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PROPERTIES OF THE MEMBRANE-BOUND RESPIRATORY CHAIN SYSTEM OF *HALOBACTERIUM SALINARIUM*

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SUMMARY

1. The membrane-bound respiratory chain system of the colourless mutant, *Halobacterium salinarium* (Strain 1 M2), was investigated by polarographic and spectrophotometric techniques.

2. Difference spectra at room (22°) and liquid-N_2 (−196°) temperature showed the participation of a predominant complex of *b*-type cytochromes (α-peaks: 555, 557 and 562 nm at −196°), a *c*-type cytochrome (α-peak: 553 nm at 22°, 549 nm at −196°) and cytochromes *a*, a_3 and *o* in electron transport.

3. Cytochrome *a* was placed as a side chain in the main postulated respiratory chain system based on the marked difference in the half-time reduction ($t_{1/2}$) value estimated for cytochrome *a* (14 sec) and a_3 (2.5 sec) measured at 606–630 nm and 444–465 nm, respectively, with ascorbate. The estimated $t_{1/2}$ values suggest that ascorbate oxidation, which was enhanced by tetramethyl-*p*-phenylenediamine (TMPD), was faster than either succinate or α-glycerophosphate oxidation.

4. The succinate–phenazine methosulphate reductase activity was inhibited by amytal, *p*-chloromercuribenzoate (PCMB) and *N*-ethylmaleimide. Succinate and α-glycerophosphate oxidation was also blocked by amytal, PCMB and *N*-ethylmaleimide in addition to CN^-.

5. The antimycin A-insensitive ascorbate and ascorbate–TMPD oxidase activities were inhibited by 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide, CN^-, N_3^- and CO. CO was a competitive inhibitor of ascorbate oxidation which has a K_m of 2.0 mM.

INTRODUCTION

Previous studies[1–3] on the electron transport systems of three different carotenoid-containing extreme halophiles belonging to the Halobacterium group, which grow best in a medium containing 25–30 % NaCl[4], suggest that these bacteria have more than one oxidase during their logarithmic growth phase. Isolated electron transport particles from *Halobacterium cutirubrum*[1] and membranes from *H. sali-*

Abbreviations: HQNO, 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide; TMPD, tetramethyl-*p*-phenylenediamine; PCMB, *p*-chloromercuribenzoate: TN, turnover number.

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narium[2] have substrate-reducible cytochromes a_3 and *o*, and the membrane-bound ascorbate oxidase system of *H. halobium* has cytochromes a_1 and o[3]. These findings differ from those reported for *H. cutirubrum* by LANYI[5,6] who was unable to demonstrate the existence of any substrate-reducible cytochromes *a* and a_3 and an *o*-type cytochrome.

It is thought worthwhile to look at the respiratory chain system of the colourless mutant of *H. salinarium* (Strain 1), designated *H. salinarium* (Strain 1 M2)[7], in order to determine firstly whether there are differences between the carotenoid-containing strains and the colourless mutant and secondly to obtain more information so that a general conclusion about the Halobacterium electron transport system could be drawn from these investigations.

MATERIALS AND METHODS

Growth of bacteria and preparation of membranes

The procedure for growing and preparing the membranes from the colourless mutant, *H. salinarium* (Strain 1 M2), harvested at the mid-logarithmic growth phase, was similar to that described for the carotenoid-containing *H. halobium*[3]. Unless specifically stated, all membranes were freshly prepared and used on the same day. From here onwards, *H. salinarium* (Strain 1 M2) will only be referred to as *H. salinarium* except in DISCUSSION.

O_2 uptake was measured polarographically and the membrane-bound cytochromes were detected from difference spectra at room (22°) and liquid-N_2 (−196°) temperature as previously described[1–3]. Other experimental details are given in the legends to figures. Protein was estimated by the method of LOWRY *et al.*[8] with bovine serum albumin as standard.

RESULTS

Distribution of cytochromes and properties of the membrane-bound oxidase systems of H. salinarium

As in the case of *H. halobium*[3] the cytochromes of *H. salinarium* were located only in the membrane fraction (Fig. 1).

Fig. 2 shows that both α-glycerophosphate (A) and succinate (B) reduced the same *c*-(553 nm) and probably different *b*-type cytochromes (558 nm for α-glycerophosphate (A) and 557 nm for succinate (B)). Neither substrate appeared to reduce cytochrome ($a + a_3$) (606 nm). Ascorbate reduced cytochrome ($a + a_3$) and a *b*-type component (560 nm), the latter was different from that associated with either α-glycerophosphate or succinate oxidation. Ascorbate, like ascorbate–tetramethyl-*p*-phenylenediamine (TMPD) (not shown), could also have reduced the α-glycerophosphate and succinate-reducible *b*-and *c*-type cytochromes and that their absorption maxima were masked by the predominant peak at 560 nm.

