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Hybrid polar compounds produce a positive shift in the surface dipole potential of self-assembled phospholipid monolayers

Roberto Herrero [a,1], Maria Rosa Moncelli [a], Rolando Guidelli [a,*], Marcello Carlà [b], Annarosa Arcangeli [c], Massimo Olivotto [c]

[a] *Dipartimento di Chimica, Università di Firenze, Via Gino Capponi 9, 50121 Florence, Italy*
[b] *Dipartimento di Fisica, Università di Firenze, 50134 Florence, Italy*
[c] *Istituto di Patologia Generale, Università di Firenze, 50134 Florence, Italy*

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Abstract

Hybrid polar compounds (HPCs) are powerful inducers of terminal differentiation of various types of tumors, including Friend murine erythroleukemia cells (MELCs). They are known to act synergistically with an increase in the extracellular concentration of cations, which causes a positive shift in the negative value of the ionic surface potential. Two HPCs, hexamethylenebisacetamide (HMBA) and suberoylanilide hydroxamic acid (SAHA), were adsorbed on self-assembled phospholipid monolayers supported on a mercury drop and the shift in the surface dipole potential χ of the lipid film due to their adsorption was estimated from charge measurements. At their optimal concentrations for inducing MELC terminal differentiation (5 mM for HMBA and 2.6 μM for SAHA), these HPCs cause a χ shift of about 15–20 mV, positive toward the hydrocarbon tails, both on neutral phosphatidylcholine films and on negatively or positively charged phosphatidylserine films. This strongly suggests that the nonspecific effect of HPCs of different structure in inducing cancer cells to rescue their differentiation program is related to a positive χ shift on the extracellular side of the cell membrane.

Keywords: Phosphatidylserine; Phosphatidylcholine; Lipid self-assembled phospholipid monolayer supported on mercury

1. Introduction

A modern promising approach to cancer therapy attempts to induce tumor cells populations to rescue normal patterns of tissue differentiation usually impaired, but often not definitely abolished, upon the neoplastic transformation [1]. This approach, which offers the great advantage of avoiding the use of generally toxic antiblastic agents, is supported by clinical indications and sound experimental evidence that the differentiated phenotype can be restored in cancer cells by various inducers, ending malignant in vitro and in vivo behavior. Among these inducers, a class most widely effective on transformed cell lines and primary tumors are the so called hybrid polar compounds (HPCs) [2,3] (formerly named polar apolar inducers). These constitute a family of substances, whose prototypes are dimethylsulfoxide (DMSO) and hexamethylenebisacetamide (HMBA), which are powerful inducers of terminal differentiation of

* Corresponding author. Fax: +39-055-244-102; E-mail: guidelli@unifi.it

[1] On leave from University of La Coruna.

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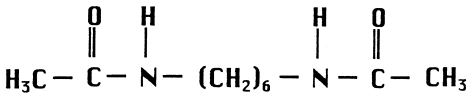
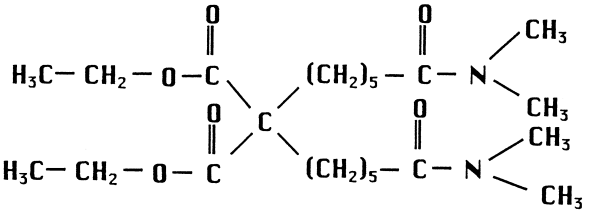
various types of tumors, including Friend murine erythroleukemia cells (MELCs). HPCs have been extensively studied by groups led by Marks and Rifkind [1,2,4,5], who synthesized two generations of variously designed compounds, gaining a fundamental insight into their structure–function relationships. To be active, HPCs must possess two or, even better, three or four uncharged polar groups of limited size connected by apolar chains of about six carbons, usually a hexamethylene chain [6]. Among the first HPCs, HMBA has been the most extensively investigated, being 30-fold more active than DMSO in inducing MELC terminal differentiation (optimal concentration: 5–10 versus 280 mM), but less active than diethyl bis-(pentamethylene-*N*,*N*-dimethylcarboxamide) malonate (EMBA) (optimal concentration 0.3 mM). In turn, the prototype of the second generation of HPCs, *N*-hydroxy-*N*′-phenyl-octane-1,8-diotic acid diamide (suberoylanilide hydroxamic acid, SAHA) is 2000-fold more active than HMBA, producing the maximum differentiating activity at 2.5 μM. Fig. 1 shows the structural formulas of HMBA, EMBA and SAHA.

HMBA



EMBA



SAHA

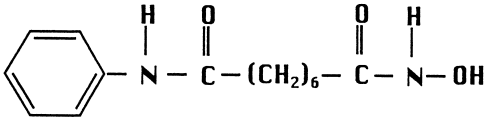


Fig. 1. Structural formulas of HMBA, EMBA and SAHA.

The mechanism of action of HPCs has been the subject of innumerable studies, with only the substantial agreement that their target is the plasma membrane (see [7] and references therein). It was observed that MELC commitment to terminal erythroid differentiation is brought about by HPCs of either the first (DMSO, HMBA, EMBA) or the second generation (SAHA) synergistically with cation addition to the culture medium, depending on the valence but not on the nature of the cations [8]. It was therefore suggested that HPCs and cations have a similar interfacial effect at the plasma membrane level, consisting in a change of the surface potential in the same direction and in the resulting modulation of voltage-sensitive proteins signaling the cell commitment.

