Study of the Adriamycin–Cardiolipin Complex Structure Using Attenuated Total Reflection Infrared Spectroscopy†

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ABSTRACT: Adriamycin plays a prominent role in the treatment of leukemia and solid tumors in man. The mode of interaction of adriamycin with its nuclear target, responsible for its therapeutic effect, is known [Berman, H. M., & Young, P. R. (1981) *Annu. Rev. Biophys. Bioeng. 10*, 87–114]. The planar anthracycline moiety of adriamycin intercalates between the base pairs whereas the sugar moiety fits into the DNA large groove. However, the cardiotoxicity of adriamycin places a limit on the total dose that may be given [Minow, R. A., Banjamin, R. S., & Gottlieb, J. A. (1975) *Cancer Chemother. Rep. 6*, 195–202]. Much evidence suggests that the mitochondrial membrane could be the target responsible for adriamycin cardiotoxicity. The formation of a very stable complex between adriamycin and cardiolipin, a phospholipid specific to the inner mitochondrial membrane, has been shown to inhibit several mitochondrial membrane enzymes whose activities depend on the presence of cardiolipin. Using attenuated total reflection infrared spectroscopy, we demonstrate here that, in the adriamycin–cardiolipin complex, both cardiolipin and adriamycin structures are modified as compared with the pure substances. Dichroism values indicate a slight reorientation of the cardiolipin molecule toward a normal to the plane of the bilayer whereas adriamycin, which shows no ordering in a pure phase, is highly ordered in the complex, the anthracycline moiety tilted at about 40° with respect to the normal to the plane of the bilayer. The partial disappearance of NH_3^+ characteristic bands indicates the involvement of the positively charged amino group of adriamycin in the complex formation.

Adriamycin (ADM)[1] plays a prominent role in the treatment of leukemia and solid tumors in man (Di Marco et al., 1969; Oldham & Pomeroy, 1972; Lippman et al., 1972). However, its cardiotoxicity places a limit on the total dose that may be given (Minow et al., 1975). This cardiotoxicity could be related to a disturbance of heart mitochondrial functions and ATP synthesis (Bachmann et al., 1975). The mode of interaction of ADM with its nuclear target has been previously reviewed (Harteel et al., 1975; Calendi et al., 1975; Berman & Young, 1981). Both X-ray measurements (Quigley et al., 1980; Patel et al., 1981) and conformational analysis (Neidle & Taylor, 1979) indicate that the planar anthracycline moiety of ADM intercalates between the base pairs whereas the sugar moiety fits into the DNA large groove. Much evidence suggests that the mitochondrial membrane could be the target responsible for the ADM cardiotoxicity. The formation of a very stable complex between adriamycin and cardiolipin (CL), a phospholipid found almost exclusively in the inner mitochondrial membrane, has been shown to inhibit numerous mitochondrial membrane enzymes whose activities depend on the presence of CL, i.e., cytochrome *c* oxidase (Goormaghtigh et al., 1982a), NADH:cytochrome *c* oxidoreductase (Goormaghtigh et al., 1986), and the phosphate carrier (Cheneval et al., 1983), and to stimulate the formation of free radical $O_2^{\bullet -}$ and $OH^{\bullet}$ (Goormaghtigh et al., 1984) with subsequent membrane damages observed both in vitro (Goormaghtigh et al., 1983b) and in vivo (Praet et al., 1984). In a recent review (Goormaghtigh & Ruysschaert, 1984), we suggested that those effects could be responsible for the development of heart failure, which occurs when the total dose of ADM given exceeds ~550 mg/m^2 (Praga et al., 1979). We believe that the knowledge of the structure of the ADM–CL complex at the molecular level is essential for the understanding of the ADM effects on the mitochondrial membrane. Furthermore, such an understanding would allow a modulation of those effects by subtle changes of the molecule structure that could in turn be of pharmacological interest. In previous communications, we have shown that ADM specifically complexes CL in a ratio of two ADM for one CL, i.e., a charge–charge complex, with an association constant of 1.8×10^6 L/mol (Goormaghtigh et al., 1980a). Importantly, these values obtained on model membranes are in good agreement with those obtained on mitochondria (Cheneval et al., 1985). Surface potential data obtained on monolayers (Goormaghtigh et al., 1980a) and fluorescence measurements performed on liposomes (Goormaghtigh et al., 1980a) show that the anthracycline moiety of the ADM molecule does not penetrate deeply into the hydrocarbon chain region of the bilayer. Differential scanning calorimetry measurements indicate that the ADM–CL complex segregates in a separate phase in the plane of the membrane when CL/DMPC liposomes are used (Goormaghtigh et al., 1986). Furthermore, ^{31}P NMR demonstrates that the phosphorus motion of the CL remains typical of a bilayer structure and that the bilayer structure is stabilized in the complex in the presence of Ca^{2+} (Goormaghtigh et al., 1982b). Finally, the stacking of the anthracycline rings of the ADM molecules concentrated at the surface of CL liposomes was established by visible absorbance spectroscopy (Goormaghtigh et al., 1980b). Since it occurs spontaneously in solution for ADM (Barthelemy-Clavey et al., 1974), we suggested that it

