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The effect of free radicals on the conductance induced by alamethicin in planar lipid membranes: activation and inactivation

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Abstract

Exposure to ionizing radiation of planar lipid membranes doped with alamethicin gives rise to an increase and to a subsequent decrease of the membrane conductance. Both effects are due to the presence of radiation-induced free radicals of water radiolysis as was shown by addition of various radical scavengers. The increase of the conductance was found to be a consequence of free radical-initiated lipid peroxidation favouring the formation of active ion channels. The decrease of the conductance observed at larger radiation doses is due to an inactivation of alamethicin monomers. The characteristic D_{37} dose of inactivation was found to be about two orders of magnitude larger than in the case of gramicidin A. The comparatively high sensitivity of the latter is due to the presence of its four tryptophan residues. Inactivation of trichorzianine AIIIc, an analogue of alamethicin with a C-terminal tryptophanol residue, occurs at radiation doses two orders of magnitude lower than observed with alamethicin.

Keywords: Ion channel; Alamethicin; Free radical; Ionizing radiation; Tryptophan

1. Introduction

The investigation of the cellular effects of free radicals may be considered at present as an important part of biomedical research [1]. The experiments presented deal with a model system and are part of a study on effects of radiation-induced free radicals on ion transport through biological membranes. The primary and secondary radicals of water radiolysis, especially the hydroxyl radical $OH^{\cdot}$, and the oxygen radicals $O_2^{\cdot-}$ and $HO_2^{\cdot}$, have been discussed in connection with many deleterious cellular phenomena. We have been using water radiolysis as a convenient method of radical generation. Our study aims at the consequences of radical actions for the functional properties of ion-translocating systems in lipid membranes. Substances like valinomycin, nonactin, gramicidin A, amphotericin B or alamethicin have been studied in much detail throughout the last two decades and have been considered as simple models for the more complicated structures of ion transport systems of biological membranes. We use these models, which act as ion carriers or pore-formers in lipid membranes, to investigate fundamental principles of the influence of free radicals on membrane transport.

Our previous studies of this series – dealing with the ion carriers valinomycin and nonactin [2], and with the pore-formers gramicidin A [3–6], amphotericin B and nystatin [7,8] – may be summarized as follows: In all cases a strong change, by several orders of magnitude, of the membrane conductance was observed. While valinomycin and nonactin were found to behave as sensors of free radical-induced lipid peroxidation, gramicidin A, amphotericin B and nystatin became inactivated at comparatively small radiation doses (i.e., at comparatively small radical concentrations generated). In the presence of gramicidin A, the pronounced decrease of the conductance was found to be due to a subsequent interaction of $OH^{\cdot}$ and $HO_2^{\cdot}$ radicals with the tryptophan residues of the pentadecapeptide, which leads to fragmentation of the latter [3–6]. The polyene antibiotics amphotericin B and nystatin proved to be the most sensitive channel-forming structures investigated so far. A radical-chain mechanism (similar to lipid peroxidation) involving the polyene part of the molecules was found to be responsible for channel inactivation [7,8].

Alamethicin, the subject of the present communication, has been selected as a representative of membrane-active

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peptide structures devoid of special chemical groups which are known to be sensitive for radical attack. It is shown that inactivation occurs at comparatively large radiation doses in this case, whereas – at comparatively small doses – the channel is found to respond to radical-induced lipid peroxidation.

2. Materials and methods

Planar (black) lipid membranes were formed from 1% solutions of dioleoyllecithin (Avanti Polar Lipids, Birmingham, AL, USA) in n-decane (Fluka, Buchs, Switzerland; standard for gas chromatography) across a hole of 1 mm diameter in a bilayer-cell either made from polytetrafluoroethylene or from polycarbonate. The current across the membrane was amplified by using a current/voltage converter (10^6 V/A). The signal was finally transferred into a computer for further analysis of the data. The aqueous solution contained 1 M NaCl (unbuffered) and 0.1–0.2 μg/ml alamethicin (Sigma, Deisenhofen, Germany) or 0.2–0.4 μg/ml trichorzianine AIIIc (a gift from Dr. Bodo, Museum d'Histoire Naturelle, Paris). The experiments were usually performed at pH 3, where the most pronounced effects of free radicals were observed. This is believed to reflect the pH-dependent equilibrium $O_2^{\cdot-} + H^+ \rightleftharpoons HO_2^{\cdot}$. At sufficiently low pH (pH $\ll pK$, $pK = 4.8$), the superoxide radical $O_2^{\cdot-}$ is converted into the more reactive hydroperoxyl radical $HO_2^{\cdot}$.

