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Mechanism of inhibition of mitochondrial enzymatic complex I-III by adriamycin derivatives

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We demonstrate here that complex I-III of bovine heart mitochondrial membrane is inhibited by adriamycin derivatives. This inhibition is a cardiolipin-dependent process. This lipid, specific to the inner mitochondrial membrane, has been shown previously to interact specifically with adriamycin in model membranes (Goormaghtigh, E., Chatelain, P., Caspers, J. and Ruysschaert, J.-M. (1980) Biochim. Biophys. Acta 597, 1–14) and in mitochondrial membranes (Cheneval, D., Müller, M., Toni, R., Ruetz, S. and Carafoli, E. (1985) J. Biol. Chem. 260, 13003–13007). The differential scanning calorimetry data indicate that, in multilamellar liposomes, the formation of antibiotic-cardiolipin complexes induces a clustering of cardiolipin molecules. Conformational analysis of the antibiotic-cardiolipin complexes suggests that plane–plane interactions between the antibiotics aromatic moieties stabilize this complex formation. Possible mechanisms of inactivation of complex I-III by adriamycin are proposed.

Introduction

The activity of several mitochondrial enzymes has been demonstrated to be lipid-dependent after extraction of phospholipids by organic solvents, detergents or action of phospholipases. β-Hydroxybutyrate dehydrogenase requires phosphatidylcholine in its immediate environment to be active [1]. Fry and Green have demonstrated the absolute requirement of cardiolipin for three enzymatic complexes of the mitochondrial respiratory chain: cytochrome *c* oxidase [2], NADH dehydrogenase and cytochrome *c* reductase [3], although the number of cardiolipin molecules associated with these enzymatic complexes is still under discussion. This dependence of enzyme activity on the lipid environment has been demonstrated to be responsible for new mechanisms of enzyme inactivation. Cardiolipin complexation by adriamycin has been proposed as responsible for cytochrome *c* oxidase inhibition [4]. A linear relationship was found between the affinity of the antibiotics for cardiolipin and the concentration inhibiting 50% of the cytochrome *c* oxidase activity on mitochondria extracted from bovine heart. Moreover, the same drug (namely adriamycin) was shown to inhibit the enzymatic activity to differ-

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ent extent if purified and lipid-depleted cytochrome *c* oxidase was reactivated by cardiolipin or phosphatidic acid in proteoliposomes (phosphatidic acid is able to reactivate in vitro cardiolipin-depleted cytochrome *c* oxidase). The affinity of adriamycin for cardiolipin was about 80-times higher than for phosphatidic acid [4] and the adriamycin concentration required to inhibit 50% of the cytochrome *c* oxidase activity was precisely 80-times higher in the phosphatidic-acid-reconstituted system than in the cardiolipin-reconstituted system. These data indicate that the inactivation could not be explained in terms of a direct drug–enzyme interaction. The adriamycin specificity for cardiolipin has been used to demonstrate the role of cardiolipin on the activity of several other membrane-associated enzymes: mitochondrial phosphate carrier [5] and the membrane receptor of mitochondrial creatine phosphokinase [6]. Recently, this specificity led to the determination of the distribution of cardiolipin across the inner membrane of rat liver and heart mitochondria [7]. The authors rule out very elegantly the possibility of any significant binding of adriamycin to mitochondrial proteins. Binding experiments were performed for liposomes made of lipids extracted from mitochondria, for liposomes made of lipids extracted from microsomes and for whole mitochondria. Binding of adriamycin to microsomal liposomes (which do not contain cardiolipin) was very modest as compared with that to the mitochondrial liposomes and could be due to binding to neutral phosphatidylcholine [8,9]. Moreover, the association constant for adriamycin binding determined for the mitochondrial liposomes was in excellent agreement with that obtained in whole mitochondria and with the value published by Goormaghtigh et al. [10]. These data suggest strongly that the target for adriamycin in mitochondria was indeed cardiolipin and could be a convenient probe for detecting membrane cardiolipin and for investigating its role in membrane-bound functions [11]. In the present paper, our purpose was to study in intact mitochondrial membranes the effect of the same series of antibiotics on the activity of complex I-III (NADH : cytochrome *c* oxidoreductase), another cardiolipin-dependent enzymatic complex.

Materials and Methods

Experimental methods

Chemicals. L-α-Dimyristoylphosphatidylcholine (DMPC), cardiolipin from bovine heart, antimycin A, NADH (grade III, disodium salt), sodium deoxycholate and cytochrome *c* (type III, from horse heart) were purchased from Sigma Chemical Co; NaN_3 from Merck. Duroquinone is an Aldrich product. Adriamycin and derivatives were a gift from the National Cancer Institute. Coenzyme Q_1 was a gift of Dr. Goffeau (Laboratoire d'Enzymologie, Université de Louvain). All chemicals were of analytical grade and water was triple distilled.

Enzymatic assays. Mitochondria were extracted from bovine heart according to the procedure of A.L. Smith [12] and were stored at −20°C for several weeks without loss of respiratory chain enzymatic activity. The activity of complex I-III was determined in a medium (total volume = 1.0 ml) containing 50 μmol Tris-HCl buffer (pH 7.4), 141 nmol NADH, 56 nmol cytochrome *c* (oxidized form), mitochondria (300 μg protein), 50 μmol NaN_3 and 600 nmol sodium deoxycholate. Complex I-III was assayed for its NADH-cytochrome *c* oxidoreductase activity by following cytochrome *c* reduction at 550 nm or NADH oxidation at 340 nm on a Shimadzu U.V. 190 spectrophotometer. The mitochondrial complex I-III activity was rotenone- and antimycin-sensitive.

