SHORT COMMUNICATIONS

BBA 43250

The membrane-bound carbon monoxide-reactive hemoproteins in the extreme halophiles

The halobacteria grow optimally in a 25–30% NaCl-containing medium and lyse when the salt concentration is less than 5–10% (ref. 1). Previous studies[2] on one of these microorganisms, *Halobacterium cutirubrum*, harvested at early logarithmic growth phase, showed that isolated electron transport particles possessed two CO-reactive pigments, cytochromes *o* and a_3. One of the difficulties encountered was the identification of the overlapping absorption spectra of the CO complexes in the Soret region. This communication reports the membrane-bound CO-reactive hemoproteins in two other strains of the extreme halophiles, *Halobacterium halobium* and *Halobacterium salinarium*, and also describes a method for differentiating between the two CO complexes in the Soret.

The membranes were isolated from cells in mid-logarithmic growth phase by the method of BROWN *et al.*[3] but using 20 mM $MgCl_2$–50 mM Tris–HCl (pH 8.2) and at 0°. The CO difference spectrum (CO + ascorbate *minus* ascorbate) of freshly isolated membranes from *H. salinarium* (Fig. 1, A) has a minimum at 445 nm and maxima at 425 and 595 nm The α-peak at 595 nm and the trough at 445 nm in the CO difference spectrum indicates the presence of cytochrome a_3 rather than cytochrome a_1, as the reduced (ascorbate or ascorbate–tetramethyl-*p*-phenylenediamine) *minus* oxidized spectrum showed an α-peak at 604 nm instead of 589–595 nm, the α-peak of reduced cytochrome a_1 (ref 4). The peak at 425 nm was at significantly lower wavelength than that expected for the Soret peak of the cytochrome a_3–CO complex at about 427–430 nm (ref. 5), and was at higher wavelength than 419 nm, the peak of cytochrome *o*–CO complex alone[2,6]. In fact, the 425-nm maximum results from the overlapping maxima of the CO complexes of cytochrome *o* and cytochrome a_3 The spectrum of cytochrome *o*–CO alone is gradually seen if cytochrome a_3–CO is eliminated from the spectrum by adding cyanide before (not shown) or after CO treatment (Fig. 1): the CO difference spectrum recorded at 8 min after cyanide addition (Fig. 1, B) has a trough at 443 nm and a peak at 420 nm, a shift to shorter wavelength of 2 and 5 nm, respectively, observed in the absence of cyanide (Fig. 1, A). The marked shoulder at about 431 nm observed at 8 min (Fig. 1, B) was replaced by a trough at 433 nm, and the 420-nm peak had shifted to 419 nm at about 15 min after cyanide treatment (Fig. 1, C). The peak of cytochrome *o*–CO complex at 419 nm was clearly seen at 25 min (Fig. 1, D), and was free from interference by cytochrome a_3–CO complex In addition, the 595-nm peak of cytochrome a_3–CO complex (Fig 1, D) was no longer detected after cyanide treatment. Similar results were obtained with the electron transport particles of *H. cutirubrum* which also have cytochromes *o* and a_3 (ref. 2) The same technique was also used to

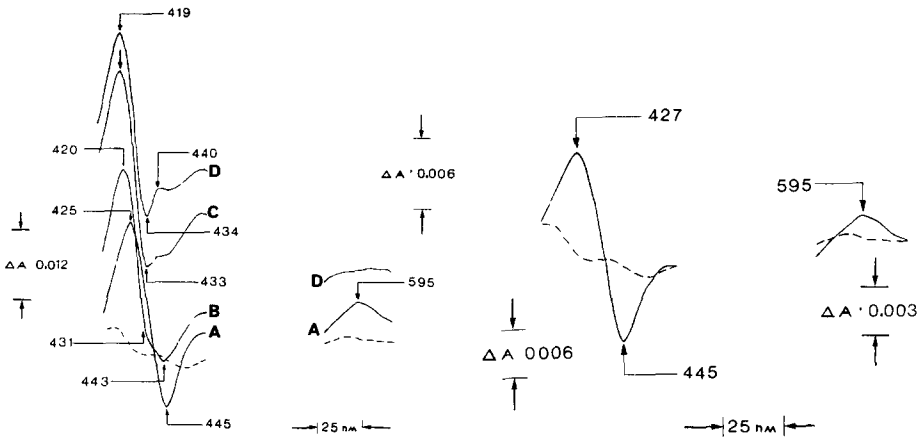

Fig 1 CO difference spectrum (CO + ascorbate *minus* ascorbate) of membranes from *H salinarium* Both the sample and reference cuvettes (10-mm light path) contained 2 5 ml membrane preparation (1 8 mg protein per ml) in 3 4 M NaCl–0.1 M Tris–HCl (pH 7 6) The membrane suspension in the sample cuvette was bubbled with CO for 3 min before 2 mM ascorbate (final concn) was added simultaneously to both the sample and reference cells. – – – –, baseline, ———, CO difference spectra were recorded in the absence (A) and presence (B–D) of 2 mM cyanide (final concn) added after CO treatment A, 2 min after ascorbate addition, B, 8 0 min; C, 15 min, D, 25 min after introduction of cyanide to both cuvettes All spectra recorded at room temperature (22°) with a Phoenix Precision Instrument (PPI) dual/split-beam spectrophotometer

