golgi appartus.

Inhibition of 'trimming' glycosidases

Glycoprotein processing can be arrested at various stages by selective inhibition of the 'trimming' glycosidases and the consequences of altered glycan structure on the intra- and extracellular fate of the glycoproteins can be studied. Inhibitors suitable for this kind of experiments (see ref. 31 for a review) are 1-deoxynojitimycin (3, dNM) and its N-methyl derivative for glucosidase I, 1-deoxy-manno-nojirimycin (dMM) for mannosidase I and the indolizine alcaloid swainsonine (5) for mannosidase II. Another useful inhibitor for glucosidase I is castanospermine (6), also an indolizine alcaloid. The inhibitory properties of the two indolizines can be rationalized when we consider their basic character and the resemblance of their hydroxylation pattern with mannose and glucose, resepctively. The selectivity of swainsonine against mannosidase II vs. mannosidase I (which is not inhibited) may be due to steric effects at the active site of mannosidase I. A selective inhibition of glucosidase II vs. glucosidase I is not possible with the inhibitors described here.



5



6

Nojirimycin (2) and its D-manno analog are only moderately effective inhibitors of trimming glucosidase I and mannosidase I (I_{50} 0.1 mM, ref. 9 and unpublished data) but they have, nevertheless, profound effects on glycan structure. Glycoproteins synthesized in their presence appear to have only one or two N-acetylglucosamine residues per glycan attachment site instead of the complete $Glc_3Man_9GlcNAc_2$-oligosaccharide (ref. 32). Presumably they interfer with one of the early steps of glycoprotein biosynthesis. We could show that nojirimycin can be phosphorylated with ATP and hexokinase to its 6-phosphate but it was not possible to demonstrate its further activation and incorporation into glycoproteins as discussed in ref. 32 (Legler and Kappes, unpublished data).

Effects that could not be explained by an inhibition of trimming glycosidases were also observed with 1-deoxynojirimycin. High concetrations of dNM applied to suppress an incomplete inhibition of glucosidase I did not only cause the desired inhibition but also resulted in the formation of glycoproteins with a smaller number of glycan chains than normal (ref. 33 and 34). N-methyl-dNM which is a tenfold better inhibitor than dNM (ref. 9) does not cause these side effects.

An important point for a correct interpretation of results from inhibition experiments with whole cells is the specificity of the inhibitors. Concentrations of 100 K_i are required to keep the activity of the inhibited enzyme below 1% of its normal value and one has to make sure that only the desired enzyme is affected by the concentrations employed. An incomplete inhibition observed with concentrations above 100 K_i (usually around 1 mM) may be due to the presence of glycosidases different from those described. The presence of several enzymes acting on the same substrate is indicated by a non-linear Dixon-plot for the inhibition by crude preparations as shown for glucosidase I from calf liver microsomes (ref. 9). The Inhibition of this enzyme by excessively high concetrations of the mannosidase I inhibitor dMM (K_i 190 µM vs. 1 µM for dNM) shows that its ability to discriminate between the two configurations at C-2 of the inhibitor is limited (ref. 35).

Effects of 'trimming' inhibitors on glycan structure

Analysis of glycans synthesized in the presence of dNM or N-methyl-dNM reveals that the main portion has the $Glc_3Man_9GlcNAc_2$-structure but one also finds glycans with less than 12 hexoses. All of them, including those with the full hexose complement, can be cleaved from the peptide cahin with endoglucosaminidase H (endo H). In addition, a minor portion is found which is resistant to endo H. This enzyme is specific for the chitobiose linkage of branched glycans, provided they do not contain sialic acid (see Fig.5 and ref. 36). The occurrence of glycans with less than nine mannose residues and of the so-called hybrid structures with added sialic acid and thus resistant to endo H is taken as evidence that removal of mannose residues from the upper two branches (Fig. 5) by mannosidase I does not require prior deglucosylation (confirmed by studies with purified mannosidase I, ref. 37). We can also conclude that N-acetylglucosamine, galactose, and sialic acid can be added to partially

trimmed glycans. As the glycosyltransferases of the processing reactions are located in the Golgi compartment we conclude from the occurrence of 'hybrid' structures that the transport of proteins from the rough endoplasmatic reticulum to the Golgi vesicles does not depend on the removal of glucose residues.

Glycans synthesized in the presence of 1-deoxy-manno-nojirimycin mainly have the $Man_9GlcNAc_2$-structure but some $Man_8GlcNAc_2$-glycans are also found. The reason for an incomplete inhibition of mannose trimming might be the presence of mannosidases with different susceptibility to dMM as discussed above for glucosidase I.

Intra-and extracellular fate of glycoproteins with altered glycan structure

Glycoproteins synthesized in the rough endoplasmatic reticulum and processed in the Golgi compartment can have three destinations: they are transferred to the plasma membrane and stay there until they are recycled, they are secreted, or they are routed to lysosomes and peroxysomes. The signals which determine the fate of individual glycoproteins are of current interest. In this respect the structure of the glycans which in turn could be determined by specific interaction of the protein with the processing enzymes may play an important role.

