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Active-site-directed inhibition of asparagine *N*-glycosyltransferases with epoxy-peptide derivatives

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The hexapeptide Arg-Asn-Gly-epoxyethylglycine-Ala-Val-OMe specifically inactivates membrane-bound *N*-glycosyltransferases. The specificity is demonstrated by the inability of peptides containing 2,3-epoxypropyl-, allyl- and vinyl-glycine in the epoxyethylglycine position to function as inhibitors. The inhibition is concentration-dependent and follows first-order kinetics, but requires disruption of the membrane vesicles by detergents to achieve accessibility to the transferase. The enzyme can be protected partially against inactivation by the addition of the acceptor peptide Arg-Asn-Gly-Thr-Ala-Val-OMe, pointing to an active-site-directed reaction. Exhaustion of the endogenous pool of glycosyl donor molecules by preincubation of the membrane vesicles with the acceptor peptide before inhibitor application is accompanied by an additional decrease in the inhibition rate. This suggests that inactivation occurs only under conditions where glycosyl transfer is catalysed. A mechanism of inactivation is proposed in which the transferase catalyses its own inactivation by a kind of 'suicide' mechanism.

N-Glycosyltransferases are key enzymes of a complex pathway leading to the biosynthesis of *N*-linked glycoproteins (for review, see Struck & Lennarz, 1980). They catalyse the Dol-*PP*-mediated transfer of oligosaccharides from their glycolipid stage on to particular asparagine residues, which must be part of the triplet sequence Asn-Xaa-Thr(Ser) (Marshall, 1974; Bause, 1979). Fairly recent studies demonstrated that the presence of a hydroxy amino acid in this specific position of the peptide chain is unambiguously connected with its direct involvement in the catalytic step of glycosyl transfer (Bause & Legler, 1981).

Taking into account this catalytic participation of the hydroxy amino acid in the process of transglycosylation, two hexapeptide derivatives have been designed and synthesized as potential active-site-directed inhibitors for *N*-glycosyltransferases. Both peptides are derived from the basic sequence Arg-Asn-Gly-Yaa-Ala-Val and contain, in position Yaa, amino acids with epoxy functions in their side chains. Since epoxides are known to be compounds with high alkylating power, it was expected that these derivatives, carrying an alkylating group at a strategically favoured position for a reaction with an amino acid side chain at the active site, might lead to a covalent inhibition of *N*-glycosyltransferases. The results presented here show that the peptide having an epoxyethyl function in the side chain of the amino acid Yaa is indeed an efficient inhibitor of the enzymes under investigation, in contrast with that with a 2,3-epoxypropyl structure. In addition, evidence is presented indicating that this inactivation is occurring by a kind of 'suicide' mechanism in which the enzyme catalyses its own inactivation.

Abbreviations used: Dol-*P*, dolichyl monophosphate; Dol-*PP*, dolichyl diphosphate; Xaa, Yaa, 'unknown' or 'variable' amino acids; OMe, methylester; Boc-ON, *N*-t-butoxycarbonyloximino-2-phenylacetonitrile.

Materials and methods

Materials

The materials used and their sources were: UDP-*N*-acetyl[^{14}C]glucosamine (sp. radioactivity 323 Ci/mol), The Radiochemical Centre; Dol-*P*, DL-2-aminopent-4-enoic acid (allylglycine), trifluoroacetic acid, Triton X-100, *p*-chloroperbenzoic acid and Boc-ON, Sigma; *N*-t-Boc amino acids and dicyclohexylcarbodi-imide, Serva; Bio-Beads S-X1, chloromethylated, Bio-Gel P-4, 200–400 mesh, Bio-Rad Laboratories; silica-gel thin-layer plates, Merck. All other chemicals were purchased from commercial sources in the highest purity available.

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Preparation of calf liver microsomal membranes

These were prepared as described by Bause *et al.* (1982). The protein concentration of the final enzyme preparation was between 10 and 20 mg/ml when determined by the procedure of Lowry *et al.* (1951).