Preparation of liposomes. To prepare multilamellar liposomes, lipids were dissolved in chloroform. The solution was evaporated under nitrogen to dryness, and further dried under a high vacuum overnight. Multilamellar liposomes were formed by mechanical stirring (vortex mixer) of the lipid film in buffer (50 mM Tris-HCl (pH 7.4)).

Antibiotics were added in two ways. Water-soluble antibiotics were dissolved in buffer and added to the lipid film before mechanical stirring. Poorly water-soluble antibiotics (steffimycin) were dissolved in chloroform/methanol with lipids. After evaporation under nitrogen, the lipid-antibiotic film was dried further under high vacuum. Multilamellar liposomes were then prepared as before.

Differential scanning calorimetry. Differential scanning calorimetry measurements were performed on a Setaram 111, using 120 μl inox cells.

The heating rate was 2 Cdeg/min. For each spectrum 100 μl of solution was used. The mixture contained 75% (w/w) of DMPC (5.0 μmol) and 25% (w/w) of cardiolipin (0.9 μmol). Final antibiotic concentration was 18 mM (twice that of cardiolipin). The reference was composed of 100 μl of buffer solution (50 mM, Tris-HCl (pH 7.4)). Prior to measurement, samples were allowed to stand at room temperature (20°C) for 6 h.

Theoretical methods [13–16]

The conformational energy (E_{tot}) is calculated according to an empirical scheme as the sum of two terms:

$$E_{tot} = E_{loc} + E_{int}$$

The first term (E_{loc}) contains all contributions resulting from local interactions in the isolated molecule. The second term (E_{int}), which describes the interactions between molecules, was calculated only for conformations corresponding to the minima of E_{loc}.

The total conformational energy is calculated as the sum of:

The London-van der Waals energy of interaction between all pairs of non-mutually-bonded atoms, using the Buckingham's pairwise atom-atom interaction functions.

The electrostatic interaction between atomic point charges.

The potential energy of rotation of torsional angles.

The transfer energy of each part of the molecule.

The procedure of assemblage of isolated molecules oriented at the lipid/water interface has been described elsewhere [13–16]. Calculations were made on a CDC-Cyber 170 Computer coupled to a Calcomp 1051 drawing table with a Pluto drawing program (Motherwell, S. and Clegg, W. (1978) PLUTO, Cambridge, U.K.).

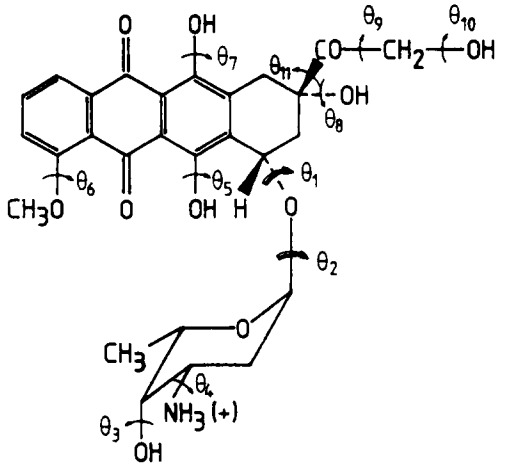
Results

Influence of the antibiotics on complex I-III activity

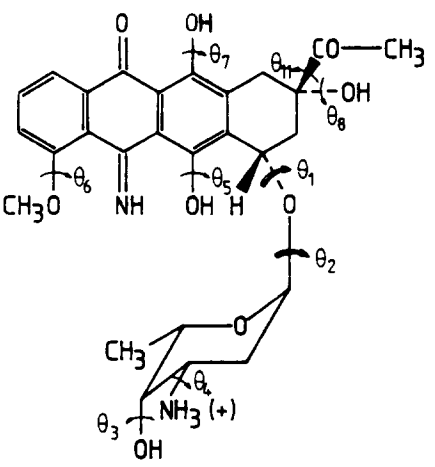
Complex I-III (NADH : cytochrome *c* oxidoreductase) activity has been measured for a series of antibiotics (Fig. 1) at concentrations varying between 10^{-7} and 10^{-3} mol/liter. A linear relationship between the inhibitory capacity of acridine orange, rubidazone, 5-iminodaunorubicin and adriamycin on the complex I-III activity and their affinity for cardiolipin is obtained (Fig. 2). This observation supports the idea that these antibiotics inactivate complex I-III only by interacting with cardiolipin, a phospholipid specifically required for complex I-III activity [3]. Furthermore, recent data strongly indicated that the target for adriamycin in heart mitochondria was indeed cardiolipin [7]. Further evidence is provided by the fact that *N*-acetyladriamycin and steffimycin, which do not bind cardiolipin [17], were found to be completely ineffective inhibitors of complex I-III activity at all concentrations tested (results not shown). In complete opposition to this pattern is the behavior of ethidium bromide (Fig. 1). Although this molecule has a high affinity for cardiolipin (association constant = $2 \cdot 10^6$ liter/mol) [18], no inhibition was detected on the complex I-III activity even at the highest concentration tested (10^{-3} mol/liter) (results not shown). This discrepancy in the general trend drawn above raised the point that the cardiolipin-mediated inhibition requires not only a high affinity of the antibiotic for cardiolipin but also another factor that was further identified as a specific molecular arrangement for the complex formed with the cardiolipin molecules. This molecular arrangement was found to consist of a clustering of the complexed cardiolipin molecules. The following sections describe the use of differential scanning calorimetry and conformational analysis in order to explain this clustering at the molecular level.