Fig 2 CO difference spectrum (CO + ascorbate *minus* ascorbate) of cytochrome a_3 in the membrane preparation of *H salinarium* The procedure was similar to that described in Fig 1 except that the CO difference spectrum was recorded at 4 min after 2 mM ascorbate (final concn) was added to both cuvettes No cyanide was present in this experiment

TABLE I

APPROXIMATE CONCENTRATIONS OF THE CO-REACTIVE HEMOPROTEINS IN HALOBACTERIA

The concentrations of cytochromes were calculated from room temperature CO difference spectra (CO + ascorbate *minus* ascorbate) The following millimolar extinction coefficients and wavelength pairs were used 80 0 (*cf* the Soret cytochrome *o*–CO complex of *Staphylococcus albus*[10]) for the cytochrome *o*–CO complex ($\Delta A_{419-434\ nm}$), 60 0 (*cf* the Soret cytochrome a_1–CO complex of *Acetobacter pasteurianum*[11]) for the cytochrome a_1–CO complex ($\Delta A_{427-445\ nm}$) and 148 0 (*cf* the Soret cytochrome a_3–CO complex of mammalian tissue[12]) for the cytochrome a_3–CO complex ($\Delta A_{427-445\ nm}$) or 10 1 for the α-peak of cytochrome a_3–CO complex[12] Cytochrome *o* concentration was estimated from the cytochrome *o*–CO complex after elimination of the cytochrome a_3–CO complex in the presence of cyanide Cytochrome a_3 concentration was calculated from the cytochrome a_3–CO complex free from interference of cytochrome *o*–CO complex (*e g* Fig 2 for *H salinarium*)

Respiratory components	*Concn (nmoles/mg protein)*		
	H salinarium	*H halobium*	*H. cutirubrum*
Cytochrome *o*	0 20*	0 23*	0 27**
Cytochrome a_1	—	0 21*	—
Cytochrome a_3	0 09* (0 08***)	—	0 10**

* Detected in membrane preparations
** Detected in electron transport particles[2]
*** Concentration of cytochrome a_3 estimated from the α-peak of cytochrome a_3–CO (Fig 2).

differentiate the cytochrome *o*–CO complex from that of cytochrome a_1 using membranes from *H. halobium* which has cytochromes *o* and a_1. It may be concluded from these observations that in the presence of cyanide, cytochrome *o* of halobacterium, unlike the cytochrome *o* of the free living cultured cells of *Rhizobium japonicum*[7], can form a CO complex while cytochrome a_3 (or a_1) cannot

The spectrum of the cytochrome a_3–CO complex can be observed free from cytochrome *o*–CO if the difference spectrum is recorded relatively early. Fig. 2 shows that after ascorbate is added to a CO-saturated membrane preparation, an α-peak appears at about 595 nm and a Soret peak at 427 nm, both attributable to the cytochrome a_3–CO complex. The success in demonstrating the CO complex of cytochrome a_3 free from interference by cytochrome *o*–CO complex was based on previous reports[8,9] that cytochrome *o* binds slowly with CO, and on the observation that the Soret peak of cytochrome a_3 (442 nm) was seen before that of cytochrome *o* following ascorbate addition to the membrane preparation of *H. salinarium* and to the electron transport particles of *H. cutirubrum*.

Table I shows the content of the CO-reactive pigments in three strains of halobacteria. The concentration of cytochrome *o* reducible by ascorbate was approximately the same in each of the halobacteria investigated. Variations were only observed with the *a*-type CO-reactive pigment. *H salinarium* and *H. cutirubrum* have cytochrome a_3, and *H. halobium* has a_1, in addition to cytochrome *o*.

The author is indebted to Dr. A. R. Wasserman for providing laboratory facilities and to the Canadian Medical Research Council for a Research Fellowship (1968–1969).

Biochemistry Department, McGill University, Montreal (Canada) K. S. CHEAH

1 H LARSEN, *Advan Microbiol Physiol*, 1 (1967) 97
2 K S CHEAH, *Biochim Biophys Acta*, 180 (1969) 320
3 A D BROWN, C D SHOREY AND H P TURNER, *J Gen Microbiol*, 41 (1965) 225
4 R LEMBERG, *Rev Pure Appl Chem*, 15 (1965) 125
5 R LEMBERG, in F F NORD, *Advan Enzymol*, 23 (1961) 265
6 H W TABER AND M MORRISON, *Arch. Biochem. Biophys*, 105 (1964) 367
7 C A APPLEBY, *Biochim Biophys. Acta*, 172 (1969) 88
8 P L BROBERG AND L SMITH, *Biochim Biophys. Acta*, 131 (1967) 479
9 K. S CHEAH, *Biochim. Biophys Acta*, 153 (1968) 718
10 B CHANCE, in J E FALK, R LEMBERG AND R. K MORTON, *Haematin Enzymes*, Part 2, Pergamon Press, New York, 1961, p 433
11 L SMITH, in S P COLOWICK AND N O KAPLAN, *Methods in Enzymology*, Vol 2, Academic Press, New York, 1955, p 732
12 W H VANNESTE AND M-T VANNESTE, *Biochem Biophys. Res. Commun*, 19 (1965) 182.

Received September 29th, 1969

Biochim Biophys Acta, 197 (1970) 84–86