N-Glycosyltransferase assay

N-Glycosyltransferase activity was routinely measured with Dol-*PP*-[^{14}C]chitobiose as the glycosyl donor and the hexapeptide Arg-Asn-Gly-Thr-Ala-Val-OMe (**I**) as acceptor. Standard incubation mixtures contained, in a final volume of 100 µl: (3–5) × 10^3 c.p.m. of Dol-*PP*-[^{14}C]-chitobiose, 0.8% Triton X-100, 50 mM-Tris/HCl, pH 7.2, 10 mM-$MnCl_2$, 1–2 mM-acceptor peptide and 200–400 µg of membrane protein. Incubations were carried out at room temperature and terminated after 15 min by the addition of 1 ml of methanol. [^{14}C]Glycopeptides were then isolated and determined as described by Bause *et al.* (1982).

Inhibition studies

The particulate membrane fraction was preincubated at room temperature in the presence of 5 mM-$MnCl_2$ with various amounts of detergents and inhibitor peptides as indicated. At given times the remaining *N*-glycosyltransferase activity was measured by the standard assay procedure.

Synthesis of DL-*vinylglycine*

DL-Vinylglycine was prepared from acroleine via the cyanhydrin addition product, which was hydrolysed with HCl to give the DL-2-hydroxybut-3-enoic acid (Glattfield & Hoen, 1935). The hydroxy acid was converted into the α-bromoderivative with PBr_3, from which, by treatment with aq. 34% NH_3, DL-vinylglycine was obtained. The amino acid was separated from unchanged bromo or hydroxy acid by cation-exchange chromatography on Dowex 50 (X4). The oily product was crystallized from water/ethanol mixtures yielding white needles with an m.p. of 218–220°C dec. (literature value: m.p. 216–218°C dec.; Friis *et al.*, 1974). N.m.r. data are in agreement with the expected structure.

*Preparation of Boc-*DL-*vinylglycine and Boc-*DL-*allylglycine*

Butoxycarbonylation of DL-vinylglycine and DL-allylglycine was carried out with Boc-ON in dioxan/water (1:1, v/v). Boc amino acids were isolated as described previously (Bause & Legler, 1982).

*Synthesis of Arg-Asn-Gly-Thr-Ala-Val-OMe, Arg-Asn-Gly-*DL-*vinylglycine-Ala-Val-OMe and Arg-Asn-Gly-*DL-*allylglycine-Ala-Val-OMe*

These peptides were synthesized by the solid-phase method as described by Merrifield (1963) and Erickson & Merrifield (1976), with chloromethylated Bio-Beads S-X1 as polymer. Cleavage from the resin was done by transesterification in 50 mM-sodium methylate in methanol for 1 h at room temperature. Peptides were purified by gel chromatography on Bio-Gel P-4 with 0.5 M-acetic acid as solvent.

Epoxidation

Purified peptides **II** and **IV** (30 µmol) were dissoved in 300 µl of acetic acid and a 10-fold molar excess of *p*-chloroperbenzoic acid was added. Epoxidation was run at room temperature and its progress continuously checked by t.l.c. in propan-1-ol/aq. 34% (w/v) NH_3 (7:3, v/v). After completion, the reaction mixture was diluted with 5 ml of water and the *p*-chloroperbenzoic acid extracted by five additions of diethyl ether. The aqueous solutions containing the epoxypeptide derivatives were then freeze-dried and the residue dissolved in water to give final peptide stock solutions of about 40 mM. After adjusting the pH to 7.2, these preparations were directly used for the inhibition studies.

General procedures

Dol-*PP*-[^{14}C]chitobiose was prepared and purified as described previously (Bause & Hettkamp, 1979). T.l.c. of peptides and derivatives was performed on silica-gel thin-layer plates with butan-1-ol/acetic acid/water (4:1:1, by vol.), butan-1-ol/acetic acid/water/pyridine (15:3:12:10, by vol.) and propan-1-ol/aq. 34% NH_3 (7:3, v/v) as solvents. Radioactivity was measured in a liquid-scintillation counter (Delta 300, Searle Analytic) using Bray's (1960) reagent as counting fluid. Amino acids were analysed on a Beckman amino acid analyser (model 119 CL) after hydrolysis of peptides with 5.7 M-HCl for 20 h at 105°C.