Differential scanning calorimetry

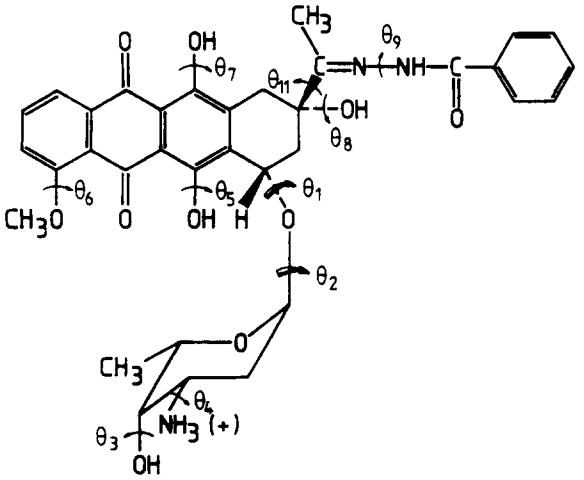
Multilamellar DMPC liposomes dispersed in an excess of water show a transition peak at 23.9°C, associated with the phase transition from the gel to the liquid-crystalline state (Fig. 3a, full line). Presence of cardiolipin (DMPC/cardiolipin, 6 : 1 molar ratio) in DMPC multilamellar liposomes abolishes this transition peak (Fig. 3a, dotted line) as a consequence of a modification of the DMPC–DMPC interactions. However, subsequent addition of adriamycin (cardiolipin/adriamycin, 1 : 2 molar ratio) restores the transition peak characterizing a pure DMPC phase (Fig. 3b, full line). This result demonstrates that the adriamycin-cardiolipin complex segregates in the lipid matrix. Differential scanning calorimetry



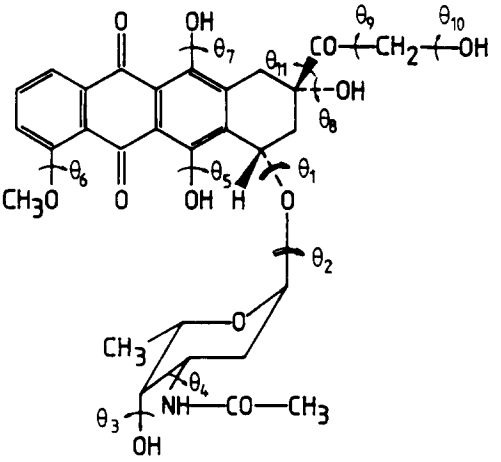
ADRIAMYCIN



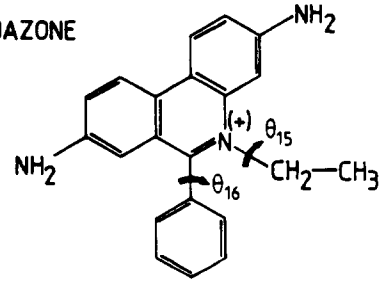
5-IMINODAUNORUBICIN



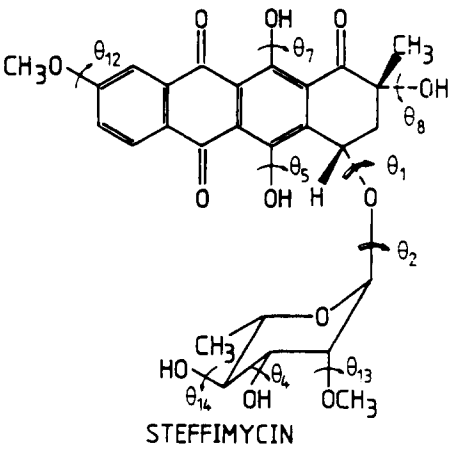
RUBIDAZONE



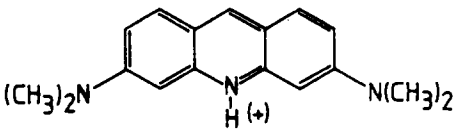
N-ACETYL-ADRIAMYCIN



ETHIDIUM BROMIDE



STEFFIMYCIN



ACRIDINE ORANGE

Fig. 1. Chemical structure and numbering of torsional angles of antibiotics.

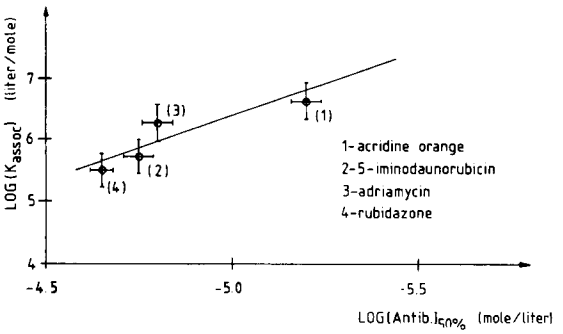


Fig. 2. Relation between the logarithm of the antibiotic concentration inhibiting 50% of NADH:cytochrome *c* oxidoreductase activity on extracted bovine heart mitochondria and the logarithm of the association constant of the cardiolipin-antibiotic complex. Steffimycin and *N*-acetyladriamycin, which do not bind to cardiolipin, were ineffective inhibitors of NADH:cytochrome *c* oxidoreductase in the 10^{-7}–10^{-3} mol/liter antibiotic concentration range. No inhibition was obtained, even at 10^{-3} mol/liter, with ethidium bromide, which binds very efficiently to cardiolipin (association constant = $2 \cdot 10^6$ liter/mol). Association constants were those determined experimentally by Goormaghtigh et al. [17,18].

