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EFFECT OF ACIDIC PHOSPHOLIPIDS ON THE ACTIVITY OF LYSOSOMAL PHOSPHOLIPASES AND ON THEIR INHIBITION INDUCED BY AMINOGLYCOSIDE ANTIBIOTICS—II

CONFORMATIONAL ANALYSIS

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Abstract—In a companion paper (Mingeot-Leclercq *et al. Biochem Pharmacol* **40**: 489–497, 1990), we showed that the inhibitory potency of gentamicin on the activity of lysosomal phospholipases, measured towards phosphatidylcholine included in negatively-charged liposomes, is markedly influenced by the nature of the acidic phospholipid used (phosphatidylinositol, phosphatidylserine, phosphatidic acid), whereas the binding of the drug to the three types of liposomes is similar. This result challenged previous conclusions pointing to a key role exerted by drug binding to phospholipid membranes and presumably charge neutralization, for phospholipases inhibition (Carlier *et al. Antimicrob Agents Chemother*, **23**: 440–449, 1983; Mingeot-Leclercq *et al., Biochem Pharmacol* **37**: 591–599, 1988). Conformational analysis of mixed monolayers of gentamicin and each of the three acidic phospholipids shows that gentamicin systematically adopts an orientation largely parallel to the hydrophobic–hydrophilic interface, but that (i) the energies of interaction are largely different (phosphatidylinositol > phosphatidylserine > phosphatidic acid), and (ii) the apparent accessibility of the bound drug to water varies in an inverse relation with the energies of interaction. Amikacin, a semisynthetic derivative of kanamycin A with a lower inhibitory potential towards phospholipases than gentamicin in the three types of liposomes used, also showed similar differences in energies of interaction and accessibility to water, but constantly exhibited an orientation perpendicular to the hydrophobic–hydrophilic interface. We conclude that impairment of lysosomal phospholipase activities towards phosphatidylcholine included in negatively-charged membranes by aminoglycoside antibiotics is indeed dependent upon drug binding to the bilayer, but is also modulated by (i) the nature of the acidic phospholipid, which influences the energy of interaction and the accessibility of the drug with respect to the hydrophilic phase, and (ii) the orientation of the drug, which is itself related to its chemical structure. Inasmuch as phospholipases inhibition is related to aminoglycoside nephrotoxicity, these findings may help in better defining the molecular determinants and mechanisms responsible for this adverse effect.

The activity of lysosomal phospholipases towards phosphatidylcholine included in unilamellar liposomes is markedly increased upon addition of phosphatidylinositol in the bilayer, and aminoglycoside antibiotics inhibit this activity presumably by binding to and neutralizing the negative charges carried by the membrane [1]. The latter effect has been related to the nephrotoxicity induced by aminoglycosides in animals and humans (see Refs 2 and 3 for review). In a companion paper [4], however, we showed that the activity of phospholipases was considerably more enhanced when phosphatidic acid rather than phosphatidylserine or phosphatidylinositol was included in liposomes (in a range of 10 to 30% of total phospholipids), whereas the inhibitory potency of aminoglycosides increased when tested towards phosphatidic acid-, phosphatidylserine- and phosphatidylinositol-containing liposomes, respectively. Equilibrium dialysis studies failed to reveal significant differences in the binding parameters of gentamicin towards these three types of negatively-charged liposomes, which suggests that charge neutralization is not the only parameter governing the drug-induced phospholipases inhibition. Previous studies have applied the method of conformational analysis to examine the interactions and mode of insertion of several aminoglycosides with phosphatidylinositol monolayers, and to correlate these data with inhibitory potency and nephrotoxicity [5–7]. We therefore have explored and report here in more detail, the interaction between two aminoglycosides (gentamicin and amikacin) of different nephrotoxicity potencies with the three types of negatively-charged phospholipids used for the biochemical analysis reported in the companion paper (phosphatidylinositol, phosphatidylserine and phosphatidic acid). This approach which has been successfully used to obtain information on the interaction between various compounds including drugs and proteins, and membranes [8–11], could

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help us to better define which molecular parameters govern the activity of lysosomal phospholipases and influence the inhibitory potency of aminoglycoside antibiotics, and thereby may be responsible for and/or modulate their toxicity.