Results

Synthesis and characterization of inhibitor peptides

Table 1 presents structural details of the various peptides which were synthesized and investigated in the present study. All compounds can be regarded as derivatives of the hexapeptide sequence Arg-Asn-Gly-Yaa-Ala-Val-OMe, differing in the amino acid Yaa only. The epoxyethyl and epoxypropyl peptides fulfil, in principle, the basic requirements of being active-site-directed inhibitors, as they represent substrate analogues containing a chemically reactive group. The former feature determines the binding affinity to the enzyme that is to be inhibited; the latter ensures a covalent reaction with specific residues in the active site. The epoxide function in this particular position of the peptide chain was selected (i) on the basis of our present knowledge of

the catalytic mechanism of the N-glycosyltransferases (see the Discussion section) and (ii) because it is capable of alkylating nucleophilic groups in an acid-catalysed reaction.

The epoxyethyl and epoxypropyl derivatives were prepared from their vinyl- and allyl-glycine-containing peptide precursors. The synthesis of the latter derivatives was by the solid-phase procedure described by Merrifield (1963). The double-bond-containing peptides were cleaved from the resin by treatment with sodium methylate in methanol, yielding the corresponding peptide esters. The decreased solubility in water due to this ester formation was balanced by the incorporation of arginine into the basic sequence. The epoxidation of the double bond was carried out on the purified peptides with p-chloroperbenzoic acid in solution. In contrast with the olefinic peptides, the epoxy derivatives proved to be quite unstable during the longer purification procedures, decomposing slowly into peptide fragments containing the N-terminal Arg-Asn-Gly and the C-terminal Ala-Val sequences. This cleavage, though interesting from a chemical point of view, and obviously due to the epoxide function in the molecule, has not been studied in further detail. The epoxy peptides were used without further purification for the inhibition studies, since t.l.c. showed them to be approx. 90% homogeneous. Amino acid-analysis data for all peptides are summarized in Table 2. Neither intact nor cleavage products of vinylglycine or epoxyethylglycine were detectable after acid hydrolysis of peptides **IV** and **V**, whereas a number of unidentified components resulting from 2,3-epoxypropylglycine were observed for peptide **III**. Allylglycine, on the other hand, is stable under the hydrolytic conditions.

The olefinic peptides **II** and **IV** were synthesized with racemic allyl- and vinyl-glycine respectively. It is tentatively assumed that both enantiomers are incorporated into the peptide chain with equal efficiency. A new chiral centre is introduced during the epoxidation, and it is assumed that no discrimination between the two possible modes of the oxygenation occurs. The concentrations given in the inhibition experiments are based on half the total peptide concentration.

Table 1. *Side-chain structures of synthetic peptide derivatives*

Peptide*	Yaa	Side-chain structure
I	Threonine	$-\underset{OH}{\underset{\vert}{CH}}-CH_3$
II	Allylglycine	$-CH_2-CH=CH_2$
III	2,3-Epoxypropylglycine	$-CH_2-\underbrace{CH-CH_2}_{O}$
IV	Vinylglycine	$-CH=CH_2$
V	Epoxyethylglycine	$-\underbrace{CH-CH_2}_{O}$

* Basic sequence: Arg-Asn-Gly-Yaa-Ala-Val-OMe.

Effect of epoxide-containing peptide derivatives on the N-glycosylation of Arg-Asn-Gly-Thr-Ala-Val-OMe

In order to monitor possible inhibitory effects on the N-glycosyltransferases, the hexapeptide **I** was

Table 2. *Amino acid composition of synthetic peptides*

Hydrolysis was carried out with 5.7M-HCl for 24h in evacuated tubes. Values represent mean values of two separate determinations and are not corrected. For amino sequences of peptides, see Table 3.

Amino acid	Residues				
Peptide ...	I	II	III	IV	V
Aspartic acid	0.95 (1)	0.90 (1)	0.97 (1)	0.90 (1)	1.03 (1)
Threonine	1.05 (1)	—	—	—	—
Vinylglycine	—	—	—	*	—
Epoxyethylglycine	—	—	—	—	*
Alanine	1.02 (1)	0.99 (1)	0.85 (1)	1.04 (1)	0.88 (1)
Allylglycine	—	0.95 (1)	—	—	—
2,3-Epoxypropylglycine	—	—	†	—	—
Glycine	1.04 (1)	1.01 (1)	1.06 (1)	1.01 (1)	1.19 (1)
Valine	1.10 (1)	1.06 (1)	0.91 (1)	1.05 (1)	0.87 (1)
Arginine	0.92 (1)	1.00 (1)	0.96 (1)	1.00 (1)	1.01 (1)

* Decomposition during acid hydrolysis; no cleavage products detectable.

