NUCLEOTIDE TRANSLOCATION ACROSS THE CYTOPLASMIC MEMBRANE IN THE PHOTOSYNTHETIC BACTERIUM *RHODOPSEUDOMONAS CAPSULATA*

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1. Introduction

The cell envelope of Gram-negative bacteria such as *Rhodopseudomonas capsulata* contains an inner cytosplasmic membrane and an outer membrane [1]. The space between the two membranes is the periplasma, which contains the periplasmic proteins and enzymes [2]. Several of the reactions catalyzed by the periplasmic enzymes, such as cell wall and capsule synthesis, require energy. The possible function of specific transport systems for UDP-glucose was suggested [3]. However, a general mechanism for energy transfer from the cytoplasm to the periplasm was not found. In photosynthetic bacteria both oxidative and photosynthetic phosphorylation systems are located in the cytoplasmic membranes [4] and the reversible ATPase is attached on the cytoplasmic side of this membrane [5]. A system which could translocate nucleotides across the cytoplasmic membrane would provide energy for the periplasmic enzymes.

The outer membrane was found to exclude macromolecules [1] and even oligosaccharides having molecular dimensions larger than 600 daltons [6]. Except for the case of *Bdellovibrio bacteriovarus* [7] nucleotides were found to be excluded from bacteria [8]. It is possible, therefore, that the outer membrane could serve as a barrier to prevent the diffusion of nucleotides from the periplasmic space to the outer medium.

The possible existence of nucleotide transport across the cytoplasmic membrane was indicated from the finding of photophosphorylation of exogenous ADP to ATP by heavy chromatophores [9]. These vesicles, prepared from *Rhodopseudomonas capsulata*, retain the membrane polarity of the intact cell as indicated from the direction of light-induced proton movement [9], the location of cytochrome c_2 [10] and their capacity to accept electrons from hydrophilic electron donors [11]. In this work we show that nucleotides are translocated across the membrane of heavy chromatophores possibly, as was found in mitochondria [12], through an exchange diffusion mechanism. The outer membrane is probably a barrier for nucleotides transport since only bacteria having damaged outer membrane can photophosphorylate exogenous diphospho-nucleotides and hydrolyze triphospho-nucleotides.

2. Materials and methods

Rhodopseudomonas capsulata were grown anaerobically in the light on the medium in [13] and cells were harvested and stored as in [10].

Heavy chromatophores were prepared by breaking frozen cells by a Yeda press in a medium containing: 30 mM tricine–NaOH, pH 7.5 and 10 mM ADP, pH 7.5. Following precipitation by centrifugation the heavy chromatophores were treated with DNAase (mg protein/mg bacteriochlorophyll) for 10 min and then washed by resuspension in 30 mM tricine–NaOH, pH 7.5 (final conc. bacteriochlorophyll 50 μg/ml). The procedure for preparation of heavy chromatophores is detailed in [10]. Regular chromatophores were prepared by sonication of cells [13]. Freshly harvested cells were treated with ethylenediamine tetracetic acid and lysozyme in order to increase the outer membrane permeability [14].

Measurements of [^{14}C]ADP distribution between the internal, osmotic space and the outer space were

done essentially following the method in [15] for measurement of amine distribution in chloroplasts. Heavy chromatophores containing 120 μg bacteriochlorophyll were incubated in a medium of 200 mM tricine–NaOH, pH 7.5, 15 mM NaCl, 0.1 mM ADP (0.66 μCi [^{14}C]ADP/μmol) and 0.6 μCi $^{3}H_2O$ in total vol. 1.5 ml at 30°C. Three aliquots of 0.5 ml were transfered at each indicated time interval into small plastic tubes and the chromatophores were precipitated by centrifugation in a Beckman Model 152 microfuge for 3 min. The pellet was cut and nucleotides extracted in the presence of 14% perchloric acid. Dextran impermeable space was used to measure the inner space enclosed by the cytoplasmic membrane. For these determinations heavy chromatophores were suspended in a medium containing 200 mM tricine–NaOH, pH 7.5, 15 mM NaCl, 0.1 mM ADP and 1.5 mg [^{14}C]dextran (mol. wt. 40 000) 0.5 μCi and 3 μCi $^{3}H_2O$ in total vol. 1.5 ml at 30°C.

