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Role of Vitamin K_2 in the Organization and Function of *Staphylococcus aureus* Membranes

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A mutant of *Staphylococcus aureus* auxotrophic for menadione (a vitamin K_2 precursor) was used to study the effects of menadione deprivation on the structure and function of the cell membrane. The phospholipid composition and metabolism was essentially unaltered by menadione deprivation. Removal of this precursor caused cellular levels of the cytochromes, protoheme, vitamin K_2, and several membrane-bound flavoprotein dehydrogenase activities to decrease as a function of growth dilution. The cytochromes were enzymatically reducible and maintained in the same proportions as menadione-supplemented cells. Oxidative phosphorylation, however, was reduced more than 10-fold and membrane vesicles obtained from menadione-deprived cells were unable to couple glycine transport to L-lactate oxidation. Succinic dehydrogenase and adenosine 5′ triphosphate hydrolysis appeared unaffected by menadione deprivation. These data suggest that menadione deprivation in the mutant stops the synthesis of vitamin K_2 and other electron transport chain components and prosthetic groups. Although individual electron transport chain members remained fully functional during menadione deprivation, the overall efficiency of the chain, measured in terms of its function in electron transport, oxidative phosphorylation, and electron transport chain-linked transport, dropped greatly. This suggests that the synthesis of vitamin K_2 is modulated to the synthesis of other components of the electron transport system, and that their organization into a functional system requires a specific concentration of vitamin K_2 with respect to total membrane lipid.

Glycerol and fatty acid auxotrophs of bacteria have been found to be useful tools for altering the complex structure-function relationship of cell membranes (13, 14, 17). Their usefulness stems from the fact that these molecules are integral components of the phospholipids and hence play a major structural role in the membrane. Vitamin K_2 functions in the bacterial membrane as a part of the electron transport chain, passing reducing potential from certain membrane-bound flavoprotein dehydrogenases to the cytochromes. Its role is therefore more functional than structural in nature, and it was of interest to determine what effects the deprivation of this molecule would have on both structural and functional parameters of the membrane. *Staphylococcus aureus* provides a model system for studying such effects since it contains only one class of quinones (vitamin K_2) and much data involving the metabolism of this naphthoquinone are available (4, 5, 9).

A double mutant, auxotrophic for both glycerol and menadione (a vitamin K precursor), was selected. Experiments were conducted in which the double auxotroph was grown in the presence of both glycerol and menadione and then deprived of either or both growth requirements. Phospholipid metabolism, active transport, neutral lipids, and structure and function of the electron transport chain were examined to determine the effects of deprivation conditions on these aspects of the membrane.

MATERIALS AND METHODS

Materials. Adenosine 5′-monophosphate (AMP), type III, sodium salt; adenosine 5′diphosphate (ADP), grade I, sodium salt; adenosine 5′-triphosphate (ATP), Sigma grade, disodium salt (all from equine muscle); firefly lanterns (desiccated tails); pyruvate kinase (ATP:pyruvate phosphotransferase, EC no. 2.7.1.40), type II, from rabbit muscle; myokinase (adenylate kinase, ATP:AMP phosphotransferase, EC no. 2.7.4.3) grade IV, from pig muscle; 2,6-dichlorophenol-indophenol, grade I, sodium salt;

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phenazine methosulfate and 2-methyl-3-phytyl-1,4-naphthoquinone (vitamin K_1) were obtained from Sigma Chemical Co. (St. Louis, Mo.). Menadione (1, 4-naphthoquinone, vitamin K_3) was purchased from Calbiochem (San Diego, Calif.). Tricyclohexylammonium phosphoenolpyruvate was obtained from Boehringer Chemical Co. (Mannheim, Germany). Carrier-free $H_3{}^{32}PO_4$ and $H_3{}^{33}PO_4$ were supplied by New England Nuclear (Boston, Mass.); [U-^{14}C]glycine, 80 mCi/mmol, was obtained from ICN Chemical and Radioisotope Division (Irvine, Calif.); 2-[*methyl*-^{14}C]1,4-naphthoquinone, 8.9 mCi/mmol was purchased from Amersham/Searle (Arlington Heights, Ill.); and [1-^{14}C]1-naphthol, 19.6 mCi/mmol from Nuclear Chicago (Des Plaines, Ill.). All other materials are as described in cited references.

Growth of bacteria. The mutant, *S. aureus*, strain S2M, auxotrophic for both menadione and glycerol, was derived from the glycerol auxotroph, strain S2 (14) by kanamycin treatment and selection on nutrient agar (18) with and without 3 μM menadione. The cultures were grown in the defined medium under the conditions previously described (14), except that 3 mM menadione dissolved in ethanol was added to a final concentration of 1.5 μM where indicated.