MATERIALS AND METHODS

The method of conformational analysis used consists of a two-step procedure which involves the successive calculation of (i) the conformation and orientation of the isolated molecule at the lipid water interface, and (ii) the conformation of the molecule inserted in a lipid monolayer. The principles of this method have been reviewed by Brasseur [12]. It has been used earlier to examine the interactions between aminoglycosides and phosphatidylinositol [5, 6, 11]. Application to the present analysis, and the improvements over our previous publications, can be summarized as follows.

The total energy of a monolayer is equal to the sum of two terms

$$E^{\text{tot.}} = E^{\text{isol.mol.}} + E^{\text{int.monolayer}} \quad (1)$$

where $E^{\text{isol.mol.}}$ is the energy of the isolated molecule at the lipid water interface which is calculated as the sum of the following terms defined in Brasseur *et al.* [6, 12]

$$E^{\text{isol.mol.}} = E^{\text{VdW}} + E^{\text{cb}} + E^{\text{tor}} + E^{\text{tr}} \quad (2)$$

in which E^{VdW} is the London–Van der Waals energy of interaction between all pairs of non-mutually-bonded atoms, E^{cb} the generalized Keeson–Van der Waals interaction, E^{tor} the potential energy of rotation of torsional angles, and E^{tr} the transfer energy of each part of the molecule. $E^{\text{int.monolayer}}$ is the interaction energy between an amphiphilic molecule within a monolayer and is equal to the sum of three terms

$$E^{\text{int.monolayer}} = E^{\text{VdW}} + E^{\text{cb}} + E^{\text{tr}}. \quad (3)$$

Isolated molecule. In the calculation procedure, the values used for the angles and bond lengths are those commonly used in conformational analysis [13]. We have adopted an *all trans* conformation as the initial configuration. Each molecule has n rotational angles corresponding to the n torsional angles previously defined for phospholipids [5, 14] and aminoglycosides [5]. Thus, if systematic 60° changes were applied, 6^n conformations would be generated. To avoid such a large number, the structure tree technique where six consecutive changes of 60° each were imposed to m ($m \leqslant n$) torsional angles, yielding 6^m conformers in each branch of the structure tree was selected. The conformational energy was calculated for each of these conformers and the most probable configurations were taken as those yielding the lowest energy by the following equation:

$$P_i = \frac{e - E_i/kT}{\Sigma_j e^{-E_j/kT}} \quad (4)$$

with T being set at 298°K, and E_i and E_j corresponding to the internal energy of the ith conformer and to the energies of all generated conformers, respectively. The effect of the entropy was considered as negligible at this stage and, hence, the selection of the conformers was based on their energy rather than on the free energy. A structure tree includes the most probable of all configurations (a selection based upon the Boltzmann statistical weight of all configurations) together with their probability of existence as obtained after successive systematic analysis. At each step, the conformations for which the probability of existence was less than 5% were discarded. After systematic analysis, the sheets of the tree were submitted to a simplex minimization procedure in order to further reduce their total energy [15] with a precision of 10° on each conformational angle. The systematic analysis and the first minimization procedure were carried out in a medium of intermediate dielectric constant representative of the membrane/water interface. The total conformational energy was calculated as the sum of the London–Van der Waals energy (E^{VdW}), the electrostatic interaction (E^{cb}) and the potential energy of rotation of torsional angles (E^{tor}). A second minimization procedure was then performed taking into account the interface properties. At this step, the total conformational energy was calculated as the sum of four terms: the London–Van der Waals energy (E^{VdW}), the electrostatic interaction (E^{cb}), the potential energy of rotation of torsional angles (E^{tor}) and the transfer energy (E^{tr}) of each part of the molecule from the hydrophilic to the hydrophobic phase. At each step, the molecule was oriented with the line joining the hydrophilic and hydrophobic centers perpendicular to the interface [6, 16].