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[1] Abbreviations: ATR, attenuated total reflection; SUV, small unilamellar vesicles; MLV, multilamellar vesicles; 1,4-$(OH)_2$AQ, 1,4-dihydroxyanthraquinone; 1,4-$(OD)_2$AQ, deuteriated 1,4-dihydroxyanthraquinone; ADM, adriamycin; CL, cardiolipin; DPPC, DL-α-dipalmitoylphosphatidylcholine.

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brings a significant stabilization to the ADM–CL complex. It is the purpose of this paper to characterize the molecular structure of the ADM–CL complex by attenuated total reflection (ATR) infrared spectroscopy, which allows spectra to be recorded on ordered bilayers and information to be gained about the orientation of different parts of the whole molecule.

Materials and Methods

For general information about internal reflection infrared spectroscopy, see Harrick (1967). Details about the application of infrared attenuated total reflection (ATR) spectroscopy to lipid membrane research were reviewed by Fringeli and Günthard (1981). Spectra were recorded with a Perkin-Elmer infrared spectrophotometer 983G equiped with a Perkin-Elmer microspecular reflectance accessory (Ref. P.E. 221-0357) and a polarizer mount assembly with a silver bromide element (Ref. P.E. L 106-0249). The internal reflection element was a KRS-5 ATR plate (50 × 20 × 2 mm, Harrick EU 2121) with an aperture angle of 45°, yielding 25 internal reflections. The mean scan rate was 174 cm^{-1}/min, and the spectrophotometer was purged with dry air. Spectra were encoded every 1 cm^{-1} and transferred at the end of the scan from the memory of the spectrophotometer to an Olivetti M40 computer through an RS232C interface. All the encoded points were used in subsequent mathematical treatments of the spectra. Neither spectral deconvolution nor contour smoothing procedures were applied. The samples were scanned with parallel (0°) and perpendicular (90°) polarized incident light with respect to a normal to the ATR plate. A base line recorded with the same ATR element and the same polarization was substracted from each spectrum. Polarization was expressed as the dichroic ratio $R^{ATR} = A^{\parallel}/A^{\perp}$, where $A^{\parallel}$ and $A^{\perp}$ are the absorbances measured with the parallel and the perpendicular incident light, respectively. The angle between the oscillating dipole moment of a transition and a normal to the bilayer support was calculated according to Fringeli and Günthard (1981). For CL, the vibrational assignment as well as the orientation of the transition moments with respect to the molecular axis was taken from the compilation made by Fringeli and Günthard (1981). For ADM, assignments and orientation of transition moments were determined after comparison of the spectrum of ADM before and after removal of its amino sugar moiety by acid hydrolysis (Goormaghtigh & Ruysschaert, 1983) and examination of polarized infrared spectra of single crystals of the model compound 1,4-dihydroxyanthraquinone [1,4-$(OH)_2$AQ] and of its deuteriated analogue 1,4-$(OD)_2$AQ (Smulevich et al., 1982). Information about the nonaromatic part of the ADM molecule were taken from Bellamy (1985). The technique applied to achieve the deposition of oriented multilayers is the following: In a first step, small unilamellar vesicles (SUV) were formed. For this purpose, a methanolic solution of CL or DPPC containing 10 mg was evaporated to dryness under a nitrogen flow. Traces of solvent were removed by storing the vial under vacuum overnight. Multilamellar vesicles (MLV) were formed by mechanical stirring (vortex mixer) of the lipid film in 2 mL of triple-distilled water. When present, ADM was added and dissolved in the water before the mechanical stirring, so that the CL/ADM ratio was 2.5:1 (mol/mol) (unless otherwise indicated). The small unilamellar vesicles were obtained by sonication of the MLV suspension by a Branson sonicator for 15 min at a nominal power output of 60 W under a N_2 stream. Oriented multilayers were obtained as described by Fringeli and Günthard (1981) by slowly evaporating 100 μL of the SUV suspension on one side of the ATR plate. The ATR plate was then sealed in a universal sample holder (Perkin-Elmer 186-0354), which was flushed with pure N_2 overnight. This precaution took care of any variation in the hydration state of the bilayer and allowed very reproducible data for different preparations to be obtained. The actual number of H_2O molecules bound per lipid in those conditions was however not evaluated. H/D exchange was obtained by using D_2O-saturated N_2 (room temperature) in the same conditions. To avoid recording of spectra of multilayers not included in the cavity of the universal sample holder, which is atmospher controlled, only a 10-mm window was left open on the middle of the aperture of the ATR plate; the two side bonds (3 and 7 mm) were masked by aluminum foil.