Free radicals were produced by absorption in the aqueous phases of either 80 kV X-rays from a conventional source or of 14 MeV electrons from a linear accelerator (LINAC). Radiolysis of water molecules generates the primary radicals $OH^{\cdot}$, $H^{\cdot}$, and $e_{aq}^{\cdot-}$ [9–11]. In the presence of oxygen, $H^{\cdot}$, and $e_{aq}^{\cdot-}$ are converted into the secondary superoxide radical $O_2^{\cdot-}$ and into the perhydroxyl radical $HO_2^{\cdot}$. As a consequence, air-saturated solutions of sufficiently low pH essentially contain $OH^{\cdot}$ and $HO_2^{\cdot}$ radicals. In the presence of inorganic anions (e.g., Cl^-) $OH^{\cdot}$ radicals are converted into inorganic radicals (e.g., $Cl_2^{\cdot-}$) [12].

Radiolysis of water allows generation of well-defined concentrations, c_R, of radicals R. At a given radiation dose, D (in Gy), c_R is determined by the G_R value of the radical ($G_R(OH^{\cdot}) = 2.7$, $G_R(O_2^{\cdot-}/HO_2^{\cdot}) = 3.2$) according to

$$c_R = 1.03 \cdot 10^{-7}\, M \times G_R \times (D/\mathrm{Gy}) \qquad (1)$$

Two different experimental procedures have been used throughout the present study:

(a) The membrane and its aqueous environment were exposed to 80 kV X-rays. The procedure allows investigation of the response of the conductance at a constant dose rate, i.e., at a constant concentration of free radicals. (b) Alternatively the method of pulse radiolysis was applied. Irradiation of the membrane by pulses of 14 MeV electrons enables one to study the time dependence of the conductance following a concentration jump of free radicals. There is a further advantage of this method: It allows reduction of the oxygen concentration to considerably smaller values as compared with the continuous irradiation method. Decrease of the oxygen concentration is achieved by flushing the cuvette (used for bilayer formation) and its outer casing with argon. There is, however, a practical limit of this method. In view of the high amount of oxygen adsorbed within the cuvette material (PTFE) and its long lasting diffusion into the aqueous phase, O_2 concentration can be decreased to a few percent of its normal value at best. Application of radiation pulses of sufficient intensity will consume the residual concentration of O_2. This holds in view of the reaction of the primary radicals $H^{\cdot}$ and $e_{aq}^{\cdot-}$ with O_2 (see above). Using $G_R(O_2^{\cdot-}/HO_2^{\cdot}) = 3.2$ and Eq. (1), a maximum decrease of $3.3 \cdot 10^{-7}$ M O_2/Gy is obtained. Thus O_2 – concentration is reduced at the expense of the production of oxygen radicals $HO_2^{\cdot}$ and $O_2^{\cdot-}$. The concentration of the latter will, however, be considerably smaller than at the normal oxygen concentration of $2.5 \cdot 10^{-4}$ M in air-saturated aqueous solutions (at sufficiently high radiation doses applied). If the membrane and its environment are continuously irradiated from a conventional X-ray source instead, the dose is applied at rates many orders of magnitude smaller than with the pulse method. In this case, because of the comparatively long irradiation time, oxygen diffusion from the cuvette material will enhance both, the concentrations of O_2 and of the radicals $O_2^{\cdot-}/HO_2^{\cdot}$.

Details of the two experimental arrangements have been described in previous publications [3,5,7,11].

3. Results

3.1. The response of the membrane conductance following continuous irradiation by 80 kV X-rays

The conductance of lipid membranes, which are doped with alamethicin ion channels and which are exposed to ionizing radiation at a constant dose rate, shows a characteristic increase followed by a pronounced decrease to values far below the initial membrane conductance (Fig. 1, curve 1). Both, activation and inactivation of the conductance, are due to radiation-induced free radicals. This may be inferred from the conductance behaviour in the presence of some well-known radical scavengers. Their action is discussed in detail in the literature [1,10,13]. *t*-Butanol and formate reduce the concentration of $OH^{\cdot}$ radicals, while H_2O_2 lowers the formation of the secondary oxygen radicals $O_2^{\cdot-}/HO_2^{\cdot}$. The radiation effect on the membrane conductance is considerably reduced in the presence of all three kinds of substances (curves 2–4 of Fig. 1).

