A laser-T-jump study of the adsorption of dipolar molecules to planar lipid membranes

I. 2,4-dichlorophenoxyacetic acid

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Abstract. The adsorption of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) as well as of other dipolar molecules to the interface of artificial lipid membranes gives rise to a change of the dipole potential between the membrane interior and water. As a consequence of the adsorption of the neutral species, the conductance of planar membranes, observed in the presence of the macrocyclic ion carriers nonactin or valinomycin, may change by many orders of magnitude. Using this effect in combination with a laser-T-jump technique, the kinetics of the adsorption process were measured and were interpreted on the basis of a Langmuir-isotherm. A partition coefficient (at small concentrations) of \( K_{pA} = 4.7 \times 10^{-4} \) cm, a rate constant of desorption \( k_D = 100 \text{ s}^{-1} \) and a maximum surface density \( N_D = 7.7 \times 10^{13}/\text{cm}^2 \) were found. The concentration at half saturation is \( K_p = 2.7 \times 10^{-4} \) M.

Using these values the membrane conductance induced by the ion carrier nonactin and the shape of the current-voltage relationship as a function of the ligand concentration in water was analyzed. A maximum dipole potential of \( V_{D_{\text{max}}} = -239 \text{ mV} \) and a contribution of \( b = 3.1 \times 10^{-15} \text{ Vcm}^2 \) per single adsorbed 2,4-D molecule was found. 74% of the dipole potential acts on the inner membrane barrier separating the two interfacial adsorption planes of nonactin. The remainder (26%) favours interfacial complex formation between nonactin and \( \text{K}^+ \) from the aqueous phase. The data hold for membranes formed from dioleoyllecithin in n-decane.

Key words: Adsorption, lipid membranes, laser-T-jump, Langmuir isotherm, 2,4-D

Introduction

The adsorption of lipophilic molecules to biological membranes is of great importance for their permeability properties. The measurement of the concentration of adsorbed molecules as a function of the free concentration in water has, however, turned out to be difficult. Therefore, starting with the pioneering work of Collander and Bärlund (1933) the membrane concentration on nonelectrolytes is usually estimated via the oil/water partition coefficient, thus avoiding a direct determination.

The present study deals with adsorption to an artificial membrane system. Planar lipid membranes have proven to be an excellent in vitro-system for the investigation of mechanisms of ion transport across biological membranes. It was found, however, that these membranes can also be used to study the behaviour of nonelectrolytes which, as a consequence of their adsorption to the membrane interface, modify the electrical properties of the membrane. The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) (Smejtek and Paulis-Ilangasekare 1979 a,b) and phloretin, the inhibitor of certain routes of transport across biological membranes (Andersen et al. 1976; Melnik et al. 1977; Reyes et al. 1983) as well as other dipolar molecules (McLaughlin 1973; Szabo 1974) were found to modify the dipole potential at the membrane/water interface. As a result, the membrane permeability for hydrophobic ions and for alkali ions in the presence of macrocyclic ion carriers may be changed by many orders of magnitude.

We use this effect to study the kinetics of adsorption of dipolar molecules using a laser-T-jump method. The data are analyzed on the basis of Langmuir's equation. The maximum number of available sites at the interface, the aqueous concentration at half-saturation and the contribution of a single adsorbed molecule to the dipole potential were obtained. In favourable cases the method also allows one to determine the rate constants of adsorption and desorption.

The present paper concentrates on the methodological aspects, illustrating the procedure with the herbicide 2,4-D. In a second paper the adsorption of...
phloretin will be considered and compared with a series of analogues which show only small structural differences (Reyes et al. 1983). The method allows one to study the effect of substituents on membrane adsorption in a more detailed way than has so far been possible.

Experimental

The experiments were performed on optically black lipid membranes made from a 1% solution of 1,2-dioleoyllecithin (Avanti Polar Lipids) in n-decane (Fluka, standard for gas chromatography). The aqueous solutions normally contained 0.1 M KCl, 0.9 M LiCl, 10 mM buffer and 10⁻⁷ M nonactin (Boehringer). In some experiments valinomycin (Boehringer) was used instead of nonactin. It was added to the membrane forming solution (10⁻³ M). The solutions also contained appropriate concentrations of either 2,4-dichlorophenoxyacetic acid (2,4-D) (Aldrich) or phloretin (Sigma) added from stock solutions in ethanol. Most of the experiments in the presence of 2,4-D (pK ≈ 2.7) were performed at pH 2 (phosphate buffer). In the case of phloretin (pK = 7.35) the pH was adjusted to 5 (acetate buffer). The pH was selected to ensure that the substances were largely in the protonated (neutral) form. The PTFE-cuvette used for bilayer formation was cleaned after every experiment (ethanol in case of phloretin, chromic acid in the case of 2,4-D). The laser-T-jump method as applied to planar lipid membranes was described in previous publications (Brock et al. 1981; Stark et al. 1986). In brief, the temperature of the membrane and of the surrounding aqueous phase is increased by a few tenths of a degree (typically 0.2°-0.3 °C) by absorption of a high intensity infrared flash (wavelength 1.06 μm), which is produced by a Nd-glass laser (JK Lasers Ltd. GB). The response of the electric current was observed at constant applied voltage. The current transient after amplification was stored in a digital oscilloscope (Tektro-nix 7603/7D20, 1024 words memory size, 8 bit resolution). The time range of the method is limited by the width of the laser flash (400 μs in the fixed Q mode of the laser) and at long times by the temperature stability of the membrane and its environment (limited by heat conduction and convection to about 5 s). The heating time can be reduced to 40 ns by using the Q-switch mode of the laser. The problems of signal distortion associated with this technique (due to the presence of shock waves) have been solved recently. For 2,4-D and phloretin the comparatively low time resolution of the fixed Q-mode was sufficient. For some of the analogues of phloretin the high time resolution of the Q-switch-technique was necessary (cf. the subsequent paper). Details of the fast technique will be published elsewhere (R. Awiszus, G. Stark, in preparation).

The measurement of the current-voltage characteristics was carried out as described previously (Stark and Benz 1971). All experiments were performed at 25°C.

Theory

The theoretical treatment of adsorption of dipolar lipophilic molecules is based on a model proposed by Ketterer et al. (1971) to explain saturation effects in the presence of lipid soluble ions. The model is illustrated in Fig. 1. The molecules adsorb to the membrane/water-interface, where they find a fixed surface density, \( N_p \), of binding sites. The rate constants of adsorption and desorption are \( \beta \) and \( k \). The rate of adsorption for a symmetrical problem is given by

\[
\frac{dN}{dt} = \beta k C_0 \left(1 - \frac{N}{N_B}\right) - k N.
\]

At equilibrium

\[
N = \beta C_B/(1 + q),
\]

with

\[
q = \beta C_B/N_B.
\]

\( \beta \) is the concentration independent partition coefficient at low surface density

\[
\beta = N/C_B, \quad (N \ll N_B).
\]