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INTERACTIONS OF AMINOGLYCOSIDE ANTIBIOTICS WITH NEGATIVELY CHARGED LIPID LAYERS

BIOCHEMICAL AND CONFORMATIONAL STUDIES

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Abstract—Previous studies [Laurent *et al.*, *Biochem. Pharmac.* **31**, 3861 (1982)] have demonstrated that aminoglycoside antibiotics bind to negatively charged phospholipid bilayers and inhibit the activity of lysosomal phospholipases. This inhibition also occurs *in vivo* in animal and man. It is considered to be an early and significant step in the development of aminoglycoside-induced nephrotoxicity. The binding of 6 aminoclycosides in current clinical use (dibekacin, gentamicin, tobramycin, kanamycin A, amikacin and streptomycin) to phosphatidylinositol has been studied by gel filtration technique and by conformational analysis. Variation of the phosphatidylinositol content from 0 to 27% of total phospholipids causes a cooperative increase in aminoglycoside binding. At fixed phosphatidylinositol concentration, the binding of the different aminoglycosides is related to the number of aminogroups carried by the drug (viz., gentamicin > kanamycin A > streptomycin) and is largely, but not entirely dependent upon electrostatic interactions. Conformational analysis of the interaction of aminoglycosides with phosphatidylinositol monolayers was made by a step-wise computation approach. We first have taken into account the Vander Waals, torsional and electrostatic energies and we have calculated the hydrophobic and hydrophilic centers of each molecule. Assembly was then computed by successive association of one molecule of drug and up to 4 molecules of phosphatidylinositol. The calculated interaction energies varied from −8.5 kcal/mol (gentamicin) to −4.9 kcal/mol (amikacin) and −3.9 kcal/mol (streptomycin). Electrostatic interactions were observed between the phospho groups of phosphatidylinositol and up to 3 of the 5 aminogroups in gentamicin, dibekacin or tobramycin (N1, N2, N'6) but only 2 of the 4 aminogroups in kanamycin A or amikacin (N3, N'6). A higher energy of interaction was also associated with a deeper penetration of the drug into the monolayer, due to significant, albeit minor hydrophobic interactions. The insertion of gentamicin, dibekacin and tobramycin in the bilayer was deep enough to allow the inositol groups to freely move over the aminoglycoside. Since we previously showed a correlation between the binding of aminoglycosides and the phospholipase inhibition [Carlier *et al.*, *Antimicrob. Ag. Chemother.* **23**, 440 (1983)], this approach may contribute to the rational design of less toxic aminoglycosides.

Nephrotoxicity is a well-known complication of the use of aminoglycoside antibiotics such as gentamicin [1]. These drugs are taken up by proximal tubular cells from the glomerular filtrate and accumulate in lysosomes [2, 3, 4]. Within these subcellular structures, they induce a conspicuous phospholipidosis [5–7]. Recently, a mechanism has been proposed to explain the development of such metabolic disorder. *In vitro*, aminoglycosides bind to negatively charged phospholipid bilayers and this binding is associated with an inhibition of the activities of lysosomal phospholipases [7, 8]. Specifically, inhibition of lysosomal phospholipase A1 has also been observed both in animals [7] and humans [9] following injection of aminoglycosides. Inhibition of lysosomal sphingomyelinase has also been reported [7, 10]. Comparative studies of several aminoglycosides have demonstrated a parallelism between the capacity of these drugs to cause phospholipidosis and their nephrotoxic potential [8, 11, 12].

An interesting aspect of the *in vitro* experiments using gentamicin and phospholipid bilayers (liposomes) is that phosphatidylinositol is required for both gentamicin binding *and* gentamicin-induced inhibition of the breakdown of the phosphatidylcholine present in the bilayer [8]. This observation has an important biological and toxicological significance, since phosphatidylcholine is abundant in cells and any impairment of its degradation would be expected to cause a rapid, conspicuous phospholipidosis, as is indeed the case *in vivo* [5, 6]. Based upon this line of reasoning, we have assumed that the formation of aminoglycoside–phosphatidylinositol complexes could perturb the bilayer and/or the phospholipases acting on the other phospholipids. In this paper, we analyze the interactions between gentamicin and phosphatidylinositol. First, we examine the binding of aminoglycosides to liposomes with varying content in phosphatidylinositol. Next, we use conformational analysis to identify the most probable configuration of the phosphatidylinositol–gentamicin complexes. We have included other aminoglycosides with de-

creasing inhibitory potency towards lysosomal phospholipases *in vitro* and decreasing affinity for negatively charged liposomes or phospholipid monolayers [8, 13, 14] for comparison with gentamicin (dibekacin ≃ gentamicin ≃ tobramycin > kanamycin A ≃ amikacin > streptomycin), on a molar basis. Since streptomycin causes little nephrotoxicity [15], this comparative work may enhance our understanding of the structure–toxicity relationship for aminoglycoside antibiotics.