There are further arguments which support the assumption of radical-induced phenomena:

(1) The radiation effect depends on the nature of the

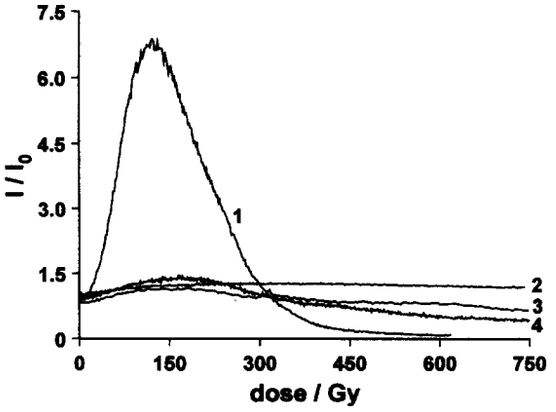

Fig. 1. Membrane current as a function of radiation dose in the presence of various radical scavengers. The value of the constant voltage (35 mV) was chosen within the voltage-dependent range of the membrane conductance (initial conductance $\lambda_0 = 4.2 \cdot 10^{-4}$ S/cm^2). The membrane – formed in an aqueous solution containing 1 M NaCl (pH 3) and 0.2 μg/ml alamethicin – was exposed to 80 kV X-rays at a dose rate of 36.3 Gy/min. The aqueous solution in addition contained the following radical scavengers: (1) control, (2) 50 mM formiate, (3) 50 mM H_2O_2, (4) 100 mM *t*-butanol.

aqueous solutions on both sides of the membrane (Fig. 2). The activation of the conductance is only observed at comparatively low pH (pH < 5.6). Activation also disappears, if Cl^- anions are replaced by SO_4^{2-}.

(2) Activation is strongly augmented by addition of Fe^{3+} ions (Fig. 3).

The findings may be explained as follows (see Section 4 for detailed arguments): Activation is a consequence of lipid peroxidation induced by primary ($OH^{\cdot}$) or certain secondary ($Cl_2^{\cdot-}$) free radicals of water radiolysis. It is a well-known experience that lipid peroxidation is amplified in the presence of Fe^{3+} or of $HO_2^{\cdot}$ radicals (formed at low pH) [11,14]. Further evidence for lipid oxidation as the basis for activation is obtained from the finding of a strongly reduced conductance increase, if the membrane is

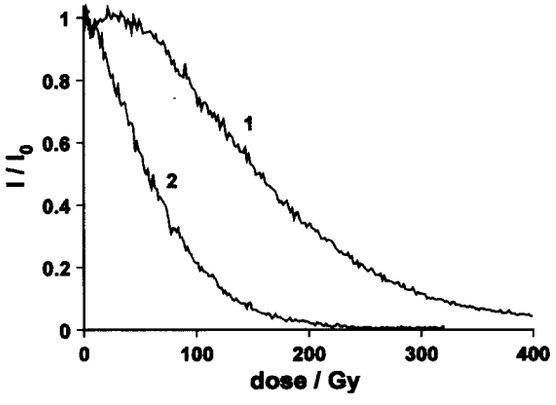

Fig. 2. The influence of different aqueous media on the radiation induced conductance changes: (1) 1 M NaCl (pH 5.6, unbuffered), (2) 0.5 M Na_2SO_4 (pH 3, unbuffered). For further experimental details see legend to Fig. 1.

