JOURNAL OF BACTERIOLOGY, Oct. 1974, p. 562-564

Vol. 120, No. 1
Printed in U.S.A.

Respiratory Activities Associated with Mesosomal Vesicles and Protoplast Membranes of *Staphylococcus aureus*

T. S. THEODORE AND E. C. WEINBACH

Laboratory of Microbiology and Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20014

Received for publication 5 April 1974

Analysis of oxidase and dehydrogenase activities, cytochrome content of mesosomal vesicles, and protoplast membranes showed that the respiratory chain in *Staphylococcus aureus* is associated predominantly with the protoplast membranes.

Although most studies pertaining to the localization of respiratory activities in either mesosomal vesicles or protoplast membranes have been done with bacilli (2-4, 10, 12), it is the consensus that succinate and nicotinamide adenine dinucleotide, reduced form (NADH), dehydrogenase activities are primarily associated with the protoplast membrane. The precise location and distribution of cytochromes, however, has not yet been completely resolved (13). To date the most consistent results obtained on the localization of respiratory activities have been by Owen and Freer (9), who have shown that in *Micrococcus lysodeikticus* the major content of cytochromes, except for cytochrome *b* (556 nm), is present in the protoplast membrane fraction. Because an apparent discrepancy still existed, we examined the oxidase, dehydrogenase, and cytochrome activities of purified mesosomal vesicles and protoplast membranes of *Staphylococcus aureus*.

Mesosomal vesicles and protoplast membranes were isolated from *S. aureus* ATCC 6538P after late log-phase growth in AOAC Synthetic Broth (Difco). Except for one modification in the isolation of protoplast membranes, procedures for the preparation of *S. aureus* strain LS muralytic enzyme, protoplasting conditions, and the purification and isolation of mesosomal vesicles and protoplast membranes have been described in detail elsewhere (16). Protoplast membranes were prepared by osmotic lysis in hypotonic buffer in the absence of Mg^{2+} and treated with 20 μg of ribonuclease per ml. Membranes prepared in this manner contained only a few visible free or membrane-associated ribosomes or mesosomal vesicles (11). For all subsequent studies, the membrane fractions were resuspended in 0.05 M tris(hydroxymethyl)aminomethane buffer, pH 7.4. Protein content was estimated by the method of Lowry et al. (7).

The results in Table 1 summarize the oxidase and dehydrogenase activities of staphylococcal mesosomal vesicles and protoplast membranes. With each substrate tested the highest activity always was associated with the protoplast membrane. On the other hand, mesosomal membranes were not devoid of activity and had approximately one-eighth (glycerol-3-phos-

TABLE 1. *Oxidase and dehydrogenase activities of mesosomal and protoplast membranes of Staphylococcus aureus*

Substrate	Oxidase activity[a]		Dehydrogenase activity[b]	
	Mesosomal vesicles	Protoplast membranes	Mesosomal vesicles	Protoplast membranes
L-Lactate	124	338	0.170	1.140
DL-Glycerol-3-phosphate	20	155	0.041	0.362
NADH	25	105	0.222	0.428
L-Malate	20	97	0.031	0.100
Succinate	5	13	0.360	0.638

[a] Oxidase activities at nonlimiting substrate concentrations were determined polarographically at 35 C with the Clark oxygen electrode. Results are expressed as nanoatoms of oxygen per minute per milligram of protein.

[b] Succinate dehydrogenase was determined by the method of King (5), NADH dehydrogenase by the method of King and Howard (6), and glycerol-3-phosphate and malate and lactate dehydrogenases by the method of Barnes and Kaback (1) with 2,6-dichlorophenolindophenol as the artificial electron acceptor. Dehydrogenase activity was defined as Δ optical density, 600 nm per min per mg of protein.