MATERIALS AND METHODS

Biochemical studies and materials. Liposomes were prepared as previously described [8], in 4 mM Na acetate buffer pH 5.4. Standard composition was cholesterol–egg yolk phosphatidylcholine–bovine brain sphingomyelin–wheat germ phosphatidylinositol (5.5 : 4 : 4 : 3, molar ratio). When the phosphatidylinositol content was decreased, it was replaced by an equimolar amount of sphingomyelin. Aminoglycosides were mixed with liposomes in a molar ratio drug : phospholipids of 1 : 100 and incubated under N_2 at 37° for 1 hr. Following incubation, the proportion of drug bound to liposomes was thereafter determined by gel permeation [7]. Elution was performed with buffers of composition, pH and ionic strength specified in the Results section. Phospholipids were measured by microassay of phosphorus and aminoglycosides by reaction with fluorescamin [7]. Streptomycin, which does not possess free amino groups was measured by a microbiological assay [16]. The recoveries of the phospholipids and the drugs eluted from the gel consistently ranged between 90 and 110%. The hydrolysis of phosphatidylcholine present in the bilayer by lysosomal phospholipase A1 (EC 3.1.1.32) was measured using liposomes containing 1-palmitoyl-2-[1-^{14}C]oleoyl-*sn*-glycero-3-phosphocholine (140 μCi/mmol of phosphatidylcholine) exposed to a soluble extract from purified liver lysosomes, as described earlier [7, 8]. The antibiotics were obtained from the following suppliers: gentamicin, Schering Corp., Kenilworth, NJ (we used the commercial gentamicin made of a mixture of gentamicin C_1, C_{1a} and C_2; mass ratio approx. 30 : 30 : 40); tobramycin, E. Lilly Bénélux, Brussels; dibekacin and kanamycin A, Continental Pharma, Brussels; amikacin, Laboratoires Bristol Bénélux, Brussels; streptomycin, Laboratoire Wolfs, Antwerp. Phospholipids were obtained from Lipid Products, nr Redhill, U.K. and other reagents from Sigma Chem. Co., St. Louis, MO or E. Merck, AG. Darmstadt, F.R.G.

Conformational studies. A step wise computation approach was used to predict the configuration of mixed monolayers of aminoglycosides and phosphatidylinositol. In a first step, the conformations of isolated molecules of phosphatidylinositol or of aminoglycosides, and their orientation at a simulated hydrophilic–hydrophobic interface were established by methods described elsewhere [17–19]. Briefly, the total conformational energy was calculated from the Van der Waals, torsional and electrostatic energies. The latter was calculated for a dielectric constant of 16, a value intermediate to those currently used for the aqueous and hydrophobic phases at the simulated interface [17]. Selected conformers were then submitted to a simplex minimization procedure [24] with a precision of 10° on the rotational angles, and their orientation at the interface defined by calculations of the hydrophobic and hydrophilic gravity centers. The hydrophilic and hydrophobic gravity centers were established taking into account the transfer energy for each part of the molecule. The values for the transfer energies used are those determined experimentally by numerous authors and summarized elsewhere [25]. The hydrophobic and hydrophilic centers have been defined previously [18, 19].

The second step of the conformational studies involved the molecules in the monolayer as previously described [21, 23]. The position of molecule B relative to the reference molecule A was assessed by step-wise and successive changes of the hydrophilic centers of A and B (from 0.05 to 5 nm, by steps of 0.05 nm each), the rotation of molecule B around its own z axis (by steps of 30° each), the gravitation of molecule B around molecule A (also by steps of 30° each), the up-and-down migration of molecule B along the z axis perpendicular to the lipid–water interface (by steps of 0.05 nm each) and the oscillation of molecule B around its z axis (by steps of 2°30′ each). In each case, the interaction between molecule A and B was calculated from the Van der Waals and electrostatic energies. The configuration of the A and B pair yielding the lowest energy was then used as a reference to assess the position of third molecule C, and the same procedure was then repeated up to a total of 4 phosphatidylinositol molecules, at which point the mean molecular area was found to reach a fairly stable value. When the final configuration of the cluster had been established, the mean molecular area was calculated from both the area occupied by each molecule and the intermolecular area, estimated by projection on the X–Y plane using a grid of squares, each with 0.1 nm side [22, 23]. Calculations were made on a CDC-CYBER 170 computer coupled to a Calcomp 1051 drawing table (Computing Center of Brussels University). The drawing program (PLUTO) was kindly provided by Dr. A. Englert (26).

