The effect of flavomycin on the synthesis and transfer of lipid-linked saccharides in pig brain

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Particulate membrane fractions from pig brain catalyse the synthesis of lipid-linked sugar derivatives of the dolichyl phosphate pathway. Flavomycin, a phosphoglycolipid antibiotic produced by various species of streptomycetes, interferes with the formation of these glycolipids to a different extent. The formation of dolichyl phosphate glucose was shown to be most susceptible to the antibiotic, being blocked by about 50% in the presence of 0.2 mM-flavomycin, whereas the synthesis of dolichyl diphosphate *N*-acetylglucosamine, dolichyl diphosphate chitobiose and dolichyl diphosphate chitobiosyl mannose required higher concentrations to achieve a comparable inhibition. Although the formation of dolichyl phosphate mannose was hardly affected, the accumulation of oligosaccharides with five to seven sugar units was observed, when dolichyl diphosphate oligosaccharides were synthesized with GDP-[¹⁴C]mannose in the presence of 1 mM-flavomycin. This indicates that the inhibition of the synthesis of larger-sized oligosaccharides, known to be mediated by lipid-bound mannose, was not caused by an actual deficiency in dolichyl phosphate mannose. At flavomycin concentrations that inhibited the formation of dolichyl phosphate glucose by 50%, the transfer of lipid-linked saccharides to either the hexapeptide Tyr-Asn-Gly-Thr-Ser-Val or endogenous protein acceptors was hardly influenced. The mode of action of flavomycin is still obscure, but seems not to be of a competitive nature, since the inhibition was unaffected by increasing concentrations of dolichyl phosphate. Some evidence indicates that, besides a direct interaction of the antibiotic with some transferases, a non-specific incorporation into the membrane and alteration of its properties might be responsible for those inhibitory effects on all enzymes which were observed at high concentrations of flavomycin.

Flavomycin is a phosphoglycolipid antibiotic of the moenomycin group that is produced by various species of streptomycetes. The antibiotic is mainly active against Gram-positive bacteria, presumably by interfering with the synthesis of peptidoglycans of the bacterial cell wall (Huber, 1979). Moenomycin A, the main component of flavomycin, was recently shown to contain the C₂₅ lipid alcohol, moenocinol, which is bound via an ether bridge to the C-2 hydroxy group of 3-phosphoglyceric acid, the phosphate group of which is linked by a phosphoacetal ester bond to a branched carbohydrate moiety (Welzel *et al.*, 1981). The limited, but apparent, structural similarity between flavomycin and those glycolipids that are synthesized in the dolichyl phosphate pathway during the biosynthesis of

N-linked glycoproteins prompted us to investigate the ability of flavomycin to interfere with the synthesis and turnover of these glycolipids in a mammalian system.

Our studies, which were carried out with a particulate membrane fraction from pig brain as the enzyme source, showed indeed that flavomycin exerts a distinct influence on the synthesis of the various glycolipid derivatives of the dolichyl phosphate cycle as well as on their use as glycosyl donors for the *N*-glycosylation step. So we found that the antibiotic efficiently blocked the formation of Dol-*P*-glucose and, although to a lesser extent, that of Dol-*PP*-*N*-acetylglucosamine and lipid-bound oligosaccharides, as well as the transfer of lipid-linked saccharides to exogenous acceptor peptides. In contrast with this, the formation of Dol-*P*-mannose was hardly affected.

Abbreviations used: Dol-*P*, dolichyl phosphate; Dol-*PP*, dolichyl diphosphate.

Materials and methods

Materials

UDP-[^{14}C]glucose (sp. radioactivity 240 Ci/mol), GDP-[^{14}C]mannose (sp. radioactivity 108 Ci/mol) and UDP-*N*-acetyl[^{14}C]glucosamine (sp. radioactivity 323 Ci/mol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Dol-*P* and unlabelled sugar nucleotides were purchased from Sigma G.m.b.H., Taufkirchen, Germany. Bio-Gel P-4 (–400 mesh) was from Bio-Rad Laboratories. Silica-gel thin-layer plates were from Merck, Darmstadt, Germany. Flavomycin was a gift from Dr. G. Huber, Hoechst A.G., Frankfurt, Germany. All other chemicals were purchased from commercial sources in the highest purity available.