Fig. 3, A–D, represents a series of difference spectra of the membrane of *H. salinarium* recorded at −196°. Both succinate (A) and α-glycerophosphate (B) reduced an additional *b*-type cytochrome (562 nm) and also cytochrome ($a + a_3$), all of which were not observed at 22° (Fig. 2). The absorption maxima (22°) of the succinate and α-glycerophosphate-reducible *b*- and *c*-type cytochromes were also shifted a

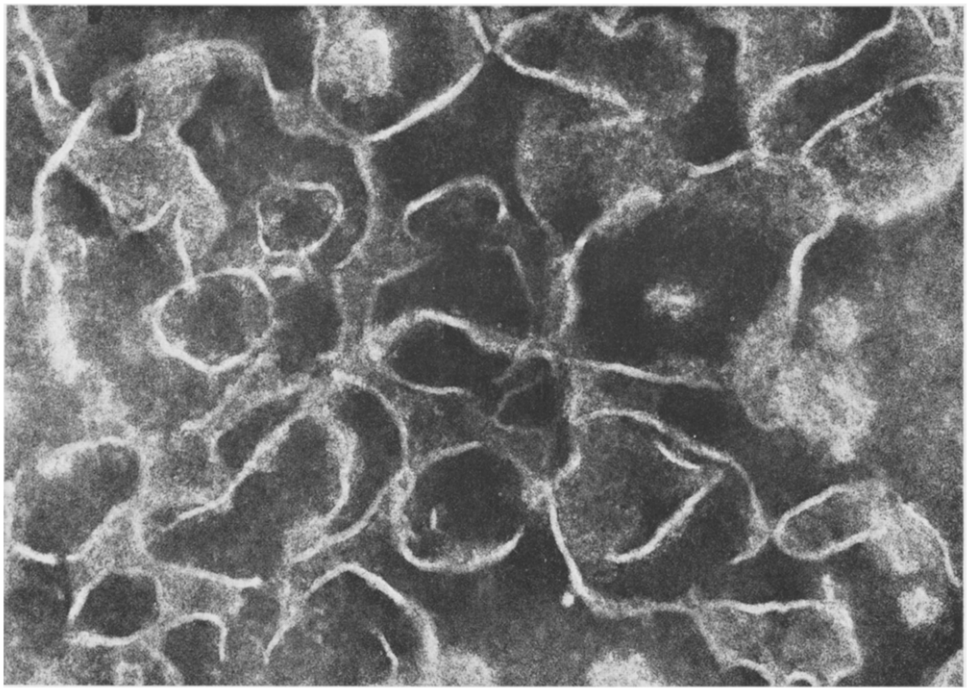


Fig. 1. Electron microscopy of the negatively stained membranes of *H. salinarium*. The membranes were stained for 2 min with 2% phosphotungstic acid (pH 6.8) using Formvar carbon-coated grids before examination under a Philips (Model EM 300) electron microscope as previously described[1]. Magnification 100000 ×.

few nm towards the blue end of the spectrum at –196°. Thus, the *b*-type component was seen at 555 nm (Fig. 3, A and B) instead of 558 and 557 nm with α-glycerophosphate and succinate, respectively (Fig. 2, A and B), and the *c*-type at 549 nm instead of 553 nm. Ascorbate (Fig. 3, C), like ascorbate–TMPD (not illustrated), also reduced the *c*-type cytochrome which was not observed previously at 22° (Fig. 2, C), in addition to cytochrome $(a + a_3)$ and the *b*-type pigment. The maximum of the latter component was shifted to 557 nm from 560 nm (Fig. 2, C). The *a*-, *b*- and *c*-type cytochromes were reduced by dithionite (Fig. 3, D).

The addition of CN^- prior to reducing agents to the membranes resulted in the appearance of a new absorption band at 587 nm (Fig. 4, A–D), probably contributed by cytochrome a_1 (ref. 11). The CN^--inhibited α-glycerophosphate oxidase pathway (B) involved the same pathway as the ascorbate oxidase system (C) but was different from the succinate oxidase pathway (A).

The 549-nm band (Figs. 3 and 4) was proved to be that of a *c*-type pigment with 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide (HQNO) (Fig. 5). The addition of 1.5 μg HQNO per mg membranous protein *plus* O_2 to membranes pretreated with either ascorbate (A) or ascorbate–TMPD (B) caused the reoxidation of cytochrome $(a + a_3)$ and cytochrome c_{549} (–196°) but not cytochrome b_{556} (–196°). A marked shoulder at 561 nm (–196°) was also observed in the presence of HQNO.

