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TOPICAL REVIEW

Temperature- and pH-Induced Structural Changes in the Membrane of the Hyperthermophilic Archaeon *Aeropyrum pernix* K1

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Abstract The influence of pH and temperature on the structural organization, fluidity and permeability of the hyperthermophilic archaeon membrane was investigated *in situ* by a combination of electron paramagnetic resonance (EPR) and fluorescence emission spectroscopy. For EPR measurements, *Aeropyrum pernix* cells, after growing at different pHs, were spin-labeled with the doxyl derivative of palmitic acid methylester (MeFASL[10,3]). From the EPR spectra maximal hyperfine splitting ($2A_{max}$) and empirical correlation time (τ_{emp}), which are related to mean membrane fluidity, were determined. The mean membrane fluidity increases with temperature and depends on the pH of the growth medium. Computer simulation of the EPR spectra shows that membrane of *A. pernix* is heterogeneous and consists of the regions characterized with three different types of motional characteristics, which define three types of membrane domains. Order parameter and proportion of the spin probes in the three types of domains define mean membrane fluidity. The fluidity changes of the membrane with pH and temperature correlate well with the ratio between the fluorescence emission intensity of the first and third bands in the vibronic spectra of pyrene, I_1/I_3. At pH 7.0 a decrease of I_1/I_3 from 2.0 to 1.2, due to the penetration of pyrene into the nonpolar membrane region, is achieved at temperatures above 65°C, the lower temperature limit of *A. pernix* growth.

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Introduction

Archaea, the third domain of living organisms, have cell structures and biocomponents that are markedly different from those found in bacteria and eukaryotes. Their unique features include distinct rRNA motifs, ether-linked membrane lipids and unique cell-wall components (Bernander, 1998). Hyperthermophiles grow optimally at temperatures higher than 80°C and are unable to grow below 60°C (Stetter, 1998, 1999). *Aeropyrum pernix* is a thermophile that has been isolated from a coastal solfataric thermal vent on Kodakara-Jima Island of Japan (Sako et al., 1996). It is the first reported obligately aerobic and neutrophilic hyperthermophilic archaeon, with an optimal growth temperature between 90°C and 95°C; and it was classified as a member of the Crenarchaeota.

The glycerol ether lipid is one of the most remarkable features that distinguish members of Archaea from those of Bacteria and Eucarya (Woese, Kandler & Wheelis, 1990). In Bacteria (and Eucarya), the membrane phospholipids feature an *sn*-glycerol-3-phosphate scaffold, which is ester-linked to the fatty acid (acyl) tails (C_{14}-C_{20}). In contrast, archaeal lipids use the opposite glycerol stereoisometry, *sn*-glycerol-1-phosphate, with isoprenoid groups connected via ether linkages (Peretó, López-Garcia & Moreira, 2004; Pakchun, Simpson & Codd, 2006). The lipids of *A. pernix* are different from those of the anaerobic sulfur-dependent hyperthermophyle in terms of the lack of tetraether lipids and the direct linkage of inositol and sugar moieties (Morii et al., 1999). The isolated core lipids of *A. pernix* consist

solely of 2,3-di-*O*-sesterterpanyl-*sn*-glycerol ($C_{25,25}$-archaeol). The major two polar lipids are 2,3-di-*O*-sesterterpanyl-*sn*-glycerol-1-phospho-1′-(2′-*O*-α-D-glucosyl)-*myo*-inositol ($C_{25,25}$-archaetidyl[glucosyl]inositol) about 91 mol% and 2,3-di-*O*-sesterterpanyl-*sn*-glycerol-1-phospho-*myo*-inositol ($C_{25,25}$-archaetidylinositol), about 9 mol% (Morii et al., 1999). C_{25}-isoprenoid chain-containing ether lipids were first reported for the haloalkalophiles including $C_{20,25}$-archaeol and $C_{25,25}$-archaeol (Tindall, 1985; deRosa et al., 1983). However, in halophiles, the $C_{25,25}$ molecular species were present in small amounts and the bulk of the lipids were composed of the $C_{20,25}$ or $C_{20,20}$ species. The chain length of the C_{25}-isoprenoid hydrocarbon is 20% greater than that of the C_{20}-isoprenoid and C_{18} straight-chain fatty acids. Therefore, it being composed of only the $C_{25,25}$-archaeol-based lipids, the thickness of the membrane of *A. pernix* is assumed to be 20% greater than the membranes of the $C_{20,20}$-archaeol-based lipids of other archaea. Since the physiological significance of this state is unknown, we have investigated the influence of environmental factors (pH and temperature) on the structural properties of the membranes of *A. pernix in vivo* by electron paramagnetic resonance (EPR) and fluorescence emission spectroscopy.

