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Temperature Dependence of Membrane-Bound Enzymes of the Energy Metabolism in *Rhodospirillum rubrum* and *Rhodopseudomonas sphaeroides*

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Abstract. *Rhodospirillum rubrum* grown either chemotrophically or phototrophically at 14° C and 30° C, was employed to study the effect of temperature on fatty acid composition as well as on several membrane-bound functions involved in energy metabolism. Upon growth at both temperatures the fatty acid composition of membranes showed differences, which could be attributed to an incomplete formation of photosynthetically active membranes rather than specifically to the growth temperature. Activities of NADH dependent respiration and light induced proton extrusion by cells did not show discontinuities in Arrhenius plots down to temperatures of 15° C and 5° C, respectively. In contrast, coupling factor Mg^{2+}- and Ca^{2+}-ATPase as well as succinate cytochrome *c* oxidoreductase showed significant breaks at 20° C and 18° C, respectively. Similarly, in *Rhodopseudomonas sphaeroides*, NADH dependent respiration and light induced proton extrusion by cells was continuous over the entire range of temperatures applied. ATPase as well as succinate cytochrome *c* oxidoreductase, on the other hand, featured discontinuities in Arrhenius plots at 20° C and 18° C. The implication of the data on growth rates and membrane structure are discussed.

Key words: *Rhodospirillum rubrum* – *Rhodopseudomonas sphaeroides* – Respiratory reactions – ATPase – light induced proton extrusion by cells – Arrhenius plots – Activation energies – Fatty acid patterns.

Fatty acids undergo thermotropic phase transitions from an ordered gel state to a disordered liquid-crystalline state. Discontinuities in Arrhenius plots of membrane-bound activities have been interpreted to represent largely the midpoint of thermotropic phase transitions in the membrane lipids. The same interpretation has been proposed to explain discontinuities in Arrhenius plots of complex physiological activities like the growth rate (Raison, 1973; McElhaney, 1974; Melchior and Steim, 1976). On the other hand, it is also known that some enzymes undergo temperature dependent changes in activity which, although featuring discontinuities in Arrhenuis plots, cannot be related to lipid phase transitions (Raison, 1973; Ayala et al., 1976; Madden and Quinn, 1979).

Both species of the phototrophic bacteria, *Rhodospirillum rubrum* and *Rhodopseudomonas sphaeroides*, exhibit unusual high proportions of unsaturated fatty acids with low melting points (lit. reviewed by Kenyon, 1978). Investigations employing membranes from *R. sphaeroides* indicated that lipid phase transitions occur at temperatures below 0° C (Fraley et al., 1978). It was shown in the foregoing communication (Kaiser and Oelze, 1980), that both of the species exhibit discontinuities in Arrhenius plots of growth rate as well as of the rate of bacteriochlorophyll (Bchl) synthesis. As both of these cellular activities presumably depend on energy metabolism the question arises as to whether the temperature characteristics of the growth rate and Bchl formation reflect the temperature characteristics of the activities of the energy regenerating systems. In an attempt to answer this question the present communication presents experimental results on the temperature dependency of the relevant electron transport reactions and of the coupling factor ATPase. Investigations on the dependency on growth temperature of the fatty acid composition of membranes have been carried out using several organisms (Cullen et al., 1971; Gill and Suisted, 1978). Most of the investigations revealed an approximately inverse relationship between growth temperature and the percentage of unsaturated fatty acids. Con-

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Abbreviation. Bchl = bacteriochlorophyll

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comitantly the breaks in Arrhenius plots of membrane-bound functions changed with the fluidity of lipids (Overath et al., 1970; de Kruyff et al., 1973; McElhaney, 1974; Mabrey et al., 1977). Knowing this, the questions arose as to temperature effects on fatty acid composition and enzyme activities in an organism like *R. rubrum* which at 30° C growth temperature already exhibits a high percentage content of unsaturated fatty acids. The present communication attempts to provide information concerning this point.