As a first step toward the unraveling of the purely interfacial activity of HPCs, the adsorption and electrostatic effects of DMSO and HMBA were investigated at the surface of a mercury electrode, in a physical apparatus [9], leaving aside the complexity of biological membranes. The interfacial behavior of DMSO and HMBA was compared with that of a typical apolar aliphatic compound, 1-octanol, which has a hydrophobic moiety similar to that of HMBA but a much smaller dipole moment and is unable to act as a MELC inducer. Both HMBA and 1-octanol were found to adsorb on the mercury surface because of hydrophobic forces, with very similar free energies of adsorption. However, the ratio of polar to apolar moieties in HPCs turned out to be crucial in shifting their adsorption maximum toward physiological values of membrane surface charge densities (−5 to −10 μC cm^{-2}), where 1-octanol is desorbed. At their effective concentrations for cell differentiation and within the physiological range of membrane charge densities, both DMSO and HMBA produced a shift in the surface dipole potential that was positive toward the mercury surface for charge densities ≥ −5

μC cm^{-2} and negative for more negative charge densities. As a whole, it was calculated that, for cells having a surface charge density of about −5 μC cm^{-2}, addition of optimal concentrations of DMSO (280 mM) and HMBA (5 mM) was expected to produce a positive shift of 10–20 mV in the surface potential, causing an equivalent negative shift in the transmembrane potential. This effect was in the same direction and of comparable magnitude as that produced by the excess of cations of various species and valence to be added in the culture medium in order to potentiate the effects of suboptimal doses of the HPCs [8]. To fit these data to the hypothesis pointed out above, the potential shift caused by HPCs was assumed to be sufficient to modulate the signaling proteins of cell commitment to differentiation. Proteins of this sort have been identified [10–12] and may also include GTP-binding proteins.

The experimental validation of this hypothesis would open new perspectives in the interpretation of the action mechanisms of HPCs and other inducers, crucially contributing to the development of members of this family with higher therapeutic activity and lower general toxicity. An essential step in this direction is the verification of the above interfacial effects of HPCs in biomimetic membranes.

This work reports measurements of changes in surface dipole potential following the adsorption of HPCs of the first and second generation on phospholipid monolayers mimicking simplified plasma membranes. These measurements were carried out by a novel procedure that makes use of a biomimetic membrane consisting of a phospholipid self-assembled monolayer supported on a hanging mercury drop electrode (HMDE) [13–15]. This lipid monolayer behaves like a half-membrane over a potential range from −0.2 to −0.8 V versus the saturated calomel electrode (SCE), where it is impermeable to inorganic ions. The differential capacity of this lipid film is about twice the value for a black lipid membrane (BLM). The procedure consists in varying the area of the lipid-coated mercury drop in contact with an aqueous solution, while constantly maintaining the drop neck in contact with the lipid-covered surface of the solution, and in measuring the charge flowing as a consequence of such a change in area [16].

The results obtained by this technique confirm and extend the data previously obtained on a bare mercury surface, showing that HPCs produce on biomimetic membranes surface dipole potential shifts of the same sign and magnitude.

2. Experimental

The water used was obtained from light mineral water by distilling it once and by then distilling the water so obtained from alkaline permanganate, while discarding the heads. Merck suprapur grade KCl was baked at 500°C before use to remove any organic impurities. Dioleoylphosphatidylcholine (DOPC) and dioleoylphosphatidylserine (DOPS) were obtained from Lipid Products (South Nutfield, Surrey, UK). DMSO and HMBA were obtained from Sigma, St. Louis, MO, USA. EMBA and SAHA were kindly donated by Dr. R.A. Rifkind (Sloan-Kettering Institute, New York, NY, USA). All measurements were carried out in aqueous solutions of 0.1 M KCl at 25 ± 0.1°C. The solutions were buffered at pH 7.5 with a 1 mM $HPO_4^{2-}/H_2PO_4^-$ buffer when using DOPS, and unbuffered when using DOPC; in the latter case the pH was constantly found to range between 4.8 and 5.3.

Differential capacity measurements were carried out using a Metrohm Polarecord E506 (Herisau, Switzerland). The ac signal had a 10 mV amplitude and a 75 Hz frequency. The system was calibrated using a precision capacitator. All potentials were measured versus a SCE. Capacitative charge measurements were carried out by a computerized chronocoulometric apparatus described elsewhere [17]. The microprocessor used to control the operations was a Model NOVA 4X from Data General (Westboro, MA, USA), whereas an Amel Model 559 (Milan, Italy) potentiostat was employed for the potentiostatic control of the three-electrode system. The detailed scheme of the home-made electronic current integrator working under microprocessor control is described elsewhere [18].

