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Effect of growth temperature on membrane dynamics in a thermophilic cyanobacterium: a spin label study

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A strain of *Synechococcus* sp. was grown at its optimal growth temperature (58°C) and at 38°C, in order to investigate possible adaptations of membrane-related properties to growth temperature. Light-induced electron transport in thylakoid membranes from both types of cells showed linear Arrhenius plots with the same activation energy (48 kJ/mol). Membranes from cells grown at 58°C had a higher temperature optimum (53°C) than those from cells grown at 38°C (41°C). Growth at 38°C caused an increase in the proportion of unsaturated fatty acids compared to growth at 58°C. The fluidity of the membranes was investigated by measuring the temperature dependence of the parameters derived from electron spin resonance spectra of the spin-labels 5-doxyldecane, 5-doxylstearate and 16-doxylstearate. Only small differences between the dynamic properties of the membranes from cells grown at different temperatures could be detected. This suggests that the observed change in fatty acid composition of the membranes following the change in growth temperature does not serve to maintain a constant viscosity at the growth temperature.

Introduction

Cyanobacteria assigned to the genus *Synechococcus* are the most thermotolerant cells capable of oxygenic photosynthesis. They can be isolated from hot springs in several parts of the world, the maximum recorded growth temperature being 73°C [1]. The study of such thermophilic cyanobacteria allows investigation of the adaptation of the photosynthetic apparatus to an extreme environment, and provides an experimental system in which temperature-dependent effects can be studied over a wide range in an aqueous medium.

Light-induced electron transport from water to $NADP^+$ and the associated proton translocation occur in and across the intracellular thylakoid membranes. A plausible assumption is that these reactions are affected by the nature and properties of the lipid phase of the membrane. Any qualitative change in the phase of the membrane lipids from liquid crystalline to the gel phase can be expected to have dramatic effects, and it is possible that the membrane 'fluidity' or microviscosity may have to lie within a particular range for efficient operation.

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Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; ESR, electron spin resonance.

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Changes in ambient temperature will have effects on membrane properties, which may possibly be compensated by growth-temperature dependent changes in composition. Unsaturated fatty acids have lower melting points than their corresponding saturated analogues and an increase in the degree of unsaturation would be expected to increase fluidity. An extreme possibility, 'homeoviscous adaptation' [2], involves changes in lipid composition to give complete compensation of the membrane fluidity so as to maintain a constant value at the growth temperature. However, although this idea is instinctively appealing, it has not been confirmed by the majority of reported investigations in which compensation is at best partial [3].

The aim of the study reported here was to investigate the extent to which changes in growth temperature in a thermophilic cyanobacterium would elicit corresponding changes in fluidity. We have used a strain of *Synechococcus* originally isolated from a hot spring in Japan, with an optimal growth temperature of 58°C. The cyanobacteria were grown at this optimum temperature and at a value 20 degrees lower, close to the lower limit for growth [4]. We have determined the fatty acid composition of the membrane lipids of the two types of cells, and compared the temperature dependence of membrane-associated electron transport and the micro-viscosity of the membrane, measured in terms of the mobility of nitroxide spin labels.

A preliminary report of some of these observations has been published [5].

Methods

Organism. The original culture of *Synechococcus* sp. was provided by Professor S. Katoh, Tokyo, and had been isolated from a hot spring with a temperature of 57°C at Beppu, Japan [4]. An axenic clonal culture (Strain Od-24) was obtained by pour-plating in half-strength medium BG-11 [6] containing 0.5% agar.

Culture conditions. Cultures for preparation of membranes were grown in thermostatted glass fermentors (12 cm diameter) with a working volume of 1.8 litres. Each day the cultures were diluted approx. 20-fold with fresh medium. The growth medium was BG-11 with the Na_2CO_3 concentration increased to 0.2 g/l. The medium was stirred at 300 rev/min and gassed with 1% CO_2 in air at a rate of about 1 l/min. The culture was illuminated with three circular fluorescent tubes placed around the fermentor giving a photosynthetic photon flux density of 200 μmol quanta $\cdot$ $m^{-2} \cdot s^{-1}$ at the surface of the culture. Cultures for preparation of membranes were routinely harvested in the linear growth phase at a cell density around 10^8 cells/ml.

