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CONVERSION OF ACTIVE TRANSPORT VESICLES OF *ESCHERICHIA COLI* INTO OXIDATIVE PHOSPHORYLATION VESICLES

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SUMMARY

Passage of membrane vesicles of *Escherichia coli* which catalyze active transport but not oxidative phosphorylation through a French pressure cell yields vesicles which catalyze oxidative phosphorylation but not active transport. The properties of these two species of membrane vesicles are described.

INTRODUCTION

Membrane vesicles derived from *E. coli* are becoming increasingly important research tools for studies of membrane biochemistry. This communication is concerned with two species of membrane vesicles that can be prepared from this organism. One species referred to in the title as "active transport vesicles" [1] catalyzes active transport of amino acids and certain sugars in the presence of an electron donor such as D-lactate with oxygen as terminal acceptor; this transport class of vesicles shows little capacity for oxidative phosphorylation [2, 3]. We have found that "oxidative phosphorylation vesicles" prepared by the method of Butlin et al. [4] have oxidative phosphorylation capacity but little ability to transport amino acids.

RESULTS AND DISCUSSION

Two experiments, which are pertinent to the argument that two different species of vesicles can be generated from *E. coli* membranes, are described here. In the first experiment summarized in Fig. 1 the rates of active transport of [^{14}C]proline are compared between active transport vesicles, active transport vesicles ruptured in the Ribi press (20 000 lb/inch^2), and oxidative phosphorylation vesicles prepared by the procedure of Butlin et al. [4]. Note that the specific activity (nmoles proline transported/mg vesicle protein per min) of the active transport vesicles is more than ten times greater than the specific activity of the oxidative phosphorylation vesicles which possess very little transport activity.

Kaback [1] has shown that preparations of active transport vesicles, as observed in the electron microscope, consist predominantly of intact "sacs" varying from 0.5–1.5 μ in diameter. Apparently active transport vesicles are, on average, much

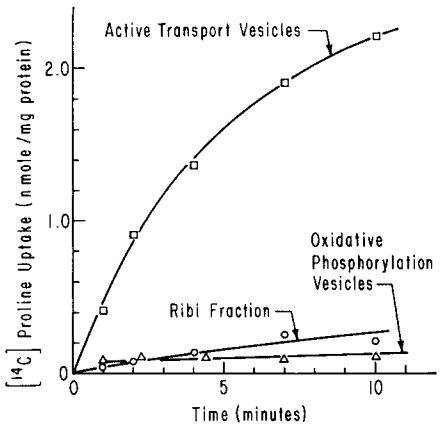

Fig. 1. Loss of active transport of [^{14}C]proline following passage of active transport vesicles through a French pressure cell (Ribi). Active transport vesicles from *E. coli* K12 (3110) were prepared as described in Table I. Vesicles were ruptured by passage through a Ribi cell fractionator. Oxidative phosphorylation vesicles were prepared according to the method of Butlin et al. [4]. Active transport of [^{14}C]proline was measured by the procedure of Kaback [1]. Each assay vessel, containing 1–4 mg protein, was preincubated at 30 °C for 10 min. To start the reaction [^{14}C]proline was added and at the times indicated aliquots of 0.3 ml were removed and collected on a Millipore filter for counting.

larger than oxidative phosphorylation particles [4] which require much greater centrifugation speeds for pelleting. It occurred to us that the rupturing of larger active transport vesicles might yield the smaller species of vesicles capable of catalyzing oxidative phosphorylation.

Table I summarizes the balance sheet of active transport and oxidative phosphorylation activities respectively, following rupturing of active transport vesicles with the Ribi fractionator. Note in Expt 1 that active transport activity of vesicles is strongly inhibited after passage through the Ribi; the ruptured vesicles retain only about 14 % of their original active transport activity. On the other hand a seven-fold net increase in oxidative phosphorylation activity was observed. Respiration capacity (lactate $\xrightarrow{e^-}$ O_2) was not significantly altered during the rupturing process. The conditions for Expts 2 and 3 were similar to Expt 1 except that following the Ribi step, oxidative phosphorylation particles were collected by centrifugation [4]. These vesicles have activities comparable with those of Expt 1. In Expt 4 active transport and oxidative phosphorylation vesicles were prepared from an uncoupled (*unc A*) mutant. Note that active transport activities of both species of vesicles prepared from the uncoupled mutant are similar to the "wild-type" vesicles. As expected, oxidative phosphorylation activities are blocked in the mutant. Data on oxidative phosphorylation particles prepared by the method of Butlin et al. [4] is included for comparative purposes; note that these particles catalyze oxidative phosphorylation whereas they exhibit only about 5 % of the active transport ability of activetransport vesicles (Expt 5).