Deprivation of glycerol and menadione. For deprivation studies, cells were grown to a density of between 0.035 and 0.058 mg (dry weight) per ml, rapidly filtered using either a 142- or 293-mm membrane filter (Millipore Corp.) (0.45 μm pore size), washed with an equal volume of warm medium devoid of glycerol and/or menadione, and suspended in a similar volume of this same prewarmed medium also lacking glycerol and/or menadione. This procedure requires less than 3 min and does not produce a growth lag. The cultures were incubated for 150 min before harvesting by centrifugation at 10,000 $\times$ *g* for 15 min and washing with cold 0.05 M phosphate buffer, pH 7.3.

Extraction and separation of lipids. The total lipids were extracted by a modification of the procedure of Bligh and Dyer (2) as previously described (11). The extraction and all subsequent manipulations involving vitamin K_2 or carotenoids were done with a minimum of illumination as these compounds are photosensitive. Phospholipids were separated and identified chromatographically on silica gel-impregnated paper (Whatman SG-81) (15). Radioactive lipids on chromatograms were detected by autoradiography (15). For neutral lipid separation total lipid extract was applied to silica gel G thin-layer plates and developed in chloroform-isooctane, 2:1 (vol/vol) (4). This technique allows separation of vitamin K_2 isoprenologues (R_f 0.6 to 0.7) from the polar (R_f 0.1) and nonpolar (R_f 0.9 to 1.0) carotenoid pigments. The vitamin K_2 isoprenologues were separated on hexadecane-impregnated Kieselguhr G thin-layer plates by reverse-phase chromatography (6).

Assay of cytochromes. Cytochromes and protoheme were measured by difference spectroscopy using a Cary 14 split-beam spectrophotometer, as described by Frerman and White (4), except that membrane vesicles were used instead of whole cells. This modification avoided the problems of cell clumping encountered with whole cell suspensions of this strain.

Flavoprotein dehydrogenase assays. Primary flavoprotein dehydrogenases were measured in purified-membrane vesicles according to the procedure of Ells (3). The assay was modified to be performed in a reaction mixture of 3.0-ml total volume and run at 30 C using phenazine methosulfate and dichlorophenol indophenol as electron acceptors. The decrease in absorbance at 600 nm was measured in a Gilford 2400S recording spectrophotometer.

Oxidative phosphorylation. The levels of ATP, ADP, AMP, and oxygen were measured as follows. A modification of the device described by Knowles and Smith (10) was devised using a 30-ml syringe with a teflon plunger containing a Clark-type oxygen electrode. The plunger assembly was fitted to a repeating dispenser so that 0.5-ml samples of the syringe contents could be dispensed as desired during the experiment. The cell suspension within the syringe was stirred using a small teflon-coated magnetic bar. Additions to the syringe chamber were made via a three-way valve at the tip of the syringe. For these experiments washed whole cells were added to the syringe and allowed to equilibrate. Equilibration was indicated when the endogenous level of respiration was achieved. L-Lactate (20 mM final concentration) was added and the experiment continued through anaerobiosis. During the experiment, cell samples (0.5 ml) were dispensed directly into ice-cold 0.2-ml portions of 1.92 M perchloric acid in culture tubes (10 by 75 mm). After extraction and neutralization, ATP was assayed by a luciferin-luciferase assay as described (10). ADP was measured after incubation of the sample with 0.1 μmol of tricyclohexylammonium phosphoenol pyruvate and 10 μg of pyruvate kinase in buffer for 30 min at 30 C as the additional ATP generated. This value, minus the value for ATP, represents the ADP level. AMP was determined by adding 5 μg of myokinase per reaction tube to a portion of the ADP reaction mixture. The difference of this value minus the ADP (determined after pyruvate kinase) minus the ATP level represents the AMP level of the sample. ATP, ADP, and AMP standards were prepared fresh for each experiment.

Membrane vesicles-amino acid transport. Membrane vesicles of *S. aureus* were prepared and their ability to transport glycine was tested as described by Short et al. (16). Glycine was used because of the favorable V_{max} and K_m of its transport kinetics. Uptake was initiated by addition of L-lactate, 0.05 M final concentration.

Measurements of radioactivity. Samples were assayed for radioactivity in a scintillation spectrophotometer (model 2405, Packard Instrument Co.) in a scintillation fluid of 9.28 mM 2,5-bis[2(5-terbutyl benzoazol)]-thiophene in toluene. Radioautograms were prepared with Kodak no-screen X-ray film as previously described (19).

Measurement of protein and phosphorus. Protein was measured by the procedure of Lowry et al. (12). Phosphorus was determined as described by Bartlett (1) as adapted to the autoanalyzer (19).

RESULTS

Effect of glycerol and menadione deprivation on growth of the organism. Glycerol deprivation caused growth to slow and cease after 20 to 30 min, and an immediate cessation in net phospholipid synthesis (Fig. 1). In contrast, deprivation of menadione caused a decrease in the growth rate after 60 to 80 min, or about two generations of cell growth. Net lipid phosphate incorporation paralleled growth, rather than ceasing immediately as with glycerol deprivation. When glycerol and menadione were simultaneously removed from the culture, the kinetics of growth and lipid phosphate incorporation followed those of glycerol deprivation alone.