Preparation of the particulate membrane fraction

Pig brain from freshly killed animals was supplied from the local slaughterhouse by courtesy of Dr. Krebs. Membranes were prepared from the whole brain in 50 mM-Tris/HCl, pH 7.2, containing 0.25 M-sucrose by the use of a Potter homogenizer as described by Moulé *et al.* (1960). The homogenate was filtered through cheesecloth and centrifuged at 12 000 *g* for 20 min. Recentrifugation of the supernatant at 48 000 *g* for 60 min gave the pellet, which, when resuspended in the homogenizing buffer, served as the enzyme source. Protein concentration was between 10 and 20 mg/ml when assayed by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Glycosyltransferase assays

Standard incubation mixtures for the synthesis of Dol-*P*-mannose, Dol-*P*-glucose and Dol-*PP*-*N*-acetylglucosamine contained, in a final volume of 50 μl : 3 μg of Dol-*P*, 0.6% Triton X-100, 10 mM-MgCl₂, nucleotide ^{14}C -labelled sugar as indicated, 50 mM-Tris/HCl, pH 7.2, and the particulate membrane fraction (100–200 μg of protein). The reactions were run for the indicated times and terminated by the addition of 1.0 ml of methanol, 1.5 ml of chloroform and 0.5 ml of water. The layers were separated by centrifugation and the Dol-*P*(*P*)-linked monosaccharides were isolated from the lower chloroform/methanol phase by the multiple-extraction procedure as described by Folch *et al.* (1957). Dol-*P*-linked oligosaccharides were extracted from the interface with chloroform/methanol/water (10:10:3, by vol.). The residual pellet was solubilized in 10% Triton X-100 by sonication and counted for radioactivity to estimate the extent of endogenous glycosylation.

Transfer of *N*-acetylglucosamine from UDP-*N*-acetylglucosamine to Dol-*PP*-*N*-acetyl[^{14}C]glucosamine and mannose from GDP-mannose to Dol-*PP*-[^{14}C]chitobiose to give the corresponding lipid-

linked di- and tri-saccharide respectively, was measured with assays containing the following components in a final volume of 80 μl : 10 000–40 000 c.p.m. of Dol-*PP*-*N*-acetyl[^{14}C]glucosamine or Dol-*PP*-[^{14}C]chitobiose, 20 μl of 2% Triton X-100 in 100 mM-Tris/HCl, pH 7.2, 10 μl of 80 mM-MgCl₂, 10 μl of unlabelled 10 mM-UDP-*N*-acetylglucosamine or GDP-mannose and 40 μl of the particulate membrane fraction (200–400 μg of membrane protein). After the time intervals given, the incubations were stopped with 1 ml of methanol, 1.5 ml of chloroform and 0.4 ml of water. The Dol-*PP*-linked sugars were isolated by solvent extraction as described above and analysed by chromatography on silica gel with chloroform/methanol/0.5 M-NH₃ (65:25:4, by vol.) as solvent. Radioactive bands were localized by autoradiography on Sakura X-ray film and the glycolipids were extracted from the silica gel with chloroform/methanol/water (10:10:3, by vol.). Portions were counted for radioactivity.

N-Glycosylation of the hexapeptide Tyr-Asn-Gly-Thr-Ser-Val with Dol-*PP*-[^{14}C]chitobiose or Dol-*PP*-[^{14}C]chitobiosyl mannose as the glycosyl donor was measured by incubating the particulate membrane fraction (100–200 μg of protein) with the glycolipid (2000 c.p.m.) in the presence of 0.6% Triton X-100, 50 mM-Tris/HCl, pH 7.2, 10 mM-MnCl₂ and 1 mM-hexapeptide. The incubations were stopped with 1 ml of methanol and centrifuged. The supernatant containing the water-soluble glycosylated peptide derivative was separated from the pellet and made biphasic with 2 ml of chloroform and 0.4 ml of water to extract small amounts of unreacted Dol-*PP*-linked ^{14}C -labelled derivatives. Portions of the upper phase were then counted for radioactivity. Controls were run without peptide to correct for the water-soluble radioactivity that was present in the water phase probably as a result of a partial hydrolysis of the glycosyl donor. Generally, these values were below 5% of the total counts used in the incubations.

Inhibition studies

To determine the effect of flavomycin on the various glycosyl-transfer reactions, the antibiotic was, unless stated otherwise, added from a 20 mM stock solution in 50 mM-Tris/HCl, pH 7.2, to the incubation mixtures before the reactions were started by the addition of the particulate membrane fraction. In all cases controls were run with buffer alone.

