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PULSE RADIOLYSIS AT PLANAR LIPID MEMBRANES DOPED WITH ION CARRIERS OR PORE FORMERS

GÜNTHER STARK [a], MANUEL STRÄSSLE [a] and MARTIN WILHELM [b]

[a] *Fakultät für Biologie, Universität Konstanz, D-7750 Konstanz (F.R.G.) and* [b] *Hahn-Meitner-Institut für Kernforschung Berlin, Bereich Strahlenchemie, D-1000 Berlin 39 (Germany)*

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The effect of 14 MeV electrons on ion transport through planar lipid membranes was investigated. The membranes were formed in the presence of well defined ion carriers or pore forming substances. In the presence of the ion carriers valinomycin or nonactin or in the presence of the pore formers nystatin or amphotericin B, irradiation produced a transient increase of the membrane conductance followed by a long lasting decrease. The effects are interpreted on the basis of a time-dependent chemical modification of the membrane structure caused by exposure to high energy radiation. The pore former gramicidin A shows an exponential inactivation with increasing dose. At pH 3 and in the presence of oxygen the pore is highly sensitive to radiation ($D_{37} \approx 10$ Gy) whereas at pH 9.5 a considerably lower radiation sensitivity ($D_{37} \approx 1000$ Gy), was found. In the absence of oxygen, gramicidin A is virtually insensitive to irradiation. This is considered an evidence that the inactivation of this ion channel is primarily caused by the perhydroxyl radical HO_2.

Studies on the effect of high-energy radiation on biological membranes have been scarce compared to the extensive research dealing with the primary cellular radiation target DNA. Though membranes were repeatedly suggested as important for the development of cellular radiation damage [1–3], only few attempts were made to understand the molecular basis of radiation effects in cell membranes. Our present study has been performed with an artificial membrane system. In recent years, planar (black) lipid membranes have considerably contributed to clarify the principles of ion transport through biological membranes [4–7]. We report on the first attempts to study this membrane system using pulse radiolysis. Such experiments may provide a clue to radiation inactivation of transport proteins in native membranes.

Studies on detergent micelles and lipid vesicles have shown that the radiation induced primary radicals OH and the hydrated electron e_{aq}^- adsorb very fast to the interface of these systems [8,9]. An adsorption to phospholipid membranes was also reported for the superoxide anion O_2^- [10]. The radicals were found to interact with appropriate solutes inside the membrane such as pyrene. In addition, radiation caused alterations of the membrane structure which resulted in changes of solute mobility inside the membrane [9].

Radiation effects on ion transport through planar lipid membranes may be detected through conductance measurements. We investigated the effect of 14 MeV electrons applied as a sequence of 20-ns pulses. The membranes were doped with ion carriers or pore formers of well defined structure and transport mechanism [4–7,11]. The current response, following an irradiation of the membrane, strongly depends on the kind of trans-