The CO complexes indicative of both cytochromes a_3 and *o* are illustrated

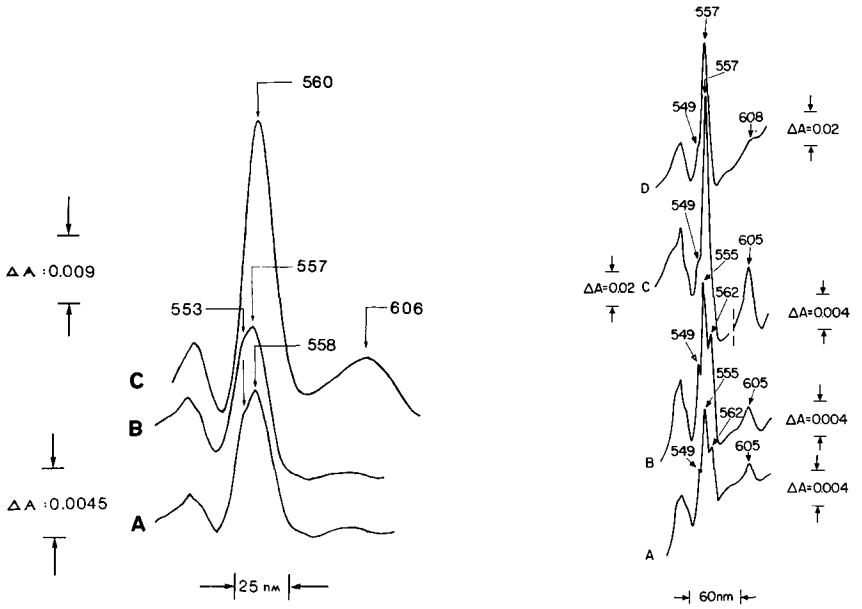


Fig. 2. Difference spectra (22°) of the substrate-reducible membrane-bound cytochromes of *H. salinarium*. Both the sample and reference cuvettes (10 mm light path) contained 2.5 ml membrane suspension (4.0 mg protein/ml) in 3.5 M NaCl, 0.05 M Tris–HCl buffer (pH 7.6). A, α-glycerophosphate-reduced *minus* oxidized; B, succinate-reduced *minus* oxidized; C, ascorbate-reduced *minus* oxidized. The difference spectrum was recorded at 3 min after each substrate addition using a Phoenix Precision Instrument (PPI) dual/split-beam spectrophotometer. Final concn. (mM): succinate, 10; α-glycerophosphate, 10; ascorbate, 4.0.

Fig. 3. Difference spectra (−196°) showing the substrate- and dithionite-reducible cytochromes in the membranes of *H. salinarium*. Both the sample and reference cells (2.0 mm light-path) contained 0.35 ml membrane suspension in 3.5 M NaCl, 0.05 M Tris–HCl buffer (pH 7.6). The substrate-reduced difference spectra (A–C) were recorded at 3 min after substrate addition and the dithionite-reduced spectrum 1 min after dithionite treatment. The difference spectra were recorded by the quick-freezing technique[9,10] with a Johnson Research Foundation split-beam spectrophotometer using membranes which had been suspended in 3.5 M NaCl, 0.05 M Tris–HCl buffer (pH 7.6), frozen in liquid N_2 and stored in dry ice for 4 days. A, succinate-reduced *minus* oxidized; B, α-glycerophosphate-reduced *minus* oxidized; C, ascorbate-reduced *minus* oxidized; D, dithionite-reduced *minus* oxidized. Final concn. (mM): α-glycerophosphate, 10; succinate, 10; ascorbate, 5; dithionite, 1 mg. Protein concn. (mg/ml): A and B, 12.8; C, 8.5; D, 6.5.

in Fig. 6. The peaks at 571, 536 and 415 nm were contributed by cytochrome *o*–CO complex and the trough at 558 nm was that of the reduced form of cytochrome *o* in the reference cuvette. Cytochrome a_3–CO complex was represented by the maximum at 591 nm. The γ-peak (427–430 nm) of cytochrome a_3–CO complex was not observed, as the reduced form of cytochrome *o* (reference cuvette) had more absorption in this region than that due to cytochrome a_3–CO complex alone. The trough at 431 nm thus represents the net absorbance changes due to the γ-peak of the cytochrome a_3–CO complex (sample cuvette) and the reduced form of cytochrome *o* (reference cuvette). The marked shoulder at 437 nm was probably the γ-peak of cytochrome *a*. The substrate-reduced CO difference spectrum (not illustrated) was different from the (dithionite + CO *minus* dithionite) difference spectrum only