Materials and Methods

Growth of *A. pernix*

A. pernix K1 was purchased from the Japan Collection of Microorganisms (number 9820; Wako-shi, Japan). The culture medium contained 37.4 g Bacto marine broth 2216 (Difco, Detroit, MI) and 1.0 g $Na_2S_2O_3 \times 5H_2O$ per liter. The concentration of NaCl in the growth medium was 3.3%. The buffer systems used were 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, pH 7.0 and 8.0), 20 mM morpholinoethanesulfonic acid (MES, pH 6.0) and 20 mM citrate (pH 5.0). The pH of the medium was adjusted to the desired values at ambient temperature using 1 M NaOH or HCl prior to autoclaving. The *A. pernix* cells were grown in 800 ml growth medium inside 1,000-ml heavy-wall flasks using a magnetic stirring hot plate and forced aeration (0.5 l min^{-1}) at 92°C, as described before (Milek et al., 2005). The growth of the cells was monitored spectrophotometrically at an optical density of 650 nm (OD_{650}) using a Hewlett-Packard (Waldronn, Germany) 8453 UV-VIS spectrophotometer. After reaching the mid- to late exponential phase of growth, the suspensions were centrifuged (Eppendorf, Hamburg, Germany; 5415C) at 14,000 rpm for 2 min at 25°C. The pellets of cells grown at pH 6.0, 7.0 and 8.0 were washed twice with the corresponding working buffers (20 mM HEPES or MES containing 3% NaCl). Cell suspensions at $OD_{650} = 0.2$ were prepared in the corresponding buffers and used for EPR and fluorescence measurements.

EPR Measurements

Freshly prepared *A. pernix* cells were used for EPR measurements, which allowed us to study the membrane characteristics and the activity of the oxy-redoxy system *in situ*. A lipophilic spin probe, the methylester of 5-doxyl palmitic acid (MeFASL[10,3]), was selected as the spin probe due to its moderate stability in the membrane and its relatively high resolution capability for local membrane ordering and dynamics (Schara, Pečar & Svetek, 1990). Forty microliters of 0.1 mM ethanol solution of MeFASL(10,3) was added to the glass tube, and the ethanol was evaporated on a rotary evaporator to obtain a uniform distribution of MeFASL(10,3) on the walls of the tube. A suspension of *A. pernix* cells (2 ml of 0.015 g l^{-1}) was placed in the glass tube with the spin probe. After 15-min incubation at room temperature, with gentle shaking, the suspension was centrifuged at 3,000 × *g* for 5 min and the pellet of cells introduced into a glass capillary with a 1-mm inner diameter for EPR measurements. The measurements were performed on a Bruker ESP 300 X-band spectrometer (Bruker Analytishe Messtechnik, Rheinstten, Germany); the parameters used were as follows: center field, 332 mT; scan range, 10 mT; microwave power, 20 mW; microwave frequency, 9.62 GHz; modulation frequency, 100 KHz; modulation amplitude, 0.2 mT; temperature range, 20–95°C. The spectra of ten replicates were determined for each sample of spin-labeled *A. pernix* cells, and the experiments were repeated three times at each pH. In the control experiments, the spectra of nonlabeled *A. pernix* cells and the spectra of growth media were measured.

To determine if the spin probe was incorporated into the membrane or whether it remained adhered on the surface of the *A. pernix* cells, in some experiments 20 µl of the paramagnetic broadening agent, potassium trioxalatochromiate (CrOx, 120 mM) was added to 20 µl of the spin-labeled cell suspension. Due to the charge, CrOx does not penetrate into the membrane, and it broadens the EPR spectral lines due to the exchange interaction between the spin probes and the paramagnetic complex at the water-lipid interfaces. Therefore, in presence of CrOx, only the spectra of the spin probe in the membrane remain unperturbed (Pečar et al., 1982).