Preparation of membranes. Cells used for membrane preparations had been grown at the respective temperature for more than one week. Membranes were obtained essentially according to the procedure of Schatz and Witt [7]: after washing twice with 10 mM potassium phosphate buffer (pH 7.8) the cells were resuspended in buffer A (20 mM Hepes-NaOH (pH 7.8), 10 mM $MgCl_2$, 2 mM K_2HPO_4 and 500 mM mannitol) at a final concentration of 1 mg Chl/ml. The cells were then incubated with 0.1% (w/w) lysozyme in the dark for 1 h at 50°C for cells grown at 58°C, while the lysozyme treatment was extended to 1.5 h at 35°C for cells grown at 38°C. All further manipulations were carried out at room temperature. After washing in and resuspension with buffer B (20 mM Hepes-NaOH (pH 7.8), 10 mM $MgCl_2$ and 2 mM K_2HPO_4) membranes were prepared by Yeda press treatment of the spheroplasts at 1.0 MPa N_2. After dilution in buffer B the membrane fraction was collected by centrifugation at 24000 $\times g$ for 10 min and finally resuspended in a mixture of 80% (v/v) buffer A and 20% (v/v) glycerol and stored at −60°C.

Photosynthetic activity. Photosynthetic activity was measured as light-induced oxygen uptake using a Spectramass Dataquad quadrupole mass spectrometer with an inlet covered with silicone rubber tubing as described by Jensen and Cox [8]. The signal due to O_2 was calibrated at different temperatures using water in equilibrium with air saturated with water vapour at the same temperature. Oxygen solubility values were taken from Wilhelm et al. [9]. The assay medium contained buffer A, 125 μM benzyl viologen, 1 mM NaN_3, 125 μM gramicidin and membranes corresponding to 25–30 μM Chl *a*. The thermostatted reaction

chamber was illuminated by saturating yellow light (1.5 mmol quanta $\cdot m^{-2} \cdot s^{-1}$) provided by two Schott fiber optic light sources.

Lipid extraction. Lipids were extracted from the membranes according to Bligh and Dyer [10] except that 0.1 M HCl was used instead of water to ensure complete extraction of acid lipids. During the extraction procedures 0.1% butylated hydroxytoluene was present as an antioxidant. A polar membrane lipid fraction was obtained as described by Mannock et al. [11].

Fatty acid composition. The lipid extracts were methanolyzed at 100°C for 2 h in methanol containing 7% H_2SO_4. Fatty acid methyl esters were analyzed by gas chromatography using a Perkin-Elmer 3920 gas chromatograph with a 2 m column (4.1 mm i.d.) packed with 5% Silar 10 C and Diatomite M 85–100 mesh. The oven temperature was programmed from 180°C to 240°C at 4°C/min. Identification of the individual fatty acid methyl esters was done by comparison with known standard mixtures and verified by combined gas chromatography-mass spectrometry.

ESR measurements. Measurements of spin label spectra were made as described previously [12]. Measurements with the isolated membranes were made in the presence of NaCN and DCMU to decrease the rate of loss of signal due to destruction of the spin labels by the membrane preparation. The spin-broadening agent tris(oxalato) chromium (III) was synthesised as the potassium salt [13]. Parameters were calculated using the formulae given by Marsh [14].