RESULTS

Biochemical studies. A previous study [7] had demonstrated that gentamicin binds to liposomes containing phosphatidylinositol. Figure 1 shows the influence of the phosphatidylinositol content of the bilayer on such binding. Whereas at a PI/(PC + SM) ratio of either 3/8 or 2/9, almost all gentamicin present in the mixture is associated with liposomes, the binding sharply decreases at lower PI concentration and is negligible with neutral liposomes. Amikacin binding is considerably less than gentamicin, especially at low PI/(PC + SM) ratio. This binding is quantitatively greater than that reported earlier for sphingomyelin-free liposomes (46%; ref. 7); the effect of sphingomyelin was not investigated further. Streptomycin binding to liposomes is trivial. Binding of the other aminoglycosides was only studied with liposomes of PI/(PC + SM) ratio of 3 : 8 and results are given in Table 1.

Figure 2 shows that the binding of gentamicin and

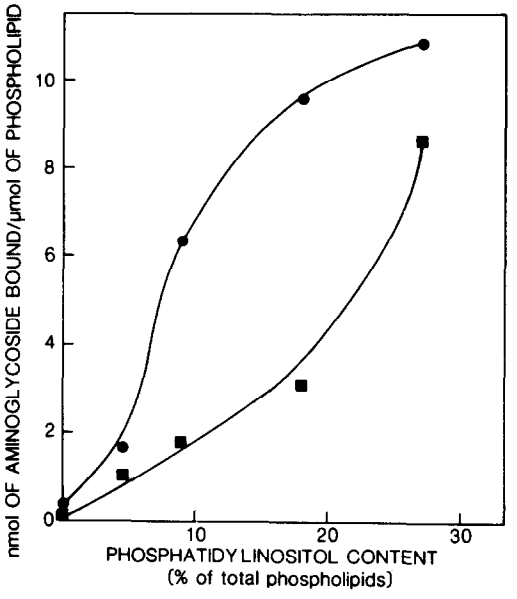


Fig. 1. Binding of gentamicin (●) and amikacin (■) to liposomes. All liposomes contained for 16.5 μmoles of lipid, 5.5 μmoles of cholesterol, 4 μmoles of phosphatidylycholine and a mixture of phosphatidylinositol/sphingomyelin to obtain the final percentage of phosphatidylinositol shown in the abscissa. Mixtures of aminoglycoside–liposomes (molar ratio drug : phospholipids, 1/100) were prepared in 4 mM acetate buffer pH 5.4 and passed through Sepharose 4B in the same buffer. Liposomes were collected in the void volume and the next fractions. The ordinate shows the molar ratio aminoglycoside–phospholipids in the liposome peak. This ratio was not much different from that of adjacent fractions. The free aminoglycoside was recovered from the column after the eluant was changed to 50 mM cacodylate buffer–0.15 M NaCl (see ref. 7).

amikacin are both pH and ionic strength dependent. Increasing the salt concentration at pH 5.4 results in a parallel decrease of the binding of both drugs. At pH 7.4, binding of both gentamicin and amikacin is only half of that recorded at the acid pH. Binding of amikacin is more susceptible to an increase of ionic strength at neutral pH than it is at acid pH. A small but consistent amount of both gentamicin and amikacin remains bound at neutral pH and/or high ionic strength.

CONFORMATIONAL ANALYSIS

Isolated molecules. The values used for valence angles, bond lengths, atomic charges and torsional potentials were those in current use for conformational analysis [29]. For phosphatidylinositol, the initial systematic study analyzed the torsional angles located in the polar head (α_4, α_3, α_2, α_1, θ_1) (Fig. 3), which were given successive increments of 60°. This yielded 6^5 different conformations from which 2 structures with maximal probability, i.e. the lowest energy, were selected. In a second systematic study, the angles θ_3, γ_1, γ_2, γ_3, β_1, β_2, β_3 located in the hydrocarbon chain were increased by steps of 60°, yielding 6^7 different conformations from which 3 more structures were selected. Combination of the 2 structures obtained for the polar head group and the 3 structures obtained for the hydrocarbon chain yielded 6 structures for the entire isolated lipid molecule. The energy minimum attributed to each structure was obtained by application of a simplex minimization procedure [24]. Figure 3 shows the most probable conformer after minimization and orientation at the lipid–water interface. For aminoglycosides, the torsional angles located between the cycles (θ_1', θ_2', θ_3', θ_4') (Fig. 4) for various aminoglycosides were given successive increments of 60°, yielding 1296 different conformations from which one structure with a probability over 99% was retained. For amikacin, in which the N_1 amino group is substituted by a L-4-amino-2-hydroxy-1-oxobutyl radical, the torsional angles (β_1', β_3', β_4', β_5') in this side chain were widened by 60° increments, yielding 6^4 different conformations from which one structure was selected with a probability of 95%.