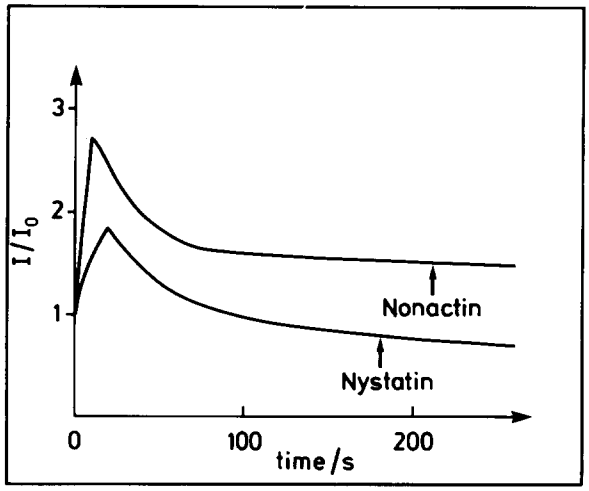

Fig. 1. Effect of 14 MeV electrons on the conductance of planar lipid membranes containing the ion carrier nonactin or the pore former nystatin. Radiation was supplied from the linear accelerator (Linac) of the Hahn-Meitner-Institut as a sequence of 100 20-ns pulses in case of the nonactin-experiment and of 500 10-ns pulses in case of nystatin. The time interval between two pulses was 100 ms and 40 ms, respectively. The total radiation dose delivered to the aqueous solutions surrounding the membrane was 590 Gy (nonactin) and 830 Gy (nystatin) as determined by Fricke dosimetry. In the nonactin-experiment, the membrane was formed from a 1% solution of dioleoylphosphatidylcholine in *n*-decane in an aqueous solution containing 1 M KCl and 10^{-6} M nonactin (Böhringer) (membrane area $5 \cdot 10^{-3}$ cm^2, voltage 50 mV, initial current I_0 before radiation exposure 246 nA). In case of the nystatin-experiment, the membrane was formed from a mixture of 0.66% dioleoylphosphatidylcholine and 1.32% cholesterol in *n*-decane. The aqueous solution contained 0.1 M NaCl and 1.75 μg nystatin/ml (Squibb) added from a 0.1% stock solution in dimethylsulfoxide. The current I_0 before application of radiation was 82 nA.

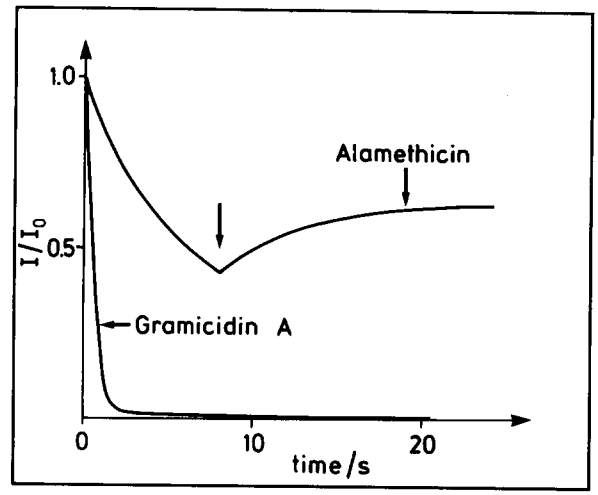

Fig. 2. Effect of 14 MeV electrons on the conductance of planar lipid membranes containing the pore-formers alamethicin or gramicidin A. In both cases radiation was applied in form of 200 pulses of 10 ns duration with an interval of 40 ms. The end of the irradiation is indicated by an arrow. The radiation dose was 250 Gy (alamethicin) and 300 Gy (gramicidin A). The membranes were formed from a 1% solution of dioleoylphosphatidylcholine in *n*-decane in an aqueous solution of 1 M NaCl containing 10^{-7} g/ml alamethicin (Upjohn) (plus 0.1% ethanol) or $2 \cdot 10^{-7}$ M gramicidin A. In case of gramicidin the presence or organic solvents was carefully avoided. The initial current I_0 before radiation exposure was 294 nA (alamethicin) and 1.4 μA (gramicidin A) at an applied voltage of 50 mV (membrane area $5 \cdot 10^{-3}$ cm^2).

port system present in the membrane. A qualitative identical result was found for the ion carriers nonactin and valinomycin (data not shown) and for the pore formers nystatin and amphotericin B (not shown), as illustrated in Fig. 1. In all these cases radiation induced a transient conductance increase followed by a decrease over several minutes after irradiation was stopped. Membranes doped with the pore former alamethicin showed an inverse behaviour (cf. Fig. 2). Conductance was found to decrease during irradiation and to increase after the cessation of the electron bombardment. The strongest effect was observed in the presence of gramicidin A. The pores formed by this peptide are irreversibly inactivated at comparatively small doses. On the other hand, the large, ion-permeable membrane channel formed by the matrix protein (porin) of *Escherichia coli* (isolated from the cell envelope of this bacterium and incorporated into planar membranes of oxidized cholesterol [12]) revealed to be completely radiation insensitive up to doses of 0.5 Mrad (data not shown).

The very different results obtained for the various substances indicate that the radiation sensitivity of a transport system can hardly be predicted a priori. The results are discussed along the following lines. The membrane conductance induced by a specific transport system can be influenced in different ways:

A transport system may become inactivated through

(a) direct radiation action on the transporting molecules,

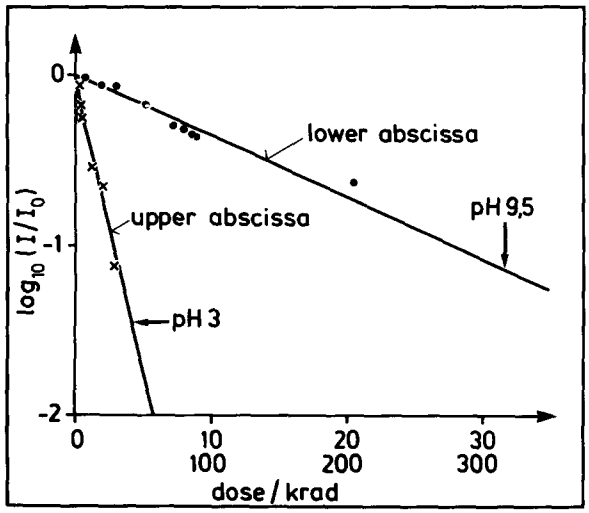

Fig. 3. Radiation inactivation of gramicidin A in planar lipid membranes at different pH of unbuffered aqueous solutions. The experimental conditions correspond to those of Fig. 2. The radiation dose was varied through the number of applied pulses (about 140 rad/pulse). The D_{37} dose was 11.5 Gy at pH 3 and 1230 Gy at pH 9.5.

(b) radiation action on the membrane lipids and subsequent energy transfer to the transporting molecules,
(c) interaction with free radicals produced in the aqueous phases adjacent to the membrane.

On the other hand, the transport system itself may not be influenced by the irradiation, but responds to a chemical modification of the membrane lipids. The latter may be caused either
(d) by direct radiation action on the lipids, or
(e) through a chemical reaction with free radicals produced in water.

The irreversible inactivation of the gramicidin A-pores is preferentially due to a reaction with free radicals (mechanism c). This follows from arguments based on Fig. 3. An exponential inactivation was found for the gramicidin-channel, according to $J = J_0 \exp(-D/D_{37})$. The D_{37}-dose strongly depends on the pH of the aqueous phases and the oxygen concentration in the system. The experiments shown in Figs. 1–3 were performed in the presence of oxygen (normal atmosphere). The radiation inactivation of the gramicidin-channels decreased strongly, if the cuvette was flushed with argon, i.e. if the oxygen concentration was reduced to (at most) a few percent of the original value as was tested by an oxygen electrode. The ratio $D_{37}(\text{air})/D_{37}(\text{Ar})$ of D_{37}-dose values observed in the presence and absence of oxygen was larger than 100. This finding, in combination with the pronounced pH-dependence (D_{37}(pH 3)/D_{37}(pH 9.5) $\approx$ 100), supports the assumption that the perhydroxyl radical HO_2 is primarily responsible for the radiation effect on gramicidin A doped lipid membranes. The protonated and deprotonated forms of the superoxide anion O_2^- are produced through association of the primary radicals H and e_{aq}^- of water radiolysis to molecular oxygen O_2. The pK of the reaction $HO_2 \rightleftarrows O_2^- + H^+$ is 4.88 [13], so that at pH 3 the perhydroxyl radical and at pH 9.5 the superoxide anion are the predominant species. Thus, gramicidin A is assumed to be indirectly inactivated by fast electrons via a chemical reaction with oxygen radicals. A direct inactivation (mechanism a) through flash photolysis of the peptide tryptophans with ultraviolet light was reported recently [14].

In contrast to gramicidin A, the radiation effects found in the presence of the ion carriers nonactin and valinomycin and in the presence of the pore formers nystatin and amphotericin B rather point to a structural modification of the membrane lipids (mechanism d and/or e). The transport properties of the potassium carriers nonactin and valinomycin have been found to depend on structural properties of the membrane, such as the surface charge density or the dipole potential at the membrane/water interface, and on the microviscosity of the membrane interior [6,7]. The pores formed by the polyene antibiotics nystatin and amphotericin require the presence of stabilizing membrane components (cholesterol) for their activity [11]. In summary, the conductance induced by all these compounds differs from that found in the presence of gramicidin A (or alamethicin) by a stronger dependence on structural factors of the membrane. The increase of the current found during the irradiation period might, therefore, arise as a consequence of a radiation damage of the lipids. The same interpretation could also hold for the conductance decrease observed after the irradiation period. This would mean to postulate different radiation induced chemical processes involving the membrane lipids, which occur at different time scales after an irradiation

and which have an opposite effect on the conductance. There are, however, alternative interpretations of the current decrease, which cannot be excluded at present. Radiation inactivation of the transporting molecules through long-lived radical species of the lipids might occur according to mechanism b. Finally, the radiation damage of the lipids might be reversible. A kinetic analysis of the single reaction steps of carrier-mediated cation transport [7] as a function of time after an irradiation could help to clarify the problem.

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