Molecule inserted into the lipid monolayer. The method used for monolayer formation uses the hypermatrix approach. After orienting a first molecule A (usually the aminoglycoside) at the air/water interface, its position and orientation were fixed. A second molecule B (usually the first phospholipid) was also oriented at the interface and it was allowed to move towards molecule A along an x axis in steps of 0.05 nm. For each position, molecule B was rotated in steps of 30° around its long axis z' and around the first molecule. l is the number of positions along the x axis, m the number of rotations of the second molecule around the first one, and n the number of rotations of the molecule itself. For each set of value of l, m and n, the intermolecular energy of interaction was calculated as the sum of the London–Van der Waals energy of interaction (E^{VdW}), the electrostatic interaction (E^{cb}) and the transfer energy of atoms or groups of atoms from a hydrophobic to a hydrophilic phase (E^{tr}). Then, the second molecule was allowed to move in steps of 0.05 nm along the z' axis perpendicular to the interface and the position of the z' axis was varied in steps of 5° with respect to the z axis, to obtain the lowest interaction energy state for each set of values l, m and n. The energy values together with the coordinates associated to each set of l, m and n, were stored in a hypermatrix in order of decreasing value of the interaction energy. A third molecule C (usually a second molecule of phospholipid) was then approached from the group formed by molecule A and B, and its position was defined as the first energetically favourable orientation stored in the hypermatrix taking into account the sterical and energetic

constraints imposed by the presence of molecule B. Thus, orientations were disregarded in which overlap of atomic coordinates of two molecules occurred and in which the interaction energy between the two molecules was positive. In order to further minimize the conformational energy, the position of molecules B and C were alternatively modified in steps according to the energy order classification of the hypermatrix. For the next molecules of phospholipids (molecule D, E . . .), the same process was repeated in succession but, for each of them, the positions of the all molecules surrounding the drug (molecule A) were modified alternatively in order to find the lowest energy state. In this calculation, the interaction energy between all monomers in the aggregate were considered and were minimalized until the lowest energy state of the entire aggregate was reached. We limited this approach to the number of phospholipid molecules sufficient to surround one molecule of each aminoglycoside.

Materials. All calculations were performed on an IBM-compatible PC microcomputer equipped with a mathematical co-processor (Intel 8087, 80287 or 80387), using the PC-TAMMO+ (Theoretical Analysis of Molecular Membrane Organization) software, and the PC-MSA+ (Molecular Structure Analysis) procedures. Graphs were drawn with the PC-MGM+ (Molecular Graphics Manipulation) program. Information on these programs and procedures can be obtained from their author (R.B.).

RESULTS

Previous studies have demonstrated that aminoglycosides bind to phosphatidylinositol included in bilayer membranes and thereby impair the activities of lysosomal phospholipases [17, 18], presumably by decreasing the quantity of available negative charges carried by the bilayer and which are critical for enzyme activity [1, 19]. The results reported in a companion paper [4] showed, however, that binding parameters and inhibitory potencies of gentamicin and amikacin are not systematically correlated when comparing bilayer membranes containing different acidic phospholipids, namely phosphatidylinositol vs phosphatidylserine vs phosphatidic acid. We therefore have examined the interactions of gentamicin and amikacin with phosphatidylserine and phosphatidic acid by computer-aided conformational analysis, in comparison with phospatidylinositol for which such an analysis was performed earlier [5, 11]. Aminoglycosides were analysed under their fully protonated form to mimic their behaviour in lysosomes, the pH of which is estimated to be around 5.4 [20, 21], i.e. below the pK_a values of the aminogroups of the aminoglycosides [22]. The main characteristics of the isolated drug molecules at a hydrophobic–hydrophilic interface, along with the corresponding stereoviews of the most probable conformers have been published [5, 11]. Full atomic size views of the most probable conformers of the three acidic phospholipids studied are shown in Fig. 1. The torsional angles of all relevant critical bonds in the fatty acid chains and in glycerol, and that of the P—O bond of phosphatidylserine and phosphatidic acid were similar to those reported earlier for phosphatidylinositol [5] and the relevant torsional angles in the seryl moiety of phosphatidylserine have also been published [14]. Phosphatidylinositol and phosphatidic acid were considered to carry one negative charge on their phospho group at the pH (5.4) at which analysis was simulated, assuming a pK_a of approx. 2.5 for the free acid function of phosphatidylinositol, and pK_a values of approx. 2 and 9 for those of phosphatidic acid [23]. Phosphatidylserine was considered to display two negative charges (phospho- and carboxyl-groups) and one positive charge (alpha amino group). As shown in Fig. 1, the distance between the hydrophobic–hydrophilic interface (at the level of the carbonyl group of the ester bond between the fatty acids and the glycerol) and the atom pointing the farthest to the aqueous phase was comparable for phosphatidylinositol and phosphatidylserine, but somewhat smaller for phosphatidic acid.