Material and Methods

Rhodospirillum rubrum, strain FR1 (DSM No. 1068, Göttingen) and *Rhodopseudomonas sphaeroides*, strain 2.4.1 (ATCC No. 17023) were grown either chemotrophically in the dark or phototrophically in the light on a complex malate medium (Drews, 1965). At the end of the exponential phase of growth the cultures were harvested and subjected to standard procedures for membrane isolation (Oelze and Kamen, 1975). Activities of the NADH and succinate horse heart cytochrome *c* oxidoreductase as well as of NADH dependent respiration were determined with freshly prepared membranes using published methods (Oelze and Kamen, 1975). Rates of light dependent proton extrusion by whole cells calculated on the pH changes for the first 10 s of illumination were measured according to Edwards and Bovell (1970) as described by Oelze and Post (1980). Membrane-bound ATPase was assayed in the presence of either Ca^{2+} (Ca^{2+}/ATP = 0.5) or Mg^{2+} (Mg^{2+}/ATP = 1) as detailed by Lücke and Klemme (1976). The reactions were terminated by addition of ice-cold 0.6 M trichloroacetic acid after 5 – 25 min, dependent on the incubation temperature (the optimum incubation time was determined for each temperature tested). Following sedimentation of the denaturated protein, inorganic phosphate was determined colorimetrically.

Protein was determined by the method of Lowry et al. (1951). Methods for the determination of the fatty acid composition of membranes have been described proviously (Oelze et al., 1975).

Results

Rhodospirillum rubrum, strain FR1, was grown chemotrophically and phototrophically at either 14° C or 30° C. Membrane preparations were employed to study the temperature dependencies of succinate and NADH cytochrome *c* oxidoreductase, as well as of NADH dependent respiration. The results representative of cells grown phototrophically at 30° C are depicted as Arrhenius plots in Fig. 1. A constant slope, i.e. temperature coefficient or activation energy (EA) can be observed for NADH respiration and NADH cytochrome *c* oxidoreductase within the range from 15 – 35° C. Succinate cytochrome *c* oxidoreductase, however, exhibits a clear cut discontinuity (TK) at about 18° C. Comparable experiments performed with membranes from cells grown phototrophically at 14° C and chemotrophically at both 14° C and 30° C led to similar results. For conciseness the activation energies (EA = kJ mol^{-1}) and temperatures of discontinuities (TK),

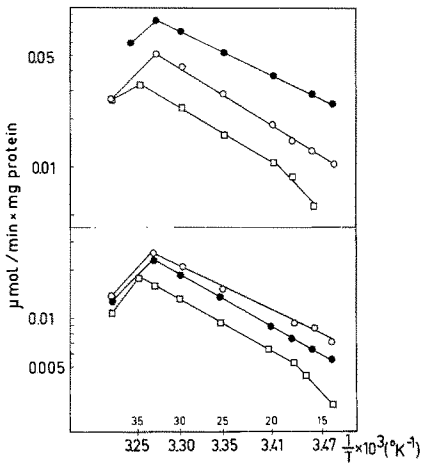

Fig. 1. Arrhenius plots of membrane bound respiratory activities of *Rhodospirillum rubrum* grown chemotrophically (*upper plots*) and phototrophically (*lower plots*) at 30° C. NADH dependent respiration (●——●); NADH- (○——○) and succinate- (□——□) cytochrome oxidoreductase

respectively, are compiled in Table 1. This shows clearly that, regardless of the mode of cultivation, activation energies of NADH dependent respiration are largely identical. The same applies to NADH cytochrome *c* oxidoreductase. Nevertheless, the activation energies of the two reactions differ from each other. In contrast, activities of succinate cytochrome *c* oxidoreductase exhibit a discontinuitiy at about 18° C with significantly different activation energies above and below this temperature (Table 1).