Measurement of other constituents. The chlorophyll content of the membranes was determined in 80% acetone [15], and total carotenoid according to Wellburn and Lichtenthaler [16]. The myxoxanthophyll content of the isolated polar membrane fraction was determined in acetone using the specific absorption coefficient $E_{cm}^{1\%} = 2160$ at 478 nm [17]. The carbon and protein content of membranes was measured after washing twice with a large volume of 10 mM potassium phosphate buffer (pH 7.0). Carbon was measured with a Beckman Model 915 total organic carbon analyser using malonic acid as a standard. Protein was determined following solubilisation of the membranes with sodium dodecyl sulphate, using both the Bio-Rad protein assay [18] and the Lowry method [19]. Bovine serum albumin was used as standard in both cases.

Results

Characterisation of the membrane preparation

Cyanobacteria usually contain three types of membrane: the cell envelope, the cytoplasmic membrane and the internal thylakoid membranes containing the photosynthetic apparatus. Electron micrographs of *Synechococcus* cells showed 4 to 9 layers of thylakoids arranged concentric with the cell wall, so these are the predominant membrane type in these cells. In some cyanobacteria, yellow membrane fractions not containing chlorophyll can be separated by centrifugation on a sucrose density gradient [20,21]. This was not possible with the preparation used in these studies, and we conclude that it is comprised mainly of thylakoid membranes with possibly a minor contribution from the cytoplasmic membrane and the cell envelope.

Photosynthetic activity of isolated membranes

The effect of temperature on photosynthetic electron transport involving both Photosystems I and II was measured as oxygen uptake with benzyl viologen as electron acceptor. Fig. 1 shows that membranes isolated from *Synechococcus* cells grown at 58°C have a higher temperature optimum than those from cells grown at 38°C. The optimum temperature for activity in the isolated membranes are thus close to the temperature of growth, showing that the temperature stability of the photosynthetic apparatus in the living cells is conserved in the isolated membranes. On the lower temperature side of the optimum the Arrhenius plot for both membranes shows a linear dependency in the temperature range investigated. There is no evidence for discontinuities or break points. The two curves are parallel with a difference corresponding to 12°C. The apparent activation energy calculated from the slopes is 47 kJ/mol. The same value for the apparent activation energy was obtained when the temperature dependence of *P*700 reduction following a single turnover flash was examined (results not shown).

Hirano et al. [22] obtained a similar value for the activation energy of light-induced electron

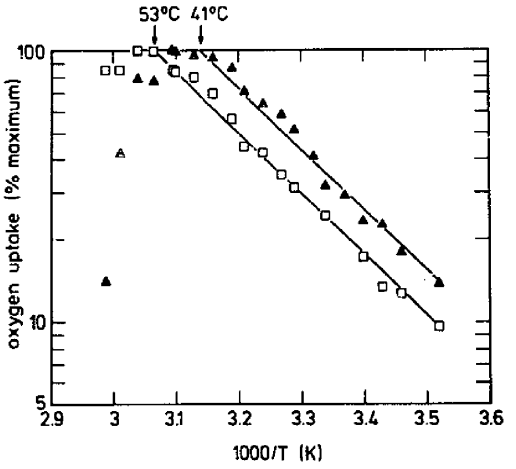

Fig. 1. Effect of temperature on light-induced oxygen uptake by membranes isolated from *Synechococcus* cells grown at 38°C (▲) or 58°C (□). Maximum rates were 290 and 135 μmol O_2·(mg Chl)$^{-1}$·h^{-1}, respectively. The assays were performed as described in Methods.

transport from water to methyl viologen in intact cells of the same strain of *Synechococcus*, at temperatures above 30°C. At lower temperatures a higher activation energy was observed, in contrast to the results reported here. It is noteworthy, however, that the doubly charged methyl viologen ion has been used in studies of membrane orientation because of its low rate of diffusion across membranes [23], so it is possible that the rate limiting step in measurements with whole cells at low temperatures is movement across the cytoplasmic membrane.