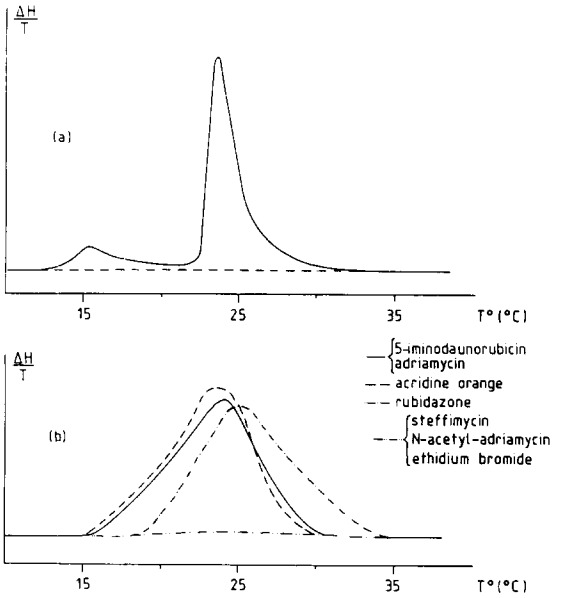


Fig. 3. Differential scanning calorimetry heating curves of: (a) DMPC multilamellar liposomes in the absence (full line) and in the presence of cardiolipin (dotted line); (b) DMPC-cardiolipin multilamellar liposomes in the presence of the different antibiotics used. The mixture contained DMPC (59 mM) and cardiolipin (9 mM). Final antibiotic concentration is twice those of cardiolipin (18 mM). For each spectra, 100 μl of solution were used. The reference is constituted by 100 μl of buffer solution (50 mM Tris-HCl (pH 7.4)). The heating rate is 2 Cdeg/min. Before the measurement, the samples were allowed to stand at room temperature (20°C) for 6 h. Addition of antibiotic (18 mM) to DMPC (59 mM) multilamellar liposomes did not modify the differential scanning calorimetry patterns (data not shown).

spectra, obtained for other antibiotics (Fig. 3b) can be classified into two groups:

(1) Steffimycin, *N*-acetyladriamycin and ethidium bromide do not restore the DMPC transition peak, indicating that they do not induce cardiolipin clustering.

(2) Adriamycin, 5-iminodaunorubicin, acridine orange and rubidazone restore the DMPC transition peak and induce the clustering of cardiolipin molecules in the lipid phase.

There is thus a direct relationship between the cardiolipin cluster formation and the complex I-III inactivation. Since, at this moment, we cannot evaluate the number of molecules constituting a cluster, this relationship must be considered only as qualitative.

Conformational analysis

The conformational analysis procedure developed by R. Brasseur was used to analyse the structure of the adriamycin-cardiolipin complex; it provides a molecular description of the mode of organization of amphiphilic molecules (phospholipids, drugs, etc.) assembled in mono- or bilayers. The validity of this approach has been illustrated for instance by the excellent agreement obtained for DL-α-dipalmitoylphosphatidylcholine (DPPC) between the conformational prediction and the neutron diffraction data [13]. It may be argued that in the conformational analysis interactions between molecules are not allowed to perturb the minimum energy conformation. However, the interaction energy between molecules can modify each structure's probability, as has been demonstrated for DPPC. In this case, the most stable structure was different for the isolated lipid molecule and after assemblage in monolayer. It was obvious that the monolayer packing stabilizes a structure characterized by the close proximity of the phosphate residue associated to the hydrophilic moiety of one lipid and the choline residue

TABLE I

TORSIONAL ANGLES OF THE CARDIOLIPIN MOLECULE AFTER SIMPLEX MINIMIZATION AND ORIENTATION AT THE LIPID/WATER INTERFACE

Torsional angles	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Conformer	300	184	300	178	181	149	58	90	0	205	173	187	169	188	182	180	180	181	182	181
Torsional angles	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Conformer	180	179	179	180	136	180	179	176	180	174	180	179	177	183	177	178	180	176	184	183
Torsional angles	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Conformer	180	61	294	180	179	58	120	0	180	180	179	180	180	180	177	180	181	180	179	
Torsional angles	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78		
Conformer	180	280	172	179	177	136	179	180	179	181	180	181	180	181	180	181	180	181		

Probability of existence at 25°C, 89%

associated to the adjacent lipid, the electrostatic interaction between the two residues stabilizing the lipid structure. A saturated cardiolipin molecule has here been considered for the sake of the numerical analytical method. This simplification is not expected to modify the structure of the complex obtained. Indeed, since the fluorescence spectrum of the anthracycline drugs is strongly dependent on the dielectric constant, ε, of the medium surrounding the dye, penetration of the anthra-

TABLE II

TORSIONAL ANGLES OF THE ADRIAMYCIN DERIVATIVES AFTER SIMPLEX MINIMIZATION AND ORIENTATION AT THE LIPID/WATER INTERFACE

θ_i	Adriamycin	5-Iminodaunorubicin	Rubidazone	Steffimycin	*N*-Acetyladriamycin
1	211	212	212	174	211
2	147	147	158	127	149
3	213	213	205	–	209
4	132	122	143	206	181
5	243	235	81	236	71
6	73	181	78	–	75
7	110	114	109	273	76
8	290	180	294	298	235
9	172	–	177	–	171
10	144	–	–	–	151
11	181	151	174	–	161
12	–	–	–	111	–
13	–	–	–	104	–
14	–	–	–	167	–