Use was made of a home-made HMDE described elsewhere [19], which allows appreciation of the changes in the drop area of as little as 0.04 mm^2 and to obtain highly reproducible drops throughout the piston movement. The capillary and the mercury reservoir were thermostated at 25 ± 0.1°C by the use

of a water-jacketed box to avoid any changes in the drop area due to a change in temperature. A homemade glass capillary with a finely tapered tip, about 1 mm in outer diameter, was employed [16]. The coating of the HMDE with a lipid monolayer was carried out following the procedure of Lecompte and Miller [20,21]. The tip of the capillary was positioned just above the surface of the electrolyte solution, on which the lipid material dissolved in pentane had been previously spread. The contact between the mercury drop and the solution was then realized by simply extruding a mercury drop manually from the capillary while taking care to maintain the contact of the drop neck with the lipid reservoir on the solution surface by avoiding wetting the glass tip. This allows a free exchange of lipid material between the lipid monolayer that coats the mercury drop and the lipid film spread on the solution. This procedure ensures that the monolayer maintains its properties, including its thickness, as the drop is expanded or compressed. The best conditions for obtaining reproducible results were achieved by spreading on the solution surface an amount of lipid corresponding to about 6–7 monolayers. This amount is greater than that normally required, 3–4 monolayers, in preparing lipid-coated mercury drops fully immersed in the solution by the procedure of Nelson and Benton [14].

3. Results

The effect of the adsorption of three different HPCs on self-assembled monolayers of DOPC and DOPS supported on mercury was investigated by two different procedures. The first procedure consists of measuring the charge density σ_M on the mercury surface at a constant applied potential E both before and after adding a given concentration c of the HPC to the aqueous solution. The charge density was measured by shrinking the drop area manually at a constant applied potential E by means of the micrometric head and by integrating analogically the capacitative current flowing along the external circuit as a consequence of this contraction with the chronocoulometric apparatus [16]. Values of the charge Q as a function of time t were stored in the microcomputer memory and then plotted. A typical Q versus t curve obtained at −0.500 V/SCE by shrinking a DOPS-coated mercury electrode in a pH 7.5 buffered solution of 0.1 M KCl is shown in Fig. 2. In this figure, the abrupt increase in charge occurring in a period of about 100 ms is the capacitative charge that accompanies the manual contraction of the mercury drop. The sloping straight lines preceding and following this abrupt increase in charge are due to the integration of a modest constant faradaic current flowing across the lipid monolayer while the drop

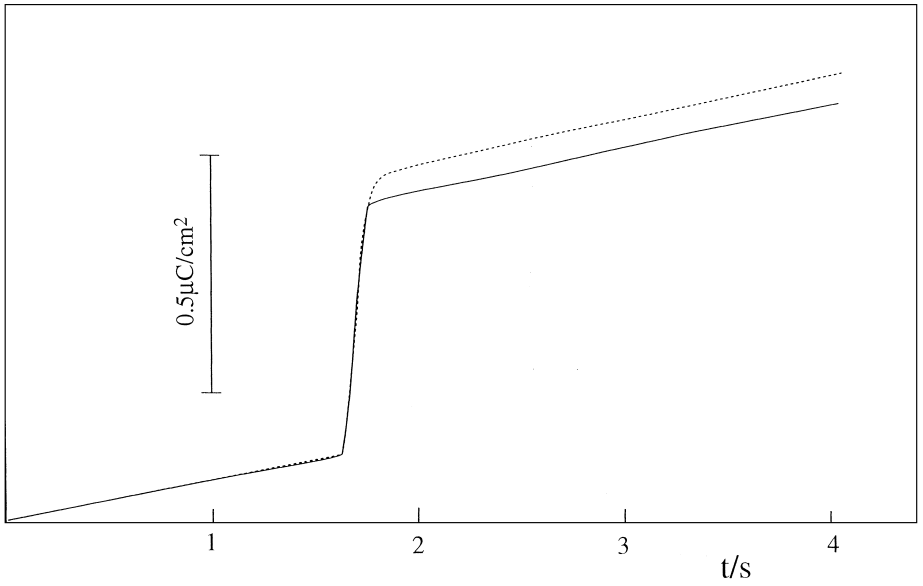


Fig. 2. Charge versus time curves obtained at −0.500 V/SCE by shrinking a DOPS-coated mercury electrode in a pH 7.5 buffered solution of 0.1 M KCl both in the absence (solid curve) and presence of 5 mM HMBA (dashed curve).