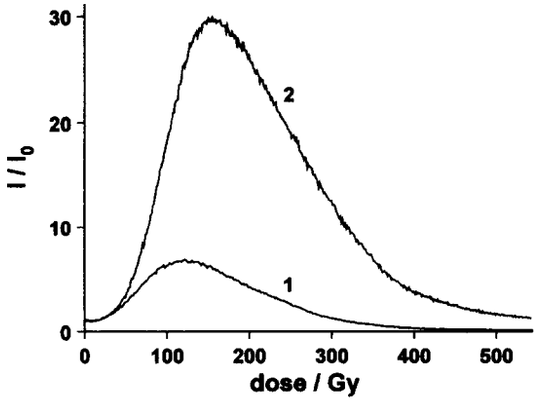

Fig. 3. The effect of Fe^{3+} on the dose effect relationship of alamethicin-doped lipid membranes: (1) control, (2) presence of 20 μM $FeCl_3$. For further experimental details see legend to Fig. 1.

formed from diphytanoyllecithin, i.e., in the absence of unsaturated fatty acid residues [17]. Alamethicin channels act as functional probes of this radiation effect. The decrease of the membrane conductance on the other hand is believed to reflect a radical-induced modification of alamethicin molecules leading to the inactivation of the ion channels. This is supported by the following finding: Aqueous solutions containing alamethicin were irradiated by 400 Gy and were subsequently used for bilayer experiments. It was found that the channel forming activity of these solutions was considerably reduced. The concentration of alamethicin had to be increased by a factor of 2–4 in order to obtain roughly the same membrane conductance as compared to nonirradiated solutions (at equal voltage applied).

The D_{37} dose of inactivation estimated from Fig. 1 is of the order of 260 Gy (cf. Section 4). This is about two

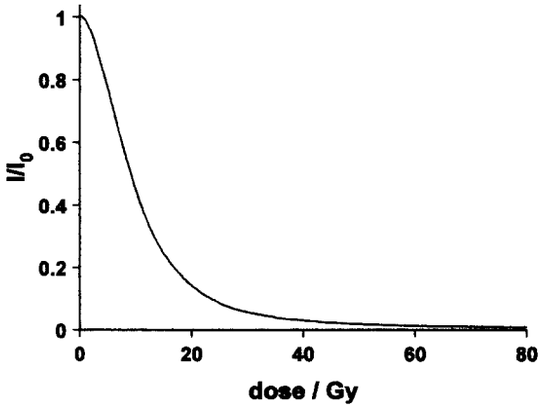

Fig. 4. The effect of 80 kV X-rays on the conductance induced by trichorzianine A IIIc in dioleoyllecithin membranes. The aqueous phase in which the membrane was formed contained 1 M NaCl (pH 3, unbuffered), 0.2 μg/ml trichorzianine A IIIc and about 100 mM ethanol. The dose rate was 4.3 Gy/min. The data refer to a constant voltage of 35 mV (initial conductance $5 \cdot 10^{-3}$ S/cm^2). Trichorzianine AIIIc exhibits a nonlinear current–voltage relationship similar to alamethicin (cf. Fig. 5 and [25]).

orders of magnitude larger than the D_{37} value observed for ion channels formed by the antibiotic gramicidin A under identical experimental conditions [3–6]. The comparatively high sensitivity of gramicidin A has been found to be due to its four tryptophan residues per monomer. In order to study the sensitizing effect of tryptophan residues at the alamethicin channel, some supplementary experiments were performed with trichorzianine AIIIc, an alamethicin-like peptaibol, which has a tryptophanol residue in position 19 [15]. Inactivation of the conductance induced by this compound is shifted to 10-times lower doses (Fig. 4). No activation is observed in this case (which might be buried under the pronounced decrease of the conductance to virtually zero at radiation doses below 100 Gy). The sensitivity of trichorzianine AIIIc as compared with alamethicin is greater than expressed by the ratio of D_{37} values (12 Gy versus 260 Gy). While alamethicin was directly dissolved in water, trichorzianine AIIIc – due to its low solubility in water – had to be added in form of an ethanolic solution to the aqueous phase in which the membrane was formed. The D_{37} value of trichorzianine AIIIc is within the same order of magnitude as observed with gramicidin A, if one accounts for the presence of the radical scavenger ethanol.

In order to investigate the molecular basis of the radiation effects, the current–voltage characteristics as well as the single channel behaviour of alamethicin-doped lipid membranes were compared before and after irradiation. To study the gating behaviour of alamethicin channels, a triangular voltage signal of 0.01 Hz frequency was applied to the membrane. The current–voltage curves were normalized to their respective maximum currents and were subsequently superimposed. No effect of irradiation could be detected (cf. Fig. 5). A virtually identical behaviour before and after irradiation of the membrane was also found at the level of single alamethicin channels. Following Boheim [16], single channel fluctuations of phosphatidylserine membranes were investigated at 4°C (in order to enhance the life time of the channels). Neither the amplitude (up to the 6th substate) nor the life time of the channels were found to be affected by radiation doses of 560 Gy [17].

