OXIDATIVE PHOSPHORYLATION IN BACTERIA

I. OXIDATIVE PHOSPHORYLATION OF GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

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Oxidative phosphorylation in particulate fractions of eleven bacteria was examined without adding any soluble factor. The bacteria examined which could oxidize both NADH and succinate were divided from their properties of phosphorylation into the following three groups; (i) phosphorylation is scarcely coupled to the oxidation of both substrates, (ii) the P : O ratio in NADH oxidation is nearly equal to that in succinate oxidation, and (iii) the P : O ratio in NADH oxidation is lower than that in succinate oxidation. The bacteria whose P : O ratio in NADH oxidation is higher than that in succinate oxidation were not observed.

In small particles of strains from group (ii) 0.1 mM of cyanide inhibited NADH and succinate oxidation at a similar rate, while in small particles of strains from group (iii) cyanide inhibited succinate oxidation more strongly than NADH oxidation.

These groups agree well with the groups classified by the quinones present. The bacteria in group (ii) contain benzoquinones alone or, at least, mainly, and those in group (iii) contain naphthoquinones alone. From these observations, the role of quinones in oxidative phosphorylation of bacterial particles was discussed.

Studies on oxidative phosphorylation in bacteria have been described by a number of investigators using such strains as *Alcaligenes faecalis* (1-4), *Azotobacter vinelandii* (5-7), *Mycobacterium phlei* (8-10), *Micrococcus lyso-deikticus* (11-13), *Micrococcus denitrificans* (14, 15), and so on (16-18).

Properties of oxidative phosphorylation systems in bacteria were found to be different from those of mammalian mitochondrial systems. In general, bacterial systems show lower P : O ratio than mammalian mitochondrial systems and fail to exhibit respiratory control, though few exceptions were reported (14, 19-23). These observations may be partly derived from loose coupling of oxidative phosphorylation to respiration and partly from lack of

phosphorylation sites and occurrence of by-pass reactions in a respiratory chain.

Some bacteria have the same cytochrome pattern as those of yeast and mammalian mitochondria, whereas a number of bacteria lack cytochrome *a* which is replaced by cytochrome *a*₁ or by a mixture of cytochrome *a*₁ and *a*₂ (24). In addition, bacterial quinones are not always the same as those in the respiratory chain of mammalian mitochondria. Naphthoquinones are found in gram-positive bacteria and sometimes in gram-negative bacteria in place of benzoquinones (25). These lack and replacement of respiratory chain components will result in the lack of phosphorylation sites and non-phosphorylative by-passes.

The object of the present study is to investigate the specific properties of oxidative phosphorylation systems in eleven bacteria which cover various taxonomical groups.

MATERIALS AND METHODS

Bacterial strains. *Pseudomonas aeruginosa* IAM 1202, *Azotobacter vinelandii* IAM 1078, *Achromobacter cycloclastes* IAM 1013, *Escherichia coli* K 12 IAM 1264, *Aerobacter aerogenes* IAM 1183, *Serratia marcescens* IAM 1223, *Micrococcus lysodeikticus* IAM 1056, *Micrococcus luteus* IAM 1097, *Brevibacterium helvolum* IAM 1637, *Bacillus megaterium* IAM 1166, and *Bacillus subtilis* were used and stocked on bouillon-agar slants.

Preparation of particulate fractions. The cells cultured to stationary phase in 0.2% succinate-bouillon broth with shaking at 30° were collected by centrifugation, washed twice with 50 mM Tris-HCl buffer (pH 7.4) containing 250 mM sucrose and 5 mM MgCl₂, and suspended in 50 mM Tris-HCl buffer (pH 7.4) containing 1M sucrose to the concentration of 1 g wet cells per 20 ml.

Cell-free extract was prepared mainly by the method of ISHIKAWA and LEHNINGER (11). To every ml of the cell suspension, 0.1 mg of crystalline lysozyme and 0.5 mg EDTA were added and the mixture was incubated for 30 min at 30°. The cells were recovered by centrifugation at 17,000 × *g* for 30 min, suspended in 50 mM Tris-HCl buffer (pH 7.4) containing 250 mM sucrose and 5 mM MgCl₂ to a concentration of 1 g wet cells per 10 ml, and incubated for 20 min at 20° to induce osmotic lysis. After disruption of the cells by sonic vibration at 10 kC for 5 min at 2°, the suspension was centrifuged at 8,000 × *g* for 15 min to remove intact cells and large cell debris. The supernatant fraction obtained was centrifuged at 17,000 × *g* for 30 min, giving large particles (LP) and supernatant fraction. LP was washed with 50 mM Tris-HCl buffer (pH 7.4) containing 250 mM sucrose and 5 mM MgCl₂. The supernatant was further fractionated by centrifugation in the Hitachi 55P ultracentrifuge at 105,000 × *g* for 120 min, giving a red gelatinous sediments of small particles (SP) which were washed with the same buffer. LP and SP

were re-suspended in Tris-HCl buffer (pH 7.4) to a concentration of 25–50 mg protein per ml. These particles were prepared daily.