Composition of the membranes

Table I shows the contents of protein and photosynthetic pigments in membranes from the two types of cells, using the amount of membrane carbon as a basis for comparison. There is possibly a small decrease in the relative membrane protein content, and a significant decrease in the amount of chlorophyll in cells grown at the lower temperature. Although there is little change in the total amount of carotenoid, qualitative analysis by thin-layer chromatography showed that cells grown at 38°C contained considerably larger amounts of myxoxanthophyll.

TABLE I

COMPOSITION OF WASHED MEMBRANES ISOLATED FROM *SYNECHOCOCCUS* GROWN AT DIFFERENT TEMPERATURES

Carbon, protein and photosynthetic pigments were measured as described in Methods. 1.0 unit of protein corresponds to the result given by 1.0 mg of bovine serum albumin in the particular protein assay. The results are given as average values from membrane preparations from four different cell batches at each temperature.

Growth temperature (°C)	Content per mg membrane carbon			
	Protein		Chl. *a* (μg)	Carotenoids (μg)
	Bio-Rad (units)	Lowry (units)		
58	0.89	1.30	97	18
38	0.79	1.27	72	21

Myxoxanthophyll contains a sugar residue [17] and is found in the polar membrane lipid extract. There is a large difference in the content of this carotenoid in the polar lipid fractions from the two types of cell. Cells grown at 58°C contained 12 μg myxoxanthophyll/mg total polar lipid; the corresponding value for the cells grown at 38°C was 29 μg/mg lipid.

Synechococcus sp. resembles other cyanobacteria and contains three major lipids; mono- and digalactosyl diglyceride, phosphatidyl glycerol, and a trace of sulfoquinovosyl diglyceride (results not shown). The fatty acid composition of total lipid extract from photosynthetic membranes isolated from cells grown at 58°C and 38°C is compared in Table II. The cells do not contain any polyunsaturated fatty acids but only saturated and

TABLE II

FATTY ACID COMPOSITION OF TOTAL MEMBRANE LIPIDS IN *SYNECHOCOCCUS* SP GROWN AT 38°C OR 58°C

The results are given as average values obtained from membrane preparations from four different cell batches at each growth temperature.

Growth temperature (°C)	Fatty acids (%)			
	16:0	16:1	18:0	18:1
38	48.4	22.3	1.4	27.3
58	60.9	15.5	4.6	19.0

monounsaturated fatty acids. Lowering the growth temperature causes an increase in the amount of the 16 : 1 and 18 : 1 unsaturated fatty acids. The ratio of unsaturated to saturated fatty acids was 0.53 in cells grown at 58°C and increased to 0.99 in cells grown at 38°C.

The fatty acid composition of the isolated polar membrane lipid fraction was the same as that of the total membrane lipid extract (not shown).

These results can be compared with those reported by Yamaoka et al. [24], working with the same strain grown at 55°C under different conditions and apparently not in axenic culture. They found a lower content of the 18 : 1 fatty acid. They also reported some polyunsaturated fatty acid, in contrast to our findings and other reports that thermophilic strains of *Synechococcus* contain only saturated and monounsaturated fatty acids [25,26].

Spin-label mobility in isolated membranes

The fluidity of the photosynthetic membranes was measured using ESR spectroscopy of three spin labels: 5-doxyldecane, 5-doxylstearate and 16-doxylstearate.

The motion of the nitroxide groups of 5-doxyldecane and 16-doxylstearate approximates to motion in all three dimensions and the ESR spectra can be analysed to give apparent rotational correlation times for isotropic motion. Fig. 2A shows the temperature dependence of the rotational correlation time τ_B for 5-doxyldecane in membranes isolated from *Synechococcus* cells grown at 58°C and 38°C. The temperature dependence of the fluidity of the membranes from the two types of cells is identical above 30°C. Below this value, membranes from cells grown at the lower temperature were more fluid at any particular measuring temperature. Similar results were observed in experiments using 16-doxylstearate as the spectroscopic probe (Fig. 2B). The equations used to calculate the correlation times are not valid for slow isotropic motion, which corresponds to τ_B values greater than a few nanoseconds [14]. Therefore the high τ_B values observed at low temperatures cannot be interpreted as evidence for a change from a liquid-crystalline to a gel phase. However, the results do suggest a clear difference between the membranes from the two types of cells.