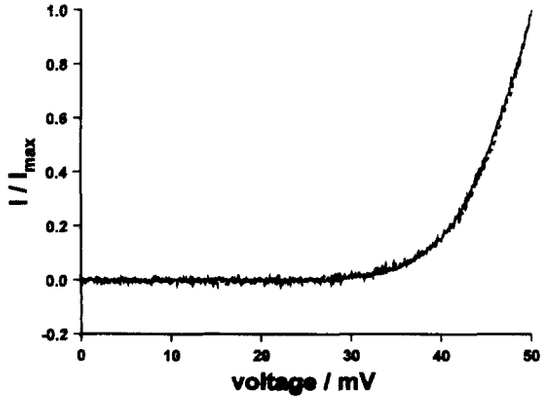

Fig. 5. Current–voltage relationship before (solid line) and after (dotted line) application of a radiation dose of 400 Gy. The curves were normalized to the maximum current observed and are virtually indistinguishable. The data were obtained by application of a triangular voltage signal of frequency 0.01 Hz. For further experimental details see legend to Fig. 1.

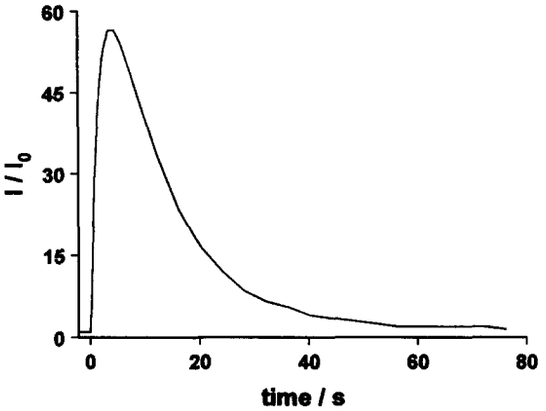

Fig. 6. The effect of 14 MeV electron pulses on the conductance of alamethicin-doped lipid membranes. The value of the constant voltage (20 mV) was chosen within the voltage-dependent range of the membrane conductance (initial conductance $\lambda_0 = 1.4 \cdot 10^{-3}$ S/cm^2). The membrane was irradiated by 15 pulses (100 ns width, 20 ms interval) supplied by a linear accelerator (LINAC). The total dose was 548 Gy (see Refs. [3,7,11] for details of the experimental setup). Membrane and aqueous solutions were as described in the legend to Fig. 1.

In summary, neither the gating properties of the channels nor their single channel characteristics seem to be influenced by the presence of radiation-induced free radicals. Therefore, activation is proposed to be due to an increase of the number of unmodified open channels (induced by lipid peroxidation), while inactivation – as a consequence of a radical-induced chemical modification of alamethicin – seems to proceed via a complete elimination (irreversible closing) of active ion channels.

3.2. The time dependence of the conductance following pulses of 14 MeV electrons

As outlined in Section 2, there was a twofold purpose of pulse radiolytic investigations: (i) to study the kinetics of the conductance changes after short pulses of radiation (i.e., after fast changes of the concentration of free radicals of water radiolysis) and (ii) to study radiation effects at very small oxygen concentrations. Fig. 6 shows a typical result obtained in air-saturated aqueous solutions. The conductance, after an irradiation period of 280 ms, shows a steep increase (rise time $\approx$ 1 s) followed by a slower decrease over a time period of about 1 min. The data may be compared with the continuous irradiation experiments shown in Fig. 1 (curve 1). A radiation dose of roughly 600 Gy was applied within 16 min in this case (i.e., at a dose rate more than 3000-fold smaller than in Fig. 6). Though the shape of the two curves shows principal agreement