For each drug the values of the torsional angles obtained after application of the simplex minimization procedure and orientation of the molecule at

Table 1. Binding of aminoglycosides to negatively charged liposomes (cholesterol : phosphatidylcholine : sphingomyelin : phosphatidylinositol, molar ratio 5.5 : 4 : 4 : 3) and inhibitory potency of these drugs on phospholipase A1 activity (hydrolysis of phosphatidylcholine present in the liposomes)

	Binding*		Inhibition of phospholipase A1 (IC_{50})§	
	% bound†	nmoles drug/μmole‡ of phospholipid		
Gentamicin‖	92 ± 4	11.40 ± 0.99	112.8 ± 19.5	(N = 10)
Dibekacin	95.5	9.47	97.6 ± 22.2	(N = 6)
Tobramycin	91.5	9.75	113.5 ± 6.4	(N = 4)
Kanamycin A	79.5	8.08	142.6 ± 22.7	(N = 6)
Amikacin	74.5	7.21	146.0 ± 6.8	(N = 4)
Streptomycin	20.0	2.25	409.2 ± 51.6	(N = 3)

* Drug association to liposomes after chromatography through Sepharose 4B. Mean of 2 experiments, except for gentamicin where N = 3; 0.1 μmoles of drug was mixed with liposomes (10 μmoles of lipid phosphorus) in 1 ml before being applied to the column.

† % of the amount of drug applied to the column.

‡ Ratio in liposome peak

§ Adapted from ref. 8; concentrations causing 50% inhibition (in nmoles/ml).

‖ For commercial mixture of C_1, C_{1a} and C_2 (molar ratio approx. 30 : 30 : 40).

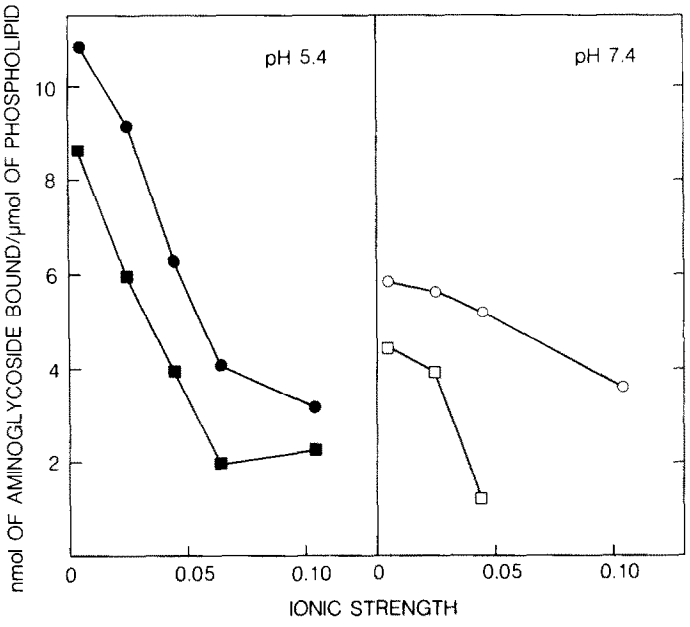


Fig. 2. Release of gentamicin (●, ○) or amikacin (■, □) bound to liposomes upon increase of ionic strength. The drugs were mixed with liposomes (cholesterol : phosphatidylcholine : sphingomyelin : phosphatidylinositol; molar ratio 5.5 : 4 : 4 : 3) at pH 5.4 in 4 mM acetate buffer at a molar ratio antibiotic : phospholipids of 1 : 100. They were thereafter passed through Sepharose 4B eluted with 4 mM acetate buffer (left panel : ●, ■) or 4 mM cacodylate buffer (right panel : □, ○) supplemented with NaCl to obtain the ionic strengths indicated in the abscissa. The ordinate shows the molar ratio aminoglycoside : phospholipids in the liposome peak, as in Fig. 1.

the simulated air–water interface are listed in Table 2, along with the distance between the hydrophobic and hydrophilic centers, and the hydrophobic–hydrophilic balance. Figure 5 shows the stereo view of all aminoglycosides after minimization and orientation at the air–water interface.

Monolayer formation. For each aminoglycoside, the most probable conformers, obtained as indicated above, were inserted in a phosphatidylinositol monolayer. The approach was made stepwise, until a minimum of energy was reached. The drug conformers selected during the first analysis (isolated molecules) were not modified in this process but only the assembling modes corresponding to the minimal energy were retained. The structures shown in Fig. 6 correspond to a probability of 99%. The interaction