† Acid hydrolysis gave a number of unidentified cleavage products.

used as the standard acceptor in the enzyme assays. A typical time-course experiment characterizing the sugar transfer from Dol-*PP*-[^{14}C]chitobiose on to this peptide, catalysed by an enzyme preparation from calf liver, is given in Fig. 1 (×). The time course shows linearity up to about 10min. The apparent deviation with longer incubation time was shown to be mainly due to the action of endogenous proteinases, which, as revealed by chromatographic studies, hydrolyse the acceptor peptide. The loss of acceptor ability correlated well with the appearance of cleavage products (E. Bause, unpublished work). Addition to the incubation mixtures of peptide **V** is followed by a dramatic decrease in the relative glycosylation rate of peptide **I** (Fig. 1, ●), indicating a time-dependent inhibitory effect of the epoxyethyl derivative on the *N*-glycosyltransferases. In contrast with that, the inhibitory effect on the glycosylation of peptide **I** caused by the 2,3-epoxypropyl derivative is rather negligible (▲) as are those exerted by either the allyl- or vinyl-glycine derivatives **II** and **IV** (results not shown). The marginal inhibitory effects found with concentrations of peptides **II**, **III** and **IV** that are much higher than K_m values observed previously for acceptor peptides, indicate that the hydroxyethyl side chain of threonine is important not only for catalysis (Bause & Legler, 1981), but also for binding the peptide to the transferase.

Glycosyl-acceptor properties of the vinyl- and allyl-glycine-containing peptides and their epoxy derivatives

Except for the standard peptide **I**, no glycosyl transfer to any of the other peptide derivatives was detectable when a crude microsomal fraction from calf liver was incubated in their presence under standard assay conditions (Table 3). This indicates that neither allyl- and vinyl-glycine, nor their epoxy

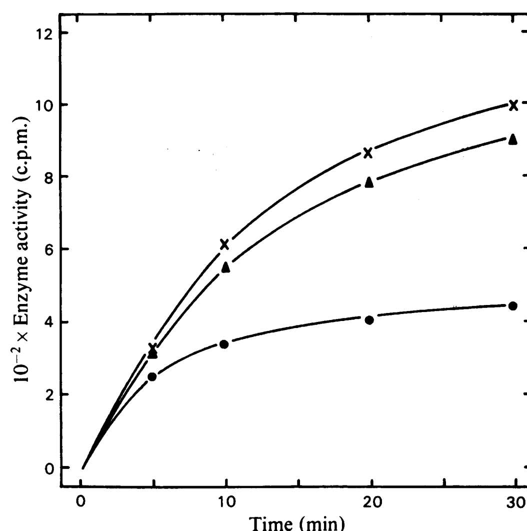

Fig. 1. *Time course of* [^{14}C]*chitobiosyl transfer on to the standard acceptor Arg-Asn-Gly-Thr-Ala-Val-OMe in the absence and presence of epoxy-peptides*

Calf liver microsomes (microsomal fractions) were incubated under standard conditions with 3000c.p.m. of Dol-*PP*-[^{14}C]chitobiose in the presence of 2.2mM-peptide **I** (×), 2.2mM-peptide **I** and 10mM-peptide **III** (▲) or 2.2mM-peptide **I** and 6mM-peptide **V** (●). Incubations were terminated at given times by the addition of 1ml of methanol, and [^{14}C]glycopeptides were isolated and quantified as described by Bause *et al.* (1982).

Table 3. *Glycosyl acceptor properties of synthetic peptides*

Glycosyl acceptor properties of the various derivatives were tested by the standard assay procedure given in the Materials and methods section.