Enzymes may change their substrate affinities as a response to changes in the temperature of the assay system. If this is not taken into account erroneous breaks can occur in Arrhenius plots (Silvius et al., 1978). Therefore K_m values were determined for succinate cytochrome *c* oxidoreductase at either 16° C or 25° C assay temperature (Table 2). The results indicate no significant change in K_m. For the determinations of ATPase ($F_0 - F_1$) activities the findings were taken into account that, dependent on temperature, this enzyme complex reaches its maximum specific activity after different times of incubation. Arrhenius plots of the activities obtained on this basis for Ca^{2+} and Mg^{2+}-ATPase show distinct discontinuities at 20° C (Fig. 2). The corresponding activation energies (EA) are included in Table 1. Essentially identical K_m-values could be determined for Mg^{2+}- or Ca^{2+}-ATPase regardless of the two temperatures applied for enzyme tests.

It is generally assumed that in the course of electron transport protons are moved from one side of the membrane to the other (Wraight et al., 1978). Rates of light-induced proton extrusion by whole cells were determined in order to gain an insight into the temperature dependency of the formation of the electrochem-

Table 1. Activation energies (EA = kJ mol^{-1}) of membrane bound functions of *Rhodospirillum rubrum* and *Rhodopseudomonas sphaeroides* grown either phototrophically (P) or chemotrophically (C) at 30°C and 14°C as indicated. Temperatures of discontinuities (TK) in Arrhenius plots were determined in the range of temperatures given in the Figs. 1–5

		Rhodospirillum rubrum			*Rhodopseudomonas sphaeroides*		
		EA (30/14°C)	TK	EA (30/14°C)	EA (30°C)	TK	EA (30°C)
NADH Oxidase	P		–	59/58.5			44
	C		–	62/59			61
NADH cytochrome *c* oxidoreductase	P		–	50/42			41
	C			46/44			44
Succinat cytochrome *c* oxidoreductase	P	113/113	18	54/55	118	18	49
	C	121/101	18	53/54	117	17	58
ATPase (F_0-F_1) Ca^{2+}	P	108/–	20	54/–			
Mg^{2+}		109/112	20	63/62	107	21	53
Rate of cellular proton extrusion (light induced)				39/–			34/–

Table 2. Michaelis-constants (K_m) of ATPase (F_0-F_1) and succinate cytochrome *c* oxidoreductase of membranes from phototrophically grown [a] *Rhodospirillum rubrum* and [b] *Rhodopseudomonas sphaeroides*. Enzyme assays were performed at either 16°C or 25°C

Activity	Temp. (°C)	K_m [a] (mM)	K_m [b] (mM)
Succinate cytochrome *c* oxidoreductase	16	0.057	0.42
	25	0.033	0.32
ATPase (Mg^{2+})	16	0.30	0.22
	25	0.33	0.25
ATPase (Ca^{2+})	16	0.58	–
	25	0.58	–

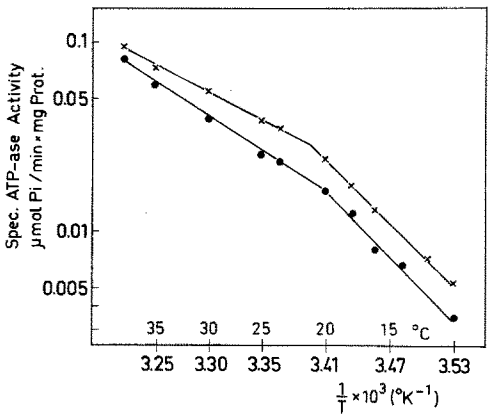

Fig. 2. Arrhenius plots of coupling factor ATPase (F_0-F_1) of membranes of *Rhodospirillum rubrum* grown phototrophically at 30°C. Ca^{2+}-ATPase (×——×); Mg^{2+}-ATPase (●——●)

ical proton gradient as well as of light dependent electron flow. The rates as depicted in Fig. 3 show no discontinuity, and furthermore the activation energy is rather low (Table 1).