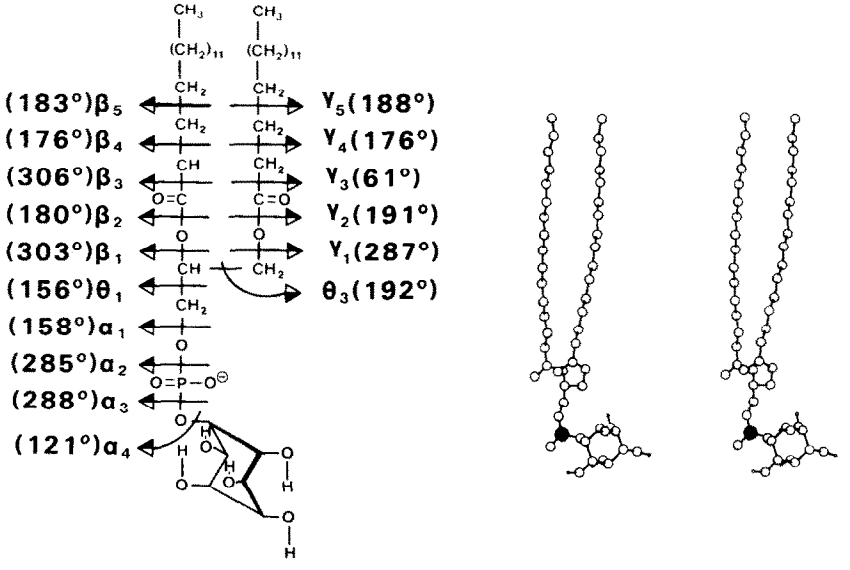
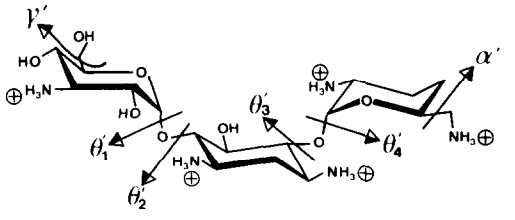
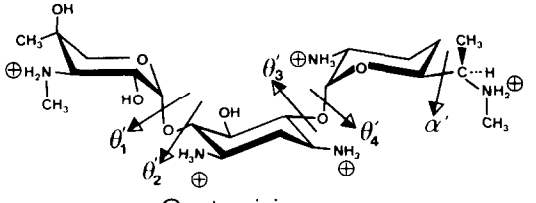


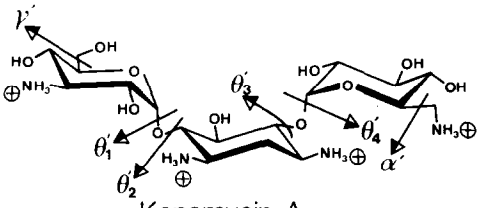
Fig. 3. Definition of torsional angles in phosphatidylinositol, values of the torsional angles and the most probable conformer after minimization procedure. Stereoview of this conformer (○ carbon and oxygen atoms; ● phosphorus atom).



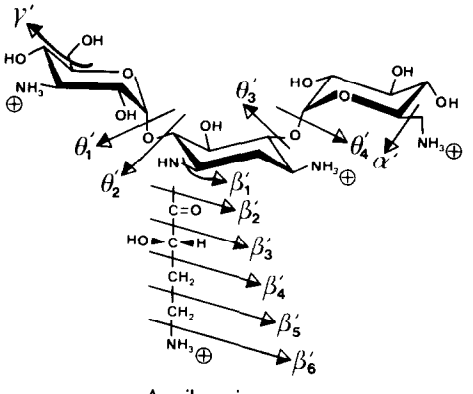
Dibekacin



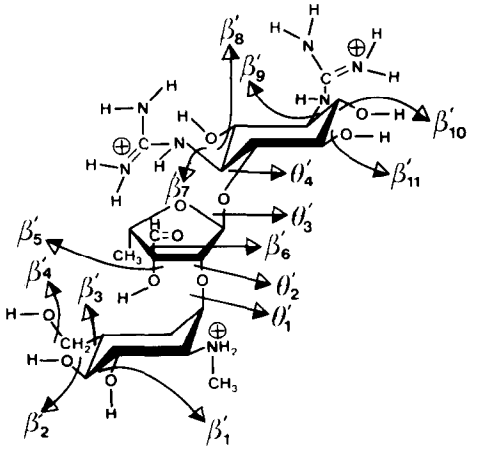
Gentamicin



Kanamycin A



Amikacin



Streptomycin

energy, the relative position and the mean area occupied by the molecule are listed in Table 3. The same table also indicates that part of the molecule which is more oriented towards the hydrophobic part of the phosphatidylinositol monolayer, taking as reference the plane of the deoxystreptamine ring. Dibekacin, and gentamicin are above the position of the phosphorus. They establish a close association with the hydrophobic part of phosphatidylinositol through the deoxygenated sugar moiety (2',3',4',6'-tetradeoxy-2',6'-diamino-D-*erythro* hexopyranose [purpurosamine]). Their position is such that the inositol moiety is probably able to move freely and to "bury" the aminoglycoside molecule in the bilayer. Tobramycin interacts in a similar fashion with the phosphatidylinositol layer, but is located closer to the plane of phosphorus. Three of the five amino groups of gentamicin, dibekacin and tobramycin (N'2, N3, N1) establish electrostatic interactions with the phospho group of 4 molecules of phosphatidylinositol. In contrast to these aminoglycosides, kanamycin A and amikacin adopt an inverted orientation with respect to the phosphatidylinositol monolayer. The association of the drug with the hydrophobic part of the monolayer is established through the 3''-deoxy-3''-aminoglucosyl moiety. This leaves the 6'-deoxy-6'-aminoglucosyl group slightly above the phospho groups (kanamycin A) or even below the phospho groups (amikacin) (see Fig. 6). The almost vertical position of amikacin is stabilized by a hydrophobic interaction of the 4-amino-2-hydroxy-1-oxobutyl moiety (which substitutes the N1 amino group of the 2-deoxystreptamine). Only 2 amino groups (N3 and N'6) are involved in electrostatic interactions with the phospho groups of phosphatidylinositol. The inositol moieties are no longer able to freely interact with each other, because of the interposed amikacin molecule. Streptomycin interaction with the monolayer was the weakest of all aminoglycosides studied. Only the 2 guanidinium groups are involved in electrostatic associations with the phospho groups. The molecule adopts a bented conformation so that its 2-deoxy-2-methylamino-L-glucose extends backward to the hydrophilic part of the monolayer. Again the inositols are no longer able to move over the antibiotic.