Peptide	Amino acid sequence	Concn. (mM)	[^{14}C]Chitobiosyl transfer (c.p.m.)
Control		—	59
I	Arg-Asn-Gly-Thr-Ala-Val-OMe	2.5	699
II	Arg-Asn-Gly-Gly(allyl)-Ala-Val-OMe	2.5*	62
III	Arg-Asn-Gly-Gly(allylepoxide)-Ala-Val-OMe	5.0*	59
IV	Arg-Asn-Gly-Gly(vinyl)-Ala-Val-OMe	2.5*	63
V	Arg-Asn-Gly-Gly(vinylepoxide)-Ala-Val-OMe	5.0*	60

* Concentrations are given as half the total peptide concentration present in the incubations.

analogues, are capable of replacing threonine in its specific catalytic function.

Inhibition studies

The inhibitory influence of the epoxyethylpeptide **V** on the N-glycosyltransferase activity, indicated in Fig. 1, as well as possible effects by the other derivatives, were studied in more detail by preincubating the microsomal enzyme fraction with these peptides under a variety of reaction conditions. A typical time-course inhibition pattern, obtained by preincubating the membrane fraction with the peptide derivatives in the presence of 0.2% Triton X-100, is shown in Fig. 2. From all derivatives tested, only the epoxyethyl peptide causes a rapid and time-dependent decrease of transferase activity, pointing to a specific inhibition of the enzyme by this compound. Fig. 3 illustrates that the extent of inactivation caused by the epoxyethyl peptide **V** is critically affected by increasing concentrations of Triton X-100. This stimulating effect of the detergent is obviously due to a disruption of the membrane vesicles, resulting in a corresponding facilitated accessibility of the N-glucosyltransferases to the inhibitor peptide. This interpretation is supported by previous studies revealing a similar enhancing influence on the glycosylation rate of acceptor peptides in the presence of increasing amounts of detergents (Bause, 1979). The use of the zwitterionic detergent 3-14 or the anionic detergent deoxycholate instead of Triton X-100 gave comparable results (results not shown). Again, as in the aforementioned experiment, the inhibiting effect on the transferase by the 2,3-epoxypropyl peptide is very low.

In order to characterize further the nature of the inactivation reaction, the inhibitory potential of the epoxyethyl derivative **V** on the N-glycosyltransferase was measured as a function of its concentration. The preincubation was carried out at room temperature in the presence of 0.2% Triton X-100 and 5mM-$MnCl_2$. The latter addition was shown to activate the transferase and consequently stimulated its inactivation. The kinetic data outlined in Fig. 4 demonstrate that the time course of inactivation follows a first-order reaction and that the degree of inhibition is linearly dependent on the inhibitor concentration. This linear dependency on the inhibitor concentration indicates that its effective concentration is still far below the dissociation constant of any preceding non-covalent complex with the enzyme. The slight deviations from linearity observed in both cases are due to a partial hydrolysis of the epoxyethyl inhibitor by endogenous proteinases (see Fig. 1).

The inhibitory effect exerted by the epoxyethyl derivative **V** on the N-glycosyltransferases is partially overcome when the preincubation mixture

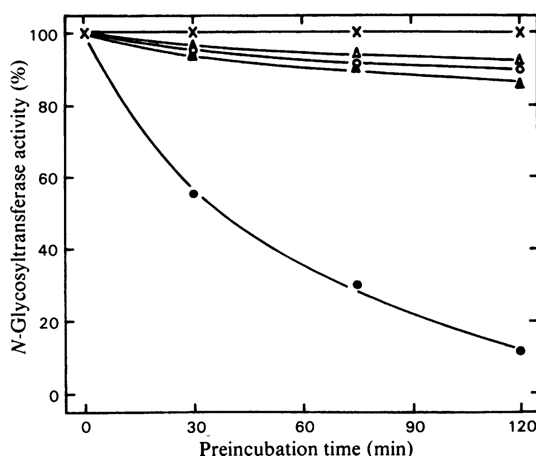

Fig. 2. *Effect of allyl- and vinyl-glycine-containing peptides and their epoxy derivatives on N-glycosyltransferase activity*

Calf liver membranes were preincubated at room temperature in the presence of 0.2% Triton X-100 and buffer (×), 3.5mM-peptide **II** (△) and -peptide **III** (▲), 2.8mM-peptide **V** (●) and 2.5mM-peptide **IV** (O). Portions were withdrawn at the times indicated and the residual N-glycosyltransferase activity tested for with peptide **I** as acceptor as described in the Materials and methods section.