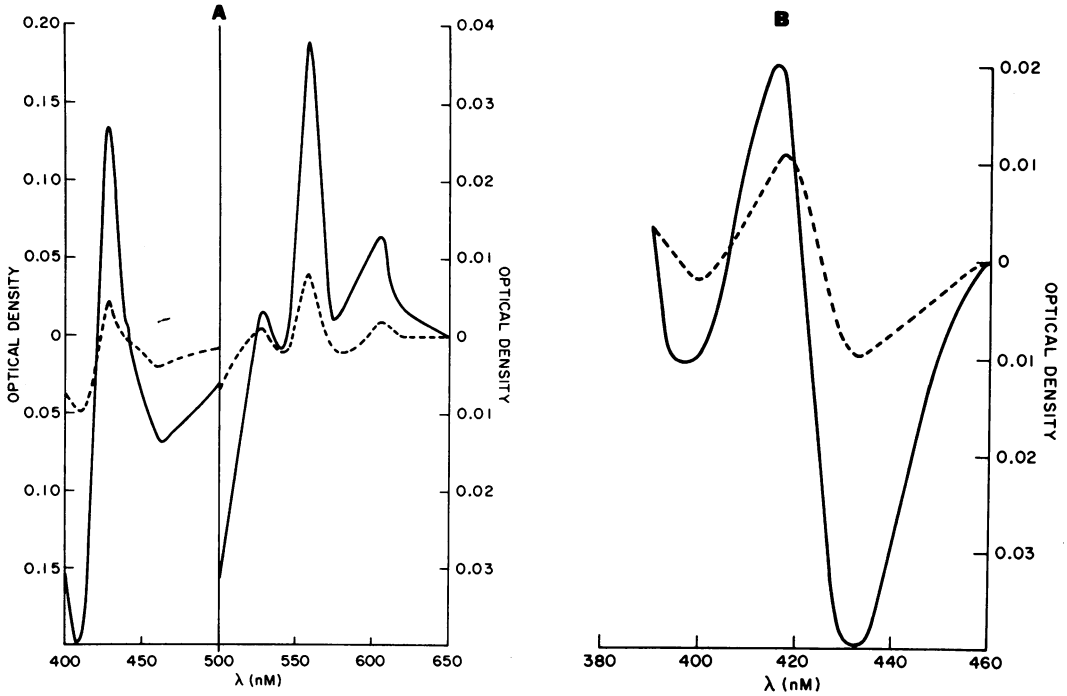

FIG. 1. *Reduced minus oxidized (A) and carbon monoxide reduced minus reduced (B) difference spectra of mesosomal and protoplast membranes of Staphylococcus aureus. Each preparation contained 3 mg of protein per ml. Symbols: Mesosomal membranes (-----); protoplast membranes (——). Cytochromes were analyzed by difference spectroscopy with the Cary model 14 recording spectrophotometer. Reduced minus oxidized difference spectra were obtained by reducing a portion of the sample with either 20 mM L-lactate or* $Na_2S_2O_4$. *Carbon monoxide reduced minus reduced difference spectra were obtained by reducing both cuvettes with* $Na_2S_2O_4$ *and bubbling carbon monoxide through the sample for 3 to 5 min.*

phate oxidase) to one-third (succinate oxidase) of the oxidase activity found in protoplast membranes. The substrate most readily oxidized by both membrane fractions was lactate, followed by glycerol-3-phosphate, NADH, malate, and succinate. Glucose, glycerol, ethanol, pyruvate, and glutamate were not oxidized. Similarly, the dehydrogenase activities of mesosomal vesicles varied between one-ninth (glycerol-3-phosphate dehydrogenase) and one-half (succinate dehydrogenase) of the activity found in the protoplast membranes. These data strongly support the findings of MacLeod et al. (8), who showed that *Bacillus licheniformis* mesosomal vesicles were not involved in active transport of amino acids. The extremely low levels of glycerol-3-phosphate dehydrogenase activity that we observed with mesosomal vesicles largely excludes their involvement in amino acid transport. More recently, Short et al. (14) have demonstrated that the oxidation of glycerol-3-phosphate is required for amino acid transport by *S. aureus* membranes.