It was determined from further growth studies that after 180 min of menadione deprivation, the addition of menadione would not stimulate further growth. A procedure was standardized so that cultures were routinely grown at a predeprivation generation time of 40 min and then deprived of menadione for 150 min before sampling the culture to assay a particular function.

Effect of glycerol and menadione deprivation on phospholipid metabolism. Cells were labeled with $H_3{}^{32}PO_4$ before deprivation and $H_3{}^{33}PO_4$ (both at 0.5 μCi/ml) after the filtration and final suspension. Samples were removed from the cultures before and after the deprivation and the phospholipids were extracted, separated, and analyzed as described. The rates of synthesis (^{32}P incorporation) and turnover (^{32}P loss) of the total and individual phospholipids were determined (data not shown). The effects of glycerol and glycerol-menadione deprivation were identical to those reported previously on glycerol deprivation (14). The deprivation of menadione alone had only a minor effect on phospholipid metabolism. The percentage of composition of the phospholipid of the membrane was unchanged by menadione deprivation (data not shown). These data indicate that the growth inhibition by menadione deprivation was not caused by change in membrane phospholipid metabolism.

Biosynthesis of vitamin K_2 isoprenologues from menadione. The alkylation of menadione was distinguished from direct incorporation into the membrane by growing the mutant

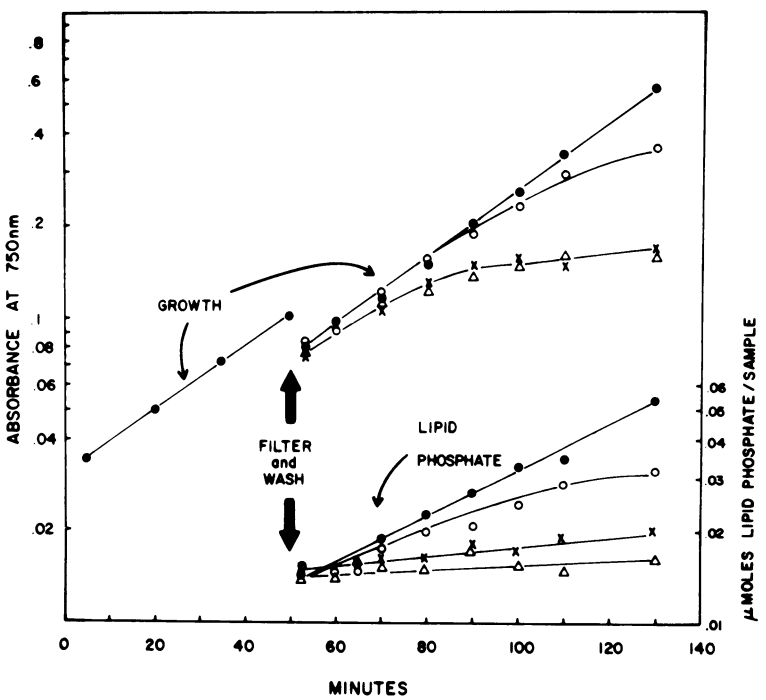

FIG. 1. *Effect of deprivation of menadione and glycerol on the growth and phospholipid synthesis in S. aureus S2M. The culture (1 liter) was grown to an absorbance of 0.1 at 750 nm (0.058 mg of cells [dry weight]/ml), filtered, and washed as described in Materials and Methods, and was divided into four equal parts for further growth, with each of the following additions: (●) glycerol and menadione; (○) menadione; (×) glycerol; (△) none. At the points indicated, 10-ml samples were removed from the cultures, total lipids were extracted (11), and lipid phosphate was determined.*

(250-ml cultures) for seven to eight generations in the presence of 25 μCi of [*methyl*-^{14}C]menadione. The cells were harvested, the vitamin K_2 was extracted and purified, and the isoprenologues were separated and detected by autoradiography as described previously (6). It was found that strain S2M does make the normal array and distribution of isoprenologues from the menadione provided. Results obtained were identical to those reported by Hammond and White (5). Interestingly, α-naphthol would not support growth in the absence of menadione, but could be used to label a full array of isoprenologues when the cells were grown in the presence of [^{14}C]α-naphthol (0.1 μCi/ml) and nonradioactive menadione. Presumably the cells were alkylating the α-naphthol ring.

The vitamin K_2 formed from the menadione was extracted and examined spectrophotometrically in isooctane and had an absorption spectrum identical with that of authentic vitamin K_2 extracted from *S. aureus* strain U71 (19), and dissimilar to the spectra of vitamin K_1, menadione, or α-naphthol.

