EFFECT OF K^+ AND Na^+ ON THE CYTOCHROME OXIDASE ACTIVITY OF *HALOBACTERIUM CUTIRUBRUM*

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1. Introduction

Halophilic enzymes function maximally in a high salt reaction medium [1–4]. In the absence of salt, the halophilic enzymes become inactive [1–4] and in the case of the purified halophilic malic dehydrogenase partial re-activation can be obtained by dialysis of the inactive enzyme in a high salt solution [5]. Another interesting feature is the recent claim that the halophilic NADH and glycerol dehydrogenase are partially stabilized in low concentrations of specific cations such as spermine [6].

The main aim of this communication is to show that the ascorbate-induced respiration and the reduction of cytochrome $a_3(a)$ in the isolated electron transport particles (ETP) from the extreme halophile, *Halobacterium cutirubrum*, is dependent on the concentration of K^+ and Na^+ and also to provide additional evidence that *H. cutirubrum* has cytochrome a_3 [7–8] which was not observed by Lanyi [9].

2. Experimental procedures

The ETP (Type II) from the early logarithmic growth cells of *H. cutirubrum* were prepared as described previously [7]. The ETP used for the following experiments were obtained by centrifugation of the 79,000 *g* pellet previously suspended in 100 mM tris-HCl (pH 7.6) containing 70 mM $MgCl_2$ at 6,000 *g* for 1 hr at 0° instead of allowing separation to take place [7].

Oxygen uptake was determined with a Clark oxygen electrode at 25°. Kinetic studies of the ascorbate-reducible cytochrome $a_3(a)$, measured at 444–465 nm, were carried out with a Phoenix Precision Instrument (PPI) dual/split-beam spectrophotometer and protein by Folin-phenol reagent [10].

3. Result and discussion

One of the characteristic features of the extreme halophiles belonging to the *Halobacterium* group is that ascorbate alone can reduce the CO-reactive hemoproteins [7–8] and also stimulate respiration [7–8, 11–12]. Thus, the cytochrome oxidase [EC 1.9.3.1] activity in these bacteria can be assayed without using an artifical electron mediator such as *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD), a procedure commonly employed for mammalian [13] and bacterial [14–15] systems. Fig. 1 illustrates the Lineweaver-Burk plot showing the ascorbate oxidation by the ETP of *H. cutirubrum* estimated in two different mono-cation (K^+ and Na^+) reaction media and also the inhibition of ascorbate oxidation by CO. The apparent K_m of ascorbate oxidation was 2.0 mM in both KCl and NaCl. The maximum velocity was about 66.7 μM O_2 per min, equivalent to 51 nmoles O_2 per min per mg protein. CO was a competitive inhibitor of ascorbate oxidation.

The addition of TMPD stimulated ascorbate oxidation by about 2-fold. In general, the rate of ascorbate oxidation with TMPD present was higher in KCl than

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NaCl as shown in fig. 2. Furthermore, the maximal rate was attained in about 3.0 M KCl as compared with 4.0 M NaCl. The maximal rate in KCl was also greater than that obtained with NaCl.

Cytochrome $a_3(a)$ reduction by ascorbate was also more rapid when estimated in KCl. Fig. 3 illustrates the typical kinetic reduction of cytochrome $a_3(a)$ of the ETP from *H. cutirubrum*. It can be seen that anaerobiosis occurred faster in the 3.0 M KCl than in the 3.0 M NaCl reaction medium. This phenomenon, clearly shown in fig. 4, illustrates big differences in the times obtained for anaerobiosis with low concentrations of KCl and NaCl. Thus, at 0.75 M, the time to reach anaerobiosis in NaCl was about 30 sec longer than that observed in KCl. At 0.05 M, anaerobiosis occurred at about 5.6 min in KCl as compared with 10.5 min in NaCl, the latter values are not shown in the figure.