Synthesis of Tyr-Asn-Gly-Thr-Ser-Val

This hexapeptide was synthesized by the solid-phase method as described by Merrifield (1963) and Erickson & Merrifield (1976) and purified by chromatography on Bio-Gel P-4 with 1 M-acetic acid as solvent. Purity of the peptide was checked by t.l.c.

on silica gel with *n*-butanol/acetic acid/water (4:4:1, by vol.) as solvent and amino acid analysis on a model 119 CL, Beckman amino acid analyser after hydrolysis with 5.7 M-HCl.

General procedures

Dol-PP-linked oligosaccharides were hydrolysed by treatment with 0.05 M-HCl in a mixture of tetrahydrofuran and water (1:1, v/v) for 60 min at 50°C. Bio-Gel P-4 chromatography of oligosaccharides was performed in 0.5 M-acetic acid. Standardization of the column was done with Blue Dextran, ¹⁴C-labelled oligosaccharides of known size, chitobiose and glucose. Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Radioactivity was determined in a Delta 300 scintillation counter (Searle Analyt Inc.), with, as counting fluid, a mixture of 4 g of 2,5-diphenyloxazole, 0.2 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene, 60 g of naphthalene and 20 ml of ethylene glycol in 100 ml of methanol, filled up to 1000 ml with dioxan. Autoradiograms were taken on Sakura QH X-ray film.

Results and discussion

Effect of flavomycin on the formation of lipid-linked monosaccharides

Recent studies by Waechter's group have demonstrated that particulate cell-free preparations from calf brain catalyse the synthesis of Dol-*P*-mannose,

Dol-*P*-glucose and Dol-PP-*N*-acetylglucosamine in high yield (Waechter & Harford, 1977; Scher *et al.*, 1977; Waechter & Scher, 1978). When we tested the formation of these glycolipids with a microsomal enzyme preparation from pig brain in the presence of the phosphoglycolipid antibiotic flavomycin, we found that the antibiotic had different effects on the rate of synthesis of the different glycolipids. Fig. 1 shows a typical time-course experiment comparing the transfer of mannose, glucose and *N*-acetylglucosamine into glycolipids in the absence and presence of 1 mM-flavomycin. Whereas the antibiotic stimulates the formation of Dol-*P*-mannose at the beginning of the reaction, its rate of synthesis falls below the value of the control experiment after longer reaction times. In contrast with this, the synthesis of Dol-*P*-glucose and Dol-PP-*N*-acetylglucosamine is markedly reduced in the presence of flavomycin over the whole time course. The *N*-acetylglucosamine incorporation into the corresponding glycolipid derivative, however, is somewhat less sensitive to inhibition by flavomycin than is Dol-*P*-glucose formation. In fact, 50% inhibition of glucose transfer was observed repeatedly in a concentration range between 0.2 and 0.3 mM-flavomycin, whereas about twice this concentration was required to achieve a comparable inhibition of Dol-PP-*N*-acetylglucosamine formation (Fig. 2). Sugar transfer from GDP-mannose to Dol-*P*, on the other hand, was found to be hardly influenced under these conditions.

The inhibitory effect of flavomycin varied con-

Fig. 1. Time course of sugar transfer into lipid-linked monosaccharides

The particulate membrane fraction was incubated with either GDP-[¹⁴C]mannose (13 200 c.p.m.), UDP-[¹⁴C]glucose (23 000 c.p.m.) or UDP-*N*-acetyl[¹⁴C]glucosamine (23 000 c.p.m.) in the absence (○) and presence (●) of 1 mM-flavomycin. At the times indicated, samples were removed and glycolipids were isolated by extraction according to the multiple-extraction procedure as described by Folch *et al.* (1957).

Fig. 2. Effect of various concentrations of flavomycin on the formation of Dol-P(P)-monosaccharides

Incubations were carried out as described in the Materials and methods section in the presence of various amounts of the antibiotic. Reaction time was 15 min at room temperature. Values are standardized to 100% glycolipid formation in the absence of flavomycin (●, Dol-P-glucose; O, Dol-PP-N-acetylglucosamine; x, Dol-P-mannose).