Cardiolipin (beef heart) and DL-α-dipalmitoylphosphatidylcholine (DPPC) were purchased from Sigma Chemical Co; adriamycin was a gift from the National Cancer Institute; D_2O (99.8% gold label) was from Aldrich. Water was triple distilled.

Results

Effect of ADM on CL Structure. Multilayers of pure CL and of the CL–ADM complex (2.5:1 mol/mol) were prepared as described under Materials and Methods. The vibrational spectrum of the hydrocarbon chains of CL is characterized by the two intense bands ν_{as} and $\nu_s(CH_2)$ at 2924 and 2854 cm^{-1}, respectively, and a shoulder at 2956 cm^{-1} for the antisymmetric (CH_3) stretching (Figure 1b). ν_{as}(CH=CH) at 3010 cm^{-1} is expected since beef heart cardiolipin contains large amounts of linoleic acid residues (Shah & Schulman, 1965). The presence of $\delta(CH_2)$ at 1464 cm^{-1} well below 1470 cm^{-1}, the broadness of the CH_2 rocking band at 723 cm^{-1} ($\Delta\nu_{1/2}$ = 10 cm^{-1}), and the lack of CH_2 wagging progression between 1180 and 1345 cm^{-1} indicate the absence of a major all-trans organization in the CL bilayer (Fringeli & Günthard, 1981). Successive subtraction of the pure CL spectrum (Figure 1b) and of the pure ADM spectrum (Figure 1d) from the spectrum of the ADM–CL complex (Figure 1a) yields a spectrum that contains only the modifications of both CL and ADM spectra due to their mutual interactions (Figure 1f). A positive deviation from the base line indicates an increase of the intensity of a band, and negative deviation indicates the (partial) disappearance of a band. The intermediate results a − b = e and a − d = c yield respectively the spectrum of ADM in the complex and the spectrum of CL in the complex. Both are affected by the spectral modifications of the other compound of the mixture. In Figure 1f, a small but significant increase in the intensity of all the CH_2 modes of vibration of CL can be observed, indicating that the crystallinity of the hydrocarbon chains of CL is slightly enhanced upon interaction with ADM. Since no shift of the position of the peaks is observed for the intense $\nu_{as}(CH_2)$ and $\nu_s(CH_2)$ vibrations, this effect on the crystallinity is much smaller in magnitude than the liquid to liquid-crystalline transition observed with synthetic lipids (Casal & Mantsch, 1984). The fatty acid ester region of the CL produces a strong ν(C=O) band at 1739 cm^{-1}, which upon interaction with ADM becomes more intense in the 90° polarization and less intense in the 0° polarization (Figure 2), indicating a reorientation of the mean direction of the carbonyl groups toward a normal to the plane of the bilayer. The ν(C=O) vibration of ADM that occurs at 1720 cm^{-1} is clearly resolved from the CL ν(C=O) band in Figure 2. The two intense phosphate vibration bands $\nu_{as}(PO_2^-)$ at 1243 cm^{-1} and $\nu_s(PO_2^-)$ at 1102 cm^{-1} experience a shift of 13 and 35 cm^{-1}, respectively, toward smaller wavenumbers. An attempt to estimate the modification of the $\nu_{as}(PO_2^-)$ band intensity was performed by integrating the spectra of b and c of Figure 1 between 1310 and 1152 cm^{-1}. The area of the