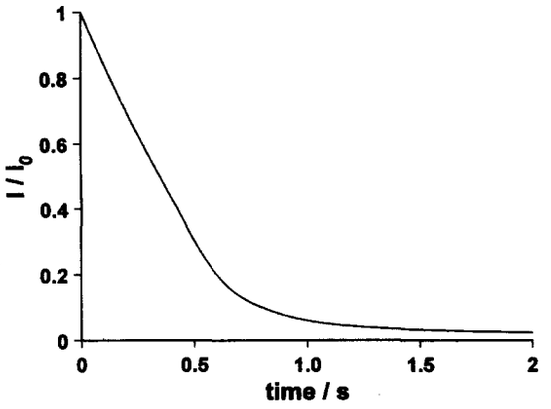

Fig. 7. Radiation effect at reduced oxygen concentration. The cuvette (used for bilayer formation) and its outer casing were flushed with argon, as described in the main text. The membrane was irradiated by 20 pulses of 14 MeV electrons (200 ns width, 20 ms interval). The total dose applied was 300 Gy. The other experimental conditions were as described in the legend to Fig. 6. The current was found to decrease to virtually zero after about 8 s.

(i.e., activation and inactivation), the time scale of the phenomena involved may be deduced only from Fig. 6. It is found that the activation process (supposed to represent an increase of the number of open channels) occurs within a time range more than one order of magnitude faster than free radical-mediated irreversible closing of alamethicin channels. The rise time (of about 1 s) of the initial conductance increase can be studied without the interference of inactivation, if the membrane is irradiated by a single pulse of radiation resulting in a dose of 30–40 Gy (data not shown).

The process of inactivation is considerably accelerated in the absence of oxygen (Fig. 7). The main difference between Figs. 6 and 7 is, however, the complete absence of the activation process at low oxygen concentration. This may be considered as a further evidence for lipid peroxidation to represent the primary cause for the increasing conductance at comparatively small radiation doses.

4. Discussion

Alamethicin and gramicidin A represent two classes of membrane-active peptides, which form pore-like ion structures in biological membranes and in lipid membranes, and which have been investigated in great detail in recent years. The gramicidin channel is based on an intrinsic property of the individual molecule, which – in its β-helical configuration – forms a cylindrical hole in the center of the molecule. A continuous aqueous pathway through the membrane is provided for ions by head-to-head association of two monomers. On the other hand current models of the alamethicin channel assume an aggregate of molecules with contributions of each individual monomer to form a water-filled structure of variable diameter. The 'barrel stave' (or helix-bundle) model was originally based on experiments by Baumann and Mueller [18] and by Boheim [16]. Various modified structures have been proposed since then, including alternative models of their voltage dependent formation (see Refs. [19–24] for a review).

The effect of free radicals on ion channels may be expected to depend on a series of different factors such as the kind of amino acids of the primary sequence, the detailed spatial structure of the channel and the properties of the surrounding lipid phase. Aromatic side chains or sulfhydryl groups are usually considered to be the most reactive parts of amino acids. In the case of gramicidin A, the four tryptophan residues determine the sensitivity of the molecule. Their replacement by other aromatic amino acids such as phenylalanine or naphthylalanine has been found to increase the D_{37} dose of inactivation by several orders of magnitude [3,4]. The primary structure of alamethicin consists of 19 amino acids and one amino alcohol:

Ac-Aib^1-Pro^2-Aib^3-Ala^4-Aib^5-Ala^6-Gln^7-Aib^8-Val^9
-Aib^{10}-...
...-Gly^{11}-Leu^{12}-Aib^{13}-Pro^{14}-Val^{15}-Aib^{16}-Aib^{17}-Glx^{18}-
Gln^{19}-Pheol^{20}

(Aib = α-aminoisobutyric acid, Glx = Glu (component R_f 30) or Gln (component R_f 50), Pheol = phenylalaninol).

The amino alcohol in position 20 represents the only aromatic side group of the sequence. In view of the importance of tryptophan residues for the inactivation of gramicidin channels, the behaviour of alamethicin was compared with trichorzianine AIIIc which has a tryptophanol residue in position 19 [15]:

Ac-Aib^1-Ala^2-Ala^3-Aib^4-Aib^5-Gln^6-Aib^7-Aib^8-Aib^9-
Ser^{10}-...
...-Leu^{11}-Aib^{12}-Pro^{13}-Val^{14}-Aib^{15}-Ile^{16}-Gln^{17}-Gln^{18}-Trpol^{19}