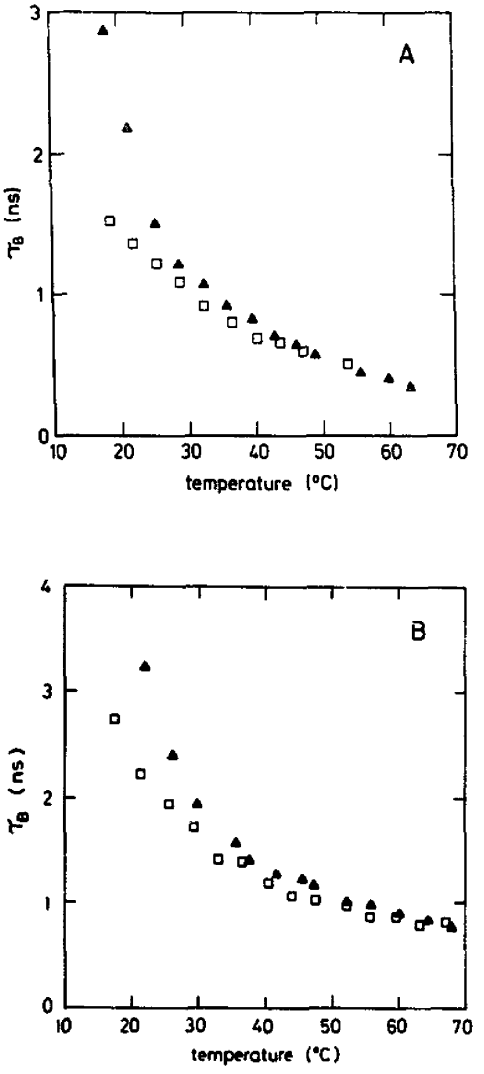

Fig. 2. Temperature dependence of the correlation time (τ_B) for 5-doxyldecane (2A) and 16-doxylstearate (2B) in membranes isolated from *Synechococcus* cells grown at 38°C (□) and 58°C (▲). Membranes corresponding to 1 mg Chl/ml were suspended in buffer A containing 20% glycerol, 50 μM DCMU, 30 mM NaCN and 25 μg/ml spin label. Tris-(oxalato)chromium (III) was added at a concentration of 30 mM to eliminate the signal of the spin-label in the water phase. ESR spectra were obtained with microwave power 10 mW and modulation amplitude 1.0 gauss.

The nitroxide group of 5-doxylstearate is localised close to the membrane surface and its motion is thus strongly anisotropic. If the mobility lies

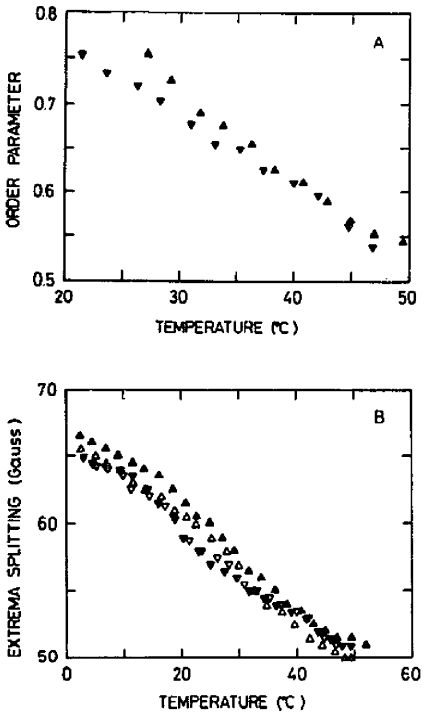

Fig. 3. The effect of temperature on the order parameter (3A) and outer hyperfine splitting (3B) for 5-doxylstearate in membranes isolated from *Synechococcus* cells grown at 38°C (▼) or 58°C (▲). Membranes corresponding to 1 mg Chl/ml were suspended in buffer A containing 20% glycerol, 54 μM DCMU, 32 mM NaCN and 5-doxylstearate (32 μg/ml). ESR instrument settings were as described in the legend to Fig. 2. The results in 3B are from membrane preparations from two different cell batches at each growth temperature.