cycline moiety of the drug into the hydrocarbon region of the phospholipid bilayer can be investigated by fluorescence titration of the drugs by small unilamellar cardiolipin liposomes. As reported previously, adriamycin [17], acridine orange and ethidium bromide [18] were embedded in a medium of dielectric constant around 50 indicating that they do not penetrate into the lipid acyl chains. Table I gives the torsional angles associated with cardiolipin after minimization and orientation at the lipid/water interface and Fig. 4 gives the numbering of the torsional angles of the cardiolipin molecule. A similar computational procedure was applied to each antibiotics. The molecular structure and the numbering of the torsional angles of antibiotics are given in Fig. 1. Torsional angles obtained after minimization and orientation at the lipid/water interface are summarized in Table II for each antibiotic. The calculated interaction energy (E_{tot}) values (Table III) between antibiotic and cardiolipin in the monolayers are the sum of the cardiolipin–antibiotic interaction energy ($E_{Cl\text{-}An}$) and the antibiotic–antibiotic interaction energy ($E_{An\text{-}An}$) ($E_{tot} = E_{Cl\text{-}An} + E_{An\text{-}An}$). It is striking that those antibiotics which inhibit strongly the enzymatic activity of complex I-III give high values of E_{tot} (acridine orange, 5-iminodaunorubicin, adriamycin and rubidazone) whereas, this value is low for ethidium bromide. In the former case, the conformational analysis shows that plane–plane interactions between the aromatic moiety of the antibiotics molecules considerably stabilize the complex with cardiolipin. The cardiolipin molecules have to be stacked in a cluster in order to maintain this plane–plane interaction. Fig. 5 illustrates this phenomenon for the adriamycin-cardiolipin complex. It must be noted that, despite the fact that a saturated cardiolipin molecule has been used for the conformational analysis, the calculated mean area occupied by one cardiolipin molecule is identical to

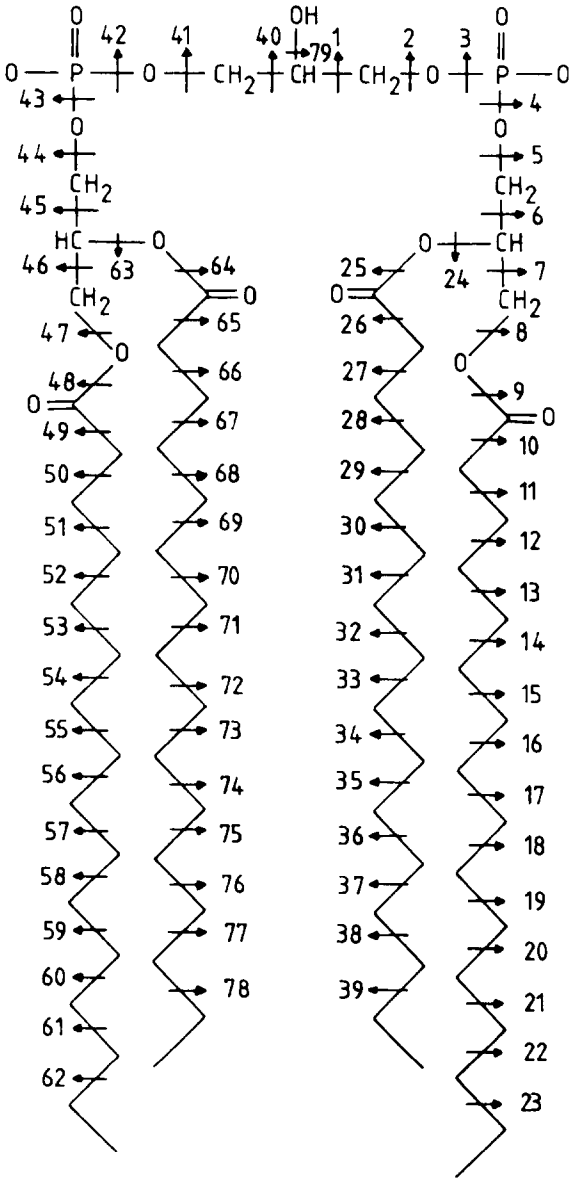


Fig. 4. Cardiolipin structure and position of the torsional angles (α).

TABLE III

VALUES OF INTERACTION ENERGY BETWEEN: ANTIBIOTIC MOLECULES, $E_{An\text{-}An}$, ANTIBIOTIC AND CARDIOLIPIN, $E_{Cl\text{-}An}$, AND THE TOTAL INTERACTION ENERGY, $E_{tot} = E_{Cl\text{-}An} + E_{An\text{-}An}$

	$E_{Cl\text{-}An}$ (kcal/mole)	$E_{An\text{-}An}$ (kcal/mole)	E_{tot} (kcal/mole)
Acridine orange	−5.1	−3.3	−8.4
5-Iminodaunorubicin	−4.2	−3.8	−8.0
Adriamycin	−4.2	−3.9	−8.1
Rubidazone	−2.2	−3.5	−5.7
Steffimycin	−0.3	−2.5	−2.8
N-Acetyladriamycin	−0.5	−3.3	−3.8
Ethidium bromide	−4.5	−0.13	−4.6