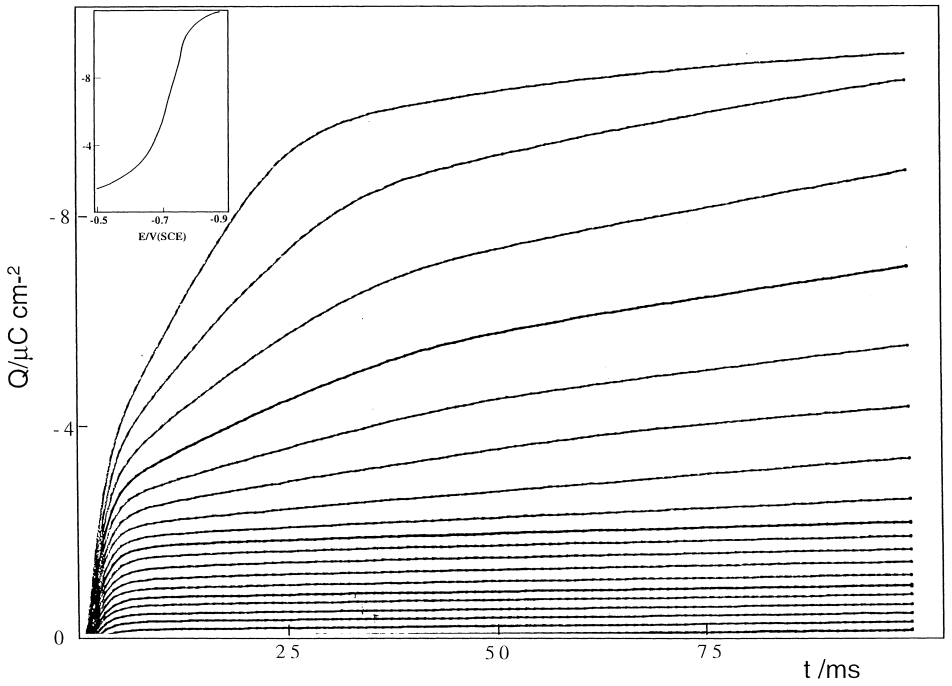


Fig. 3. Charge versus time curves at a DOPC-coated mercury electrode in 0.1 M KCl+0.4 mM EMBA obtained by stepping from a fixed initial potential $E_i = -0.300$ V/SCE to a final potential E_f varying from -0.330 to -0.880 V by -0.25 mV increments. Proceeding upwards, the curves refer to progressively more negative E_f values. The inset shows a plot of the charge versus E_f at a constant electrolysis time, $t = 100$ ms.

area is kept constant. The capacitative charge Q was measured by the vertical separation between the two sloping straight lines. The charge density σ_M was obtained by dividing Q by the corresponding change in area. The reliability of the method was tested as described in [16] by measuring the charge densities $\sigma_M(E_1)$ and $\sigma_M(E_2)$ at two different potentials E_1 and E_2, chosen over the potential range of the very flat capacity minimum of the lipid monolayer; the potential-independent differential capacity C along this minimum, as measured on a fully immersed and uncompressed HMDE, was found to be practically equal to $[\sigma_M(E_2)-\sigma_M(E_1)]/(E_2-E_1)$, thus confirming the consistency between the measurements at these two different electrodes.

Each chronocoulometric measurement was carried out on a freshly formed lipid-coated mercury drop. The high sensitivity of this method allowed us to measure charges with a precision of ± 0.01 μC/cm^2. It should be noted that with pure lipid monolayers, practically identical charge density values are obtained by expanding or shrinking the drop surface. However, with lipid monolayers adsorbing or incorporating a substance present in the aqueous solution, the shrinking mode is definitely to be preferred. In fact, one cannot be sure that the lipid material being transferred from the disordered and thick lipid reservoir spread on the solution surface to the drop surface during its expansion will immediately contain the same concentration of the compound as that originally present in the lipid monolayer at equilibrium: slow diffusion from the bulk solution may prevent such a rapid equilibration.

Charge measurements were carried out at -0.500 V, just in the middle of the potential range from ~ -0.2 and ~ -0.8 V over which the lipid monolayer behaves like a half-membrane. To ensure that adsorption equilibrium was reached, after immersing the drop in the solution with its neck in contact with the lipid film on the solution surface, the solution was stirred mildly for 3 min; in the absence of the HPC, stirring was found to have no appreciable effect. Each series of measurements consisted of six to eight charge measurements on different drops in the absence of the HPC and of an equal number of measurements after HPC addition to the solution. The

arithmetic mean of the σ_M values obtained in the presence of the HPC was then subtracted from that obtained in its absence, to yield a $\Delta\sigma_M$ value. The charge density σ_M at -0.500 V/SCE on a DOPS-coated mercury electrode in the absence of the HPC was -0.35 ± 0.02 μC cm^{-2} at pH 4, -0.52 ± 0.01 μC cm^{-2} at pH 7.5 and -0.54 ± 0.01 μC cm^{-2} at pH 8.5. On a DOPC-coated mercury electrode in a 0.1 M KCl unbuffered solution without HPC the charge density at -0.500 V/SCE was -0.75 ± 0.02 μC cm^{-2}. In over 18 series of measurements the addition of 5 mM HMBA produced a shift $\Delta\sigma_M = -0.04 \pm 0.01$ μC cm^{-2} in the charge density. This shift was found to be the same both for DOPS over the pH range from 4 to 8.5 and for DOPC in unbuffered solution, within the limits of experimental accuracy.

$\Delta\sigma_M$ shifts produced by the addition of 2.6 μM SAHA were measured by the same procedure. Sufficiently accurate results were obtained at -0.500 V on a DOPS-coated mercury electrode over the pH range from 7.5 to 8.5, where a $\Delta\sigma_M$ value of -0.03 ± 0.01 μC cm^{-2} was estimated. No $\Delta\sigma_M$ measurements could be carried out with EMBA because it readily permeates the lipid film and is electroreduced on the electrode surface. Fig. 3 shows a number of charge versus time curves following a series of potential steps from a fixed initial value $E_i = -0.300$ V/SCE, positive enough to exclude EMBA electroreduction, to progressively more negative values, E_f. The inset of Fig. 3 shows a plot of the charge versus E_f at a constant electrolysis time $t = 100$ ms. A well defined reduction wave is apparent. The slightly sigmoidal charge versus time curves in Fig. 3 are indicative of a process of nucleation and growth that accompanies EMBA electroreduction [22]. The peculiar behavior of the EMBA molecule is to be ascribed to its high number of methyl and ethyl groups, which impart it a sufficiently high lipophilicity. Its electroactivity is probably to be ascribed to a keto-enol tautomeric resonant structure with conjugated double bonds.