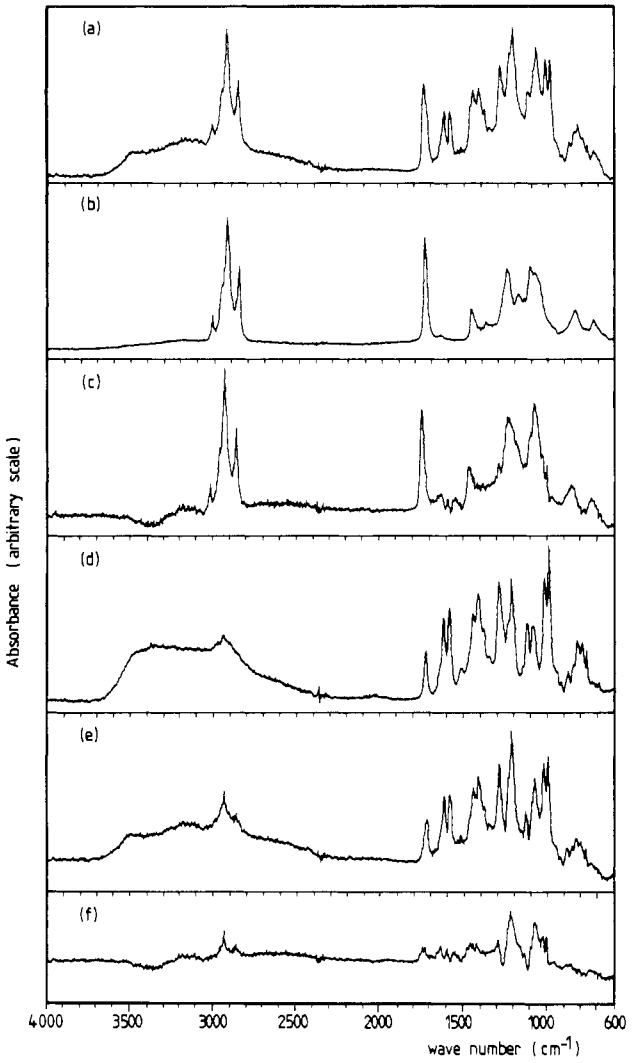

FIGURE 1: Absorbance infrared spectra recorded with 90° polarized light: (a) CL–ADM complex; (b) pure CL; (d) pure adriamycin. Samples were prepared as described under Materials and Methods. Difference spectra computed are c = a – d representing the spectrum of CL in the complex to which modifications of the ADM spectrum may be superimposed and, similarly, e = a – b representing the spectrum of ADM in the complex to which modifications of the CL spectrum may be superimposed. f is a – b – d. All spectra are represented with the same absorbance scale.

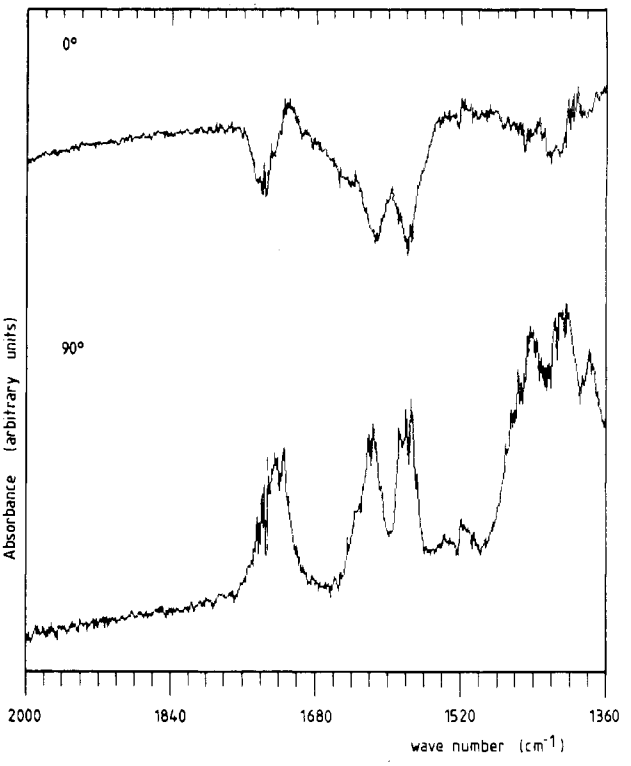

FIGURE 2: Details of spectrum f of Figure 1 obtained with the polarizer in the 0° position and of a similar spectrum obtained with the polarizer in the 90° position.

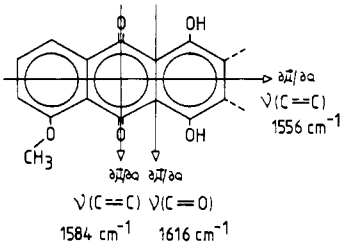

FIGURE 3: Orientation of the dipole moments of the ν(C=C) and ν(C=O) transition in the anthracycline core of ADM.