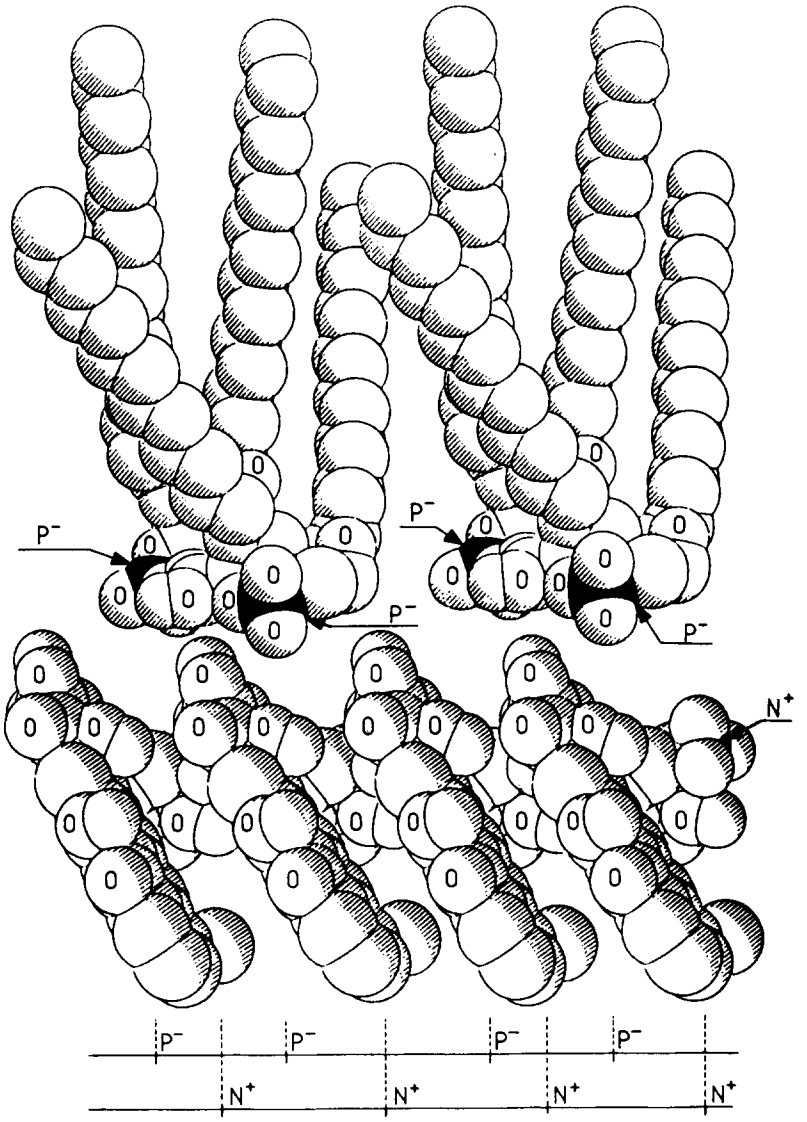


Fig. 5. Computer picture of two cardiolipin molecules assembled with four adriamycin molecules. Arrows indicate the position of the lipid phosphate groups (P^-) and of the adriamycin amino groups (N^+). The mean distance between charged phosphate groups in the same and in adjacent cardiolipin molecules is equal to the mean distance between adriamycin charged amino groups. The plane–plane interactions between adriamycin molecules stabilize the cardiolipin cluster formation responsible for the adriamycin cardiotoxicity.

that obtained experimentally (120 $Å^2$) [10]. The plane–plane interactions are made impossible by the ethidium bromide tridimensional structure and the complex obtained with cardiolipin exists as a monomer in the membrane (Fig. 6). Steric repulsion occurs when two complexed monomers are in close proximity. This repulsion is illustrated by a greater distance between the cardiolipin molecules, as shown in Fig. 6. The space between the two complexed cardiolipin molecules, which is artificially filled by a homogeneous medium of dielectric constant equal to 3 for the sake of this computation, will be filled by other phospholipid molecules in the real membrane. Both inhibiting and noninhibiting antibiotics display relatively high negative values of E_{tot} which are in agree-