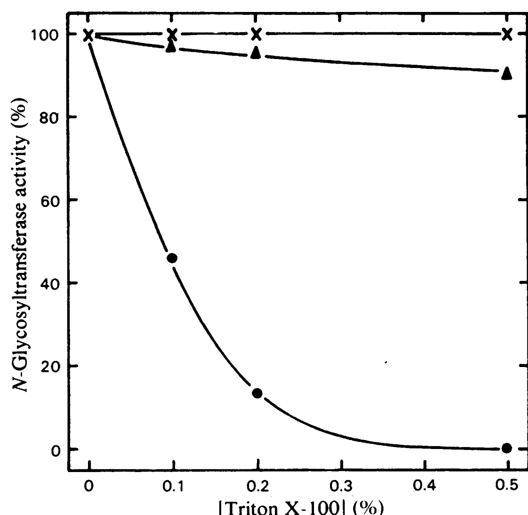

Fig. 3. *Influence of Triton X-100 on the extent of inhibition*

The particulate membrane fraction was preincubated at room temperature with various amounts of Triton X-100 in the absence (×) or presence of 6mM-peptide **III** (▲) and -peptide **V** (●). After 1h, the residual N-glycosyltransferase activity was determined using the standard assay procedure as described in the Materials and methods section.

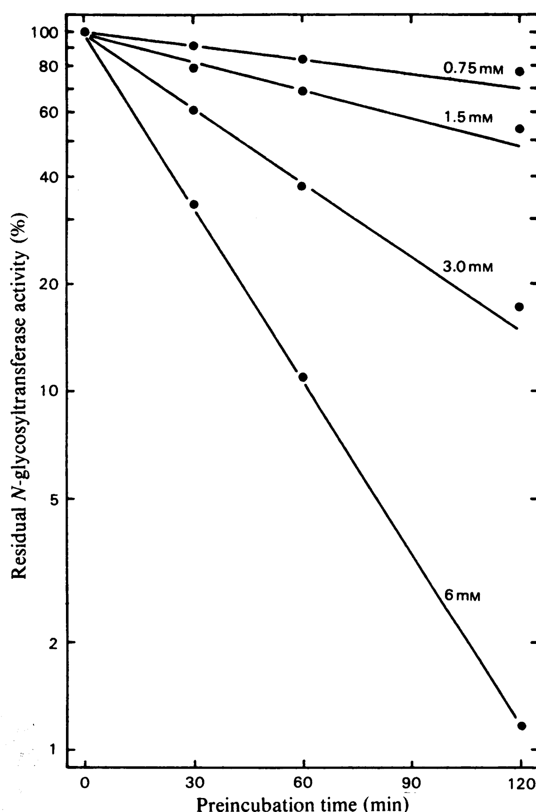

Fig. 4. *Concentration- and time-dependent inhibition of N-glycosyltransferase activity by the epoxyethyl inhibitor peptide* **V**

The membrane fraction was preincubated at room temperature in the presence of 0.2% Triton X-100 and various amounts of the epoxyethyl peptide **V**. The inhibitor concentration in the preincubation mixture was 6, 3, 1.5 and 0.75 mM respectively. At given times, portions were taken and tested for residual *N*-glycosyltransferase activity as described in the Materials and methods section.

contains, in addition to the inhibitor, the standard glycosyl acceptor peptide **I** (Table 4, *b* and *c*). Strikingly, this protecting effect by peptide **I** is even more pronounced when the membrane fraction is preincubated with this substrate before the addition of inhibitor **V** (Table 4, *e*). This observation suggests that there are two different parameters responsible for the overall protection: (i) a direct component resulting from a competition at the binding site of the transferase; (ii) a covalent inhibition of the transferase might occur only during the transferase reaction proper. Depletion of the endogenous glycosyl donor pool by preincubation with an acceptor peptide would thus prevent this 'suicide inhibition'. The specificity of the

Table 4. *Protecting effect of Arg-Asn-Gly-Thr-Ala-Val-OMe on the inhibition of calf liver N-glycosyltransferases by the epoxyethyl peptide V*