Fig. 3. Dol-P-glucose formation as a function of membrane concentration

The particulate membrane fraction was diluted with homogenizing buffer and portions were added to the standard incubation mixture. Glycolipid formation from UDP-[^{14}C]glucose (23,000 c.p.m.) was assayed in the absence (O) or presence (●) of 1.5 mM-flavomycin.

siderably depending on the amount of membranous protein present in the incubations. Fig. 3 describes this dependency for the synthesis of Dol-P-glucose. Whereas in the presence of flavomycin no glycolipid formation is detectable at low protein concentrations, the inhibitory effect of the antibiotic is

apparently less pronounced at higher amounts of protein. It is noteworthy, however, that the non-inhibited reaction deviates strongly from linearity at higher protein concentrations, thus making a realistic estimation and interpretation of the extent of the inhibition rather difficult. A similar situation was found for the formation of Dol-PP-N-acetylglucosamine, whereas the weak inhibition of Dol-P-mannose synthesis at low protein concentration was converted into a slight stimulation, when the amount of membrane protein was increased (results not shown).

To get some insight into the mode of action of flavomycin, we have studied the effect of increasing concentrations of Dol-P on the inhibition of glycolipid formation by the antibiotic. We observed that both the inhibition of glucose and N-acetylglucosamine incorporation could not be overcome by varying the concentration of exogenous Dol-P; the ratio of glycolipid synthesis in the presence and absence of flavomycin remained constant over the whole concentration range of Dol-P. This indicates that the antibiotic does not compete with Dol-P at the active site of these transferases.

In addition, the activity of both glycosyltransferases could not be restored when the particulate membrane fraction was pre-incubated with flavomycin and the antibiotic washed out by repeated centrifugation and resuspension of the membrane particles in flavomycin-free buffer (Table 1). We assume, therefore, that flavomycin does not act as a substrate analogue in terms of a competitive or uncompetitive inhibitor, as is reported for the inhibition by tunicamycin of UDP-N-acetylglucosamine:dolichyl phosphate N-acetylglucosamine 1-phosphate transferase (Keller *et al.*, 1979), but that it binds to the membrane particles in a probably unspecific and irreversible manner.

Our interpretation is supported by the recently published data on the 'specific' inhibition of glycolipid formation by diumycin in yeast (Babczinski, 1980). Diumycin is a phosphoglycolipid antibiotic that is closely related to flavomycin in that both antibiotics probably differ in the structure of their lipid alcohol part only (Huber, 1979). On the basis of their structural similarity, one would expect that both antibiotics should show a strong resemblance with respect to their effects on glycolipid formation. However, in contrast with the situation in brain, diumycin is reported in yeast to specifically block the transfer of mannose from GDP-mannose to Dol-P as well as several reactions involving Dol-P-mannose. On the other hand, despite the different reactivity of the two antibiotics with the different types of glycosyltransferases, more general parameters, such as the dependency of inhibition on the antibiotic concentration with the extent depending on the amount of membrane protein present in

Table 1. *Binding of flavomycin to the membrane particles*

The particulate membrane fraction was incubated at 4°C for 15 min in the absence and presence of 1 mM-flavomycin. The samples were diluted with 50 mM-Tris/HCl, pH 7.2, and centrifuged at 48 000 g for 30 min. The resulting pellet was resuspended in buffer and portions were assayed for Dol-*P*-glucose and Dol-*PP*-*N*-acetylglucosamine formation as described in the Materials and methods section. The procedure was repeated and glycolipid was tested for under identical conditions.

Formation of:	Pre-incubation of membranes in the presence of:	Membranes not resuspended	After first resuspension	After second resuspension
Dol- <i>P</i> -glucose (c.p.m.)	No flavomycin	3210	3100	3050
	1 mM-Flavomycin	1470	1520	1640
Dol- <i>PP</i> -GlcNAc (c.p.m.)	No flavomycin	1320	1260	1090
	1 mM-Flavomycin	890	860	870

Fig. 4. *Effect of flavomycin on the transfer of mannose from GDP-mannose to Dol-PP-[¹⁴C]chitobiose*

Lipid-linked trisaccharide formation by the particulate membrane fraction in the presence of various amounts of flavomycin was measured as described in the Materials and methods section. Values are given as percentages of Dol-*PP*-linked trisaccharide formed, based on the sum of di- and tri-saccharide.

the incubations or the non-protective effect of Dol-*P*, appear to be very similar in yeast and brain. These observations make it unlikely that both antibiotics act as substrate analogues and thus specifically.