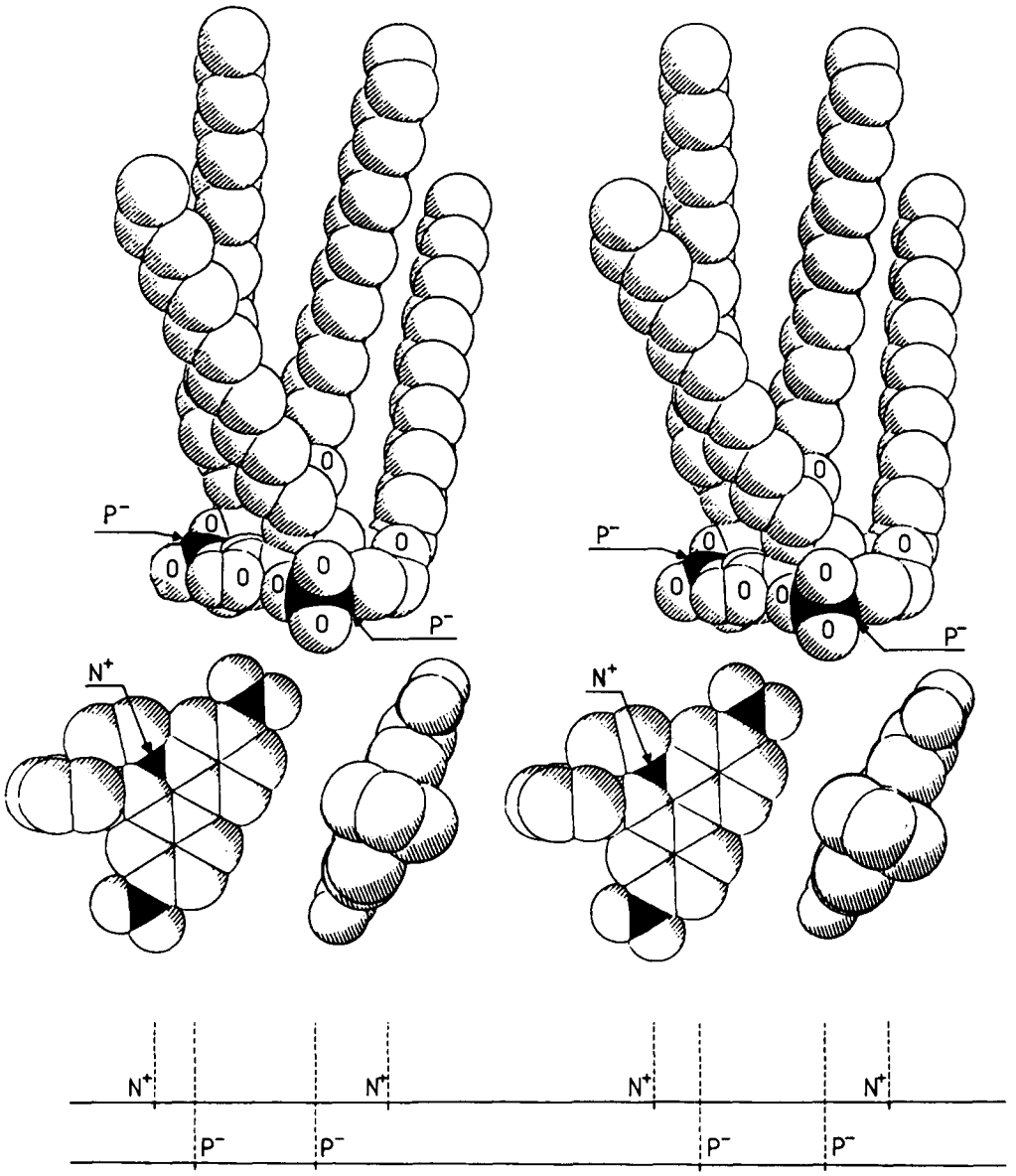


Fig. 6. Computer picture of two cardiolipin molecules assembled with four ethidium bromide molecules. P^- and N^+ have the same meaning as in Fig. 5. The plane–plane interactions are made impossible by the drug structure and the complex obtained exists as a monomer in the membrane.

ment with the high affinity constants between these antibiotics and cardiolipin previously measured [17,18]. It is worth noting that the electrostatic interactions help to stabilize the complex but are not predominant as one would have expected for those mostly very water-soluble antibiotics. *N*-Acetyladriamycin and steffimycin complexes are characterized by lower negative values of E_{tot}. Indeed, the association constants for cardiolipin–steffimycin and cardiolipin–*N*-acetyladriamycin are so low that no complex was experimentally detected [18].

Discussion

Conformational analysis indicates that two adriamycin molecules interacting with one cardiolipin molecule are sufficient close to form a cardpack dimer which would stabilize the complex and explains the high association constant. The picture presented in Fig. 6 is thus in agreement with the following observations:

(1) adriamycin does not penetrate in the hydrocarbon chain region of the cardiolipin monolayer [17];

(2) the cardiolipin bilayer is not affected by the presence of adriamycin a shown from its ^{31}P-NMR spectra [19].

For steric reasons, ethidium bromide cannot form a stable monolayer underneath the cardiolipin monolayer and therefore is not able to induce the clustering of the cardiolipin molecules as shown by differential scanning calorimetry. The three-dimensional structure of ethidium bromide calculated here is in good agreement with the X-ray structure determined in the work of Tsai et al. [20,21]. Recent infrared ATR (attenuated total reflection) measurements indicate that adriamycin molecules in the complex lie parallel, tilted at 39° with respect to the normal to the bilayer plane, in excellent agreement with the conformational data described here (36°) (unpublished data). Moreover, the infrared measurements provide evidence that the cardiolipin acyl chain orientations are not modified after complexation by adriamycin, which is in favor of their limited participation in the complex formation. This observation justifies the assumption made about the acyl chain saturation. Indeed, even if an agreement between the experimental data and the theoretical predictions was obtained for DPPC and the adriamycin-cardiolipin complex, the conformational method remains crude and does not take into account possible structural changes resulting from intermolecular interactions. This does not mean, however, that the structure of the isolated molecule is that of the same molecule organized in monolayer, since molecule–molecule interactions can modify each structure probability [13]. Work is in progress to consider the possibility of a structural change resulting from the assemblage. At this moment, only three or four molecules can be assembled using this procedure, which makes a monolayer mode of organization unrealistic. In a near future, assembly of ten molecules taking into account structural changes resulting from intermolecular interactions should be achieved. It has been recently suggested that in fact two types of binding can take place between adriamycin and cardiolipin [22]. The first is that described here and corresponds to high adriamycin-cardiolipin molar ratio (2 : 1). The second type involves the fixation of adriamycin to cardiolipin through interaction of the amino group with the phosphate moiety, but with the anthraquinone ring embedded into the bilayer; it corresponds to a lower adriamycin-cardiolipin molar ratio. It should, however, be kept in mind that the conformational analysis has been performed for an adriamycin-cardiolipin molar ratio corresponding to the experimental conditions here used (first type of complex). The possibility exists that, for lower adriamycin concentrations, the type of organization proposed by Garnier-Suillerot [23] is observed; a conformational analysis of this complex is in progress. Complex I-III of the mitochondrial membrane (NADH : cytochrome *c* oxidoreductase) is inhibited by several adriamycin derivatives. These data suggest strongly that the inhibition is mediated by interaction of the antibiotics with cardiolipin, essential for the complex I-III activity. Differential scanning calorimetry shows that antibiotic-cardiolipin complex formation results in a clustering of cardiolipin molecules in model membranes. A possible inhibition mechanism is that the adriamycin-cardiolipin complex formation induces the clustering of cardiolipin molecules into a separate lateral phase within the membrane (Fig. 7). In this environment, cardiolipin is inaccessible to complex I-III and inhibition of cardiolipin-dependent electron transfer is achieved. At the present time, the clustering has been established only in model membranes and extrapolation to the mitochondrial membrane would be premature. An inhibition resulting from the surrounding of the complex I-III by the adriamycin-cardiolipin complex cannot be excluded in mitochondrial membrane (Fig. 7). This mechanism is supported by the fact that in presence of water soluble quinone (coenzyme Q_1 or duroquinone) preliminary experiments indicated