Determination of Mean Membrane Fluidity

The EPR spectral line shape of the spin probe, which is primarily incorporated into the cell plasma membrane,

describes the properties of its surroundings. For a rough estimation of membrane fluidity, the maximal hyperfine splitting, $2A_{max}$, which is correlated with a mean ordering of membrane phospholipid chains, was measured (as indicated in Fig. 1). At higher temperatures, where these parameters cannot be resolved from the EPR spectra, an empirical correlation time (τ_{emp}) was calculated from the line heights (as indicated in Fig. 1) using the following equation:

$$\tau_{emp} = K\Delta H_0[(h_0/h_{-1})^{1/2} - 1] \quad (1)$$

where h_{-1} and h_0 are the amplitudes of the high and middle field lines of the EPR spectra, ΔH_0 is the line width of the middle field line and K is a constant typical for the spin probe (Marsh, 1981). This equation is valid for fast isotropic motion but can be taken as an approximation to evaluate the differences in the EPR spectra of different samples at higher temperatures. The empirical correlation time is inversely proportional to mean membrane fluidity.

Computer Simulation of EPR Spectra

For a more precise description of membrane characteristics, computer simulation of the EPR spectra line shape was performed using the program EPRSIM (© Janez Štrancar, 1996–2003, http://www2.ijs.si/~jstrancar/software.htm), which is described in more detail elewhere (Štrancar, Šentjurc & Schara, 2000). The model takes into account that the membrane is heterogeneous, composed of several coexisting domains with different fluidity characteristics. Therefore, the EPR spectrum is composed of several spectral components reflecting different modes of restricted rotational motion of the spin probe molecules in different membrane environments. Each spectral component is described with a set of spectral parameters, which define the line shape. These are order parameter (S), rotational correlation time (τ_c), line-width correction (W) and polarity correction of the magnetic tensors g and A (p_g and p_A, respectively). Order parameter describes the orientational order of the phospholipid alkyl chains in the membrane domains, with $S = 1$ for perfectely ordered chains and $S = 0$ for isotropic alignment of the chains. More fluid membrane domains are characterized by a small S. Rotational correlation time (τ_c) describes the dynamics of the alky chain motion, the line-width correction (W) is due to the unresolved hydrogen super-hyperfine interactions and contributions from other paramagnetic impurities (e.g., oxygen, external magnetic field inhomogeneities, etc.) and the polarity correction arises from the polarity of the spin probe nitroxide group environment. Besides these parameters, the line shape of the EPR spectra is defined by the relative proportion of each spectral component (d), which describes the relative amount of the spin probes with particular motional mode and depends on the distribution of the spin probe between the coexisting domains with different fluidity characteristics. It should be stressed that the lateral motion of the spin probe is slow on the time scale of EPR spectra (Johnson et al., 1996). Therefore, an EPR spectrum describes only the properties of a spin probe's nearest surrounding on the nanometer scale. All the regions in the mebrane with similar modes of spin probe motion contribute to one and the same spectral component. Thus, each spectral component reflects the fluidity characteristics of a certain type of membrane domain (with dimensions of several nanometers) (Štrancar, Koklič & Arsov, 2003). The mean membrane fluidity is determined primarily by the proportion between the coexisting types of domains (d) and by the order parameter S of each domain type. Fluid membranes have a high proportion of less ordered domains and are more permeant to water, solutes and ammonia (Lande, Donovan & Zeidel, 1995).

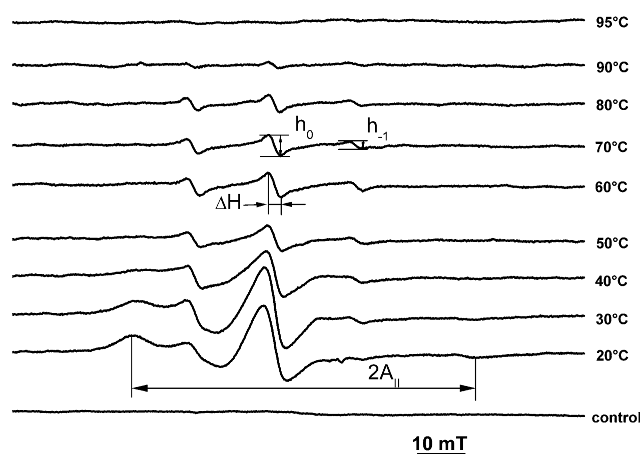

Fig. 1 Experimental EPR spectra of the spin probe MeFASL(10,3) incorporated into *A. pernix* cells grown in medium with pH 7.0 at different temperatures, as indicated, and the non-spin-labeled (control) spectrum of *A. pernix* cells