The cytochrome oxidase activity of the ETP suspended in 4.0 M NaCl was found to be stable for at least 4 days when kept in an ice-bath at 4°. This activity was not tested after 4 days. The turnover number,

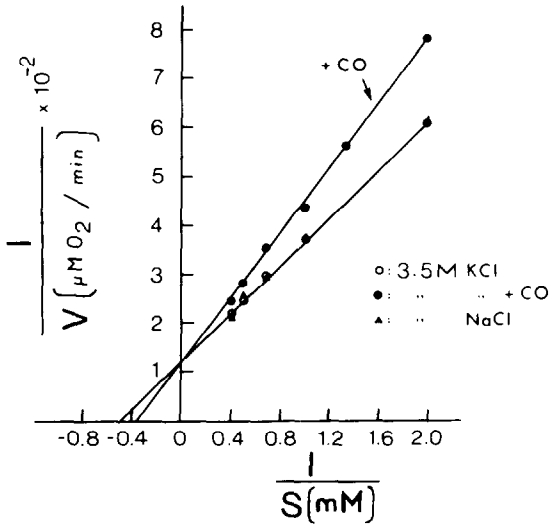

Fig. 1. Lineweaver-Burk plot showing CO inhibition of ascorbate oxidation by the ETP of *H. cutirubrum*. The respiratory rate was determined polarographically in either KCl or NaCl buffered with 0.05 M tris-HCl (pH 7.6). Total volume, 2.5 ml (3.3 mg protein); temperature, 25°. The inhibition of ascorbate oxidation by CO was carried out with a CO-saturated reaction medium.

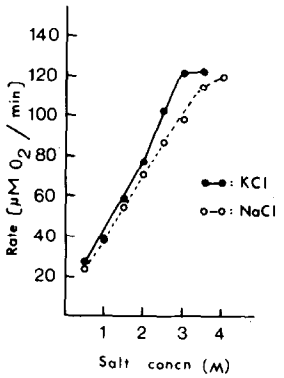

Fig. 2. Effect of various concentrations of KCl and NaCl on the cytochrome oxidase activity of the ETP from *H. cutirubrum*. The cytochrome oxidase activity was measured polarographically using ascorbate-TMPD. Other experimental details are given in fig. 1. Final concentration (mM): ascorbate, 2.0; TMPD, 0.1.

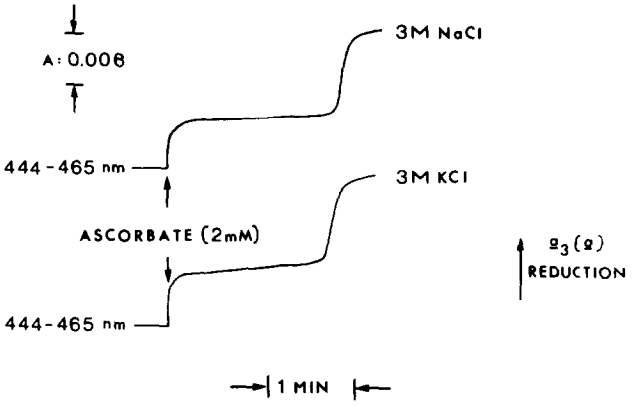

Fig. 3. Effect of KCl and NaCl on the reduction of cytochrome $a_3(a)$ by ascorbate. The kinetics of cytochrome $a_3(a)$ reduction of the ETP from *H. cutirubrum* were followed with a PPI dual/split-beam spectrophotometer using a 10 mm light-path cuvette containing 2.5 ml (1.72 mg protein) ETP suspension.

expressed in electrons per heme per second, of cytochrome a_3 in the ETP of *H. cutirubrum* was 37 with ascorbate and 76 with ascorbate-TMPD, using the previous estimated concentration of cytochrome a_3 of 0.12 nmoles per mg protein calculated from the cytochrome a_3-CO complex in the (ascorbate + CO *minus* ascorbate) difference spectrum [7].

The evidence presented shows that higher cyto-

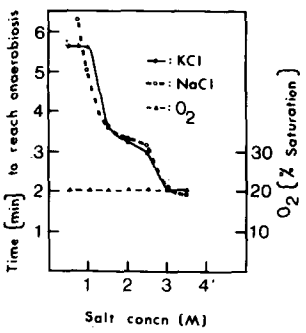

Fig. 4. Effect of various concentrations of KCl and NaCl on the time to reach anaerobiosis during reduction by ascorbate as indicated by cytochrome $a_3(a)$. Similar procedure as described in fig. 3 was used. The O_2 content in the ETP suspension was determined with a Clark O_2 electrode before starting the experiment. The O_2 content (▲) was practically identical in both the KCl and NaCl reaction medium.

chrome oxidase activity of *H. cutirubrum* was observed in KCl than NaCl. Aminoacyl transfer ribonucleic acid synthetases of *H. cutirubrum* also have a higher activity in KCl than NaCl.

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