Protein was estimated by either KALCKAR's method (26) or LOWRY's method (27).

Assay of oxidative phosphorylation. Oxygen uptake was measured by amperometry with an oxygen consumption recorder, Yanagimoto PO-100, and calculated as ΔO (total oxygen consumption minus endogenous oxygen consumption). The amount of phosphate esterification coupled to respiration was measured by HAGIHARA and LARDY's method (28) and calculated as ΔP (total esterified P coupled to oxidation of added and endogenous substrates minus esterified P coupled to oxidation of endogenous substrates).

Assay of cytochromes. The absorption spectra of cytochromes in SP were measured with Shimadzu MPS-50L spectrophotometer.

Assay of quinones. Quinones were extracted from lyophilized cells with ethanol-ether (3 : 1) (29), and purified by BRODIE's method (30). They were further purified on aluminum oxide column. The purified quinones were identified as naphthoquinone or benzoquinone by measurement with Shimadzu MPS-50L spectrophotometer. Their molecular weights were estimated with Hitachi RMU6L mass spectrometer (chamber voltage, 70 eV; temperature, 230°). The length of side chain was measured by thin-layer chromatography on liquid paraffin-impregnated silica gel, developed with acetone-water (95 : 5).

Identification of organic phosphorus compounds. ^{32}P -labeled compounds were identified by BARTLETT's method (31). Inorganic phosphate and glucose 6-phosphate were separated by HAGIHARA and LARDY's method (28).

Chemicals. ATP and ADP were obtained from Kyowa Hakko Kogyo Co., Ltd. Yeast hexokinase (Type 1), crystalline lysozyme, and NADH were obtained from Sigma Chemical Co. Other chemicals were of the purest grades commercially available.

RESULTS

Oxidation and phosphorylation by eleven bacterial systems

It is generally said that activity of phosphorylation coupled to oxidation in bacterial cell-free extracts depends on the presence of soluble factors. In most of our preparations, however, phosphorylation coupled to oxidation was observed without addition of any soluble factor, as will be seen in Table 1.

The bacteria examined in the present experiment can be divided from their properties of succinate oxidation in SP systems into two types; (1) the SPs oxidize NADH but not succinate (*Ps. aeruginosa*, *M. luteus*, and *B. megaterium*) and (2) the SPs oxidize both substrates (others). The latter can further be classified from their properties of phosphorylation into three groups; (i) the P : O ratio in oxidation of NADH and succinate is less than 0.1 (*Az. vinelandii*, and *B. subtilis*), (ii) the P : O ratio in NADH oxidation is nearly equal to that in succinate oxidation (*Ach. cycloclastes*, *E. coli*, *A. aerogenes*,

Table 1. Oxidation and phosphorylation by

The reaction mixtures consisted of 30 μ moles MgCl_2 , 15 μ moles Na_2HPO_4 (containing 600,000 cpm ^{32}P), 66 μ moles KF , 30 μ moles glucose, 0.5 μ mole ATP , 1 μ mole ADP , 0.05 mg hexokinase, 50 mM Tris-HCl buffer (pH 7.4), 2 μ moles NADH or 10 μ moles succinate (as substrate), and LP or SP (3–5 mg protein for NADH oxidation

Bacteria	SP					
	NADH			Succinate		
	ΔO	ΔP	P/O	ΔO	ΔP	P/O
	natom	nmole		natom	nmole	
<i>Ps. aeruginosa</i>	660	189	0.29	0	0	—
<i>Az. vinelandii</i>	530	8	0.02	800	0	0
<i>Ach. cycloclastes</i>	890	384	0.43	880	515	0.51
<i>E. coli</i>	910	248	0.27	500	108	0.22
<i>A. aerogenes</i>	600	154	0.26	700	126	0.18
<i>S. marcescens</i>	550	129	0.23	440	126	0.28
<i>M. luteus</i>	870	165	0.19	0	0	—
<i>M. lysodeikticus</i>	940	300	0.32	290	250	0.86
<i>Brev. helvolum</i>	710	177	0.25	430	526	1.22
<i>B. megaterium</i>	980	21	0.02	0	0	—
<i>B. subtilis</i>	630	11	0.02	590	60	0.10