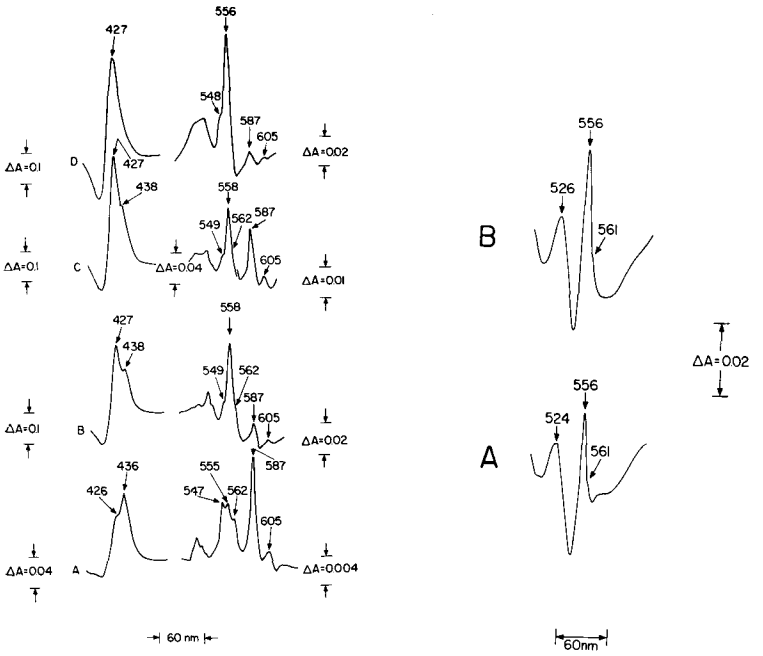


Fig. 4. Effect of CN^- on the substrate- and dithionite-reducible membrane-bound cytochromes of *H. salinarium*. The difference spectra (−196°) were recorded after substrate (3 min) and dithionite (1 min) addition to the CN^--treated membranes. A, CN^- + succinate *minus* oxidized; B, CN^- + α-glycerophosphate *minus* oxidized; C, CN^- + ascorbate *minus* oxidized; D, CN^- + dithionite *minus* oxidized. Final concn. (mM): succinate, 10; α-glycerophosphate, 10; ascorbate, 5; dithionite, 1.0 mg. Protein concn. (mg/ml): A–C, 8.54; D, 6.4.

Fig. 5. Difference spectra (−196°) showing the effect of HQNO on the ascorbate and ascorbate–TMPD oxidase systems of *H. salinarium*. Both the reference and sample cuvettes (2.0 mm light path) contained 0.35 ml membranes in 3.5 M NaCl, 0.05 M Tris–HCl buffer (pH 7.6). B, ascorbate–TMPD + HQNO + O_2 *minus* oxidized; A, ascorbate + HQNO + O_2 *minus* oxidized. Final concn. (mM): ascorbate, 2.0; TMPD, 0.16; HQNO, 1.5 μg/mg protein. Protein concn. (mg/ml): A, 6.4; B, 8.5.

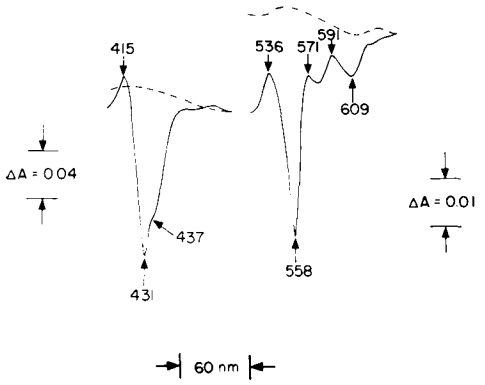


Fig. 6. CO difference (dithionite + CO *minus* dithionite) spectrum (−196°) of the membranes of *H. salinarium*. ----, base-line (dithionite *minus* dithionite); ——, CO difference spectrum recorded after 3 min CO treatment. Other experimental details as described in Fig. 5 except that the membrane suspension contained 6.5 mg protein per ml.

in the Soret region. Thus, with the (ascorbate + CO *minus* ascorbate) difference spectrum (not shown) the CO complex of cytochrome *o* had a peak at 419 nm instead of 415 nm observed with dithionite, and the trough appeared at 443 nm instead of 431 nm. The 443-nm trough was due to the overlapping troughs contributed by the reduced form of both cytochrome *o* and cytochrome a_3 in the reference cuvette[1,3].