NTPase activity was assayed in a medium containing chromatophores (100 μg bacteriochlorophyll), 4 mM $MgCl_2$, 200 mM tricine–NaOH, pH 8, 10 mM NTP in total vol. 2 ml at 37°C. The reaction was started by addition of chromatophores and terminated after 10 min by addition of cold CCl_3COOH to final conc. 5%. The inorganic phosphate released was determined spectroscopically [16]. Photophosphorylation was assayed under unaerobic conditions as in [10].

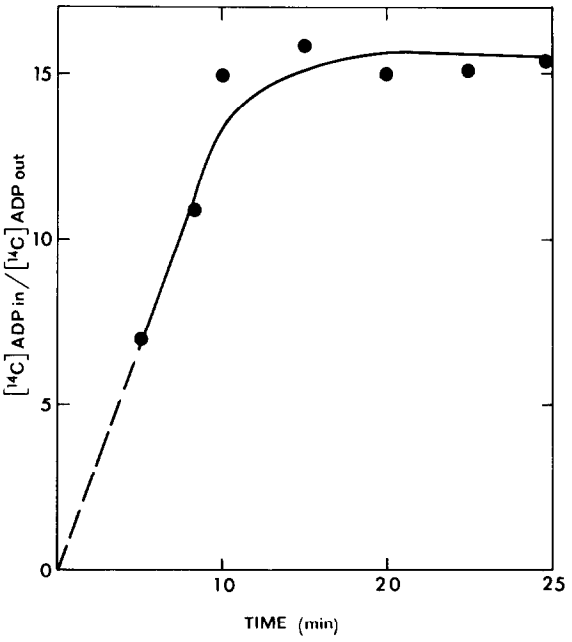

Fig.1. Distribution of [^{14}C]ADP across the cytoplasmic membrane of heavy chromatophores. Heavy chromatophores were incubated with [^{14}C]ADP, then precipitated by centrifugation within the indicated time intervals. Radioactivity was measured in the supernatant and in the pellet. The internal space enclosed within the cytoplasmic membranes was measured as dextran impermeable space in the pellet. These values were used for calculation of the ratio of $[^{14}C]ADP_{in}$ $[^{14}C]ADP_{out}$.

3. Results and discussion

Heavy chromatophores are an homogenous preparation of membrane vesicles, about one-third of bacteria in size, having broken outer membrane attached to the cytoplasmic membrane. Although we have shown that their membrane is relatively impermeable to protons [9] and to hydrophilic electron donors [11], ADP was translocated across the cytoplasmic membrane (fig.1). The time-dependent changes in the concentration of [^{14}C]ADP were probably a result of movement of ADP into the cytoplasm. In order to measure the cytoplasmic space we used dextran which was shown to be excluded from both the outer and the inner membranes of Gram-negative bacteria [6]. However, since in heavy chromatophores the outer membrane was damaged, the dextran impermeable space could serve as a measurement for the cytoplasmic space. Indeed, in control experiments the sucrose and the dextran impermeable spaces were shown to be comparable. Sucrose, however, was shown to penetrate the outer membrane in Gram-negative bacteria [6]. In analogy to the mitochondrial system [12] it can be assumed that the bacterial membrane contains a carrier which facilitates exchange diffusion of nucleotides. If this were the case, the rate of the exchange could be calculated from the initial rate of change in the distribution of radioactive nucleotides. Approximate calculations indicate that the rate of translocation can account for the rate of photophosphorylation of exogenous ADP catalyzed by these chromatophores (see below).

A leveling off of the changes in the concentration

Table 1
Specificity for nucleotides of photophosphorylation and NTPase activity in heavy and regular chromatophores

Preparation	Photophosphorylation (% control)			NTPase (% control)			
	ADP	GDP	IDP	ATP	GTP	ITP	AMP
Regular chromatophores	100	100	48.9	100	100	30	2.5
Heavy chromatophores	100	59.6	54.5	100	57	29.5	5

Rates of ATPase and photophosphorylation activities were measured in the presence of 10 mM of the indicated nucleotides in the presence of either heavy chromatophores or regular chromatophores. Assay conditions were as in section 2 and the activities of the adenine nucleotides were taken as 100. The rate of photophosphorylation of ADP were 48.9 and 56.2 and the rate of ATPase activities were 60.4 and 94.7 μmol × mg bacteriochlorophyll^{-1} × h^{-1} in regular and heavy chromatophores, respectively