It is known that during growth at extreme temperatures many organisms adjust the fluidity of membrane lipids by changing the fatty acid composition (Farell and Rose, 1967; Cronan and Vagelos, 1972; Gill and Suisted, 1978). The fatty acids patterns of the *R. rubrum* membranes were therefore determined after growth at either 14°C for 30°C (Table 3).

Analyses were carried out using membranes isolated from cells at the late exponential phase of adaptation from chemotrophic to phototrophic conditions. Because cultures growing at temperatures below 20°C do not attain the net amount of protein reached by cultures growing above this temperature (Kaiser and Oelze, 1980), fatty acid analyses were also carried out on cultures grown at 14°C but which had performed the same number (five) of doublings of cell mass as typical of cultures growing at 30°C. This reveals a decrease, especially in the proportion of palmitoleic acid, during phototrophic growth at 14°C. Thus, except for palmitic acid fatty acid patterns characteristic of both growth temperatures are very similar.

Rhodopseudomonas sphaeroides was employed to perform on a spot-check basis the same type of investigations reported above for *R. rubrum*. Respiratory activities were determined with membranes from cells grown chemo- or phototrophically at 30°C (Fig. 4, Table 1). Neither activities of NADH dependent respiration nor NADH cytochrome *c* oxido-

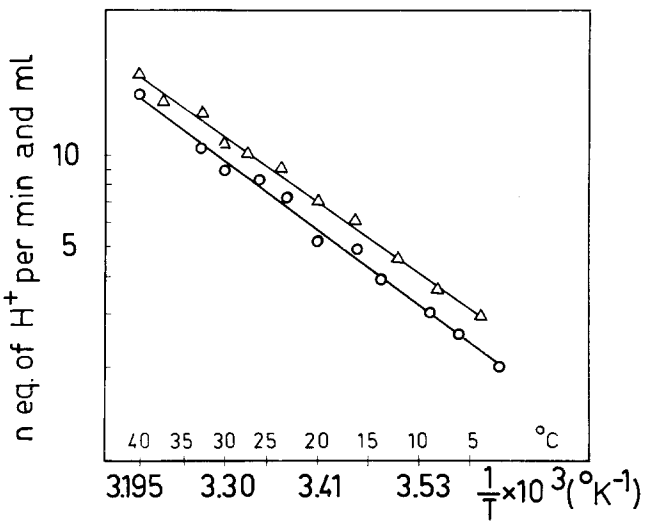

Fig. 3. Arrhenius plots of the rates of light induced proton extrusion by cells of photrophically grown *Rhodospirillum rubrum* (○——○) and *Rhodopseudomonas sphaeroides* (△——△). Bchl contents were 4 and 3.3 nmol per ml, respectively

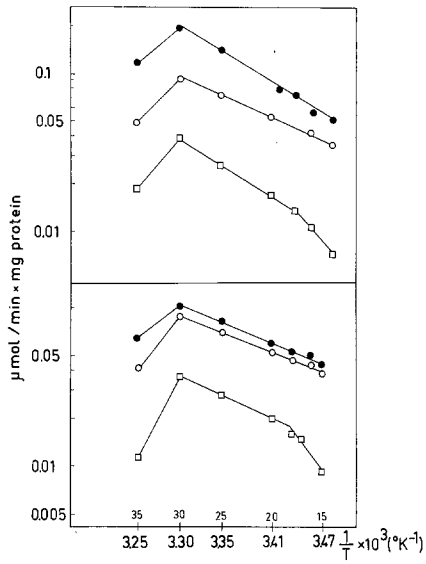

Fig. 4. Arrhenius plots of membrane bound respiratory activities of *Rhodopseudomonas sphaeroides* grown chemotrophically (*upper plots*) and phototrophically (*lower plots*) at 30° C. For symbols see Fig. 1

Table 3. Fatty acid composition of membranes from *Rhodospirillum rubrum* grown phototrophically at either 30° C or 14° C. Samples were taken after different numbers of doublings of cell mass as indicated. Values are expressed as percentage of total fatty acids