The change, $\Delta\chi$, in the surface dipole potential produced by the adsorption of a HPC can be readily estimated from the magnitude of the corresponding $\Delta\sigma_M$ value. The HPC molecules are adsorbed on top of the polar heads of the lipid film, as depicted schematically in Fig. 4, or, at most, they may slightly penetrate the outermost portion of the polar heads.

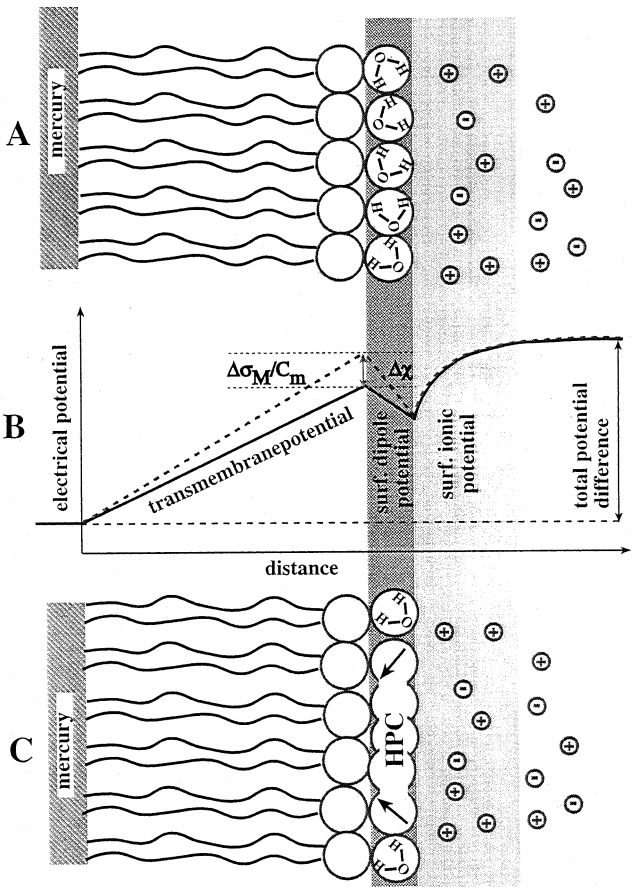


Fig. 4. Schematic picture of the adsorption of a HPC on top of a lipid monolayer supported on mercury. (A) and (C) schematize the interfacial region in the absence and in the presence of a HPC molecule. (B) schematizes the potential profile across the interphase at constant applied potential in the absence of HPC (solid curve) and in the presence of a HPC producing a positive shift, $\Delta\chi$, in the surface dipole potential (dashed curve).

Hence, the $\Delta\chi$ change produced by these molecules is located somewhere in the above region. On the other hand, by far the major contribution to the potential difference, $\Delta\phi$, across the whole interphase stems from the potential difference across the hydrocarbon tail region. If the applied potential E and the electrolyte concentration c are kept constant, the small contribution from the surface ionic potential, ψ, across the diffuse layer remains practically unchanged upon addition of the HPC. In fact, according to the Gouy–Chapman theory, ψ is only a function of c and of the overall charge density experienced by the diffuse-layer ions. The change in this charge density is practically equal to the very small $\Delta\sigma_M$ change, since the adsorbed HPC molecules are neutral; hence, under