Menadione deprivation and intracellular vitamin K_2 and carotenoid levels. The intracellular level of vitamin K_2 decreases about threefold after 150 min of menadione deprivation (Table 1). Once formed, vitamin K_2 isoprenologues do not turn over metabolically (5). Nonpolar carotenoid pigments showed a threefold increase in concentration under these same conditions.

Assay of cytochromes. When strain S2M was deprived of menadione for 150 min, the specific concentrations of cytochromes *a*, *b* + *o*, *o* and protoheme were lowered approximately threefold as compared to the menadione-supplemented cells (Table 1). The ratio of *b* + *o* to *a* (2.0 menadione-supplemented, 1.9 menadione-deprived) and the ratio of *o* to *a* (14.2 menadione-supplemented, 9.4 menadione-deprived) showed that the relative levels of all cytochromes were similarly affected by growth in the absence of menadione. Although the cytochrome level was lower in the menadione-deprived cells, cytochromes from both growth conditions were completely reduced in the presence of L-lactate.

Flavoprotein dehydrogenase activities and menadione deprivation. Menadione deprivation affected L-lactate, D-lactate and nicotinamide adenine dinucleotide, reduced form, dehydrogenases in a manner similar to the cytochromes. The specific activities of these membrane-bound primary dehydrogenases were approximately three- to fourfold lower in the deprived cultures than in menadione-supplemented cells (Table 1). Succinic dehydrogenase, however, was virtually unaffected by menadione deprivation over the experimental period, perhaps indicating that electrons from this substrate do not enter the electron transport chain (ETC) through naphthoquinones.

Vitamin K_2 and oxidative phosphorylation. The ability to link L-lactate-stimulated oxygen consumption to the synthesis of ATP in washed cells was studied in menadione-supplemented and menadione-deprived cells. With menadione-supplemented cells, ATP synthesis was initially stimulated to a high rate after the addition of L-lactate, followed in about 1 min by a somewhat lower rate, which remained constant (2.90 nmol of ATP/min per mg of protein) until all oxygen was exhausted from the test culture (Fig. 2). At this point, the level of ATP in the cells rapidly dropped. ADP concentration dropped initially and remained low throughout the experiment. The AMP level was initially high during the phase of endogenous respiration (equilibrium), after the addition of L-lactate. During the steady-state aerobic phase, AMP levels fluctuated but generally fell to a low level just before the onset of anaerobiosis. At this point, as ATP was being rapidly hydrolyzed, the AMP concentration rose sharply. The endogenous rate of oxygen utilization was increased over eightfold when L-lactate was added, going from 2.38 to 19.46 nmol of oxygen/min per mg of protein.

In contrast, the oxidative phosphorylation kinetics of the menadione-deprived cells showed a marked impairment in the ability to make ATP from the oxygen-linked oxidation of substrate (Fig. 3). The rate of oxygen utilization following L-lactate addition was 2.48 nmol of oxygen/min per mg of protein. This value was sevenfold lower than that of menadione-supplemented cells. Substrate addition did not induce the rapid and immediate synthesis of ATP as observed for menadione-supplemented cells. ATP synthesis began after a short lag during which the AMP level rose slightly. The rate of ATP synthesis (0.26 nmol of ATP/min per mg of protein) was exceeded by the rate of its degradation long before oxygen was exhausted. Just before the onset of anaerobiosis, the levels of all three adenine nucleotides were roughly the same, with AMP actually being the greatest. The rate of ATP hydrolysis was essentially the same in both cultures (Table 1).

L-lactate-stimulated glycine transport in menadione-deprived and non-deprived membrane vesicles. Membrane vesicles were prepared from menadione-deprived (150 min) and menadione-supplemented cultures using the

TABLE 1. *Levels of membrane components and activities in S. aureus S2M supplemented with and deprived of menadione*[a]

Membrane Components	(+)	(-)	Ratio (+/-)
Category 1 (effect = growth dilution)			
Vitamin K_2[b]	0.056	0.018	3.1
Nonpolar carotenoids[c]	0.864	2.64	1/3.1
Protoheme[d]	4.8	1.9	2.5
Cytochrome *a*[e]	0.026	0.009	2.9
Cytochrome *b* + *o*	0.053	0.017	3.1
Cytochrome *o*	0.369	0.085	4.3
L-lactate dehydrogenase[f]	61.5	11.6	5.3
D-lactate dehydrogenase	9.9	2.6	3.9
NADH dehydrogenase	69.6	22.5	3.1
Category 2 (effect > growth dilution)			
ATP synthesis[g]	2.90	0.26	11.1
O_2 utilization[g]	19.46	2.48	7.7
Glycine transport[h]	0.095	0.020	5.0
Category 3 (no effect)			
Succinic dehydrogenase[f]	41.7	47.3	0.9
ATP hydrolysis[g]	3.0	2.0	1.3

[a] Cell growth and menadione deprivation as described in Materials and Methods section of this paper. (+) with mendione, (-) deprived of menadione.