Though the primary structures of both peptides differ in many positions, both of them belong to the group of peptaibols, linear polypeptides rich in α-aminoisobutyric acid. Alamethicin, trichorzianines and other peptaibols have been found to form voltage dependent ion channels of similar behaviour in lipid membranes [25,26]. The voltage dependence of alamethicin channels is frequently explained by interaction of the electric field inside the membrane with the dipole moment of the N-terminal part of the sequence (up to Pro^{14}), while the C-terminal part is believed to remain in contact with the interface. If the same structure is assumed for trichorzianine AIIIc channels, free radicals of water radiolysis would have free access to the tryptophanol group in position 19. We think that the different D_{37} values of inactivation observed for the two peptaibols mainly reflect the exchange of phenylalaninol against tryptophanol, though a contribution of other positions of the peptide chains cannot be excluded. This is justified by the comparison with analogues of gramicidin A: The substitution of the only tryptophan residue in position 13 of the analogue GN^{9,11,15} against naphthylalanine (analogue GN) was found to produce considerable

differences in the inactivation behaviour of the two analogues [4]. Inactivation seems to be determined by the most sensitive amino acids of the peptide chain, if the structure allows free access for radicals from the aqueous phase. In both cases taken into consideration, gramicidin A and trichorzianine AIIIc, the tryptophan residues are located in close contact with the membrane/water interface.

The molecular changes leading to inactivation of alamethicin have not been investigated so far. In the case of gramicidin, fragmentation of the peptide chain at the tryptophan residues has been observed after photodynamic inactivation [6]. This may also hold for free radical-induced inactivation of trichorzianine AIIIc, which seems to be based on chemical degradation of Trpol[19]. Inactivation of alamethicin is certainly less specific and cannot be assigned to the cleavage of the backbone at special sites at present.

Inactivation is found to be considerably accelerated in the absence of oxygen (cf. Figs. 1 and 6). While $OH^{\cdot}$ and $O_2^{\cdot-}/HO_2^{\cdot}$ represent the dominant radical species in the presence of O_2, species $OH^{\cdot}$, $H^{\cdot}$ and $e_{aq}^{\cdot-}$ have to be considered in the absence of O_2. Two different phenomena might contribute to the oxygen effect: Radical species of the peptide produced by $OH^{\cdot}$ radicals might be transformed – by interaction with oxygen – into less reactive products thus counteracting further degradation. Alternatively, the efficiency of inactivation of alamethicin might be augmented by the radicals $H^{\cdot}$ and $e_{aq}^{\cdot-}$ which, in the presence of oxygen, are converted into the couple of (less reactive) oxygen radicals $O_2^{\cdot-}/HO_2^{\cdot}$. Cleavage of peptide bonds has been suggested for $OH^{\cdot}$ radicals (in the presence of O_2) and for hydrated electrons $e_{aq}^{\cdot-}$ (in the absence of O_2) [27,28]. Irrespective of the underlying mechanism, oxygen exerts a protective effect, i.e. counteracts alamethicin inactivation.

In the presence of high concentrations of NaCl or Na_2SO_4 in the aqueous phase, primary $OH^{\cdot}$ radicals are partly converted into the secondary radicals $Cl_2^{\cdot-}$ and $SO_4^{\cdot-}$ [29,30]. In view of the considerably longer life time of these radicals (see Ref. [12] for a compilation of relevant rate constants), radiation effects on alamethicin, attributed to $OH^{\cdot}$, are presumably due to reactions of these secondary radicals. This also seems to hold for lipid peroxidation induced by $OH^{\cdot}$ radicals and is used in the present context to explain the different activation behaviour in aqueous solutions of NaCl and Na_2SO_4. As illustrated in Figs. 1 and 2, activation of the conductance is only observed in NaCl solutions, at low pH and at comparatively small doses (where inactivation can be neglected). The effect may be explained by assuming that (contrary to $Cl_2^{\cdot-}$) $SO_4^{\cdot-}$ radicals are unable to penetrate into the nonpolar membrane interior in order to initiate lipid peroxidation. The interpretation is supported by experiments performed in the presence of ion carriers valinomycin and nonactin or in the presence of ion channels formed by polyene antibiotics amphotericin B and nystatin. Ion carriers have been found to act as sensitive probes of radiation-induced lipid peroxidation [2]. Inactivation of polyene channels is due to a radical chain mechanism similar to lipid peroxidation [7,8]. In both cases radiation effects are strongly reduced, if NaCl is replaced by Na_2SO_4, and are found to be enhanced at low pH (unpublished). The pH-dependence of the effects may be understood assuming augmentation of lipid peroxidation by $HO_2^{\cdot}$ radicals. The latter are formed at low pH and have been suggested to transform lipid hydroperoxides into alkoxy radicals thus elongating the chain lenght of the radical chain mechanism [11,14].