within a certain range the ESR spectra can be analysed to give an order parameter which gives a measure of the degree of structural order in the membrane. Such parameters could only be determined from the experimental spectra over a restricted temperature range; at the lower end of the range the spin label motion was too restricted and at high temperatures the spin label was destroyed too rapidly to allow reliable measurements. It is noteworthy that the rate of destruction of the 5-doxylstearate increased dramatically at temperatures above 50°C, whilst it was quite stable at lower temperatures. A comparison of the calculated order parameters for the two types of membrane is shown in Fig. 3A. The outer hyperfine splitting can be used as an empirical measure of mobility in cases when it is not possible to determine order parameters. The values obtained are plotted in Fig. 3B. The results are in general agreement with those obtained with the other spin labels; essentially superimposable curves above 35°C with some indications for a divergence at lower temperatures.

The data points can be fitted to a smooth curve within the limits of the uncertainty associated with determining the exact positions of the derivative peaks, and there is no evidence for sharp breaks which could be attributed to phase changes. This is in contrast to the conclusions of Yamaoka et al. [24], who measured outer extrema splittings for 5-doxylstearate in the same strain of *Synechococcus* grown at 55°C and interpreted the results as evidence for transitions at 10°C and between 30 and 40°C. Authoritative cautions against making such assignments on the basis of inadequate data have been given [27,28]. It is also noteworthy that the reported spin-label to membrane lipid ratio in these experiments was many times higher than the recommended maximum value [14].

Measurements of spin-label partitioning in membrane preparations

Measuring the relative amounts of a spin-label in the lipid phase of the membrane and the aqueous medium has been used as an alternative method of studying temperature-induced changes in membrane dynamics, particularly the transition from a liquid-crystalline to a gel phase [14].

If the spin-broadening agent used in the experiments shown in Fig. 2 is omitted, the composite ESR spectrum contains contributions from 5-doxyldecane in both aqueous and hydrophobic environments. Fig. 4 shows the temperature dependence of an arbitrary parameter, obtained from spectra of this type, which is a measure of the partition between the two environments. When comparing membranes from the two types of cell at similar concentrations of total carbon as a measure of the amount of material in the membrane, a considerable difference is seen. We have previously reported similar results when membranes from the two types of cell are compared on the basis of chlorophyll content [5]. However, the significance of this observation is called into question by the great sensitivity of the results to the

concentration of membranes in the sample, as shown by comparing the two curves for the membranes from cells grown at the higher temperature. The difference between the two membrane types is probably the effect of alterations in the volume available to the spin label as a result of the changes in composition (Table I).

Spin-label mobility in polar membrane lipid dispersions

Measurements of spin-label mobility were also made in a polar lipid fraction isolated from membranes prepared from the two types of cells. This provides a measurement of the dynamic properties of the lipid phase independent of any effects due to protein–lipid interactions. The results with 5-doxyldecane are shown in Fig. 5. No significant differences could be seen between the preparations derived from the two types of cells, in agreement with the results obtained with the isolated membranes. Measurements of the order parameter for the motion of 5-doxylstearate also gave essentially superimposable curves with membrane lipids