Fluorescence Emission Spectroscopy

Fluorescence emission measurements of pyrene in the presence of the *A. pernix* cells were performed in a 10-mm-path length cuvette using a Cary Eclipse Fluorescence Spectrophotometer (Varian, Mulgrave, Australia) in the temperature range 25–98°C. The *A. pernix* cells were washed with their corresponding buffer, at pH 6.0, 7.0 and 8.0 ($OD_{650nm} = 0.2$), as described above, and then diluted tenfold in the same buffer as that used for the washing. Before the measurements were taken, aliquots of 100 µl

saturated pyrene solution (Aldrich, Taufkirchen, Germany) were added to the cuvette with 2.9 ml cell suspension. The saturated solution of pyrene was prepared by disolving it in doubly distilled water. The solution was filtered before use. The pyrene fluorescence emission spectra were measured in the wavelength range 340–700 nm. An excitation wavelength of 330 nm was used, with the slit widths with a nominal bandpass of 5 nm for both excitation and emission. The fluorescence intensity ratios (I_1/I_3) of the first (I_1, 371 nm) and third (I_3, 391 nm) band in the pyrene vibronic spectra were determined.

Results

EPR Spectra of *A. pernix* at Different Temperatures and pH

The EPR spectra of MeFASL(10,3)-labeled *A. pernix* cells grown in medium at pH 7.0 at different temperatures are shown in Figure 1. In Figure 1 the spectrum of control, nonlabeled *A. pernix* cells measured at room temperature is also presented. For computer simulation of the EPR spectra, the spectrum of nonlabeled cells was subtracted from the EPR spectra of labeled *A. pernix*. In control experiments with the paramagnetic broadening agent CrOx, it was shown that CrOx does not influence the EPR spectra of spin-labeled *A. pernix*, proving that MeFASL(10,3) is trapped within the membrane. As evident from Figure 1, the shape and intensity of the EPR spectra of labeled *A. pernix* changed with temperature. At temperatures higher than 50°C, the signal was very low, while at temperatures around 90°C it disappeared completely. The disappearance of the EPR signal with temperature is a consequence of nitroxide reduction to hydroxylamine, which is not paramagnetic. In the biological systems, the reduction occurs due to the oxidation-reduction processes, which are more pronounced at higher temperatures due to the kinetics of the processes (Chen, Morse & Swartz, 1988).

EPR Spectra Parameters Determined Directly from the Spectra

In the temperature range 20–50°C the parameter $2A_{max}$ was determined as indicated in Figure 1. At higher temperature the maximal hyperfine splitting was no longer resolvable from the spectra; therefore, the empirical correlation time, τ_{emp}, was calculated using equation 1. The parameters ($2A_{max}$ and τ_{emp}) for the cells grown at different pH are presented in Table 1. At temperatures less than 40°C there were no significant differences between the parameters measured for the cells grown in medium with different pH. However, at higher temperatures the membranes of *A. pernix* cells grown at pH 6.0 were significantly more fluid than the membranes of the cells grown at pH 7.0 or 8.0; both parameters ($2A_{max}$ and τ_{emp}) were significantly lower, indicating smaller order and faster motion of phospholipid alkyl chains in the membrane. Our results also indicate that at higher temperature the membranes of the cells grown at pH 8.0 are slightly more rigid than the membranes of cells grown at pH 7.0.

Table 1 EPR parameters determined directly from the EPR spectra ($2A_{max}$) at 20°C and 40°C and empyrical corelation time (τ_{emp}) at 70°C and 80°C for the spin probe MeFASL(10,3) incorporated into the membrane of *A. pernix*, grown in medium of different pH

	T (°C)	pH 6.0	pH 7.0	pH 8.0
$2A_{max}$ (mT)	20	5.96 ± 0.05	6.03 ± 0.02	5.92 ± 0.02
$2A_{max}$ (mT)	40	4.98 ± 0.09	5.69 ± 0.09	5.70 ± 0.01
τ_{emp} (10^{-11} s)	70	5.7 ± 0.2	9.3 ± 0.5	10.0 ± 0.5
τ_{emp} (10^{-11} s)	80	2.5 ± 0.2	8.2 ± 0.5	9.5 ± 0.5