Influence of flavomycin on the synthesis of Dol-PP-linked oligosaccharides

Because of its complex carbohydrate structure, we expected that flavomycin might have a more pronounced effect on the synthesis of higher Dol-*PP* derivatives. Therefore, we have studied its influence on the formation of Dol-*PP*-chitobiose, Dol-*PP*-chitobiosyl mannose and Dol-*PP*-bound oligosaccharides.

Brain microsomes, incubated with Dol-*PP*-*N*-acetyl[¹⁴C]glucosamine and an excess of unlabelled UDP-*N*-acetylglucosamine, catalyse the conversion of the radiolabelled monosaccharide to Dol-*PP*-[¹⁴C]chitobiose in high yield. Extension of the lipid-linked disaccharide to the corresponding trisaccharide occurs when GDP-mannose is added. Analysis of the trisaccharide derivative by standard techniques that are usually applied to Dol-*P*(*P*)-bound saccharides revealed that it was chitobiosyl mannose. The mannose unit could be split off by treatment of the trisaccharide with a β -mannosidase from *Aspergillus niger*, whereas it was resistant to hydrolysis by an α -mannosidase from jack bean, indicating that the mannose residue is bound via a β -glycosidic linkage to the chitobiosyl core. When the synthesis of both lipid-linked saccharides from their corresponding precursor molecules was followed in the presence of various amounts of flavomycin, a concentration-dependent inhibition of chain elongation was observed. Fig. 4 shows the inhibition kinetics for the transfer of mannose from GDP-mannose on to Dol-*PP*-[¹⁴C]chitobiose. Under the conditions used in this experiment, about 0.8 mM-flavomycin was required to achieve 50% inhibition. The rate of Dol-*PP*-[¹⁴C]chitobiose formation was affected to a similar extent (results not shown).

To examine the influence of flavomycin on the synthesis of larger-sized lipid-linked oligosaccharides, these were prepared on a larger scale in the presence and absence of the antibiotic by incubating the particulate membrane fraction with GDP-[¹⁴C]mannose as the glycosyl donor as described in the Materials and methods section. The lipid-linked oligosaccharides were extracted from the pellet with chloroform/methanol/water (10:10:3, by vol.), and the oligosaccharides were released by mild acid hydrolysis and separated by gel chromatography. It was found that in the presence of flavomycin, the absolute incorporation of [¹⁴C]-mannose into the chloroform/methanol/water-soluble fraction was between 20 and 30% lower compared with the control incubations. However,

Fig. 5. Gel-chromatographic pattern of oligosaccharides formed from GDP-[^{14}C]mannose by the particulate membrane fraction

Lipid-linked oligosaccharides were synthesized with GDP-[^{14}C]mannose (270 000 c.p.m.) as glycosyl donor in the absence (○) and presence (●) of 1 mM-flavomycin. The oligosaccharides were released by mild acid and separated on Bio-Gel P-4 (–400 mesh) with 0.5 M-acetic acid as eluent. Arrows indicate the position of Blue Dextran (V_0) and of marker oligosaccharides respectively (M_9 , GlcNAc $_2$ -Man $_9$; M_3 , GlcNAc $_2$ -Man $_3$; GlcNAc $_2$, chitobiose).

the oligosaccharide pattern, obtained after chromatography on Bio-Gel P-4 showed a distinct accumulation of oligosaccharides ranging in size from five to seven sugar units, whereas in the absence of flavomycin most of the mannose was incorporated into larger oligosaccharide structures (Fig. 5). Recent studies on the synthesis of lipid-linked oligosaccharides by Schutzbach *et al.* (1980) and Rearick *et al.* (1981) indicate that GDP-mannose acts as the immediate donor for the mannosylation up to the lipid-linked heptasaccharide, whereas chain elongation to the complete Dol-PP-GlcNAc $_2$ -Man $_9$ saccharide is mediated by Dol-P-bound mannose residues. In fact, our results suggest that the glycosyltransferases using Dol-P-mannose as the glycosyl donor appear to be more susceptible to inhibition by flavomycin than those using nucleotide-bound mannose as the substrate. The inhibition of Dol-P-mannose-mediated reactions, on the other hand, might be at least one reason for the slight stimulation of the formation of this glycolipid that was reproducibly found to occur (see Fig. 1a).