In summary, the functional changes of all model systems so far investigated, which are attributed to free radical-induced lipid peroxidation, show the same dependence on the kind of radicals present in water.

In the case of alamethicin, lipid peroxidation is suggested to increase the number of open channels (cf. Section 3). The finding might be the consequence of an enhanced partition coefficient, γ, of alamethicin between membrane and water. Accumulation of polar products of lipid peroxidation has been found to increase the dielectric constant, ε, of the membrane interior (detected by corresponding changes of the membrane capacity [31]). The increase of ε (possibly in combination with further structural changes accompanying lipid peroxidation) is suggested to enhance the concentration, N, of alamethicin monomers per unit area of membrane. Though this is an ad hoc-assumption which needs further experimental justification, it allows to explain the main features of the conductance behaviour of alamethicin-doped lipid membranes, as is shown by the following simple model.

The dose dependence of γ is assumed to vary linearly with the applied dose, D:

$$\gamma(D) = \gamma_0 + aD \tag{2}$$

Alamethicin is assumed to become inactivated by a one-hit phenomenon (i.e., by attack of a single radical). This is equivalent to an exponential decrease of the concentration, c, of active monomers in water:

$$c(D) = c_0 \exp(-bD) \tag{3}$$

The probability of formation of open pores is assumed to depend on the n-th power of the monomer concentration. Thus (using $\gamma = N/c$) the membrane conductance, λ, is obtained as

$$\lambda = kN^n = k \cdot (\gamma c)^n \tag{4}$$

Combination of Eqs. (2)–(4) yields the dose dependence of the conductance as

$$\frac{\lambda(D)}{\lambda_0} = \left(1 + \frac{aD}{\gamma_0}\right)^n \exp(-bnD) \tag{5}$$

with $\lambda_0 = \lambda(D = 0)$.

Application of Eq. (5) – despite the underlying simplifying assumptions – allows approximate description of the

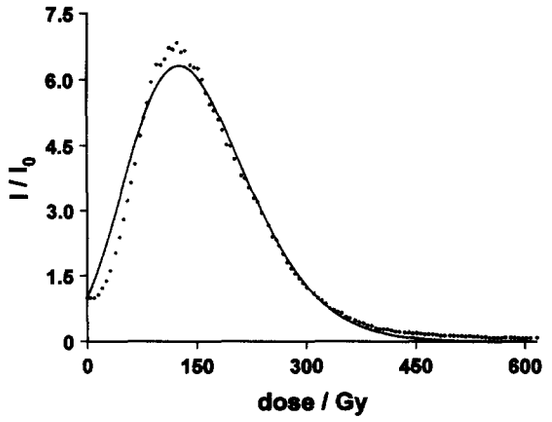

Fig. 8. Comparison of the dose response of alamethicin-induced conductance (curve 1 of Fig. 1) with Eq. (5). The solid line was obtained using $n = 10.3$, $\gamma_0 / a = 52.63$ Gy and $1/b = 261$ Gy.

experimental data (cf. Fig. 8). The discrepancy observed at low doses might be due to a delay in the dose-dependent partition equilibrium of alamethicin. Therefore the value obtained for the number of monomers per channel ($n = 10.3$) has to be considered as a rough estimate only. It is, however, similar to that obtained from the concentration dependence of the conductance ($n = 9$) [32].

Determination of b allows estimation of the D_{37} dose of alamethicin inactivation in water. Its value ($D_{37} = 1/b = 260 \pm 60$ Gy, average from nine different membranes) is about two orders of magnitude larger than for gramicidin A (under identical experimental conditions) [3]. The difference is believed to reflect the absence of chemical groups showing special sensitivity towards free radical action.

The present studies aim at an understanding of functional changes of ion transport systems in biological membranes induced by free radicals. Comparison of different model systems of well-defined structure in planar lipid membranes provides a clue for the behaviour of more complicated ion translocating proteins in native membranes.

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