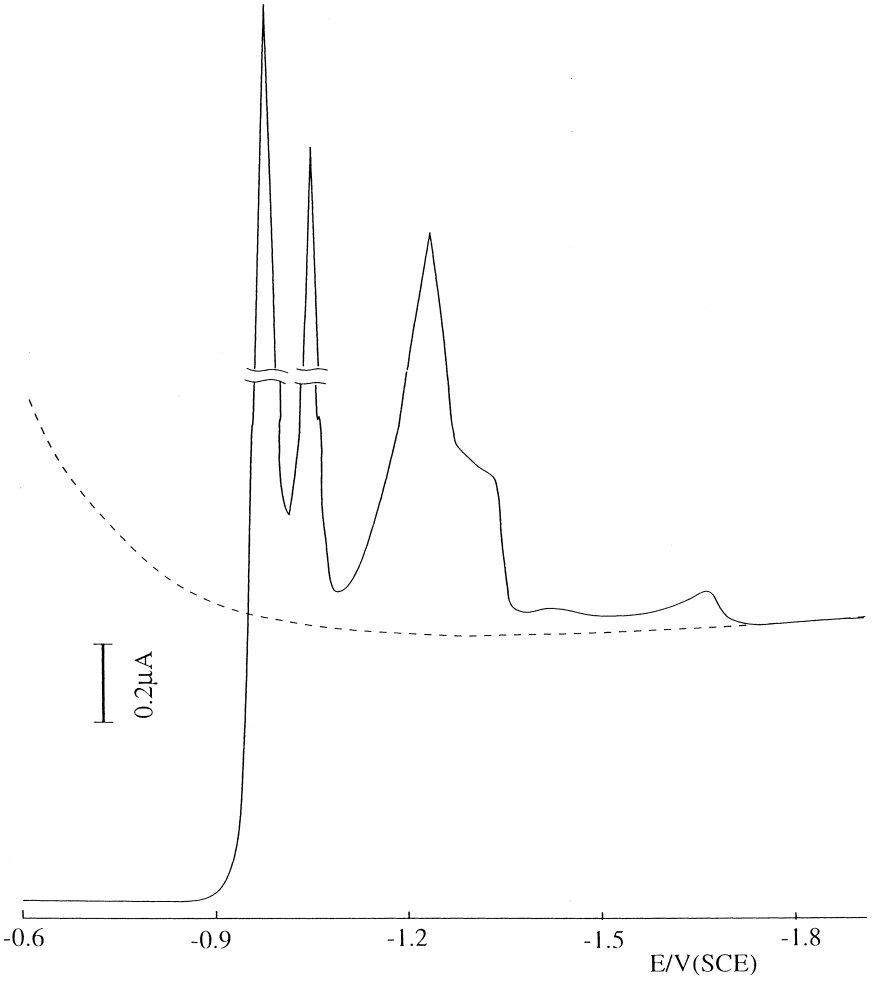


Fig. 5. Curves of the differential capacity C against E at an uncoated (dashed curve) and a DOPC-coated mercury electrode (solid curve) in aqueous 0.1 M tetramethylammonium chloride.

the most unfavorable experimental conditions (i.e. for an uncharged lipid such as DOPC), ψ in a 0.1 M KCl solution amounts to −10.3 mV for $\sigma_M = -0.75$ μC cm^{-2} and to −10.9 mV for $\sigma_M = (-0.75–0.04)$ μC cm^{-2}, with a net change $\Delta\phi = -0.6$ mV upon HMBA addition. At a constant applied potential $E = \Delta\phi$+constant, the change $\Delta\chi$ following the HPC addition is therefore practically compensated for by an equal and opposite change in the potential difference across the hydrocarbon tail region, which is given by $\Delta\sigma_M/C_m$, where C_m is the differential capacity of this region (see Fig. 4). To a satisfactory degree of approximation C_m can be identified with the differential capacity of the whole lipid film; for a DOPS monolayer it equals 1.6 μF cm^{-2} at pH 4, 1.7 μF cm^{-2} at pH 7.5 and 1.8 μF cm^{-2} at pH 8.5, whereas for a DOPC monolayer in an unbuffered aqueous medium it equals 1.7 μF cm^{-2}. Within the limits of accuracy of the $\Delta\sigma_M$ measurements, the $\Delta\chi$ change produced by 5 mM HMBA on both DOPS and DOPC monolayers can therefore be roughly estimated at +20 mV (positive in the direction of the hydrocarbon tails), whereas that produced by 2.6 μM SAHA is slightly smaller, ∼+15 mV. The comparable effect exerted by HMBA and SAHA, in spite of the large difference in their concentrations, is not surprising. In fact, the adsorptivity of a surfactant from an aqueous solution is higher the lower its solubility is and SAHA is much less soluble than HMBA. Thus, for instance, the adsorption properties of *n*-aliphatic alcohols of different chain length on mercury are similar once they are

compared for a constant ratio of their concentration to the corresponding saturation value [23].

An alternative procedure to measure σ_M and, hence, the $\Delta\sigma_M$ change accompanying HMBA adsorption, was adopted. Even though the limits of its accuracy were insufficient to appreciate the above small $\Delta\sigma_M$ changes, it served to understand some features of HPC adsorption on lipid-coated mercury. The procedure is based on the observation that the DOPC film is completely desorbed at sufficiently negative potentials. This is confirmed by the curves of the differential capacity C versus potential on a DOPC-coated mercury electrode merging with those on a bare mercury electrode in the same electrolyte, at far negative potentials. Using 0.1 M NaCl, KCl or CsCl electrolytes the merging takes place at about −1.840 V, where the quadrature component of the electrode admittance starts to be affected by an incipient faradaic current due to electroreduction of the alkali metal ions to the zero-valent state and their dissolution in the mercury with amalgam formation. Under these conditions the quadrature component of the faradaic admittance cannot, therefore, be exactly identified with ωC, where ω is the angular frequency. To realize the merging of the C versus E curves for an uncoated and a DOPC-coated mercury electrode over a satisfactorily broad potential range, the use of 0.1 M LiCl was then attempted. In fact, lithium ion is discharged on mercury at potentials by 0.2 to 0.3 V more negative than the other alkali metal ions [24]. This attempt failed because the C versus E curve on DOPC-coated mercury runs practically parallel to that on uncoated mercury over the potential range from −1.680 to −2.100 V, but remains constantly lower by 2.6 μF cm^{-2} up to Li^+ discharge. Satisfactory results were obtained using 0.1 M tetramethylammonium (TMA^+) chloride. With this electrolyte the C versus E curves for the uncoated and DOPC-coated mercury merge over a potential range from −1.740 to −2.100 V (see Fig. 5), before the onset of a detectable hydrogen evolution from water reduction.

The potential of zero charge (pzc) of an uncoated mercury electrode in aqueous 0.1 M TMACl equals −0.450 V [25]. The charge following a potential step from −0.450 to −1.850 V at this electrode, as measured by the chronocoulometric technique, amounts to -23.2 ± 0.1 μC cm^{-2}. This is, therefore, the charge density, σ_M, at −1.850 V not only at an uncoated electrode, but also at an initially DOPC-coated electrode, in view of the complete desorption of the lipid film at this potential. The charge Q following the same potential step from −0.450 to −1.850 V on a DOPC-coated electrode in 0.1 M TMACl equals -22.4 ± 0.1 μC cm^{-2}. Hence, the charge density σ_M at −0.450 V on a DOPC-coated electrode equals -23.2 μC cm^{-2} $-Q = -0.8 \pm 0.1$ μC cm^{-2}, in fairly good agreement with the value -0.75 ± 0.02 μC cm^{-2} obtained at −0.500 V by the shrinking-drop procedure. However, the present potential-step procedure has an inherently lower accuracy, since the measured quantity results from the difference between two much larger quantities. Therefore, it is unsuitable for estimating the small $\Delta\sigma_M$ change following HMBA addition to the solution.