Table I: Orientation of the Anthracycline Ring of ADM in the ADM–CL Complex with Respect to a Normal to the ATR Plate Calculated from the Dichroic Ratio R^{ATR} of Multilayers[a]

	vibration	R^{ATR}	angle (deg)
short axis	ν(C=O), 1616 cm^{-1}	1.92	37
	ν(C=C), 1584 cm^{-1}	1.69	40
long axis	ν(C=C), 1556 cm^{-1}	1.59	42

[a] The definition of the short and long axes is given in Figure 3. The angles are obtained as described in the text after H/D exchange (see Materials and Methods).

weak ν(C–O) band at 1174 cm^{-1} was assumed to be unmodified upon interaction with ADM and was subtracted from the total area. Spectral intensities were normalized with respect to $\nu_s(CH_2)$, which is subject to little modification upon interaction with ADM and is not significantly overlapped by ADM bands. An increase of 40% of the area of the $\nu_{as}(PO_2^-)$ band was calculated. If DPPC is used instead of CL, no modification of the $\nu_s(PO_2^-)$ and $\nu_{as}(PO_2^-)$ vibrations occurs (data not shown).

Structure of ADM in the Complex. The aromatic core of ADM exhibits two absorption bands in the region of 1600 cm^{-1}, i.e., at 1616 and 1584 cm^{-1}. The same pattern occurs for the model compound 1,4-dihydroxyanthraquinone (respectively 1630 and 1590 cm^{-1}) (Smulevich et al., 1982). Our confidence in the assignment of the ADM bands by comparison with 1,4-$(OH)_2$AQ is increased by their similar behavior upon H/D exchange: the high-frequency band is unmodified for both compounds, but the low-frequency band is splitted into two well resolved bands at 1584 and 1556 cm^{-1} for ADM and 1590 and 1555 cm^{-1} for 1,4-$(OD)_2$AQ. The transition dipoles are all in the plane of the molecule, and their orientation is indicated in Figure 3. When ADM is spread alone on the ATR plate, its infrared spectrum is essentially depolarized, indicating no preferential organization with respect to the ATR plate plane.

Conversely, in the presence of CL, the two absorption bands at 1616 and 1584 cm^{-1} are 90° polarized (Figure 2). An estimation of the orientation of the long and short axes of the ADM aromatic core in the ADM–CL complex was made by measuring the dichroism ratio R^{ATR} of those vibrations after H/D exchange performed as explained under Materials and Methods. R^{ATR} was found to be between 1.6 and 1.9 (Table I) from which the angle formed between the corresponding transition dipole moments and a normal to the bilayer was calculated as described by Fringeli and Günthard (1981), assuming a perfect ordering of the ADM aromatic core (Table

I). The presence of strongly polarized bands (R^{ATR} between 1.6 and 2.1) for some bands of ADM not overlapped by CL bands justifies the use of an order parameter $S = 1$ to calculate the angles. Taking an order parameter as low as 0.7 does not modify the result by more than ~7–8°. The calculated angles are therefore a maximum estimate, and the long and short axes of the ADM aromatic core could be closer to a perpendicular to the lipid membrane plane. Importantly, varying the CL/ADM ratio from 5 to 0.5 (stoichiometric charge–charge complex) yielded identical results (experiment not shown). No polarization (R^{ATR} between 1.0 and 1.1) occurs when CL is replaced by DPPC even though the hydrocarbon chains of DPPC appear to be very ordered as shown by the presence of dichroic ratios near 3 for $\gamma_w(CH_2)$ at 1200 and 1222 cm^{-1} [see Fringeli and Günthard (1981)]. In another set of experiments not shown, a DPPC–CL mixture (80%:20% w/w) was incubated in the presence of ADM to form the stoichiometric charge–charge complex. As for pure CL films, R^{ATR} varied between 1.6 and 1.9. Again, when the 20% CL was replaced by DPPC, no significant polarization was measured. The amino group of ADM is characterized by the $\delta_s(NH_3^+)$ band at 1505 cm^{-1}, whose intensity decreases by 60% upon interaction with CL (see Figures 1e and 2), and by the $\delta_s(NH_3^+)$ band at 2022 cm^{-1}, which vanishes in the complex with Cl (see Figure 1e,f) but does not vanish when ADM is mixed with DPPC. Finally, the comparison of the spectrum of pure ADM (Figure 1d) with the spectrum of ADM in the complex (Figure 1e) reveals a considerable modification of the broad peak occurring in the 3600–2400-cm^{-1} region. That peak was assigned to ν(OH). Upon interaction with CL, the maximum of this broad peak is shifted toward smaller wavenumbers. When ADM is mixed with DPPC, no shift occurs.