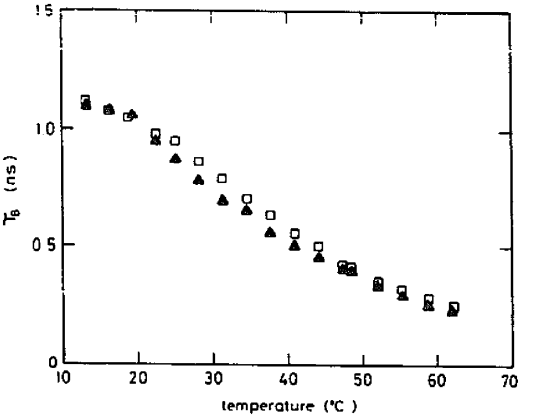

Fig. 5. Temperature dependence of the correlation time τ_B for 5-doxyldecane in polar lipids extracted from *Synechococcus* membranes isolated from cells grown at 38°C (□) or 58°C (▲). The lipids and the spin label were deposited on the walls of a small glass test tube by evaporation of the solvent. Aqueous medium composed of 100 mM Hepes-NaOH (pH 7.8), 10 mM $MgCl_2$, 30 mM tris(oxalato)chromium III was added to give a final concentration of 10 mg/ml lipid and 25 μg/ml 5-doxyldecane. A lipid dispersion was formed after vigorously shaking on a Vortex mixer and heating to 65°C for 30 min. ESR instrument settings were as described in the legend to Fig. 2.

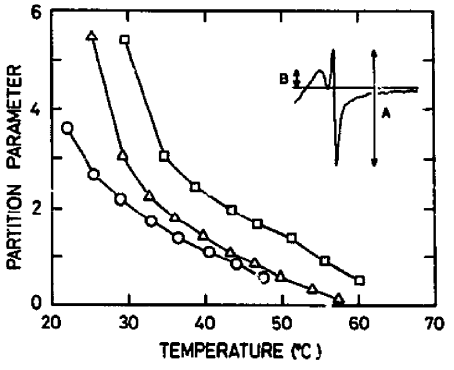

Fig. 4. The effect of temperature of the partition of 5-doxyldecane between the aqueous phase and the hydrophobic phase of *Synechococcus* membranes. The arbitrary partition parameter A/B was calculated from the composite ESR spectrum of 5-doxyldecane distributed between the aqueous phase and the hydrophobic membrane phase as shown for the high field peak in the inset. Membranes were suspended in buffer A containing 20% glycerol, 50 μM DCMU, 30 mM NaCN and 25 μg/ml 5-doxyldecane. (○) Membranes isolated from cells grown at 38°C. Concentration 10.7 mg carbon/ml. (□), (△) Membranes from 58°C cells at a concentration of 9.8 and 13.8 mg carbon/ml, respectively.

isolated from the two types of cells (results not shown).

Discussion

The fatty acid composition of cyanobacterial lipids shows considerable variation both when comparing different strains of the same genus and when the growth conditions for a particular strain are varied [25,29]. The thermophilic strains of *Synechococcus* contain no polyunsaturated fatty acids. In this they resemble some mesophilic cyanobacteria, while others contain both mono- and di-unsaturated fatty acids [25]. The proportion of monounsaturated fatty acids in the various thermophilic strains of *Synechococcus* when grown at around 50°C varies from 25% [26] to 65% [25]. These values overlap with the range of 44–66% reported by Kenyon [25] for nine mesophilic strains containing only saturated and monounsaturated fatty acids. Thus, although a general tendency towards less unsaturation (and also shorter chain length) is evident in thermophilic

TABLE III

PUBLISHED REPORTS OF THE EFFECT OF SHIFTS IN GROWTH TEMPERATURE ON THE FATTY ACID COMPOSITION OF DIFFERENT STRAINS OF *SYNECHOCOCCUS*

Note: *Agmenellum* and *Anacystis* belong to the genus *Synechococcus* as defined by Rippka et al. [6].