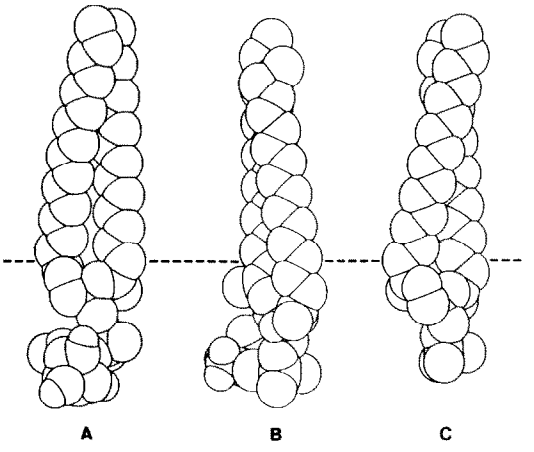

Fig. 1. Space filling view of the most probable conformer after minimization procedure of phosphatidylinositol (A), phosphatidylserine (B) and phosphatidic acid (C) at an hydrophobic–hydrophilic interface. The dotted line refers to the position of the interface, set at the mean level of the C=O group of the ester bond between fatty acids and glycerol.

Figure 2 represents the mode of assembly of gentamicin or amikacin with monolayers made of each of the three negatively-charged phospholipids studied. Each drug was assembled with an increasing number of phospholipid molecules until it was surrounded by a complete set of neighbouring lipids (i.e. an additional phospholipid could no longer be put into direct contact with the drug molecule). Because of the mode of representation used in Fig. 2 (full atomic scale), however, the phospholipid molecules located in front of the drug have not been represented in order for the latter to be seen in full, but the position of the phosphorus atom of the corresponding lipids have been indicated. Gentamicin could be surrounded by four, five and four molecules of phosphatidylinositol, phosphatidylserine and phosphatidic acid, respectively. As reported earlier [5, 11], gentamicin adopted a crescent-like shape largely opened towards the hydrophobic phase, with its N_3, N_1 and N_3'' aminogroups displayed on its