DISCUSSION

Aminoglycosides are eliminated from the body by renal filtration, but about 5–10% of the administered dose is retained in the lysosomes of proximal tubular cells [1–3]. Lysosomes display an acidic pH [30, 31] and are also rich in negatively charged phospholipids [32, 33]. These conditions promote the binding of aminoglycosides to phospholipid layers [7, 8, 13, 14, 34]. The present data show in more detail the role of phosphatidylinositol in this binding and compare different aminoglycosides. The cooperative effect of

Fig. 4. Structural formulae of the aminoglycosides studied and definition of the torsional angles. The structural formulae used are those given in refs. 44–47. The component of gentamicin shown is the C1 (C1a 6'-dihydro-6'-amino; C2 6'-methyl-6'-amino). Tobramycin is 4'-hydroxy-dibekacin.

Table 2. Conformers of aminoglycosides after minimization procedure. The precision of the torsional angles is ± 5°.

	Torsional angles (degrees)					
	θ_1'	θ_2'	θ_3'	θ_4'	ϕ	Δ(Å)
Gentamicin	223	154	217	119	0.99	0.60
Dibekacin	220	158	217	122	0.88	0.48
Tobramycin	230	162	212	122	0.78	0.24
Kanamycin A	248	152	130	146	0.80	0.28
Amikacin	231	147	148	147	0.84	0.57
Streptomycin	226	4	207	260	0.71	0.90

The hydrophobic–hydrophilic balance (ϕ) and the distance between hydrophobic and hydrophilic gravity centers (Δ) was calculated according to a previously described method [19].

an increase in phosphatidylinositol upon the binding of gentamicin suggests that the drug binds to more than one negative charge at a time, as expected from its polycationic nature. A precise calculation of the ratio phosphatidylinositol/aminoglycoside and of the affinity constants is, however, not possible from the present experiments since the proportion of phosphatidylinositol available for binding in the liposomes is unknown. The influence of pH and/or ionic strength shows that the binding of aminoglycosides to liposomes is primarily under the control of electrostatic interactions. Our data clearly delineate a decreased affinity for amikacin and streptomycin as compared with gentamicin. A similar difference was found in another study examining the binding of aminoglycosides to lipid monolayers by the Ca^{2+} replacement method [13].

The conformational analysis allows a molecular description of the mode of interaction of gentamicin and other aminoglycosides with a phosphatidylinositol monolayer. Our calculation approach is possible for drugs with up to 100 atoms. For various lipids [22] and ionophores [17], we obtained an excellent agreement between the predictions based on the same type of calculation and the experimental data. For example, atom positions in DL-α-dipalmitoylphosphatidylcholine were predicted with deviation less than 0.1 nm precision when compared to values obtained by neutron diffraction data [37]. For the aminoglycosides we observe a relation between the energy of interaction drug–phosphatidylinositol (Table 3) and the number of drug molecules fixed per phosphatidylinositol molecule (Table 1). A stronger interaction involves a deeper insertion of the drug into the lipid layer. This relationship suggests that the formation of aminoglycoside–phosphatidylinositol complexes is not limited to an electrostatic interaction between phospho groups and cationic groups. Hydrophobic interaction also exists between the drug and the lipid, explaining its incomplete release upon