An example of membrane proteins where effects of glycan structure have been studied are human class I histocompatibility antigens. Their surface expression was not impaired by dNM or dMM in spite of the fact that their glycans were of high mannose instead of complex structure

A similar lack of effect of inhibiting the removal of glucose and mannose residues from asparagine linked glycans was observed with coat proteins of a number of enveloped viruses. During maturation the virions aquire a coat consisting of lipid from the host and embedded glycoproteins which are encoded by the viral DNA and which contain complex type glycans. Strains od influenza virus (ref. 38 and 39), vesicular stomatits virus (ref. 38), and Rous sarcoma virus (ref. 40) grown in chick embryo or canine kidney cells in the presence of dNM and dMM had coat proteins with glycans of the expected structure, i. e. $Glc_{1-3}Man_{7-9}GlcNAc_2$ with dNM and $Man_{8-9}GlcNAc_2$ in the presence of dMM. In spite of these alterations there were no or only marginals effects on the number of virions, their release from the host cells, or their infectivity.

With a retrovirus causing murine leukemia, on the other hand, the glycan structure of the envelop proteins was of great importance. Fried mink cell focus inducing virus produced in cells treated with dNM was not released but the virions accumulated in intracellular vacuoles (ref. 41). Virus particles were of normal infectivity, however, after the cells were broken up.

Effects of glycosidase inhibitors on protein secretion were first studied with mouse hybridoma cell lines producing D and M type immunoglobulins (IgD and IgM). While ig M was secreted normally with glycans having a $Glc_{2-3}Man_{7-9}GlcNAc_2$-structure synthesized in the presence of 1 mM dNM, IgD was completely reteained under the same conditons (ref. 32). In the presence of 1 mM dMM, on the other hand, both types of immunoglobulins (with $Man_{8-9}GlcNAc_2$-structure) were secreted normally (ref. 42). In human hepatoma HepG2 cells the action of dNM caused the accumulation of α_1-antitrypsin and α_1-antichymotrypsin in the rough endoplasmatic reticulum (ref. 43). The same effect could be achieved by adding the antibiotic tunicamycin which prevents N-glycosylation by blocking the first step of the pathway, i. e. the formation of dolichylpyrophospate linked N-acetylglucosamine. No effect were observed with the mannosidase II inhibitor swainsonine. Secretion of transferrin, normally bearing complex glycans, was not affected. The same effects of preventing the conversion of high mannose to complex glycans has been observed with α_1-antitrypsin in rat hepatocytes (ref. 44).

The consequences of altered glycan structure on the fate of lysosomal enzyme have bee studied with cathepsins D and ß-hexosamindase in human fibroblasts (ref. 33). These proteins are routed to their lysosomal destination by aquiring a mannose-6-phospahte group after the removal of the three glucose and part of the mannose residues from the original asparagine linked glycan. In the presence of dNM there was a delay of several hours in aquiring the mannose-6-phosphate marker and also of the subsequent proteolytic processing which takes place in the lysosomes. High concentrations of dNM (5 mM) resulted, in additon in the formation of underglycosylated proteins as discussed above. Inhibition of glycan trimming of cathepsin D with dMM in hepatoma HepG2 cells caused a large part of the enzyme to be secreted and a delay of its proteolytic processing in the lysosomes (ref. 45). In fibroblasts on the other hand, these effects did not occur in spite of an identical arrest of glycan trimming at the $Man_{6-9}GlcNAc_2$-stage.

Studies on the biological importance of glycan structure of which some examples have been give here, show that alterations can have widely differing effects, even with closely related proteins like IgD and IgM. Generalisations or predictions cannot yet be made with confidence. However, the study of cellular aspects of glycan structure with glycosidase inhibitors has just begun and further studies with other systems and possibly new inhibitors will provide deeper insights.

Acknowledgement The financial support of research work carried out by the author and his co-workes by the 'Deutsche Forschungsgemeinschaft' and the 'Fonds der chemischen Industrie' is gratefully acknowledged.