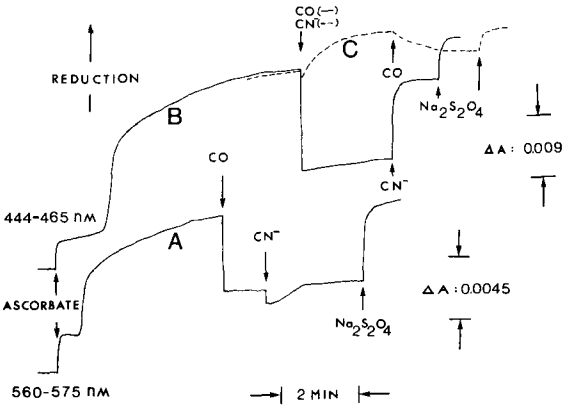


Fig. 7. Kinetic studies (22°) showing the effect of CO and CN^- on the ascorbate-reducible *b*-type cytochromes and cytochrome a_3 in the membranes of *H. salinarium*. A 10-mm light path cuvette containing 2.5 ml membranes in 3.5 M NaCl, 0.05 M Tris–HCl buffer (pH 7.6) was used. Other experimental details are given in the figure. Final concn. (mM): ascorbate, 4; CN^-, 2.0; CO, approx. 1; dithionite, 1 mg. Protein concn. (mg/ml): A, 2.26; B and C, 1.13.

Fig. 7 represents the reduction of cytochrome a_3 and the *b*-type cytochromes (including cytochrome *o*) by ascorbate measured at 444–465 and 560–575 nm, respectively, and also the effect of CN^- on the formation of cytochrome a_3–CO complex. Ascorbate (without TMPD) caused a rapid reduction of the *b*-type cytochromes (A), the aerobic steady state was reached at about 4 sec after ascorbate addition with anaerobiosis occurring after 40 sec. The aerobic steady state of cytochrome a_3 reduction was attained at about the same time as that of the *b*-type cytochromes (B), but the time for the commencement of anaerobiosis was twice as long as that observed with the *b*-type cytochromes. This interesting phenomenon was not further investigated in the present studies. CO addition (by direct bubbling of CO into the cuvette, still in the cell compartment of the spectrophotometer) caused the formation of the CO complexes of cytochrome *o* (A) and cytochrome a_3 (B); the latter was reversed almost completely by the subsequent addition of CN^- (solid trace). Cytochrome a_3–CO complex was not formed if CN^- was added before CO (C). This phenomenon was not observed with cytochrome *o* (A). The increase in absorbance changes with both the *b*-type cytochromes and cytochrome a_3 following dithionite addition was due to increase in the reduction of both types of cytochromes. In fact, the increase in absorbance measured at 560–575 nm was the difference in absorbance changes between the dithionite-reducible cytochrome *o* and other non-CO-binding dithionite-reducible *b*-type pigments in the membranes of *H. salinarium*.

Table I illustrates the approximate concentrations and the turnover numbers of the respiratory components in the membrane-bound respiratory chain system of

TABLE I

APPROXIMATE CONCENTRATION OF THE MEMBRANE-BOUND CYTOCHROMES IN *H. salinarium*

The concentration of the various cytochromes was calculated from difference spectra (22° and −196°) as previously described for other extreme halophiles[1-3]. The subscripts ascribed to the cytochromes refer to their respective α-peaks observed at −196°. Allowance was made for the enhancement factor of 10 (average value from five determinations) in estimating the concentration of cytochromes from difference spectra at −196°. Values in parentheses: dithionite-reduced components. The turnover number (*TN*) was calculated using the following formula:

$$TN = \frac{O_2 \text{ uptake (nmoles } O_2 \text{ per sec/mg protein)}}{\text{Concn. of cytochrome (nmoles/mg protein)}} \times 4$$

Respiratory components	*Concn. (nmoles/mg protein)*	*TN (electrons per cytochrome per sec)*	
		Ascorbate	*Ascorbate–TMPD*
Cytochrome b_{555}	0.04*		
Cytochrome b_{562}	0.02*		
Cytochrome b_{557}	0.44**	18	38
Cytochrome c_{549}	0.06**	67	186
Cytochrome *o*	0.09** (0.17)	86	185
Cytochrome *a*	0.04**	—	—
Cytochrome a_3	0.04**	195	410
Cytochrome a_3 (from a_3–CO)	0.04** (0.04)		

* α-Glycerophosphate-reduced components.
** Ascorbate-reduced components.

H. salinarium. The results show that there were more *b*-type cytochromes than cytochromes belonging to either the *a*- or *c*-type and cytochrome *o* concentration was greater than cytochrome a_3.