[b] Vitamin K_2 was measured after extraction from 0.32 g (+), 0.31 g (-) (dry weight) of cells, estimated by its absorbance when dissolved in isooctane using $\epsilon = 19.28 \times 10^3$ at 248 nm and expressed as micromoles per gram of cell (dry weight).

[c] Nonpolar carotenoids extracted from the cells used in (*b*) were estimated as the absorbance at 285 nm per gram of cell (dry weight).

[d] Protoheme content was determined using 1.03 mg (+) and 3.19 mg (-) of protein of membrane vesicles by the reduced minus oxidized pyridine hemochrome (4) using $\epsilon + 20.0 \times 10^3$ at 557-552 nm and expressed as nonomoles per milligram of membrane protein.

[e] Cytochromes were measured by difference spectroscopy and are expressed as absorbance increment per milligram of membrane protein with absorbance increments between 603 nm and a line connecting 630 and 595 nm for cytochrome *a*; 560 nm and a line connecting 540 and 580 nm for cytochromes *b* + *o* in the reduced minus oxidized difference spectrum; and 416 and 430 nm in the carbon monoxide-saturated minus reduced difference spectrum.

[f] Dehydrogenase activity (nanomoles/minute per milligram of membrane protein) was measured as a decrease in absorbance at 600 nm in a dichlorophenolinophenol-phenazine methosulfate assay system on the addition of the substrate to a 0.05 mM final concentration in the presence of 352 μg (+) and 366 μg (-) of membrane protein.

[g] Cell preparation and experimental conditions described in Fig. 2 and 3. Levels of ATP synthesis and degradation in terms of nanomoles of ATP per minute per milligram of protein. Oxygen concentration is nanomoles O_2 per minute per milligram of protein.

[h] Vesicle preparation and experimental conditions described in Fig. 4. Uptake expressed as nanomoles of glycine per 10 min/mg of membrane protein.

procedure of Short et al. (16) and tested for their ability to concentrate [^{14}C]glycine using L-lactate as the energy source (Fig. 4). Vesicles prepared from menadione-supplemented cells were capable of L-lactate-coupled glycine transport, whereas the vesicles prepared from menadione-deprived cells were not. An attempt was made to restore the ability to transport amino acids in the menadione-deprived vesicles by the addition of menadione, vitamin K_1, and a mixture of *S. aureus* vitamin K_2 isoprenologues. Menadione was added to a final concentration of 10 μM menadione and 1% ethanol, mixed, and preincubated at 30 C for 2 min before glycine uptake was measured. Vitamin K_2 isoprenologues were added as a part of a total lipid extract of *S. aureus* (1.1 mg) which had been taken to dryness and sonicated in 1.0 ml of 0.05 M phosphate buffer, pH 7.4. Sonication was done with a Branson model W 140 Sonifier at full power 8 × 30 s, maintaining the temperature at <10 C. An equal volume of this lipid dispersion and membrane vesicles was preincubated from 2 to 20 min at 4 C and at 30 C before glycine uptake was tested. Finally, menadione, and then vitamin K_1, were sonified (as above) with 0.15 mg of the purified-phospholipid fraction of this organism added (final concentration 50 μM) to the vesicles and preincubated, as was the total lipid extract sonicate. None of these naphthoquinone "restored" vesicles concentrated glycine within the test period.

DISCUSSION

Removal of menadione from the culture medium slowed the growth of the mutant strain S2M after about 60 min. Growth stopped after 90 and 120 min of menadione deprivation. The inhibition of growth was apparently not caused by an effect on the structural lipids of the membrane, for the composition and metabolism of the phospholipids remained unaltered throughout the 150-min deprivation period and incorporation of ^{32}P from $H_3{}^{32}P_4$ into total lipid phosphorus increased in proportion to growth.

Nonpolar carotenoid pigments accumulated in the menadione-deprived S2M membrane (Table 1). This could indicate a vitamin K_2 involvement in the oxidation of these pigments, perhaps through the heme protein, cytochrome P_{450} of the mixed-function oxidase system (8).