It is noteworthy that the accumulation of a lipid-linked heptasaccharide was also reported by Elbein (1981) in studies on the inhibition of glycolipid formation by tsushimycin in aorta, which was interpreted in terms of a substrate deficiency of Dol-P-mannose, its formation being blocked by the antibiotic. Compared with our results, it seems that the accumulation of a lipid-linked oligosaccharide intermediate of similar size is caused by an interaction of flavomycin and tsushimycin with different glycosyltransferases.

Effect of flavomycin on the Dol-PP-saccharide-mediated N-glycosylation of exogenous acceptor peptides

Recent work in our laboratory has confirmed that exogenous peptides containing the triplet sequence Asn-Xaa-Thr(Ser) represent excellent tools for the characterization of N-glycosyltransferases (Bause & Hettkamp, 1979; Bause & Legler, 1981). Since these enzymes use Dol-PP-linked saccharides as substrates, it was interesting to see if their activity was altered in the presence of flavomycin. Fig. 6 shows the time course of the transfer of [^{14}C]chitobiose from Dol-PP-[^{14}C]chitobiose to the hexapeptide Tyr-Asn-Gly-Thr-Ser-Val catalysed by a particulate membrane fraction from pig brain. In the presence of 0.36 and 1.6 mM-flavomycin, glycosyl transfer to the peptide is inhibited by about 15 and 45% respectively. A similar effect of flavomycin on the N-glycosylation of the hexapeptide and endogenous acceptor proteins is observed, when Dol-PP-[^{14}C]chitobiosyl mannose is used as the glycosyl donor. This indicates that under comparable conditions, the N-glycosyltransferases seem to be about ten times less sensitive against an inhibition by flavomycin than, e.g., the enzyme that catalyses the formation of Dol-P-glucose. These marked differences in the

Fig. 6. Time course of chitobiose transfer from Dol-PP-[^{14}C]chitobiose to the hexapeptide Tyr-Asn-Gly-Thr-Ser-Val

The particulate membrane fraction was incubated with Dol-PP-[^{14}C]chitobiose (2000 c.p.m.) and 1 mM-hexapeptide in the absence (○) and presence (●) of 0.40 and 2.0 mM-flavomycin respectively. The incubations were stopped after 15 min at room temperature and the glycosylated hexapeptide was assayed as described in the Materials and methods section.

susceptibility against the antibiotic suggest, in addition to a presumably general and unspecific effect on the membrane particles, a direct interaction of the antibiotic with the glycosyltransferases themselves.

Conclusions

Our studies demonstrate that the phosphoglycolipid antibiotic flavomycin interferes with the activity of various glycosyltransferases that are involved in the Dol-*P* pathway. However, the inhibitory effect of the antibiotic does not appear to be very specific, as follows from the relatively complex inhibition pattern and the fact that, at least to a certain degree, all enzymes tested are sensitive to high antibiotic concentrations. The most pronounced effect for the antibiotic was observed for the transfer of glucose from UDP-glucose to Dol-*P*, whereas the synthesis of Dol-*PP*-linked saccharides and the *N*-glycosylation of exogenous peptide acceptors were less affected. This rather broad specificity of flavomycin obviously restricts its applicability for the study of dolichyl-mediated reactions, a handicap that, looked at more closely, seems to be common to many other antibiotics studied by other groups, e.g., diumycin (Babczinski, 1980), tsushimycin (Elbein, 1981) or amphomycin (Kang & Elbein, 1979), to mention but a few. The mode of action of flavomycin remains unclear. We found that exogenous Dol-*P* was not able to protect the transferase against inhibition and that the inhibition by the antibiotic was irreversible when the membrane particles were pretreated with flavomycin and then re-isolated by centrifugation. Both observations suggest that flavomycin does not act as a competitive or uncompetitive inhibitor, but is rather bound to the membrane particles in an irreversible manner. This binding might occur by a direct, but unspecific, interaction with the various glycosyltransferases, as is indicated by the different reactivities of the enzymes towards the antibiotic. However, it might also be possible that the antibiotic is incorporated into the double layer of the membrane, thereby altering the membranous environment of the transferases, which itself might then cause the observed effects. The latter inter-

pretation seems to find support in the marked detergent properties of flavomycin as a phosphoglycolipid antibiotic. On the basis of this interpretation, the sensitivity of all transfer reactions against high antibiotic concentrations would be understandable.

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