Strain	Growth temperature		% unsaturated fatty acids		Reference
	upper (°C)	lower (°C)	upper temp.	lower temp.	
Synechococcus Od-24	58	38	35	50	this work
Synechococcus lividus SY-4	55	38	23	56	[26]
'Agmenellum quadruplicatum' *	43	20	36	62	[29]
'Anacystis nidulans'	38	22	47	55	[30]
Synechococcus PCC 6301	37	25	51	55	[25]

* Strain BG-1 (PCC 73109).

strains, overall fatty acid composition is in general a rather crude measure of the functionally important aspects of membrane composition.

In spite of the lack of any clear trend when comparing mesophilic and thermophilic strains of *Synechococcus*, comparison of the same strain grown under similar conditions apart from temperature consistently reveals changes in fatty acid composition in the direction of a more fluid membrane as the growth temperature is lowered (Table III). Such changes do thus appear to have a role in adaptation to temperature in both this and other strains.

Changes in fatty acid composition of the type observed in our experiments would be expected to have measurable effects on spin label mobility. Effects of similar magnitude were observed in membranes of the bacterium *Proteus vulgaris* grown at different temperatures. These caused large changes in spin label mobility in phospholipid dispersions prepared from the respective membranes [31]. Our failure to observe significant effects of growth temperature on spin-label mobility at a given temperature thus requires explanation, since it is clear that effects of small changes in measuring temperature in a given preparation can be readily detected. In the case of the polar membrane lipid fraction, the most likely explanation is that the change in fatty acid composition in the direction of a more fluid bilayer is counteracted by the increased content of myxoxanthophyll. Carotenoids have been reported to have a rigidifying effect on the fluidity of phospholipid dispersions as measured by spin label mobility [32] and other biophysical techniques [33,34]. Myxoxanthophyll may also have a rigidifying effect in the membrane itself, although differences in the interactions between lipids and membrane proteins are also possible. Although there is no evidence for any increase in the total protein content of the membranes from the cells grown at the lower temperature, there is a large increase in the ratio between the reaction centres of Photosystem II and Photosystem I (Miller, M., Simpson, D. and Cox, R.P., unpublished results). Such qualitative changes may increase the amount of lipid associated with membrane protein and decrease the average fluidity.

Our results thus lead to the conclusion that there is not a significant homeostatic adaptation of membrane fluidity to maintain a particular value at the temperature of growth. It is possible that the primary adaptations to changes in growth temperature are the changes in relative content of reaction centres. Changes in the fatty acid composition might thus be a secondary adaptation to compensate for the restricted mobility as a result of increased association with protein.

Our failure to observe 'homeoviscous adaptation' in *Synechococcus* sp. is in general agreement with the results from spin-label and fluorescent probe studies with other types of bacteria, reviewed by Cossins and Sinensky [3]. In most studies the degree of adaptation observed is much less than complete. It seems probable that there is no need for regulation of membrane fluidity within narrow limits, as long as the formation of large areas of gel-phase lipid is avoided. This has been

dubbed 'homeophasic adaptation' by Silvius and McElhaney [35,36].

The determination of phase transitions in biological membranes has been the source of much controversy. The earlier literature on this topic was dominated by sharp 'breakpoints' and 'Arrhenius plot discontinuities'. The expectation that phase transitions will be found leads to a strong temptation to interpret continuous curves as a series of straight lines [27,28]. In other cases the sharp transitions observed are apparently the result of experimental artefacts such as using correlation times for spin labels although the conditions for the equations used are not fulfilled. It now seems clear that the sharp transitions observed with artificial bilayer membranes do not necessarily take place in biomembranes.

We see no evidence for any sharp transition in any of the various types of spin-label measurement. Nor do we observe changes in the apparent activation energy for light-induced electron transport involving both photosystems. There have been reports of breakpoints in Arrhenius plots for reactions involving segments of the electron transport chain and energy transfer between the two photosystems in both this strain and another strain of thermophilic *Synechococcus* [22,26]. These may represent temperature-dependent changes in the configuration of individual membrane-protein complexes or their closely associated lipids which would neither influence the rate limiting step of whole chain electron transport nor be detectable by the spin label approach.

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