Mean value of three measurements ± standard deviation

EPR Parameters Obtained by Computer Simulation of the EPR Spectra

To get better insight on the membrane structural changes which occurred, when the cells were grown at different pH computer simulation of the EPR spectra was performed. By computer simulation of the EPR spectra, good agreement with the experimental spectra (Fig. 1) could be obtained if it is understood that the spectra are a superimposition of three spectral components with different line shapes, which reflect different modes of the spin probe motion. This indicates that the membranes are heterogeneous and composed of several coexisting domains with different fluidity characteristics. Figure 2 shows the best fits of the experimental EPR spectra of *A. pernix* grown in medium at pH 7.0 measured at 20°C together with the corresponding components of the spectra which reflect the mode of motion of spin probes in different environments. All regions in the membrane with the same fluidity characteristics are described with one spectral component, which represents one type of membrane domain. Spectrum 3 in Figure 2 describes the most ordered domain type (domain 3), with order parameter $S \sim 0.8$ at 20°C; and spectrum 1 describes the less ordered domain type (domain 1) of the membrane, with $S \sim 0.1$ at 20°C. Figure 3 demonstrates the temperature dependence of the order parameter S for the spin probes in three different domain types of *A. pernix* cell membranes grown at the different pHs, and Figure 4 shows the proportions of the spin probes in different

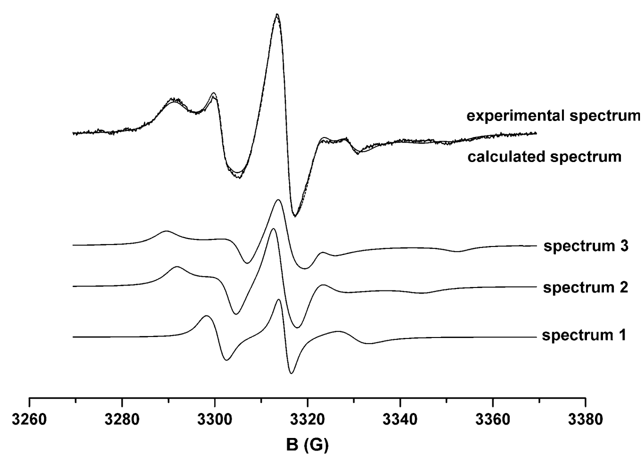

Fig. 2 Experimental EPR spectrum (*solid line*) of the spin probe MeFASL(10,3) incorporated into the membrane of *A. pernix* cells grown in the medium with pH 7.0 and measured at 20°C. The calculated spectrum, which fits best the experimental spectrum, is superimposed (*dotted line*). The corresponding spectral components of the three domain types with different motional charcteristics are also given. Spectrum 1 corresponds to the less ordered domain type D1, and spectrum 3 corresponds to the most ordered domain type D3

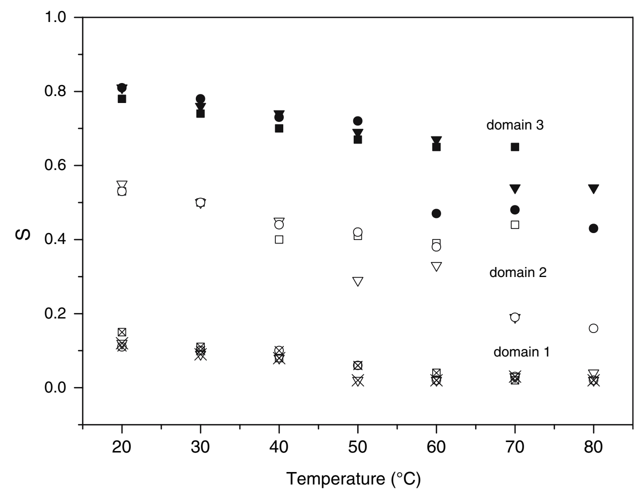

Fig. 3 The temperature-dependence of the order parameter (*S*) for the three types of domains in the membrane of *A. pernix* cells, as were detected by the spin probe MeFASL(10,3). The cells were grown in medium at pH 6.0 (⊠ - D1, ▽ - D2, ▼ - D3), 7.0 (⊠ - D1, ◯- D2, ● - D3) and 8.0 (⊠ - D1, ☐ - D2, ■ - D3). D1, D2 and D3 stand for domains 1, 2 and 3