Fatty acid	30° C (five doublings)	14° C (two doublings)	14° C (five doublings)
14:0	2.0	3.6	2.4
16:0	18.4	9.0	10.0
16:1[a]	24.5	39.0	27.8
18:0	0.2	0.5	1.6
18:1[b]	45.3	41.0	44.0
(OH) 14:0	5.0	4.1	10.2
(OH) 16:0	2.4	3.0	3.9

[a] 9-Hexadecenoic and [b] 11-octadecenoic acids (Wood et al., 1965)

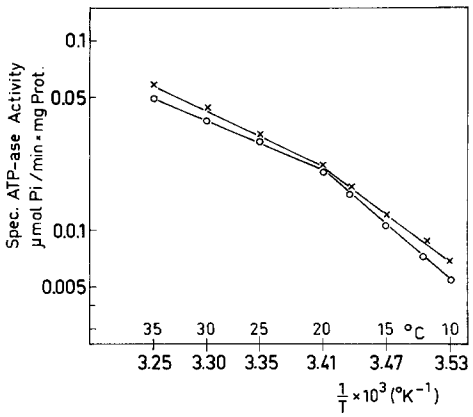

Fig. 5. Arrhenius plots of Mg^{2+}-ATPase of membranes from *Rhodopseudomonas sphaeroides* grown phototrophically at 30° C (×——×) or 14° C (○——○)

reductase show discontinuities in Arrhenius plots. Also the corresponding activation energies of membranes from chemotrophic or phototrophic cells are very similar. Arrhenius plots of light induced proton extrusion by whole cells are depicted in Fig. 3 and the activation energy is presented in Table 1.

As in *R. rubrum*, *R. sphaeroides* succinate cytochrome *c* oxidoreductase shows a significant discontinuity at about 18° C. K_m-values at 25° C and 16° C are shown in Table 2 together with K_m-values of Mg^{2+}-ATPase. The corresponding values are essentially identical. Figure 5 shows that also membrane-bound Mg^{2+}-ATPase of cultures grown at both 30° C and 14° C, exhibits a discontinuity at about 21° C.

Discussion

In agreement with results reported by Fraley et al. (1978) the data on the fatty acid composition of membranes presented in this communcation suggest that even during growth at 14° C a sufficient degree of fluidity of membrane lipids is maintained on the basis of a fatty acid pattern characteristic of cells growing at 30° C. Lack of lipid phase transition (within the range of temperatures applied) is also indicated by the lack of breaks in Arrhenius plots of NADH dependent respiratory activities as well as of the rates of cellular proton extrusion reflecting photochemical electron transport. Quite unexpectedly, however, both Mg^{2+}

and Ca^{2+}-ATPases as well as succinate cytochrome *c* oxidoreductase featured breaks in activity. Remarkably, the temperatures of the breaks differed slightly between ATPase and succinate dehydrogenase. But the respective break temperatures did not change after growth of the organisms at different temperatures.

Discontinuities in Arrhenius plots may result from phase transitions in membrane lipids (Melchior and Steim, 1976). This, however, is incompatible with the lack of a lipid phase transition above 0° C as shown with membranes of *R. sphaeroides* (Fraley et al., 1978). Other factors which might lead to changes in activity are, (i) formation of specifically composed lipid domains through phase separation; (ii) changes in the mobility of acyl chains by interaction with proteins; (iii) regulation of enzyme activities by the hydration state of polar head groups of phospholipids; (iv) the expression of high- and low-temperature forms of an enzyme independent of the physical state of the lipids (Ayala et al., 1976; Sackmann et al., 1977; Sandermann, 1978). Preliminary results from this laboratory indicate that isolated ATPase (F_0-F_1) preparations contain a lipid moiety with a fatty acid composition significantly different from that of complete membranes.