ratio of radioactive ADP will be expected when radioactive equilibrium is reached, assuming an exchange diffusion mechanism for nucleotide transport. Such an equilibrium was reached after approx. 15 min under given experimental conditions (fig.1). Similar results were obtained when ATP was used. In a separate experiment (not shown) the bacteria were broken in the presence of either a lower concentration or in the absence of ADP in the preparation medium; the equilibrium was reached at a lower ratio of distribution. A possible interpretation of this experiment suggests that the concentration of ADP inside the cytoplasmic space was lower when the heavy chromatophores were prepared in the presence of lower concentrations of ADP. Such a change would be expected if the breakage of the cytoplasmic membrane during the preparation allows an exchange between the inner and the outer medium of the bacteria. These experiments also indicate that translocation rather than binding of ADP was measured. Since the presence of ADP during the preparation would be expected to decrease rather than increase the binding of radioactive ADP to the heavy chromatophores because of a dilution effect.

The reversible ATPase is attached to the membrane facing the outer medium in regular chromatophores and the inner medium in heavy chromatophores. Nucleotides which are substrates for the reactions catalyzed by this enzyme have to cross the membrane in order to reach it in heavy chromatophores. A difference in the nucleotide specificity between the translocation and the catalysis could be reflected in the rates catalyzed by the two preparations. In table 1 it can be seen that regular and heavy chromatophores could catalyze both NTPase and photophosphorylation of nucleotides. Similarity in specificity for most of the nucleotides was found in both preparations except for the case of the guanilic nucleotides. The hydrolysis of GTP and the photophosphorylation of GDP were slower in heavy chromatophores than in regular chromatophores. It is possible, therefore, that the affinity of GTP and GDP for the nucleotide translocator were the rate limiting step for these reactions in heavy chromatophores. The finding that AMP was not hydrolyzed under these conditions supports the suggestion that both hydrolysis and phosphorylation of the various nucleotides was catalyzed by the reversible ATPase.

Another possible indication for the existence of nucleotide translocation is the observation that under certain conditions it becomes a rate limiting step in phosphorylation of exogenous ADP. Thus, more than a 3-fold increase in the rate of photophosphorylation catalyzed by regular chromatophores was observed on the addition of glucose and hexokinase to the reaction medium (table 2). Such an increase is expected since the trapping of the phosphorylated phosphate by transfering it to glucose would compete with the hydrolysis of the ATP which is catalyzed by the reversible ATPase. In heavy chromatophores the ATP is formed and hydrolyzed inside the vesicles while the hexokinase added to the medium can not cross

Table 2
Effect of hexokinase on the rate of photophosphorylation in regular and heavy chromatophores

Additions	Photophosphorylation activity (% control)	
	Heavy chromatophores	Regular chromatophores
None	100	100
Hexokinase + glucose	125	325

Photophosphorylation activities were assayed as in section 2 except for the addition of 50 mM glucose and 33 units/ml hexokinase where indicated. The rates of photophosphorylation in the absence of hexokinase were 55 and 31 μmol $\times$ g bacteriochlorophyll^{-1} $\times$ h^{-1} for regular and for heavy chromatophores, respectively

the cytoplasmic membrane. The small change in the observed rate of ATP synthesis could indicate that in the presence of a trapping system nucleotide translocation was the rate limiting step of the reaction.

The possible function of nucleotide translocation as a physiological energy transfer system requires that nucleotide would not leak from the periplasmic space through the outer cell membrane to the medium. The permeability of the outer membrane to nucleotides was assayed by measuring the hydrolysis of exogenous ATP or the photophosphorylation of exogenous ADP. Neither reaction was catalyzed by whole bacteria (table 3). However, as a result of treatment with EDTA and lysozyme, which are known to damage the outer membrane [14], the bacteria catalyzed both photophosphorylation and ATPase activities. Mechanical breakage of the outer membrane during the preparation of heavy chromatophores also removed this barrier as observed by their capacity to catalyze these activities in the presence of exogenous nucleotides.

In conclusion, it seems that the outer membrane serves as a barrier for nucleotide transport. Nucleotides can cross the cytoplasmic membrane possibly through a translocator which facilitated exchange diffusion. These are prerequisites for a function of an energy transfer system by means of nucleotide transport to the periplasm.

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Table 3
Photophosphorylation and ATPase activities in various preparations of bacterial cells

Preparation	Photophosphorylation	ATPase
	(μmol $\times$ mg bacteriochlorophyll^{-1} $\times$ h^{-1})	
Whole cells	0	2
Lysozyme + EDTA treated cells	57	48
Heavy chromatophores	81	105
Regular chromatophores	122	150

Photophosphorylation of ADP and ATPase activities were assayed and various cell preparations were obtained as in section 2

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