Kinetic studies of the membrane-bound cytochromes

Fig. 8 shows the various half-time ($t_{1/2}$) reductions of selected cytochromes in the absence of inhibitors. The kinetics of cytochrome b_{562} (−196°) were not estimated

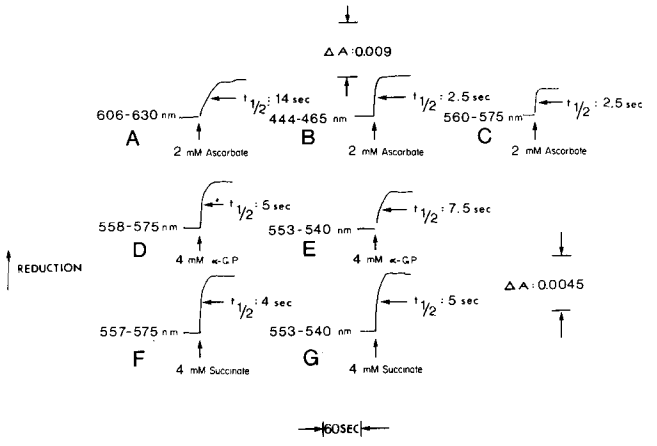


Fig. 8. The half-time ($t_{1/2}$) reduction of the membrane-bound cytochromes of *H. salinarium*. Experimental details, in addition to those in the figure, are similar to those described in Fig. 7. Protein concn. (mg/ml): A, D–G, 5.66; B, 2.26; C, 1.13. α-GP = α-glycerophosphate.

as this component was not seen in the 22° difference spectra. The $t_{1/2}$ values obtained were 14, 2.5 and 2.5 sec for cytochrome *a* (A), cytochrome a_3 (B) and the *b*-type (C) cytochromes (including cytochrome *o*), respectively. The same $t_{1/2}$ value obtained for the reduction of cytochrome a_3 and the *b*-type cytochromes could be interpreted as ascorbate donating electrons directly to both the *b*-type cytochromes (including cytochrome *o*) and cytochrome a_3. The difference in the $t_{1/2}$ value observed for the reduction of cytochrome *a* and cytochrome a_3 implied that cytochrome *a* could probably be situated as a side-chain to the main electron transport system of *H. salinarium*. This was only correct if cytochrome *a* was responsible for more of the absorbance changes measured at 606–630 nm than cytochrome a_3, an assumption based on mitochondrial cytochrome $(a + a_3)$[12]. With α-glycerophosphate and succinate the $t_{1/2}$ value for the reduction of the *c*-type cytochrome, measured at 553–540 nm, was greater than that of their respective *b*-type cytochromes associated with each particular substrate.

The rates of the *b*-type cytochromes and cytochrome a_3 reduction were greatly increased when ascorbate was used in conjunction with TMPD (Fig. 9). The aerobic steady state was also shortened considerably, with anaerobiosis occurring in a much shorter time after ascorbate–TMPD addition (*cf.* Fig. 7, A and B with Fig. 9, A and B). A further increase in reduction of the *b*-type cytochromes and cytochrome a_3 was obtained by the separate subsequent addition of CN^- and dithionite. Assuming that dithionite gave a 100% reduction in both cases, the percent reduction at the aerobic steady states and on reaching anaerobiosis (in that order) was as follows: *b*-type cytochromes, 31, 54 and 65 (with CN^-); cytochrome a_3, 20, 57 and 80 (with CN^-).

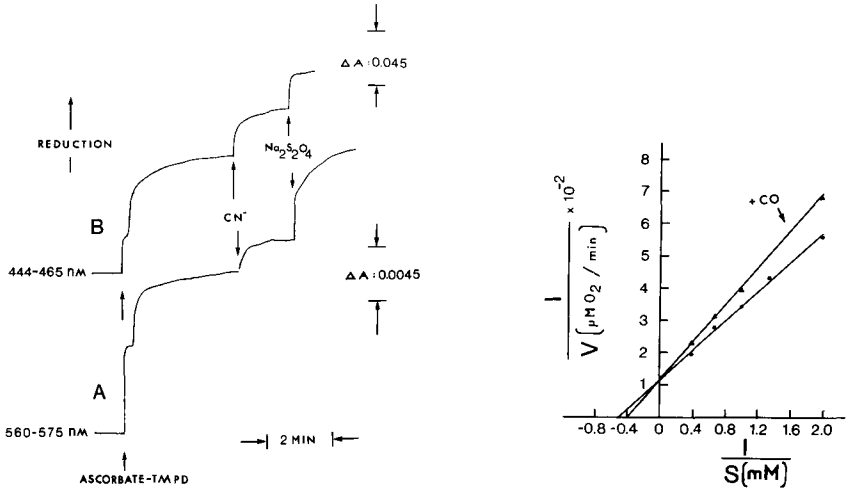


Fig. 9. Kinetic reduction of the *b*-type cytochromes (including cytochrome *o*) and cytochrome a_3 in the membranes of *H. salinarium*. Similar procedure as described in Fig. 7 was used. Final concn. (mM): ascorbate, 2.0; TMPD, 0.16; dithionite, 1 mg. Protein concn. (mg/ml): A, 2.26; B, 5.66.