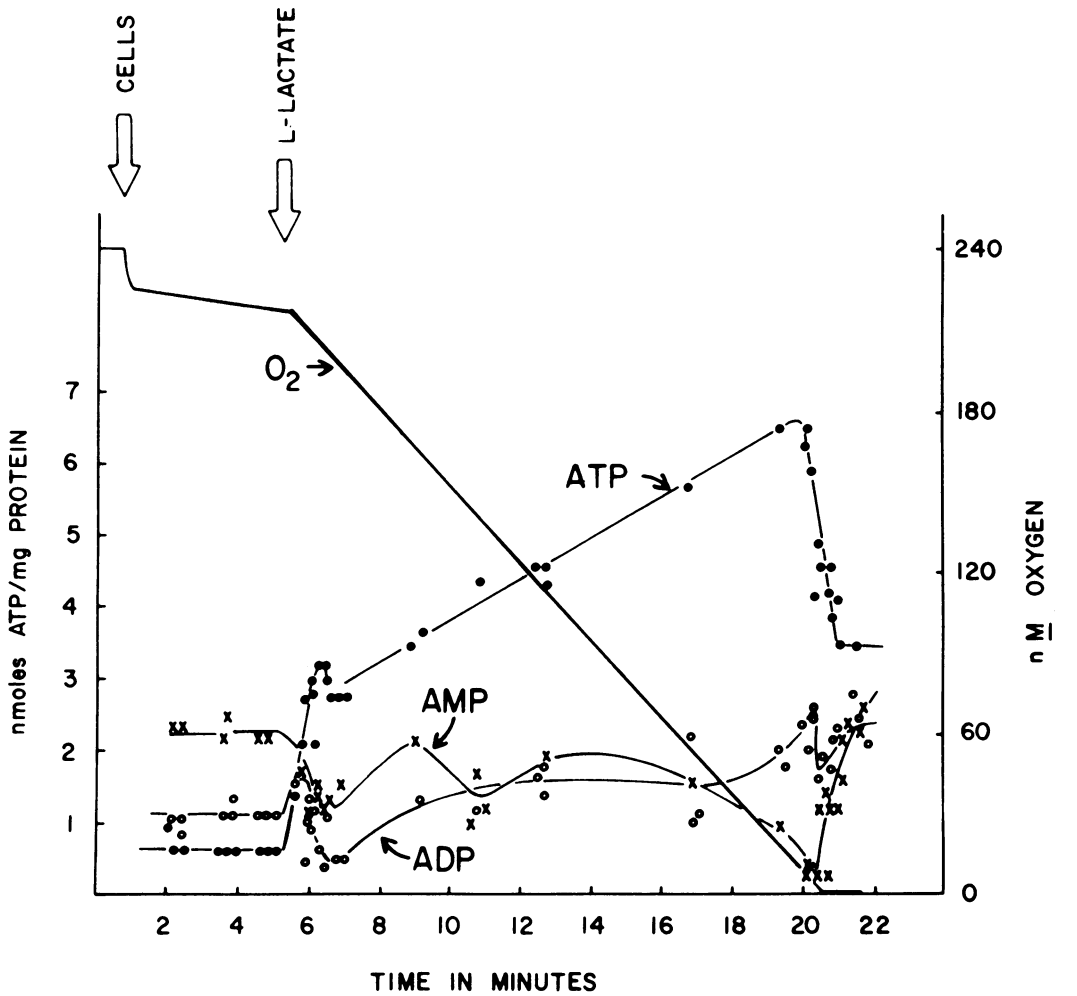

FIG. 2. *Oxidative phosphorylation kinetics in a menadione-supplemented culture of S. aureus, strain S2M. Two cultures of 1 liter each were grown, filtered and washed, and one was deprived of menadione. The washed whole cells of the menadione-supplemented culture were suspended in phosphate buffer and their ability to link* L-*lactate oxidation to the utilization of oxygen and the synthesis and/or degradation of adenine nucleotides was studied as described in Materials and Methods. Protein concentration of suspended cells was 0.77 mg/ml. Symbols: solid line represents oxygen concentration;* (×) *AMP;* (○) *ADP;* (●) *ATP concentrations.*

The increase in nonpolar carotenoids could also be a reflection of a regulatory mechanism involved in distribution of the common intermediate, isopentylpyrophosphate, which both vitamin K_2 and carotenoids require for synthesis of their isoprenoid components.

The cell mass increased an average of 3.5-fold in the cultures tested during the 150-min period of menadione deprivation. In deprived cells, the levels of vitamin K_2, protoheme, cytochromes *a*, *b*, and *o* and the specific activities of L-lactate, D-lactate, nicotinamide adenine dinucleotide, reduced form, dehydrogenase fell an average of 3.5-fold (category 1 of Table 1). This suggests that at the time of menadione deprivation, synthesis of these components ceases, and their concentrations in the membrane fall as a function of growth dilution. The cytochromes of the menadione-deprived cells remained completely reducible enzymatically. The individual members of the ETC remained fully functional during menadione deprivation when tested for their isolated function and maintained the same proportions as the amounts in the membrane were lowered by growth dilution.