domain types. With increasing temperature, the order parameter of all domain types decreases. When *A. pernix* was grown in the medium at different pH, the relative proportions of coexisting domain types changed, as shown in Figure 4. Typical for cells grown at pH 6.0 (Fig. 4a) was a very fast decrease of the most ordered domain type (domain 3), which ocupies 71% of the whole membrane at 20°C and disappears at 70°C, while the proportion of domains 1 and 2 increased from 0.01 and 0.28 at 20°C to 0.16 and 0.84 at 70°C, respectively. Also, a sharp decrease in the order parameter of domain 2 was observed between 40°C and 50°C (Fig. 3). All these observations are reasons for the rapid increase of mean membrane fluidity with increasing temperature, which was reflected in significantly smaller values of $2A_{max}$ at 40°C and of τ_{emp} (Table 1).

At pH 7.0 and 8.0, the proportion of domains 2 and 3 decreased with increasing temperature, meaning that domain 2 behaves differently at acidic pH than at neutral or slightly alkaline pH. The changes in the proportions of the domain types with temperature at pH 7.0 and 8.0 were similar.

In the temperature range 20–50°C, *S* and the proportion of the most ordered domain type (domain 3) were lowest for the cells grown in the pH 8.0 medium, while the proportion of the least ordered domain type (domain 1) was the highest (Fig. 4). This means that in this temperature region the membrane of *A. pernix* grown at pH 8.0 is more fluid than that of cells grown at pH 7.0 and 6.0. However, at 50°C and 60°C there is a sharp decrease in *S* of domains 3 and 2, respectively, for cells grown at pH 7.0 or 6.0, while at pH 8.0, the order parameter of all three domain types descreases only slightly with increasing temperature (Fig. 3). As a consequence, the membranes of the cells grown at pH 7.0 and 6.0 became more fluid than those grown at pH 8.0. Decreases in the order parameters and/or increases of the proportion of the less ordered domain type are reflected in an increase of the mean membrane fluidity. Having this in mind, the results of computer simulation agree well with the data obtained directly from the spectra. As can be seen from Table 1 for the cells grown at pH 6.0, a fast increase of membrane fluidity was observed with increasing temperature and for the cells grown at pH 8.0 slightly higher fluidity was measured at 20°C, while at 70°C slightly lower fluidity was measured than for the cells grown at pH 7.0.

Fluorescence Emission of Pyrene

It is well known that the ratio of fluorescence emission intensity of two bands in the pyrene vibronic spectra, I_1/I_3, decreases with a decrease in the polarity of the solvent (Kalyanasunduram & Thomas, 1977). Therefore, the changes in the I_1/I_3 ratio with temperature were used as indicators of the micropolarity of the pyrene environment in the *A. pernix* membrane. I_1/I_3 was thus plotted against temperature, as shown in Figure 5 for *A. pernix* cells grown in the medium at pH 6.0, 7.0 and 8.0. At pH 7.0, the optimal pH for *A. pernix* growth (Sako et al., 1996; Milek et al., 2005), I_1/I_3 decreased in the temperature range 40–65°C. Up to 40°C, the I_1/I_3 ratio was constant at around 2.0, suggesting that there was a polar environment of