Fig. 10. Lineweaver–Burk plot showing the competitive inhibition of ascorbate oxidation by CO in the membranes of *H. salinarium*. The respiratory activities were measured polarographically in 3.5 M KCl, 0.05 M Tris–HCl buffer (pH 7.6). Temp., 25°; total volume, 2.5 ml. Protein concn., 1.8 mg/ml.

TABLE II

EFFECT OF INHIBITORS ON THE RESPIRATORY ACTIVITIES OF *H. salinarium*

All respiratory activities were assayed polarographically with a Clark oxygen electrode. Where applicable, corrections were made for endogenous respiration or autooxidation of the various dyes. The percent inhibition represents the average value obtained from three experiments. The reaction medium was 3.5 M NaCl, 0.05 M Tris–HCl buffer (pH 7.6), the same as that used for suspending the freshly prepared membranes of *H. salinarium*. The percent inhibition, within brackets, refers to the carotenoid-containing membranes of *H. salinarium*. Total reaction volume (ml): 3.0 for ascorbate and ascorbate–TMPD; 2.5 for succinate–phenazine methosulphate, succinate, α-glycerophosphate. Temp., 25°. Final concn. of substrate (mM): succinate, 10; α-glycerophosphate, 10; phenazine methosulphate, 0.12; ascorbate, 2.0; TMPD, 0.16.

Inhibitors	*Final concn. (mM)*	*Inhibition (%)*				
		Succinate–phenazine methosulphate reductase	*Succinate oxidase*	*α-Glycero-phosphate oxidase*	*Ascorbate oxidase*	*Ascorbate–TMPD oxidase*
Amytal	2.0	17	40	51		
	4.0		60	57		
PCMB	1.0	48	40	33		
	2.0		100	53		
	3.0			100		
N-Ethylmaleimide	2.0	29	50	57		
	3.0		100	63		
Antimycin A	4.0 μg/mg protein			0	0	0
HQNO	1.5 μg/mg protein				0	0
HQNO	5.0 μg/mg protein				43	54
CN^-	1.0		25	20	86	100 (95)
Azide	1.0				55	
CO	1.0				26	44 (20)

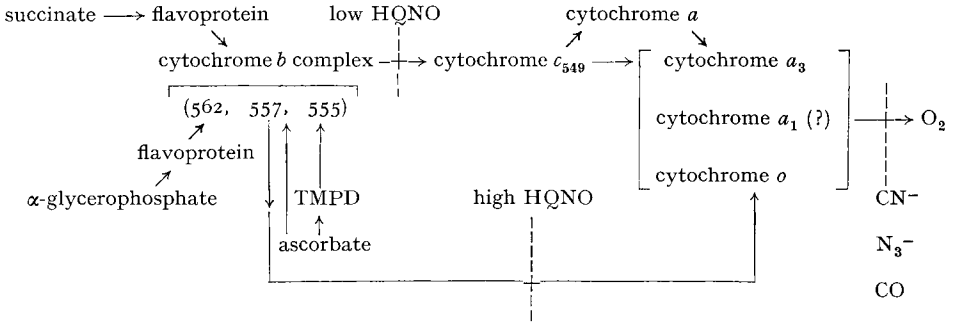
Respiratory activities of the membranes of H. salinarium

Table II summarizes the effect of various inhibitors on some of the respiratory activities observed in the membranes of *H. salinarium*. Amytal, *p*-chloromercuribenzoate (PCMB) and *N*-ethylmaleimide blocked the succinate–phenazine methosulphate, succinate and α-glycerophosphate oxidation, with variations in the extent of inhibition among the systems. Antimycin A had no effect on either the α-glycerophosphate, ascorbate or ascorbate–TMPD oxidase activity. At a concentration of 1.5 μg HQNO per mg protein, neither the oxidation of ascorbate nor ascorbate–TMPD was affected. When increased to 5 μg per mg protein, 43 and 54% of the ascorbate and ascorbate–TMPD oxidase activity were inhibited, respectively. Both the ascorbate and ascorbate–TMPD oxidase systems of *H. salinarium* were sensitive to CN^-, N_3^- and CO. With the carotenoid-containing strain of *H. salinarium*, within brackets, both CN^- and CO inhibited similar systems.

The Lineweaver–Burk plot (Fig. 10) shows that the apparent K_m for ascorbate was 2.0 mM, estimated in 3.5 M KCl, 0.05 M Tris–HCl buffer (pH 7.6) at 25°, and also the ascorbate oxidation in *H. salinarium* was inhibited competitively by CO. The same K_m value for ascorbate oxidation was found with the electron transport particles of *H. cutirubrum*[13], whether it was assayed in 3.5 M KCl, 0.05 M Tris–HCl buffer (pH 7.6) or 3.5 M NaCl, 0.05 M Tris–HCl buffer (pH 7.6), and CO was also a competitive inhibitor of ascorbate oxidation.