Although the individual members of the ETC

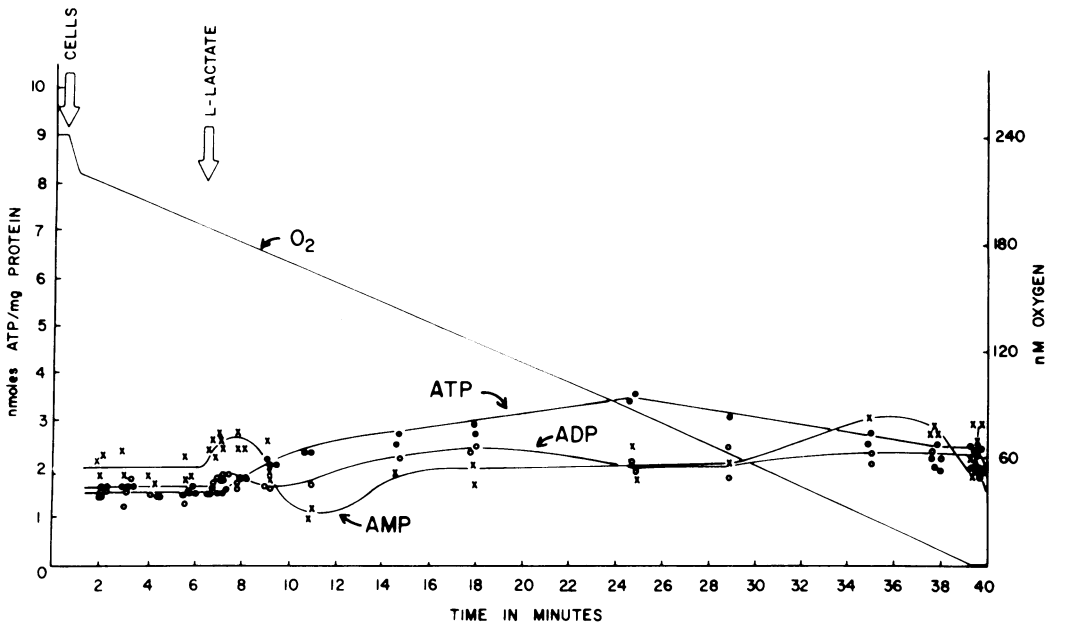

FIG. 3. *Oxidative phosphorylation kinetics of a menadione-deprived culture of S. aureus, strain S2M. Culture was deprived of menadione and tested as described in Fig. 2. Protein concentration of suspended whole cells was 1.25 mg/ml. Symbols: solid line represents oxygen concentration;* (×) *AMP;* (○) *ADP;* (●) *ATP concentration.*

maintain their efficiency during menadione deprivation, the function of the total ETC under these conditions is impaired more than can be explained by growth dilution alone (category 2, Table 1). The rate of L-lactate-linked ATP synthesis was approximately 11-fold lower, oxygen utilization eightfold lower, and net glycine uptake (Fig. 4) was absent in menadione-deprived cultures. These data show that menadione deprivation causes a loss of efficiency of the pre-existent ETC as measured by these parameters. This is not, however, a general membrane effect, for the activity of succinic dehydrogenase and the rate of ATP hydrolysis were essentially unchanged by the absence of menadione (category 3, Table 1). Further evidence showing the effect of menadione deprivation on ETC efficiency was presented by A. Sasarman and S. Sonea (Abstr. Annu. Meet. Amer. Soc. Microbiol., 1972, P235, p. 175), when they found that other menadione mutants of *S. aureus* were unable to reduce nitrate when deprived of this quinone.

In *S. aureus* the molar ratio of vitamin K_2 to cytochrome *b* is 50:1 (9). During menadione deprivation this ratio remains unchanged, for the levels of all members of the ETC fall by the same amount. Net phospholipid synthesis is not affected, however, by menadione deprivation and continues at a rate paralleling growth (Fig. 1) maintaining an average of 98 μmol of lipid

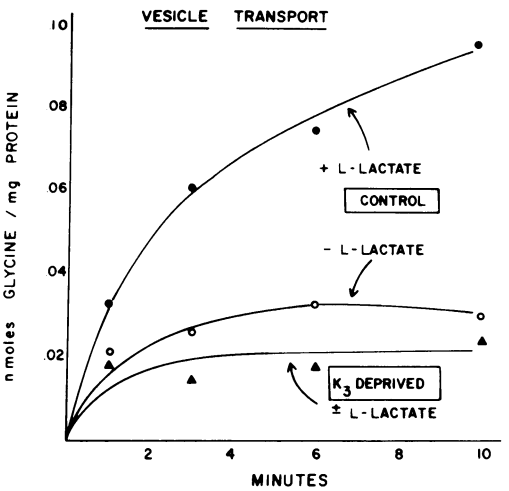

FIG. 4. *L-lactate powered transport of* L-[^{14}C]*glycine in membrane vesicles prepared from menadione-deprived and menadione-supplemented cells. Vesicles were prepared and glycine transport was tested (16). Protein values for prepared membrane vesicles were 2.09 mg/ml for the menadione-supplemented cells and 1.34 mg/ml for the menadione-deprived cells. The reaction mixture included 10 μliters of vesicles and 1 μliter of* [^{14}C]*glycine, 0.34 M (80 mCi/mmol) in addition to* L-*lactate (50 mM final concentration) and the other components as described.* (●) *menadione-supplemented, plus* L-*lactate;* (○) *menadione-supplemented, minus* L-*lactate;* (▲) *menadione-deprived with and without* L-*lactate.*

phosphate per g (dry weight). From these data and the levels of vitamin K_2 shown in Table 1, it can be calculated that the molar ratio of phospholipid to vitamin K_2 is 1,760:1 in the menadione-supplemented cells and 5,500:1 in the menadione-deprived cells.