Previous investigations have shown that respiratory reactions, particularly of the NADH dependent pathway, limit chemotrophic growth and photophosphorylation, phototrophic growth (Oelze et al., 1978). Data presented in this paper in combination with results obtained previously on the depency of growth rates on temperature, however, reveal that activities neither of NADH dependent respiratory electron transport nor of the photochemical electron flow can provide a satisfactory explanation for the occurrence of breaks in Arrhenius plots of growth rates determined with both *R. rubrum* and *R. sphaeroides* (Kaiser and Oelze, 1980). Also, succinate dependent reactions which do not significantly contribute to cellular respiration can be excluded as a cause of the limitation of growth rate (Oelze et al., 1978). ATPase, on the other hand, exhibits both, a break in activity at 20° C and also activation energies comparable to the growth rate, the latter, however, applying only to temperatures above the break. At lower temperatures growth requires considerably higher energies than ATPase. This suggests either the involvement of yet another functional system limiting growth or more likely, that growth does not depend on one single "master reaction" but rather on a combination of several interlinked reactions (Senez, 1962).

In conclusion, the data show that organisms with a naturally occuring high percentage of unsaturated fatty acids like members of the phototrophic bacteria may still feature membrane-bound functional systems with breaks in Arrhenius plots at temperatures incompatible with the lipid composition. To date, the molecular basis for this is not clear.

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References

Ayala, J., Nieto, M., Carreira, J., Muñóz, E.: Activation parameters of the adenosine triphosphatase of *Micrococcus lysodeikticus*. Eur. J. Biochem. **66**, 43–47 (1976)

Cronan, J. E., Vagelos, P. R.: Metabolism and function of the membrane phospholipids of *Escherichia coli*. Biochim. Biophys. Acta (Amst.) **265**, 25–60 (1972)

Cullen, J., Phillips, M. C., Shipley, G. G.: The effects of temperature on the composition and physical properties of the lipids of *Pseudomonas fluorescens*. Biochem. J. **125**, 733–742 (1971)

Drews, G.: Die Isolierung schwefelfreier Purpurbakterien. Zbl. Bakt., I. Abt. Orig., Suppl. **1**, 170–178 (1965)

Edwards, G. E., Bovell, C. R.: Action of phenazine methyl sulfate, inhibitors, and uncouplers on the light-induced proton transport by cells of *Rhodospirillum rubrum*. J. Membrane Biol. **2**, 95–107 (1970)

Farrel, J., Rose, A. H.: Temperature on microorganisms. Ann. Revs. Microbiol. **21**, 101–120 (1967)

Fraley, R. T., Jameson, D. M., Kaplan, S.: The use of the fluorescent probe α-parinaric acid to determine the physical state of the intracytoplasmic membranes of the photosynthetic bacterium, *Rhodopseudomonas sphaeroides*. Biochim. Biophys. Acta (Amst.) **511**, 52–69 (1978)

Gill, C. O., Suisted, J. R.: The effects of temperature and growth rate on the proportion of unsaturated fatty acids in bacterial lipids. J. Gen. Microbiol. **104**, 31–36 (1978)

Kaiser, I., Oelze, J.: Growth and adaptation to phototrophic conditions of *Rhodospirillum rubrum* and *Rhodopseudomonas sphaeroides* at different temperatures. Arch. Microbiol. **126**, 187–195 (1980)

Kenyon, C. N.: Complex lipids and fatty acids of photosynthetic bacteria. In: The photosynthetic bacteria (R. K. Clayton, W. R. Sistrom, eds.), pp. 281–313. New York, London: Plenum Publishing Corp. 1978

Kruyff de, B., Dijck van, P. W. M., Goldbach, R. W., Demel, R. A., Deenen van, L. L. M.: Influence of fatty acid and sterol composition on the lipid phase transition and activity of membrane-bound enzymes in *Acholeplasma laidlawii*. Biochim. Biophys. Acta (Amst.) **330**, 269–282 (1973)