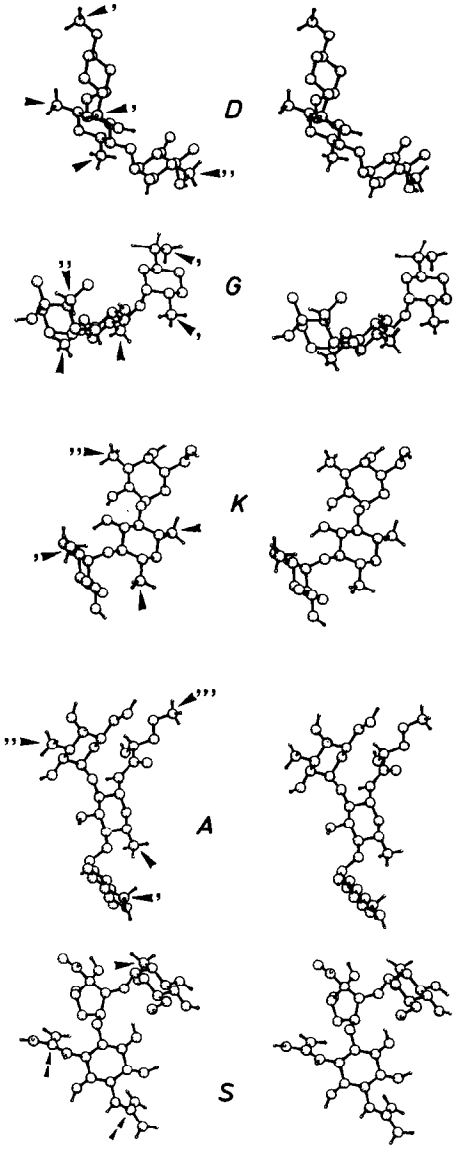


Fig. 5. Stereoviews of the most probable conformer of aminoglycosides after minimization procedure (D dibekacin; G gentamicin; K kanamycin; A amikacin; S streptomycin). The arrowheads point to the ionized atom of each amino (or guanido) function. For orientation of gentamicin, dibekacin, kanamycin A and amikacin the amino groups situated on the aminohexose linked in position 4 of the deoxystreptamine (i.e. on the right in Fig. 4) are denoted ' whereas those on the 6-linked aminohexose are denoted "; for streptomycin the guanidium groups are indicated by a double arrow. The component of gentamicin shown is the Cla (see legend of Fig. 4); no important difference was observed between gentamicin C_1, C_{1a} and C_2. The conformer of tobramycin is very similar to that of dibekacin.

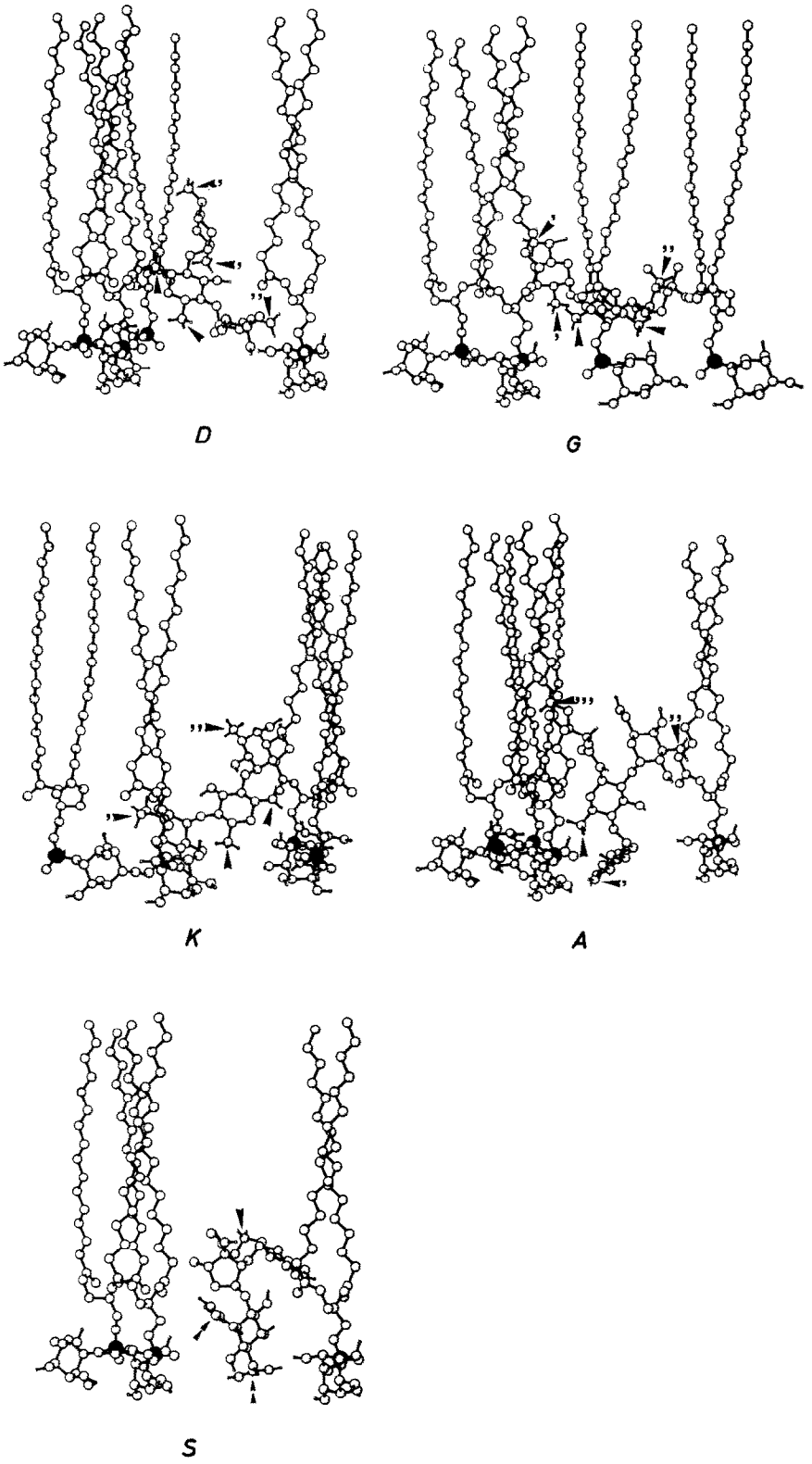
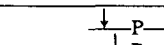
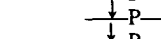
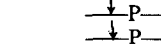
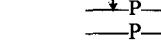
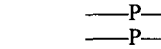
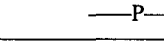