The seemingly large excess of vitamin K_2 in the ETC (as seen in the 50:1 ratio to cytochrome *b*) is perhaps the reflection of a low-binding affinity of vitamin K_2 for the ETC. This fiftyfold excess suggests a requirement for an optimal concentration of vitamin K_2 in proximity with the components of the ETC. The ratios of phospholipid to vitamin K_2 show that in the menadione-deprived cells vitamin K_2 is at a lower concentration in the membrane (and hence more diffuse). Possibly the amount of vitamin K_2 immediately available for interaction with the ETC would be low enough to impair the overall efficiency of the chain in those functions involving the oxidation-reduction cycle of vitamin K_2.

In summary, the deprivation of menadione in *S. aureus* S2M causes a rapid cessation of the synthesis of other ETC components, but has no effect on the phospholipid composition of the cell membrane. Vitamin K_2 therefore appears to play a role in the regulation of synthesis of the ETC in the organism. The overall concentration of vitamin K_2 in the membrane also seems to be a factor in determining the functional efficiency of the total ETC.

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LITERATURE CITED

1. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. J. Biol. Chem. **234**:466–468.
2. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**:911–917.
3. Ells, H. A. 1959. A colorimetric method for the assay of soluble succinic dehydrogenase and pyridine-nucleotide-linked dehydrogenases. Arch. Biochem. Biophys. **85**:561–562.
4. Frerman, F. E., and D. C. White. 1967. Membrane lipid changes during formation of a functional electron transport system in *Staphylococcus aureus*. J. Bacteriol. **94**:1868–1874.
5. Hammond, R. K., and D. C. White. 1969. Formation of vitamin K_2 isoprenologues by *Staphylococcus aureus*. J. Bacteriol. **100**:573–578.
6. Hammond, R. K., and D. C. White. 1969. Separation of vitamin K_2 isoprenologues by reversed-phase thin-layer chromatography. J. Chromatog. **45**:446–452.
7. Hammond, R. K., and D. C. White. 1970. Carotenoid formation by *Staphylococcus aureus*. J. Bacteriol. **103**:191–198.
8. Hammond, R. K., and D. C. White. 1970. Inhibition of carotenoid hydroxylation in *Staphylococcus aureus* by mixed function oxidase inhibitors. J. Bacteriol. **103**:607–610.
9. Hammond, R. K., and D. C. White. 1970. Inhibition of vitamin K_2 and carotenoid synthesis in *Staphylococcus aureus* by diphenylamine. J. Bacteriol. **103**:611–615.
10. Knowles, C. J., and L. Smith. 1970. Measurements of ATP levels of intact *Azotobacter vinelandii* under different conditions. Biochim. Biophys. Acta **197**:152–160.
11. Lillich, T. T., and D. C. White. 1971. Phospholipid metabolism in the absence of net phospholipid synthesis in a glycerol-requiring mutant of *Bacillus subtilis*. J. Bacteriol. **107**:790–797.
12. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
13. Ray, P. H., T. T. Lillich, and D. C. White. 1972. Consequences of glycerol deprivation on the synthesis of membrane components in a glycerol auxotroph of *Staphylococcus aureus*. J. Bacteriol. **112**:413–420.
14. Ray, P. H., and D. C. White. 1972. Effect of glycerol deprivation on the phospholipid metabolism of a glycerol auxotroph of *Staphylococcus aureus*. J. Bacteriol. **109**:668–677.
15. Short, S. A., and D. C. White. 1970. Metabolism of the glucosyl diglycerides and phosphatidyl glucose of *Staphylococcus aureus*. J. Bacteriol. **104**:126–132.
16. Short, S. A., D. C. White, and H. R. Kaback. 1972. Active transport in isolated bacterial membrane vesicles. V. The transport of amino acids by membrane vesicles prepared from *Staphylococcus aureus*. J. Biol. Chem. **247**:298–304.
17. Silbert, D. F., J. E. Cronan, Jr., I. R. Beacham, and M. E. Harder. 1974. Genetic engineering of membrane lipid. Fed. Proc. **33**:1725–1732.
18. Tien, W., and D. C. White. 1968. Linear sequential arrangement of genes for the biosynthetic pathway of protoheme in *Staophylococcus aureus*. Proc. Nat. Acad. Sci. U.S.A. **61**:1392–1398.
19. White, D. C., and F. E. Frerman. 1967. Extraction, characterization, and cellular location of the lipids of *Staphylococcus aureus*. J. Bacteriol. **94**:1854–1867.