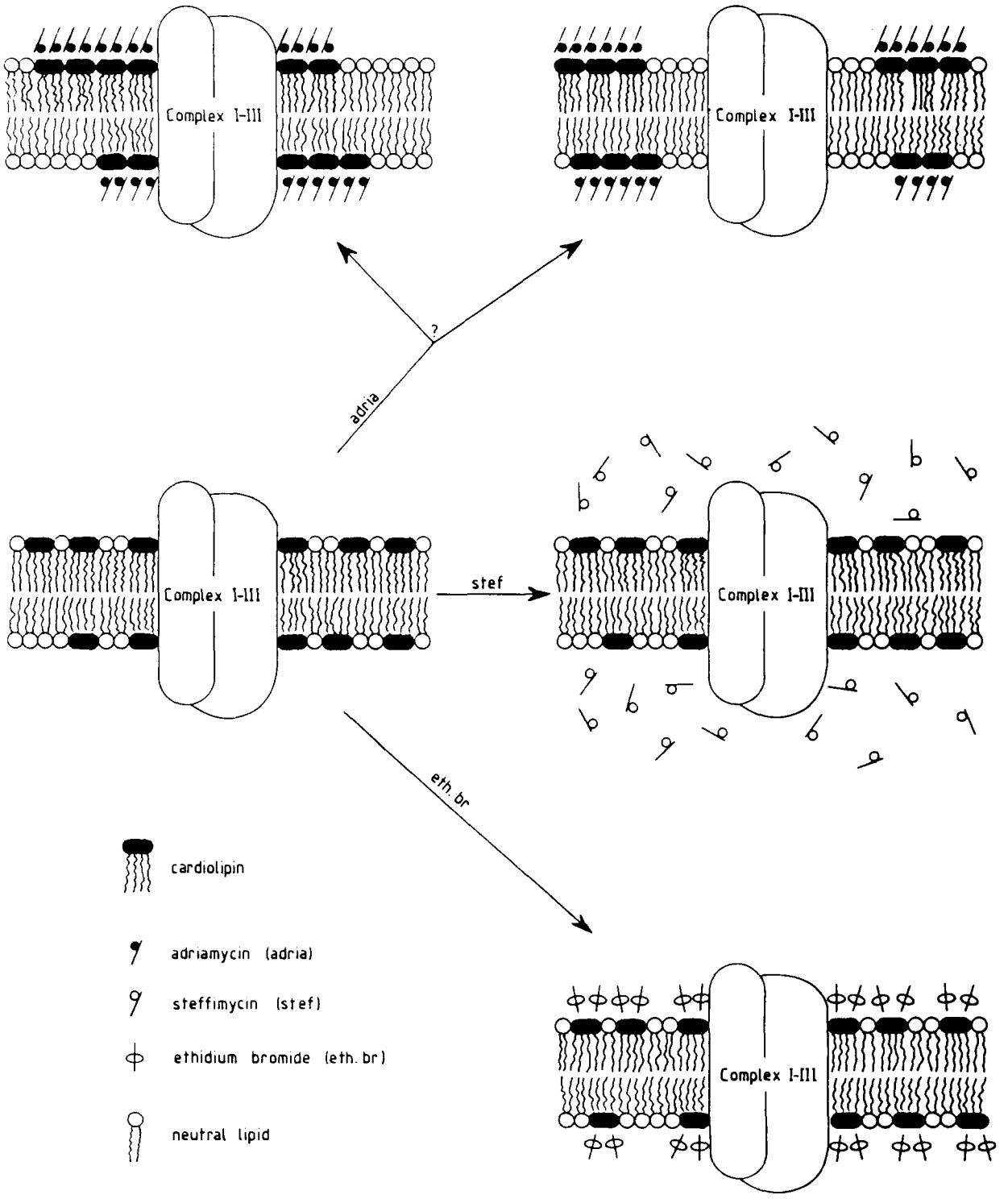


Fig. 7. Schematic representation of possible inactivation mechanisms of the complex I-III.

that no inhibition of complex I and complex III was observed in presence of adriamycin, even at 10^{-3} mol/liter (data not shown). These results suggest that the inactivation could arise from an inaccessibility to complex I or to complex III of the lipid-soluble quinone CoQ_{10} embedded in the membrane. It should, however, be mentioned that CoQ_1 is a water-soluble quinone [24], capable of interacting with enzymatic sites by using a non-lipidic external route. To gain more insight into this inhibition process, the influence of adriamycin derivatives on complex I and complex III separately should be studied using CoQ_{10}. However, because its high insolubility in water, spectrophotometric experiments are difficult to achieve for the moment. From the pharmacological point of view this work may allow the rational design of improved pharmacological agents. Indeed, adriamycin plays a prominent role in treatment of leukemias and solid tumors in man [25–27] but the total dose that may be given is limited by its cardiotoxicity. Since Goormaghtigh et al. [10,11,17] have suggested that cardiolipin may be the main target responsible for this cardiotoxicity, it is tempting to design new structures which do not induce this lipid clustering but maintain their affinity for DNA, the adriamycin nuclear target. Without affecting the positively charged amino sugar of adriamycin, it seems possible to decrease its clustering capacity and its cardiotoxicity by modifying regions which are not required for adriamycin binding to DNA.

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