Discussion

Infrared spectroscopy reveals a small but significant increase in the ordering of the CL hydrocarbon chains upon interaction with CL. Such a result is expected if no penetration of the ADM molecule into the hydrocarbon chain region of the bilayers occurs and if ADM neutralizes the negative charges of the CL polar head groups, allowing a closer packing of the molecules. Direct evidence of the involvement of the positvely charged amino group of the ADM in the complex formation is provided by the disappearance of the NH_3^+ bands at 2022 and 1505 cm^{-1} in the presence of CL (but not in the presence of DPPC). Since an electroneutral complex is formed (Goormaghtigh et al., 1983b) and since the acetylation of the ADM amino group results in a compound that presents no affinity for CL (Goormaghtigh et al., 1980a), it is likely that the positively charged amino group of the ADM interacts electrostatically with the negatively charged phosphate groups of the CL. The shift of the two phosphate stretching vibrations toward smaller frequencies and the increase of their intensity are consistent with this interpretation. When DPPC is substituted for CL, no modification of the phosphate bands or of the NH_3^+ bands occurs, demonstrating the specificity of the interaction for CL. More surprising is the reorientation of the fatty acyl C=O bond toward a normal to the membrane plane upon interaction with ADM. It could be related to an overall straightening of the hydrocarbon chains correlated with the increased packing discussed above or merely induced by a specific interaction with some parts of the ADM molecule. Under the current state of our investigations, it was not possible to decide on one of these hypotheses. The IR spectra also reveal important modifications of the hydrogen bonds of ADM. Since a stacking of the anthracycline rings must have occurred for both pure ADM (Chaires et al., 1982; Burke & Tritton, 1984; Menozzi et al., 1984) and ADM–CL complex samples owing to the high concentration on the ATR plate, the establishment of stronger hydrogen bonds must be caused by the interaction of the stacked ADM with CL. The specificity of the involvement of H bonds in the ADM–CL complex is shown by the fact that DPPC is unable to induce any measurable modification in this region of the spectrum. At this point it must be emphasized that DPPC has been shown not to interact with ADM by various techniques (Goormaghtigh et al., 1980a). Moreover, in their thorough investigation of the interaction of ADM and derivatives with small unilamellar vesicles (SUV) of synthetic phosphatidylcholine, Burke and Tritton (1985a,b) noticed that the binding of ADM onto SUV reaches a saturation when only four molecules of ADM are bound per vesicle made of about 3000 phospholipid molecules. This behavior suggested the use of DPPC as a blank to test the specificity of the spectral modifications resulting from the ADM–CL interaction. However, our data do not preclude the existence of weak interactions between ADM and DPPC or even more specific interactions involving a minute fraction of the molecules. Such a study was considered to be beyond the scope of our experiments.

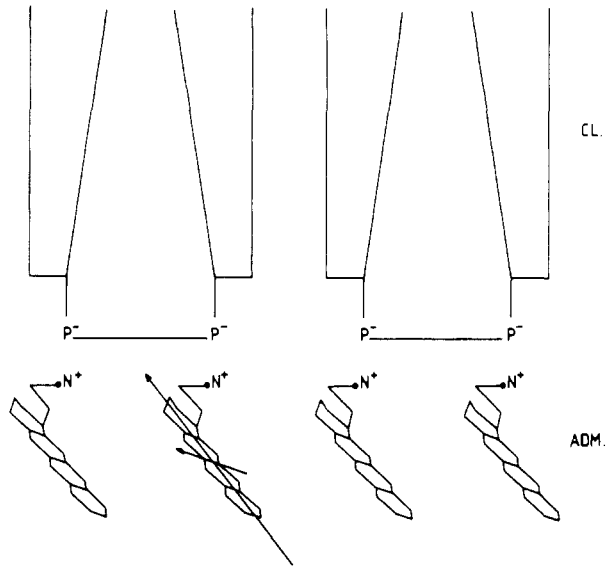

FIGURE 4: Schematic representation of the ADM–CL complex. Short and long axes have been represented by two arrows on the adriamycin molecule. Drawings of CL acyl chains delimit the hydrophobic region of the membrane and should not be considered as representative of the CL molecular structure.