It should be mentioned that a P/O ratio of 0.1–0.2 was normally obtained with freshly prepared oxidative phosphorylation particles in agreement with the finding of Butlin et al. [4]. However, the P/O ratios obtained with ruptured active transport vesicles were approximately 1/10 this value. One possible explanation of these low values is the loss or denaturation of energy transducing ATPase occurring during

TABLE I

CONVERSION OF ACTIVE TRANSPORT VESICLES TO OXIDATIVE PHOSPHORYLATION VESICLES

Active transport vesicles, prepared by the procedure of Kaback [1] (with the exception that EDTA was omitted after the lysis step), were ruptured by passage through a Ribi cell fractionator. This fraction labelled "after Ribi fractionation" was used directly for Expt 1 whereas for Expts 2 and 3 oxidative phosphorylation particles were collected from the Ribi fraction by centrifugation according to the procedure of Butlin et al. [4]. Oxidative phosphorylation vesicles for Expts 4 and 5 were prepared according to the method described by these authors [4]. The assay mixture for oxidative phosphorylation contained 0.2–0.6 mg vesicle protein, 5 mM sodium–potassium phosphate buffer at pH 7.0, 5 mM $MgCl_2$, 15 mM AMP, 3.1 μM ADP, and 18.8 mM D-lactate to a final volume of 3.2 ml. After 15 min incubation at 30 °C the reaction was terminated by addition of 0.25 ml of 1.8 M H_2SO_4, denatured protein removed by centrifugation and the sample neutralized with KOH. ATP concentrations were measured using the luciferase assay [9]. Oxygen uptake was measured using an oxygen electrode [10]. Proline uptake was measured as described for Fig. 1. The *unc A* mutant used is a derivative of *E. coli* K12 (3110; our laboratory strain *unc* 17) whose properties are described in an earlier paper [11].

Expt Number	Method of vesicle preparation	O_2 uptake (natoms/min per mg protein)	ATP synthesized (nmole/min per mg protein)	$P/O \times 10^3$	Proline uptake (nmole/10 min per mg protein)
1	Active transport vesicles	155	0.05	0.7	2.2
	After Ribi fractionation	144	0.35	6	0.3
2	Active transport vesicles	90	0.02	0.35	—
	After Ribi fractionation	233	0.47	4	—
3	Active transport vesicles	110	0.02	0.41	2.6
	After Ribi fractionation	142	0.31	4.3	—
4	Active transport vesicles (*unc A* mutant)	—	—	—	2.0
	Oxidative phosphorylation vesicles (*unc A* mutant)	70	<0.02	<0.5	0.3
5	Oxidative phosphorylation vesicles	110	5.6	102	0.12

handling of the vesicles. The extreme sensitivity of the luciferase assay [9] used plus the availability of extracts of *unc A* mutants prepared by identical procedures which show no oxidative phosphorylation (negative controls) give us confidence that the phosphorylation rates observed, though small, are nonetheless reliable.

Kaback and Barnes Sr [2], as well as other investigators [3] have reported that membrane vesicles of *E. coli* which retain their ability to transport amino acids and other nutrients show little capacity for oxidative phosphorylation. This is so even though these vesicle preparations possess an active respiratory system with oxygen as terminal electron acceptor. On the other hand, Butlin et al. [4] have described the production of oxidative phosphorylation vesicles which as reported here retain little active transport activity. It is postulated that the larger vesicles retain the right side in membrane morphology, allowing for concentration of amino acids but preventing oxidative phosphorylation since substrates such as ADP cannot reach the phosphorylation sites on the inside surface of the membrane. However, the smaller vesicles are

thought to be inside-out, exposing the oxidative phosphorylation sites and allowing substrate accessibility, but maintaining the wrong-sidedness for the concentration of amino acids. It is interesting to speculate that rupturing of the large vesicles reverses their topology and exposes their latent potential for oxidative phosphorylation.

Van Thienen and Postma [5] have recently observed that disruption of washed active transport vesicles by Triton X-100 increased the level of assayable ATPase by more than 5-fold. These workers conclude that ATPase present on the inner membrane surface of active transport vesicles is exposed to its substrate ATP after disruption with detergent. We have confirmed this finding, rupturing the active transport vesicles in a French pressure cell (instead of treatment with Triton), and have found in two separate experiments an increase in specific activity (μmole P_i released/min per mg protein) from 0.625–2.5 and from 0.64–1.5 before and after rupturing. These values are higher than those reported by Van Thienen and Postma [5] because we performed our assays at the optimum pH of the enzyme (pH 9). Using another approach, Hanson and Kennedy [6] have observed that the activity of vesicles which catalyze ATP-dependent reverse electron flow are completely blocked by antibody against coupling factor (ATPase). Though not measured in these experiments, it is probable that oxidative phosphorylation activity would be similarly inhibited. In order for antibody to block activity it seems probable that coupling factor must be located on the outer surface of these vesicles. These experiments support the view that two biochemical species of vesicles may be prepared from *E. coli* membranes, mainly active transport vesicles and oxidative phosphorylation vesicles. The reader is referred to articles by Harold [7] and Asano et al. [8] for further discussion of mitochondrial and bacterial membranes, respectively.

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