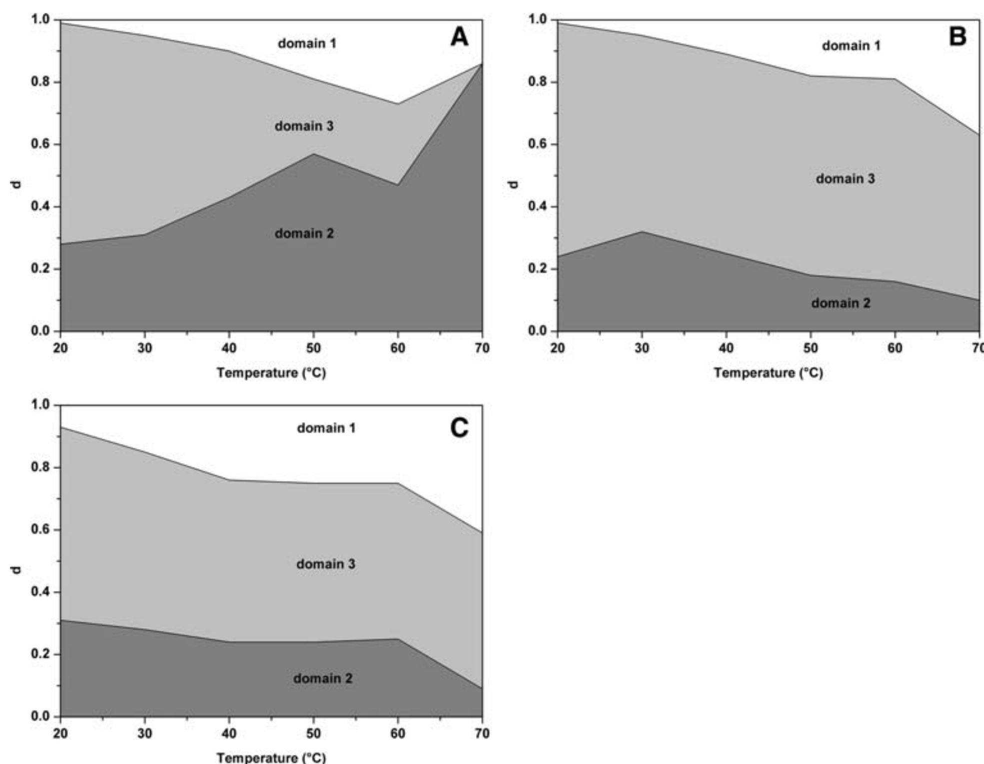

Fig. 4 Relative proportions of the spin probes in the three membrane domain types of *A. pernix* grown in medium at pH 6.0 (**a**), 7.0 (**b**) and 8.0 (**c**). Domain 1 is the less ordered domain type, and domain 3 is the most ordered domain type.

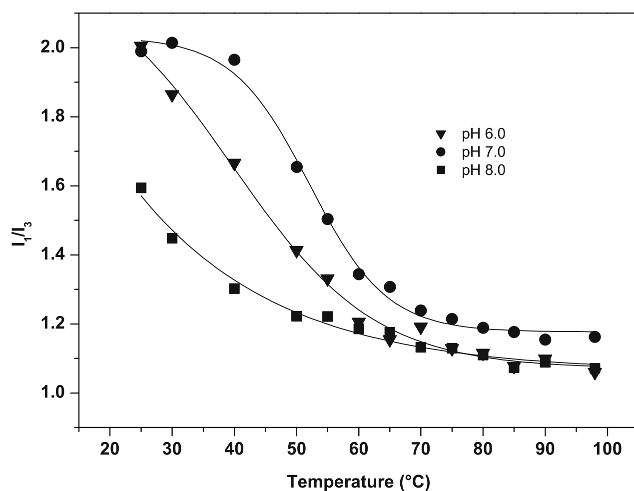

Fig. 5 Temperature dependence of the fluorescence emission intensity ratio, I_1/I_3, of the first, I_1, and third, I_3, bands in the vibronic spectra of pyrene in the presence of *A. pernix* cells grown in medium at pH 6.0, 7.0 and 8.0

pyrene molecules. At temperatures higher than 65°C, the I_1/I_3 ratio does not change significantly, and at temperatures of around 90°C the I_1/I_3 ratio was 1.15. Since the ratio of I_1/I_3 decreases with decreasing polarity, and more specifically with dielectric constant and dipole moment (Karpovich & Blanchard 1995), our results suggest that at temperatures higher than 65°C pyrene is located in a less polar environment, which was probably in the lipid bilayers of the *A. pernix* membranes. At pH 6.0 and 8.0, the I_1/I_3 ratio decreases continuously from 2.0 at 25°C to 1.05 at 60°C, and at higher temperatures it does not change significantly. It is interesting to note that for the cells grown at pH 8.0 the I_1/I_3 ratio at 25°C was 1.6, which is much lower than that for the cells grown at pH 6.0 and 7.0.

Discussion

In general, archaeal membranes appear to have properties that are well suited to the rather extreme environments in which these organisms live (Arakawa, Eguchi & Kakinuma, 2001). Thus, the ether structure should impart resistance to the hydrolysis of their lipids over a wide range of pH since ether linkages are generally more resistant to acid and alkaline hydrolysis than the ester linkages that are found in the Bacteria and Eucarya.