Figure 1 depicts the reduced minus oxidized (cytochromes *a* and *b*) and carbon monoxide reduced minus reduced (Soret region of cytochrome *o*) difference spectra of mesosomal and protoplast membranes. Qualitatively, the cytochromes of both membrane fractions were similar and showed the typical spectral patterns described previously (15). Cytochrome *a* has absorption maxima at 602 and 440 nm, cytochrome *b* at 557, 528, and 428 nm, and cytochrome *o* (terminal oxidase of the electron transport chain) at 568, 533, and 415 nm. The principal difference between the two membrane fractions was quantitative. The concentration of cytochromes *a*, *b*, and *o* was approximately three- to fourfold greater in protoplast membranes than in the corresponding mesosomal membrane fraction. The flavine content (depression at 460 nm; Fig. 1A) was also considerably less in mesosomal membranes. No differences were found when either lactate or $Na_2S_2O_4$ was used to reduce the respiratory pigments of the membranes. Our present findings with *S. aureus*, and the data of Owen and Freer with *M. lysodeikticus* (9), provide cogent

evidence that the respiratory chain is associated with the protoplast membranes.

We thank E. C. Clagett and W. W. Davis for their capable technical assistance.

LITERATURE CITED

1. Barnes, E. M., and H. R. Kaback. 1971. Mechanism of active transport in isolated membrane vesicles. 1. The site of energy coupling between D-lactic dehydrogenase and B-galactosidase in *Escherichia coli* membrane vesicles. J. Biol. Chem. **246**:5518–5522.
2. Ferrandes, B., P. Chaix, and A. Ryter. 1966. Localisation des cytochromes de *Bacillus subtilis* dans les structures mesosomiques. C.R. Acad. Sci. **263**:1632–1635.
3. Ferrandes, B., C. Frehel, and P. Chaix. 1970. Fractionnement et purification des systemes membranaires cytoplasmiques et mesosomiques de *Bacillus subtilis*. Etude de quelquesunes de leur proprietes oxydoreductrices associees a la chain respiratoire. Biochim. Biophys. Acta **223**:292–308.
4. Frehel, C., B. Ferrandes, and A. Ryter. 1971. Reactions d'oxido-reduction au niveau des membranes cytoplasmiques et mesosomiques de *Bacillus subtilis*. Biochim. Biophys. Acta **234**:226–241.
5. King, T. E. 1963. Reconstitution of respiratory chain enzyme systems. XII. Some observations on the reconstitution of the succinate oxidase system from heart muscle. J. Biol. Chem. **238**:4037–4051.
6. King, T. E., and J. Howard. 1962. The preparation and some properties of a reduced diphosphopyridine nucleotide dehydrogenase from the snake venom digest of heart muscle preparation. J. Biol. Chem. **237**:1686–1698.
7. Lowry, O., N. Rosebrough, A. Farr, and R. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
8. MacLeod, R. A., P. Thurman, and H. J. Rogers. 1973. Comparative transport activity of intact cells, membrane vesicles and mesosomes of *Bacillus licheniformis*. J. Bacteriol. **113**:329–340.
9. Owen, P., and J. H. Freer. 1972. Isolation and properties of mesosomal membrane fractions from *Micrococcus lysodeikticus*. Biochem. J. **129**:907–917.
10. Patch, C. T., and O. L. Landman. 1971. Comparison of the biochemistry and rates of synthesis of mesosomal and peripheral membranes in *Bacillus subtilis*. J. Bacteriol. **107**:345–357.
11. Popkin, T. J., T. S. Theodore, and R. M. Cole. 1971. Electron microscopy during release and purification of mesosomal vesicles and protoplast membranes from *Staphylococcus aureus*. J. Bacteriol. **107**:907–917.
12. Reaveley, D. A., and H. J. Rogers. 1969. Some enzymatic activities and chemical properties of the mesosomal and cytoplasmic membranes of *Bacillus licheniformis* 6346. Biochem. J. **113**:67–79.
13. Reusch, V. M., and M. M. Burger. 1973. The bacterial mesosome. Biochim. Biophys. Acta **300**:79–104.
14. Short, S. A., D. C. White, and H. R. Kaback. 1972. Active transport of isolated membrane vesicles. V. The transport of amino acids by membrane vesicles prepared from *Staphylococcus aureus*. J. Biol. Chem. **247**:298–304.
15. Taber, H. W., and M. Morrison. 1964. Electron transport in staphylococci. Properties of a particle preparation from exponential phase *Staphylococcus aureus*. Arch. Biochem. Biophys. **105**:367–379.
16. Theodore, T. S., T. J. Popkin, and R. M. Cole. 1971. The separation and isolation of plasma membranes and mesosomal vesicles from *Staphylococcus aureus*. Prep. Biochem. **1**:233–248.