Fig. 6. Configuration of aminoglycosides–phosphatidylinositol mixed monolayer (D dibekacin; G gentamicin; K kanamycin; A amikacin; S streptomycin). The black circles indicate the phosphorus atoms. The cationic groups of the aminoglycosides are indicated by arrows as in Fig. 5. The component of gentamicin shown is the Cla. No important difference was seen for the other components of gentamicin. The configuration of tobramycin–phosphatidylinositol complex is very similar to that of dibekacin, except that the 4-*O*-linked aminohexose moiety (2′,6′-diamino-3′-deoxyglucosamine) does not penetrate so deeply in the hydrophobic phase and the molecule is therefore slightly more horizontal.

Table 3. Energy of lipid–drug interaction; area occupied by the drug molecule; depth of insertion into the lipid monolayer; and orientation of the drug molecule

	Energy (kcal/mole)	Area (Å²/molecule)	Relative position†	Orientation‡
Gentamicin	−8.4	52		'§
Dibekacin	−8.4	44		'
Tobramycin	−7.8	53		'
Kanamycin A	−7.8	69		"
Amikacin	−4.9	81		"/"'
Streptomycin	−3.9	88		(")

† The P indicates the phosphogroup and the * the inositol moiety of the lipid; the arrow indicates the position of the aminoglycoside atom the most oriented towards the aqueous phase.

‡ The sign indicates which sugar moiety is the most oriented towards the lipophilic phase: ' : $O(1\rightarrow4)$ aminohexose (right in Fig. 4); " : $O(1\rightarrow6)$ aminohexose (left in Fig. 4); for streptomycin, " is the 2-deoxy-2 methylamine-L-glucose moiety. For amikacin, "' indicates the L-4-amino-2-hydroxy-1-oxobutyl radical substituting the N^1 amino group.

§ The molecule is almost horizontal (see Fig. 6).

increase of pH and of ionic strength. The importance of significant, albeit minor hydrophobic interactions is also indicated by the behaviour of amikacin. Its position pointing outside of the bilayer appears to be stabilized by a hydrophobic interaction of the aminohydroxybutyric acid moiety.

The calculated conformations of aminoglycosides, as isolated molecules at the hydrophilic–hydrophobic interface or assembled with phosphatidylinositol monolayer—significantly differ from those proposed for the drugs in solution, based on ^{13}C n.m.r. data [33–36]. We have not compared the energy levels of the two types of conformations since they relate to different environments. The conformations in solution are, however, unlikely to remain unchanged at a lipid–water interface and our calculations gave indeed no appreciable probability for them in those conditions. Since we previously demonstrated that binding of aminoglycosides to phosphatidylinositol is necessary to obtain phospholipase inhibition [8], we suggest that the conformations shown here are more relevant of the toxicological properties of these drugs. How the interaction of the drugs with the lipid layer determines the inhibition of phospholipases (with respect to hydrolysis of phosphatidylinositol *and* of the other phospholipids [7, 8]) remains to be elucidated. The data in Tables 1 and 3 show, however, that a higher energy of interaction *and* a deeper insertion in the lipid layer are associated with a stronger inhibitory potency. Amikacin is discrepent with this concept, since it is nearly as inhibitory as kanamycin A (on a molar basis), and yet shows a much lower energy of interaction. Studies of other N1 substitutions in kanamycin A may clarify this point.

The antibacterial activity of aminoglycosides relates mainly to an interaction of the drug with ribosomal subunits [38–40], and the molecular basis of this interaction is likely to be different from that observed for phosphatidylinositol. The rational design of aminoglycosides derivatives that maintain their antibiotic activity but have reduced binding to negatively-charged lipid layers may be attempted. This should result in lesser nephrotoxic [8, 9, 41] and perhaps also lesser ototoxic [42, 43] aminoglycosides.

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