Nonetheless, if the potential applied to a DOPC-coated electrode is first stepped from a value within the range of stability of the lipid monolayer to a value negative enough to cause complete lipid desorption and is then stepped back, the lipid film is readsorbed on the mercury electrode. The potential induced desorption of the lipid film and its subsequent readsorption are highly reproducible, even if the time elapsed between the forward and the backward step is of the order of several minutes. Thus, even though the reproducibility of the charge Q following the $-0.45 \rightarrow -1.85$ V step is about ± 0.1 μC cm^{-2} when passing from one drop to another, it is about ± 0.02 μC cm^{-2} when Q is measured a second time on the same drop after readsorbing the lipid monolayer. This behavior is analogous to that exhibited by an insoluble monolayer of stearic acid on a single crystal gold electrode [26] and by alkanethiol monolayers on mercury [27]. Optical measurements demonstrated that the potential-induced desorption of the film of stearic acid involves the formation of micelles, which can remain in the proximity of the electrode for hours and later spread back onto the electrode surface without any loss of material [26]. It was postulated that the micelles trap the charge of cations from the diffuse layer and become attracted to the electrode surface by electrostatic forces. The different behavior exhibited by the various alkali metal ions and by TMA^+ in the desorption of the DOPC film from mercury seems to support this hypothesis. Thus, it has been reported that the cation

interaction with PC vesicles increases with an increase in their hydration free energy[28–30] and hence in the order $TMA^+ < Cs^+ < K^+ < Na^+ < Li^+$. If this is also true with DOPC micelles, then their surface charge in the presence of Li^+ ions may be high enough to prevent their complete detachment from the electrode surface, with formation of hemimicelles; this results in a differential capacity lower than that on a bare electrode even at far negative potentials, as actually observed. Conversely, the low hydration free energy of TMA^+ may explain why in its presence the C versus E curve on a DOPC-coated electrode merges with that on a bare electrode at a more positive potential than in the presence of alkali metal ions.

The DOPC-coated electrode to be subjected to the $-0.45 \rightarrow -1.85$ V potential step is normally obtained by immersing the mercury drop into an aqueous electrolyte with a lipid film spread on its surface. When using this procedure, two successive Q measurements on the same drop in the same solution differ by no more than ± 0.02 μC cm^{-2} both in the absence and in the presence of 5 mM HMBA. The DOPC-coated electrode may also be obtained by a procedure whose first stage is the same as for the shrinking-drop procedure; it consists of first touching the solution surface gently with the drop so as to allow the lipid film to cover the drop surface by capillarity except for the drop neck and then immersing the drop completely into the aqueous solution. In this case, two successive Q measurements on the same drop still differ by no more than ± 0.02 μC cm^{-2} in the absence of HMBA. Conversely, when in the presence of HMBA, the second Q measurement carried out on the same drop, after desorption and readsorption of the lipid film, is systematically more negative than the first Q measurement carried on the newly formed DOPC-coated drop by about 0.05 ± 0.02 μC cm^{-2}. The above difference in behavior is to be ascribed to the two different procedures adopted to form a fully immersed DOPC-coated mercury drop. When the drop is immersed directly into the solution across the lipid film, it comes into direct contact with the aqueous solution, albeit for a very short time, before the lipid material dragged by the drop into the solution self-assembles on its surface. Hence, if some species present in the solution has a strong tendency to be adsorbed on the bare mercury surface, it may be trapped between this surface and the lipid monolayer. This event is to be excluded if the drop touches the lipid film spread on the solution surface, thus allowing this film to move along the solution surface by capillarity, before immersing the drop completely into the solution. With the latter procedure, when the lipid monolayer is desorbed at -1.850 V and then readsorbed, any strong surfactant present in the solution will be adsorbed on the bare mercury surface before complete lipid readsorption and, hence, will be trapped between the mercury surface and the lipid monolayer. The charge Q measured on a newly coated mercury drop will then be different from that measured on the same drop after readsorption of the lipid monolayer. The fact that, when in the presence of HMBA, the charge Q measured after readsorption is systematically more negative by about 0.05 ± 0.02 μC cm^{-2} than that previously measured on the newly coated drop, implies that σ_M at -0.45 V is more positive by the same amount. To produce this effect, the HMBA molecules trapped between the mercury surface and the lipid film must be adsorbed with the negative end of their dipole toward the mercury. In fact, at constant applied potential, this negative contribution must be compensated for by a positive shift of σ_M.

4. Discussion

The change $\Delta\chi$ in surface dipole potential caused by HMBA adsorption on neutral DOPC films as well as on negatively or positively charged DOPS films is always positive and amounts to about +20 mV. This behavior can be explained by considering the natural tendency of surfactant molecules to be adsorbed in those orientations that favor the formation of hydrogen bonds with the adjacent water molecules of the aqueous phase. The hydrogen bonds that can be possibly formed have the following energies [31]: 5.9 ± 0.2 kcal/mol for OH···O; 2.6 kcal/mol for C–H···O; 4–7 kcal/mol for OH···N; 2–3 kcal/mol for NH···O. Compatibly with steric constraints, the adsorbed HMBA molecules will therefore tend to direct both the $C^{\delta+}=O^{\delta-}$ and the $H^{\delta+}$–$N^{\delta-}$ dipoles with their negative ends toward the solution, creating a surface dipole potential positive toward the membrane. Analogous conclusions apply to SAHA.