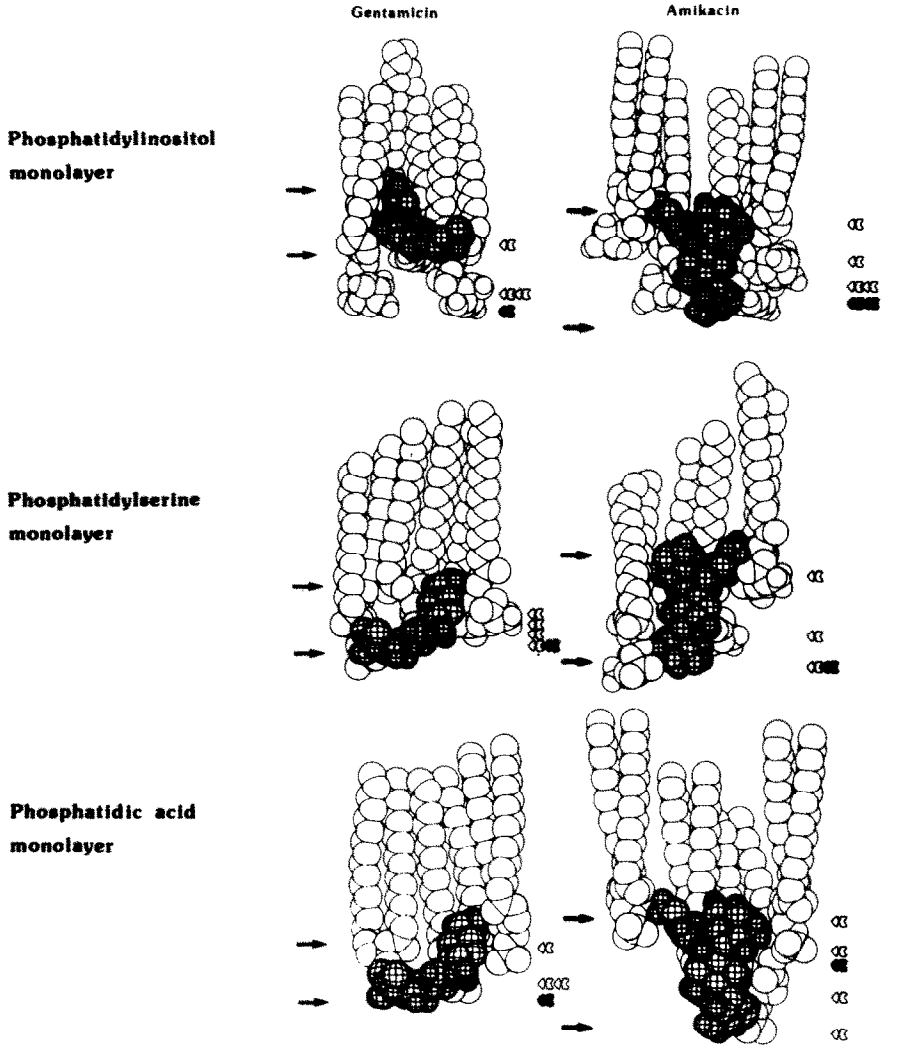

Fig. 2. Space filling view of the mode of assembly of gentamicin or amikacin (cross hatched) and acidic phospholipids (phosphatidylinositol, phosphatidylserine or phosphatidic acid). The arrows on the left of the figure refer to the amino group in the aminoglycoside molecule located nearest to the aqueous phase, and to the aminogroup most deeply inserted in the hydrophobic domain, respectively. The positions of the phosphorus atom of the acidic phospholipid molecules surrounding the drug are indicated on the right of the figure by arrows (open arrows point to those phospholipid molecules which are shown on the figure; phospholipid molecules which are located in front of the drug are not shown, but the positions of their phosphorus atoms are indicated by the closed arrows). The component of gentamicin shown is the C1a. The structural formulae of gentamicin and amikacin, and the conformation of the molecules at interface are shown in Refs 4 and 11. Note that gentamicin shows its 2′,6′-diaminohexose, deoxystrepamine and 3″-aminohexose moieties from left to right when inserted in phosphatidylinositol, but is presented in the inverse orientation when inserted in phosphatidylserine and phosphatidic acid monolayers. For amikacin, the aminosugar having access to the aqueous phase is the 6′-aminohexose moiety, whereas the 3″-aminohexose moiety and the 4-amino, 2-oxo-butyryl side chain point towards the hydrophobic phase. This side chain is oriented to the left for phosphatidylinositol and phosphatidic acid, and to the right for phosphatidylserine monolayer. These differences in drug orientation are intended to improve the visibility of the molecule, and have no meaning concerning the mode of insertion itself.

convex side and oriented towards the hydrophilic phase (see structural formula of gentamicin, full atomic stereoview and ball representation of the conformer in Mingeot-Leclercq *et al.* [11]. Whatever the nature of the phospholipid, gentamicin was located almost perpendicularly to the fatty acid chain as previously reported for phosphatidylinositol [5], although its position with respect to that of the phosphorus atoms and the misshaping of the interface it caused was largely different (see below). The behaviour of amikacin was in sharp contrast. Amikacin could be assembled with six, four and five molecules of phosphatidylinositol, phosphatidylserine or phosphatidic acid, respectively. Assembly