DISCUSSION

With the accumulated data derived from the kinetic studies of the reduction of the various cytochromes, the difference spectra recorded at 22° and −196° and the polarographic studies with various electron donors and standard respiratory chain inhibitors, the following basic scheme of the membrane-bound electron transport system of the colourless strain of *H. salinarium* is proposed.



The wavelengths ascribed to the various cytochromes refer to the α-peaks (−196°) observed without CN^-. The pronounced band at 587 nm (−196°) detected in the presence of CN^- *plus* various electron donors was tentatively suggested to be cytochrome a_1. This component was not shown conclusively to be involved with the oxidation of the various substrates tested as it was not seen in the absence of CN^-. The marked difference in the $t_{1/2}$ value estimated for the reduction of cytochrome *a* (14 sec) and cytochrome a_3 (2.5 sec) with ascorbate implied that cytochrome *c* oxidation by cytochrome a_3 might not necessarily involve cytochrome *a*. On this basis, cytochrome *a* was placed as a side chain to the postulated main electron transport pathway.

HQNO appears to act on two sites of the respiratory chain system of *H. salinarium* (Strain 1 M2). At 1.5 μg HQNO per mg protein, this inhibitor prevented only the reoxidation of the ascorbate- or ascorbate–TMPD-reducible cytochrome b_{556} (−196°) without affecting the respiration induced by either α-glycerophosphate, ascorbate or ascorbate–TMPD. This favours the existence of an alternative terminal oxidase which is not an *a*-type cytochrome. With a higher concentration of HQNO (5 μg HQNO per mg protein), 54% of the ascorbate–TMPD oxidase activity was inhibited, suggesting that the segment of the postulated branching respiratory chain without the involvement of cytochrome c_{549} (−196°) was probably sensitive only to high concentrations of HQNO.

Both ascorbate and ascorbate–TMPD reduced at least two *b*-type cytochromes, cytochrome *o* and cytochrome b_{557} (−196°). The latter component was probably identical with cytochrome b_{556} (−196°) which was found to be sensitive to HQNO. The slight shoulder at 562 nm observed in the CN^- *plus* ascorbate minus oxidized difference spectrum and that at 561 nm in the ascorbate *plus* HQNO *minus* oxidized spectrum could be contributed by a single *b*-type pigment which was different from cytochrome b_{557} (−196°). If this assumption was correct, then this *b*-type cytochrome was most likely to be the same component as that involved with α-glycerophosphate and succinate oxidation. The absorption maximum observed at

558 nm (–196°) in the presence of CN^- and that at 557 nm (–196°) without CN^- was likely to be due to two almost spectrophotometrically identical *b*-type cytochromes, one of which was cytochrome *o*. With the CN^--inhibited α-glycerophosphate oxidase pathway, the α-peak of cytochrome b_{555} (–196°), observed in the absence of CN^-, was masked by the strong absorption maximum at 558 nm (–196°).

Five common features were observed in the electron transport systems of the extreme halophiles, harvested at the logarithmic growth phase. (1) Halobacterial respiratory chain systems have a predominant complex of *b*-type cytochromes with two CO-reactive pigments. Cytochrome *o* is always present in conjunction with either cytochrome a_3 as in *H. cutirubrum*[1], *H. salinarium*[2] and *H. salinarium* (Strain 1 M2) or cytochrome a_1 as in *H. halobium*[3]. (2) The extreme halophiles have a very active ascorbate oxidase system which is sensitive to CN^-, N_3^- and CO. The latter does not completely inhibit this system, the range of inhibition varies from 20 to 40% depending on the strain used. For example, CO inhibited only 20% of the ascorbate oxidase system of *H. cutirubrum*[1] and *H. halobium*[3] but 44% of *H. salinarium* (Strain 1 M2). CN^-, on the other hand, is a more powerful inhibitor than CO. At a concentration of 0.5–1.0 mM, CN^- blocked 85–100% of the ascorbate oxidase system of *H. cutirubrum*[1], *H. halobium*[3] and *H. salinarium* (Strain 1 M2). (3) In the four strains investigated, all have a low level of cytochrome *c*. (4) Halobacterium cytochrome *o* can form a CO complex in the presence of CN^-, which prevented the formation of the CO complexes of the *a*-type cytochromes whether CN^- was added before or after the formation of the CO complexes. (5) Ascorbate alone could reduce the *b*-type cytochromes (including cytochrome *o*) and also could induce respiration.

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