REFERENCES

1. C.C.F. Blake, L.N. Johnson, G.A. Mair, A.T.C. North, D.C. Phillips, and V.R. Sarma, Proc. Roy. Soc. London, B167, 378-385 (1967)
2. I.I. Seceminski and G.E. Lienhard, J. Biol. Chem.,249, 2932-2936 (1974)
3. G.A. Levvy and S.M. Snaith, Adv. Enzymol., 36, 151-181 (1972)
4. A. Fersht, Enzmye Stucture and Mechanism, 2nd ed., p. 334-343, Freeman, New York (1984)
5. P. Lalégerie, G. Legler, and J.M. Yon, Biochimie, 64, 977-1000 (1982)
6. G. Legler, Biochim. Biophys. Acta, 524, 94-101
7. G. Legler, M.L. Sinnott, and S.G. Withers, J. Chem. Soc. (London), Perkin II, 1376-1360 (1980)
8. M.P. Dale, H.E. Ensley, K. Kern, K.A.R. Sastry, and L.D. Byers, Biochemistry, 24, 3530-3537 (1985)
9. H. Hettkamp, G. Legler, and E. Bause, Eur. J. Biochem., 142, 85-90 (1984)
10. K. Osiecki-Newman, D. Fabbro, G. Legler, T. Dinur, S. Gatt, R.J. Desnick, and G.A. Grabowski, Biochemistry (in press)
11. G. Legler and M. Herrchen, Biochim. Biophys. Acta, 616, 95-103 (1983)
12. G. Legler and H. Liedtke, Biol. Chem. Hoppe-Seyler, 366, 1113-1122 (1985)
13. R.E. Huber, G. Kurz, and K. Wallenfels, Biochemistry, 15, 1994-2001 (1976)
14. R.O. Brady, J.N. Kanfer, and D. Shapiro, Biochem. Biophys. Res. Comm., 18, 221-225 (1965)
R.J. Desnick, S. Gatt, and G.A.Grabowski, (ed.), Gaucher Disease: A Century of Delineation and Research, A.R. Liss, New York (1982)
15. R. Wolfenden, Accts. of Chem. Res., 5, 10-15 (1972)
G.E. Lienhard, Science, 180, 169-173 (1973)
16. G.W.J. Fleet, Tetrahedron Lett.,26, 5073-5076 (1985)
17. G. Hanozet, H.P. Pircher, P. Vanni, B. Oesch, and G. Semenza, J. Biol. Chem. 256, 3703-3711 (1981)
18. G.P. Ellis and J. Honeyman, Adv. Carbohydr. Chem. 10, 95-168 (1955)
19. N. Ishida, K. Kamagai, T. Niida, T. Tsuruoka, and H. Yumoto, J. Antibiotics,Ser. A, 20, 66-71 (1967)
20. D.D. Schmidt, W. Frommer, L. Müller, and E. Truscheit, Naturwiss. 66, 548 (1979)
21. H. Paulsen and K. Todt, Adv. Carbohydr. Chem., 23, 116-232 (1968)
22. S. Inouye, T. Tsuruoka, T. Ito, and T. Niida, Tetrahedron, 23, 2125-2144 (1968)
23. G. Legler and E. Jülich, Carbohydr. Res., 128, 61-72 (1984)
24. G. Legler and S. Pohl, Carbohydr. Res. (in press)
25. G. Legler u. W. Becher, Carbohydr. Res., 101, 326-329 (1982)
26. G. Kinast and M. Schedel, Angew. Chem., 93, 799-800 (1981)
27. R.C. Bernotas u. B. Ganem, Tetrahedron Lett., 26, 1123-1126 (1985)
28. G.W. Fleet, A.N. Shaw, S.V. Evans, and L.E. Fellows, J. Chem. Soc. (Lond.), Chem. Comm., 841-842 (1985)
29. G.W. Fleet, P.W. Smith, R.J. Nash, L.E. Fellows, R.B. Parekh, and T.W. Rademacher, Tetrahedron Lett. (in press)
30. S.C. Hubbard and R.J. Ivatt, Ann. Rev. Biochem., 50, 555-583 (1981)
31. U. Fuhrmann, E. Bause, and H. Plough, Biochim. Biophys. Acta, 825, 95-110 (1985)
32. N. Peyrieras, E. Bause, G. Legler, R. Vasiliv, L. Claesson, P. Peterson, and H. Plough, EMBO J., 2, 823-832 (1983)
33. P. Lemanski, V. Gieselmann, A. Hasilik, and K. v. Figura, J. Biol. Chem., 259, 10129-10135 (1984)
34. V. Groß, T.-A. Tran-Thi, R.T. Schwarz, A.D. Elbein, K. Decker, and P.C. Heinrich, Biochem. J. (in press)
35. J. Schweden, C. Borgmann, G. Legler, and E. Bause, Arch. Biochem. Biophys. (in press)
36. A.L. Tarentino and F. Maley, Biochem. Biophys. Res. Comm., 64, 411-415 (1975)
37. J. Schweden, G. Legler, and E. Bause, Eur. J. Biochem., (in press)
38. B. Burke, K. Matlin, E. Bause, G. Legler, N. Peyrieras, and H. Plough, EMBO J., 3, 551-556 (1984)
39. A.D. Elbein, G. Legler, A. Tlusty, W. McDowell, and R.T. Schwarz, Arch. Biochem. Biophys., 235, 579-588 (1984)
40. J.V. Bosch, A. Tkusty, W. McDowell, G. Legler, and R.T. Schwarz, Virology, 143, 342-346 (1985)
41. A. Pinter, W.J. Honnen, and J.S. li, Virology, 136, 196-210 (1984)
42. U. Fuhrmann, E. Bause, G. Legler, and H. Plough, Nature (Lond.), 307, 755-758 (1984)
43. H.F. Lodish and N. Kong., J. Cell Biol., 98, 1720-1728 (1984)
44. V. Gross, T. Andus, T.-A. Tran-Thi, R.T. Schwarz, K. Decker, and P.C. Heinrich, J. Biol. Chem., 258, 12203-12209 (1983)
45. A. Nauerth, P. Lemansky, A. Hasilik, K. v. Figura, E. Bause, and G. Legler, Biol. Chem. Hoppe-Seyler, 366, 1009-1016 (1985)