Lowry, O. H., Rosebrough, N., Farr, A. L., Randall, R. J.: Protein measurement with the Folin-phenol reagent. J. Biol. Chem. **193**, 269–275 (1951)

Lücke, F.-K., Klemme, J. H.: Coupling factor adenosine-5′-triphosphatase from *Rhodospirillum rubrum*. A simple and rapid procedure for its purification. Z. Naturforsch. **31c**, 272–279 (1976)

Mabrey, S., Powis, G., Schenkman, B., Tritton, T. R.: Calorimetric study of microsomal membrane. J. Biol. Chem. **252**, 2829–2933 (1977)

Madden, T. D., Quinn, P. J.: Arrhenius discontinuities of Ca^{2+}-ATPase activity are unrelated to changes in membrane lipid fluidity of sarcoplasmic reticulum. FEBS Letters **107**, 110–112 (1979)

McElhaney, R. N.: The effect of alterations in the physical state of the membrane lipids on the ability of *Acholeplasma laidlawii B* to grow at various temperatures. J. Mol. Biol. **84**, 145–157 (1974)

Melchior, D. L., Steim, J. M.: Thermotropic transitions in biomembranes. Ann. Rev. Biophys. Bioenerg. **5**, 205–238 (1976)

Oelze, J., Kamen, M. D.: Separation of respiratory reactions in *Rhodospirillum rubrum:* Inhibition studies with 2-hydroxydiphenyl. Biochim. Biophys. Acta (Amst.) **387**, 1–11 (1975)

Oelze, J., Golecki, J. R., Kleinig, H., Weckesser, J.: Characterization of two envelope fractions from chemotrophically grown *Rhodospirillum rubrum.* Antonie van Leeuwenhoek, J. Microbiol. Serol. **41**, 273–286 (1975)

Oelze, J., Fakoussa, R. M., Hudewentz, J.: On the significance of electron transport systems for growth of *Rhodospirillum rubrum.* Arch. Microbiol. **118**, 127–132 (1978)

Oelze, J., Post, E.: The dependency of proton extrusion in the light on the developmental stage of the photosynthetic apparatus in *Rhodospirillum rubrum.* Biochim. Biophys. Acta (Amst.) (in press)

Overath, P., Schairer, H. U., Stoffel, W.: Correlation of in vivo and vitro phase transitions of membrane lipids in *Escherichia coli.* Proc. Natl. Acad. Sic. USA **67**, 606–612 (1970)

Raison, J. K.: The influence of temperature-induced phase changes on the kinetics of respiratory and other membrane-associated enzyme systems. Bioenergetics **4**, 285–309 (1973)

Sackmann, E., Albrecht, O., Hartmann, W., Galla, H. L.: Domain formation in lipid bilayers and biological membranes. In: Structural and kinetic to plasma membrane functions (C. Nicolau and A. Paraf, eds.), pp. 28–43. Berlin, Heidelberg, New York: Springer 1977

Sandermann, H.: Regulation of membrane enzymes by lipids. Biochim. Biophys. Acta (Amst.) **515**, 209–237 (1978)

Senez, J. C.: Some considerations on the energetics of bacterial growth. Bacteriol. Revs. **26**, 95–107 (1962)

Silvius, J. R., Read, B. D., McElhaney, R. N.: Membrane enzyme: Artifacts in Arrhenius plots due to temperature dependence of substrate-binding affinity. Science **199**, 902–904 (1978)

Wood, B. J. B., Nichols, B. W., James, A. T.: The lipids and fatty acid metabolism of photosynthetic bacteria. Biochim. Biophys. Acta (Amst.) **106**, 261 (1965)

Wraight, C. A., Cogdell, R. J., Chance, B.: Ion transport and electrochemical gradients in photosynthetic bacteria, In: The photosynthetic bacteria (R. K. Clayton, W. R. Sistrom, eds.), pp. 471–511. New York: Plenum Publishing Corp. 1978

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