Linear dichroism data obtained by ATR demonstrate that, in the complex, ADM adopts a structure compatible with the schematic model presented in Figure 4: the long axis is oriented with respect to the normal of the bilayer plane with an angle of 42° whereas the short axis is tilted at 37–40° from a normal to the bilayer plane (Table I). It is worth noting that those angles were calculated assuming a perfect ordering of the ADM molecules ($S = 1$) for the reasons explained under Results. In practice, however, the complete ordering of such a system is not likely to be reached, allowing for uncertainties about the spread of the angles. Only a conformational analysis of the structure of the ADM–CL complex supports the idea that ordering, i.e., all the ADM molecules in the same orientation, is a prerequisite for the complex formation (Goormaghtigh et al., 1986). In the absence of more experimental data, one should accept this result with caution as well as the value of the angles given in Table I. In Figure 4 we propose a schematic representation of the ADM–CL complex on the basis of this work and the previous studies. The features of

the model are the orientation of the cyclic moiety of the ADM molecules, the stoichiometric interaction between the positively charged amino groups of ADM and the negatively charged phosphate groups of CL, and the stacking of the aromatic cores of the ADM molecules represented by their parallelism (for clarity of Figure 4, the distances between interacting groups have been exaggerated). Directing the anthracycline rings toward the hydrocarbon chain of the bilayer or toward the aqueous phase was a more controversial matter. Fluorescence measurements indicate that, among a series of ADM derivatives, ADM appeared to be the least intercalated into the hydrophobic region of the bilayer and adopts a position close to the hydrocarbon–water interface characterized by a dielectric constant $\epsilon = 20$ if a mixture of egg phosphatidylcholine–dicetyl phosphate (9/1, w/w) is used (Goldman et al., 1978) or even closer to the aqueous phase ($\epsilon = 50$) if pure CL is used (Goormaghtigh et al., 1980a). Other experiments such as fluorescence polarization (Goldman et al., 1978) or fluorescence quenching by iodide (Karczmar & Tritton, 1979) showed respectively a loss of mobility and of accessibility from the aqueous phase that are compatible with either the insertion of the molecule into the hydrocarbon chain region of the bilayer or with the structure presented in Figure 4. An interesting feature of the structure suggested in Figure 4 is that the mean area occupied by one ADM molecule on the plane of the membrane is exactly half of the area occupied by a CL molecule. A continuous array of stacked ADM molecules can therefore build up while simultaneously allowing a maximum electrostatic interaction with CL for each member of the array. A recent conformational analysis (Goormaghtigh et al., 1986) also favors this mode of organization. The increased crystallinity of the ADM–CL complex (this work) and of the ADM–dipalmitoylphosphatidic acid complex (unpublished) also supports an organization in which no intercalation of the ADM molecule into the hydrocarbon chain region of the bilayer occurs. It has been reported recently (Lorenzina-Fiallo & Garnier-Suillerot, 1986) that the accessibility of the dihydroxyanthraquinone part of the ADM molecule toward a soluble form of NADH dehydrogenase is reduced by 70–80% in the presence of egg PC–CL SUV (ADM:CL molar ratio = 2:1), arguing, in the view of the authors, for the presence of two sites for the dihydroxyanthraquinone part of the ADM molecule: one buried in the bilayer and the other in the aqueous phase. In our view, a simpler interpretation of their data is also possible: assuming an equal distribution of the CL on the outside and inside leaflets of the bilayer (in fact, it tends to accumulate inside), 50% of the complexed ADM has to be inside of the liposomes, evidently inaccessible for reduction by the NADH dehydrogenase. Additional inaccessibility could be an intrinsic property of the ADM complexed as depicted in Figure 4 or could be due to the presence of MLV, which could conceal a higher proportion of CL, and thereby a higher proportion of the ADM, from the outside environment.

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REFERENCES

Bachmann, E., Weber, E., & Zbinden, G. (1975) *Agents Actions 5*, 383.

Barthelemy-Clavey, V., Maurizot, J. C., Dimicoli, J. L., & Sicard, P. (1974) *FEBS Lett. 46*, 5–10.

Bellamy, L. J. (1975) in *The Infrared Spectra of Complex Molecules*, Vol. 1 and 2, Chapman and Hall, London.

Berman, H. M., & Young, P. R. (1981) *Annu. Rev. Biophys. Bioeng. 10*, 87–114.

Burke, T. G., & Tritton, T. R. (1984) *Anal. Biochem. 143*, 135–140.

Burke, T. G., & Tritton, T. R. (1985a) *Biochemistry 24*, 1768–1776.

Burke, T. G., & Tritton, T. R. (1985b) *Biochemistry 24*, 5972–5980.

Calendi, E., Di Marco, A., Reggiani, M., Scarpinato, B., & Valentini, L. (1975) *Biochim. Biophys. Acta 103*, 25–49.

Casal, H. L., & Mantsch, H. H. (1984) *Biochim. Biophys. Acta 779*, 381–401.

Chaires, J. B., Dattagupta, N., & Crothers, D. M. (1982) *Biochemistry 21*, 3927–3932.

Cheneval, D., Müller, M., & Carafoli, E. (1983) *FEBS Lett. 159*, 123–126.