of amikacin with a larger number of phosphatidylinositol molecules than reported earlier (four molecules [5]) is due to improvements in the mode of calculation of the mixed drug–lipid monolayer [9]. As also reported earlier [5], amikacin displayed an elongated shape, considering its three main moieties (3″aminohexose - 2 - deoxystreptamine - 6′amino-hexose), with its 4-amino-2-hydroxybutyryl side chain pointing laterally with an angle of approx. 45° from the axis linking the two sugar moieties (see structural formula of amikacin and ball representation of the conformer in Brasseur *et al.* [5]. When inserted into the phospholipid monolayer, amikacin was oriented almost parallel to the fatty acid chains, considering its main axis linking the two aminosugar moieties. This position of amikacin was largely due to the presence of its aminohydroxybutyryl side chain. As for gentamicin, the nature of the phospholipid did not markedly influence the orientation of the drug in the monolayer, but markedly influenced its position with respect to the interface and to its misshaping. When assembled with phosphatidylinositol, gentamicin was located above (i.e. towards the hydrophobic phase) three out of the four phosphorus atoms surrounding it, so that it largely appeared 'buried' in the monolayer. This was made partly possible by the marked displacement of one phosphatidylinositol molecule (close to the 6′aminogroup of gentamicin) towards the hydrophobic phase, as reported earlier [11]. In contrast, when assembled with phosphatidylserine or phosphatidic acid, gentamicin was largely located at the level of the phosphorus atoms, i.e. much closer to the aqueous phase, with which the contact seems much easier than when the drug was surrounded by phosphatidylinositol. Only part of the 2′, 6′ diaminosugar moiety of the drug, responsible for the misshaping of the interface, was located above the phosphorus atom of the lipid the most displaced towards the hydrophobic phase. Insertion of amikacin consistently misshaped the interface of all three types of monolayers. However, because of its position largely parallel to the acid chains, the 6′aminohexose moiety of amikacin was consistently located at the level of the phosphorus atoms of at least two of the surrounding lipids. Thus, even when surrounded by phosphatidylinositol, the contact of amikacin with the aqueous layer appeared much easier than that of gentamicin. The difference between gentamicin and amikacin tended to become much smaller in this respect when inserted in phosphatidylserine and phosphatidic acid monolayers, because of the increased accessibility of gentamicin to aqueous phase.

Table 1. Calculated interaction energies of the complexes formed between negatively-charged phospholipids (phosphatidylinositol, PI; phosphatidylserine, PS; or phosphatidic acid, PA) and aminoglycosides (gentamicin, amikacin)

Drug	Energy (kJ/mol) PI	PS	PA
Gentamicin	−44.7*	−33.4	−18.2
Amikacin	−35.7*	−30.5	−21.2

* The values shown here are larger than those reported in our first analysis (−35.2 and −20.5 kJ, respectively; [5]), because of improvements introduced in the analysis of the complexes [11].

The calculated energies of interaction between gentamicin and amikacin and each of the three acidic phospholipids are shown in Table 1. The differences between the energies of interaction are largely reflected by position and degree of insertion of the drugs within the monolayers, as seen in Fig. 2. Thus, gentamicin established stronger interactions with phosphatidylinositol than amikacin; the interactions between gentamicin and amikacin with phosphatidylserine were moderate, and low interactions were seen between gentamicin or amikacin and phosphatidic acid.

DISCUSSION

The present studies, combined with the results reported in a companion paper [4] extend and improve our understanding of the molecular mechanisms of aminoglycoside-induced inhibition of lysosomal phospholipase related to drug–phospholipid interactions. Using phosphatidylinositol-containing liposomes, we indeed previously concluded that critical parameters in this respect were (i) the amount of drug bound to the bilayer [17, 18] and, presumably, a corresponding decrease of the quantity of available negative charge carried at the membrane surface [1]; and (ii) the energy of interaction of the complexes formed by the aminoglycosides and phosphatidylinositol and the relative orientation and position of the drug molecule with respect to the plane formed by the phosphorus atoms in a phosphatidylinositol monolayer [5]. Natural membranes, however, contain an array of other negatively-charged phospholipids besides phosphatidylinositol, and their role was not taken into direct consideration in these studies. Aminoglycosides have been shown to bind to several of them including phosphatidylserine, phosphatidic acid, phosphoinositides and ganglioside G_{M1} [4, 24–26]. Binding of aminoglycosides to phospholipid vesicles prepared from phosphatidylcholine and phosphatidic acid, phosphatidylserine, or phosphatidylinositol was shown to cause charge neutralization [27, 28]. It was therefore anticipated that the conclusions gained from studies using phosphatidylinositol-containing liposomes would be straightly extendable to other types of negatively-charged membranes [7]. As stated in the introduction, however, the results of the biochemical studies reported in the companion paper [4] directly challenge the role played by drug binding *per se* with respect to phospholipase inhibition when comparing the inhibitory potency of gentamicin (which increase) with its binding parameters (which are not significantly different) to phospholipid bilayers made negatively-charged by the inclusion of phosphatidic acid, phosphatidylserine and phosphatidylinositol, respectively. This apparent contradiction with our earlier conclusions was already apparent from the