Interfacial tension measurements of HMBA adsorption on a bare mercury electrode also point to an orientation of the HMBA molecules with the positive end of their dipoles toward the mercury [9]. However, at charge densities σ_M on mercury negative of about -8 μC cm^{-2}, the water dipoles adsorbed on the bare mercury surface are so strongly oriented with their positive end toward the metal that their displacement by one HMBA molecule causes the net $\Delta\chi$ value to pass from positive to negative values.

When the HMBA molecules are trapped between the mercury surface and the hydrocarbon tails of the lipid monolayer, no hydrogen bonds between these molecules and water molecules can be formed. Conversely, the tendency of the mercury surface atoms to interact, albeit weakly, with oxygen atoms comes into play, causing the oxygens of the two C=O groups of HMBA to turn toward the metal. In fact, it is now generally accepted that at the mercury–water interface the adsorbed water molecules have a slight but appreciable tendency to turn their oxygens toward the mercury at $\sigma_M = 0$, i.e. in the absence of an external electric field [32]. The above considerations justify the negative shift in Q after readsorption of the lipid film in the presence of HMBA.

As already mentioned, the effect of HPCs in rescuing the differentiation program in MELCs is synergistic with that of an increase in cation concentration in the culture medium [8]. The positive shift $\Delta\chi$ in the surface dipole potential caused by the adsorption of HPCs on lipid monolayers provides a straightforward explanation for such a synergy. The increase in the cation concentration causes a decrease in the absolute value of the negative surface ionic potential ψ on the extracellular side of the negatively charged plasma membrane. This positive shift, $\Delta\psi$, in the negative ψ value has the same effect on the transmembrane potential as an equivalent positive shift $\Delta\chi$ in the surface dipole potential produced by the adsorption of a HPC on the extracellular side of the plasma membrane. Three of the present authors et al. [8,9], in comparing the experimental $\Delta\chi$ shift caused by HMBA adsorption on a bare mercury surface with the effect of this HPC on MELCs, tentatively ascribed a limiting value of about -10 μC cm^{-2} to the charge density σ_M on the membranes of these cells. For this negative charge density, the $\Delta\chi$ shift on bare mercury upon HMBA adsorption turns out to be negative for the reasons already pointed out. Even in this case, however, the synergy between HMBA adsorption and an increase in cation concentration was conceivable by assuming an equilibration of the HPC molecules on the two sides of the plasma membrane and a slightly more negative charge density on its intracellular side. These two assumptions lead to the prediction of a more negative $\Delta\chi$ shift toward the membrane interior due to HMBA adsorption on the intracellular side than on the extracellular one: this has the same effect on the transmembrane potential as a positive $\Delta\psi$ shift due to cation addition on the extracellular side.

The charge density σ_M on plasma membranes varies from cell to cell and cannot be estimated with a high degree of accuracy. The σ_M values on the DOPS monolayers supported on mercury employed in this work were estimated at different pH values from differential capacity and σ_M measurements [33] on the basis of a model of the interphase and of the Gouy–Chapman theory. The charge density σ_M so estimated is almost equal to -5 μC cm^{-2} at pH 7.5 and to -12 μC cm^{-2} at pH 8.5 in 0.1 M KCl. These values are definitely less negative than those estimated on vesicles, dispersions, monolayers and BLMs. Exhaustive reference to the literature and a possible explanation for these discrepancies can be found in Moncelli et al. [15]. However, even ascribing to the charge density of a PS monolayer its maximum value of one electronic charge/(60 Å^2) = -26.5 μC cm^{-2}, a typical 20% of PS in a biomembrane would impart a charge density of -5.3 μC cm^{-2}. Naturally, biomembranes bear further negative charges, such as those of sialic acid in gangliosides or in N-linked oligosaccharides, but these charges protrude outside the membrane body and are effectively screened by cations. Hence, they should have a minor effect on adsorbing neutral molecules such as HPCs. Moreover, even ascribing an effective negative charge density of -10 μC cm^{-2} to a biomembrane, it is quite improbable that the water dipoles to be displaced by one adsorbing HPC molecule should be oriented with their positive end pointing toward the membrane plane as regularly as on a smooth mercury surface. The negative contribution to $\Delta\chi$ resulting from their displacement by one HPC molecule is therefore expected to be smaller on a lipid monolayer

than on Hg, for the same negative σ_M value. This may explain the net positive $\Delta\chi$ shift caused by HMBA adsorption on a DOPS-coated mercury electrode in 0.1 M KCl at pH 8.5, where σ_M is about equal to -12 μC cm^{-2}.

In conclusion, the nonspecific effect of HPCs of different structure in inducing cancer cells to rescue their differentiation program is quite probably related to a positive shift in the surface dipole potential on the extracellular side of the cell membrane. This conclusion has far reaching implications for studies aimed at designing novel compounds of the HPC family and for the evaluation of the therapeutic index of some of them scheduled for clinical trials.

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