The particulate membrane fraction was preincubated at room temperature for 60 min in the presence of buffer (*a*), 5.3 mM-epoxyethyl inhibitor **V** (*b*), and 5.3 mM-**V** and 3.3 mM-Arg-Asn-Gly-Thr-Ala-Val-OMe (*c*). In a second experiment, the membrane fraction was first preincubated for 20 min with buffer (*d*) and 3.3 mM-**I** (*e*). The mixtures were then made 5.3 mM with respect to **V** and preincubated for additional 60 min. All incubations were carried out in the presence of 0.2% Triton X-100. Residual *N*-glycosyltransferase activity was assayed as described in the Materials and methods section. An activity of 100% corresponds to a transfer of 2450 c.p.m. from 5000 c.p.m. of Dol-*PP*-[^{14}C]chitobiose used in the standard assay.

Expt.	*N*-glycosyltransferase activity (%)
(*a*) Buffer	100
(*b*) Peptide **V**	<2
(*c*) Peptides **V** and **I**	18
(*d*) Buffer then peptide **V**	<2
(*e*) Peptide **I** then peptide **V**	55

inhibition pattern is underlined by the observation that preincubation conditions leading to a complete inactivation of the *N*-glycosyltransferases by the epoxyethyl inhibitor had no effect on the synthesis of Dol-*P*-mannose, Dol-*P*-glucose and Dol-*PP*-*N*-acetylglucosamine respectively (results not shown).

Discussion

The results presented here demonstrate that the epoxyethyl peptide **V** specifically inactivates membrane-bound *N*-glycosyltransferases, in contrast with the epoxypropyl derivative **III** and the allyl- and vinyl-glycine-containing analogues **II** and **IV**. The inactivation procedure requires the disruption of the microsomal membrane vesicles by detergents in order to facilitate the accessibility of the transferases for the inhibitor peptide. The necessity of relatively high detergent concentrations for obtaining full transferase activity and inactivation respectively suggests that these enzymes are rather deeply embedded in the membrane and might represent integral components of the membrane complex.

The inactivation of the *N*-glycosyltransferases by the epoxyethyl peptide occurs in a concentration-dependent fashion and follows first-order kinetics. The pronounced differences in the reactivity toward the transferases between the epoxyethyl and epoxypropyl derivatives indicates that the inhibitory effect cannot be due to a non-specific reaction of the

oxirane ring with some basic group at the surface of the enzyme. These findings, as well as the observation that the inhibition rate is decreased in the presence of the acceptor peptide Arg-Asn-Gly-Thr-Ala-Val-OMe, point to specific and active-site-directed inactivation of the enzymes under investigation.

Recent studies on the catalytic mechanism of *N*-glycosyltransferases provided evidence that the occurrence of a 'marker sequence' of the type Asn-Xaa-Thr(Ser) is mainly substantiated by the active participation of the hydroxy amino acid in the catalytic process of transglycosylation (Bause & Legler, 1981). Thus its function is likely to operate as a proton vehicle for the transfer of the β-amide hydrogen from asparagine on to a corresponding basic group in the active site of the transferase. This kind of mechanism is schematically illustrated in Fig. 5(*a*). As with the hydroxy group of threonine or serine, the oxygen in the oxirane ring of the epoxyethyl side chain is also capable of interacting by hydrogen-bonding with the β-amide of asparagine by adopting a conformation as outlined in Fig. 5(*b*). This structure seems likely to be recognized by the transferase and might thus be accepted as a potential sugar-attachment site. The initiation of the glycosylation process would then give rise to the protonation of the epoxide ring, resulting in a reactive intermediate of high alkylating potential. This intermediate might be stabilized by reaction with a properly situated basic group, e.g. the catalytically active base itself. It is evident that this course of inactivation requires the catalytic glycosylation sequence to be completed and consequently requires a sufficiently large pool of lipid-linked saccharides as substrate. In fact, the final consequence of the above inactivation reaction is that the inhibitor peptide is glycosylated at its asparagine residue and simultaneously attached to the active site of the *N*-glycosyltransferase by a covalent linkage produced via the epoxy function, thereby blocking further catalytic activities. This means that the transferase catalyses its own inactivation by a kind of suicide mechanism.