Cheneval, D., Müller, M., Toni, R., Reutz, S., & Carafoli, E. (1985) *J. Biol. Chem. 260*, 13003–13007.

Di Marco, A., Gaetani, M., & Scarpinato, B. (1969) *Cancer Chemother. Rep. 53*, 33–37.

Fringeli, U. P., & Günthard, H. H. (1981) In *Membrane Spectroscopy* (Grell, E., Ed.) pp 270–332, Springer-Verlag, Berlin.

Goldman, R., Facchinetti, T., Bach, D., Raz, A., & Shinitzky, M. (1978) *Biochim. Biophys. Acta 512*, 254–269.

Goormaghtigh, E., & Ruysschaert, J. M. (1983) *Res. Commun. Chem. Pathol. Pharmacol. 42*, 149–152.

Goormaghtigh, E., & Ruysschaert, J. M. (1984) *Biochim. Biophys. Acta 779*, 271–288.

Goormaghtigh, E., Chatelain, P., Caspers, J., & Ruysschaert, J. M. (1980a) *Biochem. Pharmacol. 2*, 3003–3010.

Goormaghtigh, E., Chatelain, P., Caspers, J., & Ruysschaert, J. M. (1980b) *Biochim. Biophys. Acta 597*, 1–14.

Goormaghtigh, E., Brasseur, R., & Ruysschaert, J. M. (1982a) *Biochem. Biophys. Res. Commun. 104*, 314–320.

Goormaghtigh, E., Vandenbranden, M., Ruysschaert, J. M., & De Kruijff, B. (1982b) *Biochim. Biophys. Acta 685*, 137–143.

Goormaghtigh, E., Pollakis, G., & Ruysschaert, J. M. (1983) *Biochem. Pharmacol. 32*, 889–893.

Goormaghtigh, E., Pollakis, G., Huart, P., Caspers, J., & Ruysschaert, J. M. (1984) *Bioelectrochem. Bioenerg. 12*, 147–153.

Goormaghtigh, E., Huart, P., Brasseur, R., & Ruysschaert, J. M. (1986) *Biochim. Biophys. Acta 861*, 83–94.

Harrick, N. J. (1967) in *Internal Reflexion Spectroscopy*, Wiley, New York.

Harteel, J. C., Duarte-Karim, M. M., Karim, D. S., & Arlandini, E. (1975) in *Adriamycin Review* (Staquet, M., & Tagnon, H., Eds.) pp 27–36, European Press, Medikon, Ghent.

Karczmar, G., & Tritton, T. (1979) *Biochim. Biophys. Acta 557*, 306–319.

Lippman, M., Zager, R., & Henderson, E. S. (1982) *Cancer Chemother. Rep. 56*, 755–768.

Lorenzina-Fiallo, M. M., & Garnier-Suillerot, A. (1986) *Biochim. Biophys. Acta 54*, 143–146.

Menozzi, M., Valentini, L., Vannini, E., & Arcamone, F. (1984) *J. Pharm. Sci. 73*, 766–770.

Minow, R. A., Banjamin, R. S., & Gottlieb, J. A. (1975) *Cancer Chemother. Rep. 6*, 195–202.

Neidle, S., & Taylor, G. L. (1979) *FEBS Lett. 107*, 348–354.

Oldham, R. J., & Pomeroy, T. G. (1972) *Cancer Chemother. Rep. 56*, 635–639.
Patel, D. J., Kozlowski, S. A., & Rice, J. A. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 3333–3337.
Praet, M., Pollakis, G., Goormaghtigh, E., & Ruysschaert, J. M. (1984) *Cancer Lett. 25*, 89–96.
Praga, C., et al. (1979) *Cancer Treat. Rep. 63*, 827–834.
Quigley, G. S., Wang, A., Ughetto, G., Van der Marel, G., Van Boom, J. H., & Rich, A. (1981) *Proc. Natl. Acad. Sci. U.S.A. 77*, 7204–7208.
Shah, D. O., & Schulman, J. H. (1965) *J. Lipid Res. 6*, 341–349.
Smulevich, G., Angeloni, L., Giovannardi, S., & Marzocchi, M. P. (1982) *Chem. Phys. 65*, 313–322.

CORRECTION

Inhibition of the Thermally Driven B to Z Transition by Intercalating Drugs, by Jonathan B. Chaires, Volume 25, Number 26, December 30, 1986, pages 8436–8439.

Page 8437. In the legend to Figure 1, the identification of the symbols used in the figure should be as follows: 40 °C (○), 25 °C (●), and 10 °C (△).