Recently, we have shown that pH affects the specific growth rate of *A. pernix* and its biomass production (Milek et al., 2005). By applying differential scanning calorimetry, we have also shown that growing *A. pernix* at different pH influences the thermal stability of the cellular components (e.g., ribosomal subunits) and, consequently, the thermal stability of the whole microorganism (Milek et al., 2007).

Preservation of membrane fluidity, which depends on the types and ordering of the liquid crystalline phase, at the environmental temperature is the principal concern that thermal extremophiles must address. For optimal membrane function, appropriately low concentrations of the

"hexagonal phase" membrane structure, which is formed from the liquid crystalline lamellar phase at elevated temperatures, must be maintained (Pakchun, Simpson & Codd, 2006). The results reported here suggest that besides temperature, the pH of the growing medium also influences the membrane fluidity.

The temperature dependence of the fluorescence emission intensity of two bands in the pyrene vibronic spectra, I_1/I_3, correlates well with fluidity changes, as measured by EPR, indicating that by increasing the mean membrane fluidity, the permeability of the membrane for pyrene increases (Table 1). In the presence of *A. pernix* cells grown at pH 7.0 the I_1/I_3 ratio of the pyrene spectra decreases with increasing temperature from 2.0 at room temperature (typical for pyrene in a polar region) and reaches a constant value of 1.2 (typical for a nonpolar environment) at temperatures higher than 65°C. This suggests that at this temperatures the membrane becomes fluid enough for the pyrene to penetrate into it, where it is in a less polar environment. These observations correlate well with the EPR results, where there is a sharp decrease in the order parameter of more ordered domains 3 and 2 (from $S = 0.71$ to 0.49 at 50°C and from $S = 0.37$ to 0.21 at 60°C, respectively) and an increase in the fraction of the less ordered domain ($d = 0.20$–0.38) (Figs. 3, 4). The increased fluidity and incorporation of pyrene into the membrane also correlate with the results reported by Sako et al. (1996), who saw that *A. pernix* grew well between 70°C (doubling time 48 h) and 97°C, with the optimal growth temperature being between 90°C and 95°C (doubling time 200 min).

A slightly different behavior of pyrene was observed in the presence of *A. pernix* cells grown at pH 8.0. The I_1/I_3 ratio of the pyrene spectra was 1.6 at 25°C. This would suggest that at pH 8.0 pyrene partially penetrates the *A. pernix* membrane already at 25°C. This corresponds to a higher fluidity measured in the cells grown at pH 8.0, which is reflected in a lower $2A_{max}$ at 20°C (Table 1), a higher fraction of the most fluid domain and a slightly lower S of all of the domain types than for the cells grown at pH 7.0 and pH 6.0 (Figs. 3, 4). Recently, we reported that *A. pernix* growing at pH 8.0 had a longer initial lag period but resulted in similar maximal cell densities being reached at similar times of cultivation to those seen at pH 7.0 (Milek et al., 2005).

For the cells grown at pH 6.0, the I_1/I_3 ratio decreased continuously from 25°C, which correlates with a fast decrease of the most ordered domain type and an increase in the less ordered domain type (Fig. 4). The maximum cell density that *A. pernix* achieved at pH 6.0 was approximately half of that achieved for the cells grown at pH 7.0 and 8.0, with the initial growth rate constant at pH 6.0 being half of the rate constant determined at pH 7.0 (Milek et al., 2005). A pH of growing medium lower than 7.0 was found to be less favorable than pH 8.0, and no growth was seen at pH 5.0. The changes in the distribution of spin probes and their motional characteristics, which reflected the changes in the membrane domain structure with temperature, were different for the cells grown at pH 6.0 than for the cells grown at pH 7.0 and 8.0. Macalady and coworkers (2004) suggested that there may be a strong correlation between the core-lipid composition and the optimal pH of growth medium. All of the known acidophilic archaea characterized to date produce extremely proton-impermeable tetraether membranes, while extreme alkalophiles have diether core lipids exclusively (Woese et al., 1990, and references citated therein). It is likely that at pH lower than 6.0 the membranes of the neutrophilic *A. pernix* composed of $C_{25,25}$-archaeol-based lipids, which are 20% thicker than membranes of the $C_{20,20}$-archaeol-based lipids of other archaea, becomes proton-permeable. The factors which influence this behavior are not yet known, but in future work, measurements are planned for isolated lipids from *A. pernix*, which should reveal these changes.

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