There is some experimental evidence which supports this latter interpretation, First of all, the inactivating potential of the inhibitor peptide is neutralized in part when the particulate membrane

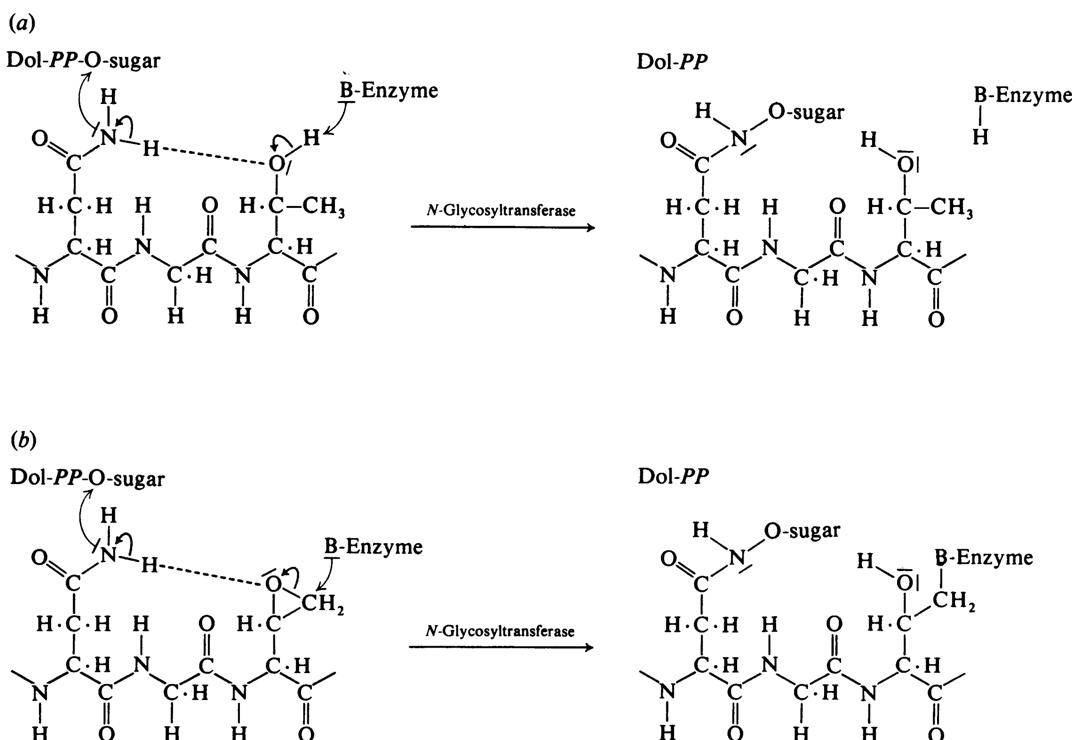

Fig. 5. *Models representing* (*a*) *the mechanism of N-glycosylation according to Bause & Legler* (*1981*), and (*b*) *the proposed reaction mechanism leading to the inactivation of N-glycosyltransferases by the epoxyethyl inhibitor peptide V*

fraction is preincubated with the acceptor peptide **I** before the addition of the inhibitor peptide. This treatment decreases the endogenous pool of lipid-linked saccharides, which consequently are not available for promoting the syncatalytic inactivation reaction. The protective effect is indeed much less pronounced when the acceptor peptide and epoxide are present simultaneously from the outset (55% as against 18% residual activity, Table 4). Secondly, the inhibitor peptide is 'inactive' as glycosyl acceptor in our standard assay, in which only soluble glycosylation products are measured. This is to be expected, as its putative glycosylation must be accompanied by the simultaneous covalent fixation to the active site of the membrane-bound transferase.

In summary, the hexapeptide derivative Arg-Asn-Gly-epoxyethylglycine-Ala-Val-OMe is likely to be acting as an active-site-directed irreversible inhibitor for membrane-bound *N*-glycosyltransferases. The inactivation presumably occurs by a kind of suicide mechanism in which the enzyme catalyses its own inactivation. The various conclusions drawn confirm and extend previous findings on the functional role of the hydroxy amino acid of the asparagine sequon Asn-Xaa-Thr(Ser) in the catalytic mechanism of *N*-glycosylation.

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