study of streptomycin derivatives such as streptomycylamine, which tightly bind to negatively-charged liposomes but are poorly inhibitory [6]. This behaviour was interpreted as reflecting a lesser degree of insertion of these derivatives in the phosphatidylinositol monolayer, compared to gentamicin. The conformational analysis presented here reveals two major differences in the interactions of gentamicin with the three types of negatively-charged phospholipids included in liposomes used to determine the inhibitory potency of aminoglycosides towards lysosomal phospholipase activity. The first concerns the calculated energies of interaction of the complexes which vary in parallel with the change of inhibitory potency. The second is the apparent drug accessibility to water which increases while the inhibitory potency decreases. Based on our previous result with streptomycylamine referred to above, we therefore suggest that the position of the drug molecule in the bilayer, and therefore its accessibility to water, plays a key role in its inhibitory potential. This conclusion is supported by the behaviour of amikacin which, thanks to its different mode of insertion in all three types of monolayers analysed, constantly remains more accessible to water than gentamicin. This would therefore explain its lower inhibitory potency. Interestingly enough, our previous data comparing gentamicin to streptomycin already demonstrated the greater accessibility of the latter to the aqueous phase [5]. Other aminoglycosides, namely 1-*N*-hydroxyaminopropionyl-gentamicin B (isepamicin), several 1-*N*-hydroxybutyryl-derivatives of kanamycin B and gentamicin C_{2b} (micronomicin, sagamicin) were also shown to be more accessible to water than their parent compounds (gentamicin B, kanamycin B or gentamicin C_{1a}) when inserted into a phosphatidylinositol monolayer (see review in Ref. 7). All these compounds are characterized by a lower inhibitory potential towards lysosomal phospholipases and, for those studied *in vivo*, by a lower capacity to induce lysosomal phospholipidosis and nephrotoxic reactions ([17, 18, 29–31]; see also review in Refs 2 and 32). It would be of great interest to determine whether differences of drug accessibility to water are associated with differences in *rates* of drug–phospholipid association and dissociation, but this would require experimental approaches different from those of equilibrium dialysis [4], measurement of charge reversal [28], or Ca^{2+} displacement [24] used so far to study aminoglycoside–acidic lipid interactions. It must, however, be stressed that accessibility to water, which has not yet been quantified at this stage, is not the only parameter to consider, and that the position of the aminoglycoside also appears critical, as evidenced by the behaviour of amikacin in phosphatidic acid monolayers. This antibiotic is indeed considerably less inhibitory than gentamicin towards lysosomal phospholipases measured with phosphatidic acid containing liposomes (see Table 2 in Ref. 4), and, yet, shows an almost similar accessibility to water than gentamicin (Fig. 2).

Our studies could be extended to other acidic lipids than those investigated here. Thus, examination of the interactions of aminoglycosides with cardiolipin and phosphoinositides, which display very specific subcellular distributions [33], or gangliosides, the biophysical properties and distributions of which are quite different from those of acidic phospholipids, would be of large interest. The use of such models, however, need to take into consideration the actual fate of gentamicin in living cells. As reviewed earlier [2, 3, 32], aminoglycosides are concentrated within lysosomes which they most likely reach by endocytosis after binding, in the case of kidney tubular cells, to the brush-border membrane. Analysis of the lipids of lysosomes from cells developing a gentamicin-induced phospholipidosis reveal an accumulation of all major phospholipids, including acidic ones [34], but also of bis (monoacyl-glycerol) phosphate [35], a phospholipid which may form itself in lysosomes and the concentration of which increases in various conditions of lysosome overloading [36, 37]. Recently, an accumulation of lipid- and protein-bound sialic acid has also been demonstrated in the kidney of gentamicin-treated rats [38]. At this stage, however, and on the basis of the present and other available data [1, 5, 6, 11, 17, 18, 28], we may suggest that critical parameters in phospholipases inhibition by aminoglycosides include: (i) the extent of the binding of the drug to the negatively charged phospholipid included in the bilayer; (ii) the energy of interaction between the drug and this phospholipid (E^{tot}); and (iii) the accessibility of the drug to the aqueous phase. The relative importance of each of these parameters will need to be established by further comparative studies using aminoglycoside derivatives such as gentamicin and amikacin for which behaviour *in vitro* and toxical potential *in vivo* markedly differ (see Ref. 2 for review). The design and study of homogeneous series of aminoglycoside derivatives may also further help in determining more precisely which parts and functions in the molecule are responsible for each of these critical properties.

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