Table 2. Effects of inhibitors and uncouplers on

The reaction mixtures were the same as given in Table 1. SP contained 0.1–0.3 mg protein for NADH oxidation and 1.0–3.0 mg protein for succinate oxidation. The reaction was started by adding the substrate after 3 min of incubation with an

Inhibitors or uncouplers	<i>M. lysodeikticus</i>			
	NADH		Succinate	
	ΔO	P/O	ΔO	P/O
Control	100 (560)	100 (0.59)	100 (420)	100 (0.58)
NaCN (0.1 mM)	51	54	0	—
NaN_3 (30 mM)	55	47	10	—
Antimycin A (33 mM)	42	8	50	24
2,4-Dinitrophenol (1 mM)	89	58	15	—
Arsenate (33 mM)	98	0	48	0
Oligomycin (50 γ)	89	61	100	79
Tributyltinchloride (0.1 mM)	93	32	78	79

eleven bacterial particulate systems.

and 30–50 mg protein for succinate oxidation); final volume 3 ml. The reaction was started by adding the substrate after 3 min of pre-incubation, and kept for 5–10 min at 28°.

LP					
NADH			Succinate		
ΔO	ΔP	P/O	ΔO	ΔP	P/O
natom	nmole		natom	nmole	
460	68	0.15	0	0	—
980	0	0	850	14	0.02
760	296	0.39	710	131	0.18
680	287	0.42	760	41	0.05
170	68	0.40	620	21	0.03
260	30	0.12	280	29	0.10
430	167	0.39	90	0	—
880	650	0.74	0	0	—
650	216	0.33	620	173	0.28
790	23	0.03	0	0	—
840	15	0.02	310	9	0.03

oxidation and phosphorylation in small particle.

inhibitor or an uncoupler. The activities of oxidation and phosphorylation were expressed as a relative activity (%). The absolute activity of the control is given in parentheses (ΔO : natom).

<i>Brev. helvolum</i>				<i>E. coli K12</i>			
NADH		Succinate		NADH		Succinate	
ΔO	P/O	ΔO	P/O	ΔO	P/O	ΔO	P/O
100 (970)	100 (0.11)	100 (660)	100 (0.47)	100 (1420)	100 (0.09)	100 (1050)	100 (0.03)
61	44	0	—	74	35	57	146
94	55	17	—	75	11	92	0
25	27	0	—	40	0	87	0
80	80	0	—	104	33	47	0
94	27	47	0	103	0	41	0
75	91	69	74	93	0	73	0
73	109	53	117	76	0	101	0

Fig. 1. Oxidative and phosphorylative activities of particles obtained at various g values.

The reaction mixtures were the same as given in Table 1. Particles from *M. lysodeikticus* contained 0.1 mg protein for NADH oxidation and 1.0 mg protein for succinate oxidation. Particles from *B. helvolum* contained 0.2 mg protein for NADH oxidation and 2.0 mg protein for succinate oxidation.

- P : O ratio with NADH, ○—○ P : O ratio with succinate
- Q_{o2} with NADH (natom/min/mg protein)
- Q_{o2} with succinate (natom/min/mg protein)

and *S. marcescens*), and (iii) the P : O ratio in NADH oxidation is lower than that in succinate oxidation (*M. lysodeikticus* and *Brev. helvolum*). The bacteria whose P : O ratio in NADH oxidation is higher than that in succinate oxidation were not observed.

In LP systems, the P : O ratio in succinate oxidation decreased and became lower than, or, at least, almost equal to that in NADH oxidation (Table 1). It has been reported that some bacteria show different oxidative phosphorylation activities in LP and SP systems (5, 10). Therefore, relationship between particle size and phosphorylative activities was next examined. As shown in Fig. 1, phosphorylative activity was observed in all the preparations, though the P : O ratio in NADH or succinate oxidation was not constant in these preparations. This agreed with the observation by TISSIERES *et al.* (5), but not with that by KASHKET and BRODIE (32).

Figure 2 shows the kind of phosphorus compounds found in the reaction

Fig. 2. Identification of phosphorus compounds in reaction mixture.

The reaction mixtures were the same as given in Table 1. SP from *M. lysodeikticus* contained 0.1 mg protein for NADH oxidation, and 1.0 mg protein for succinate oxidation. They were incubated for 20 min and the reaction was stopped by boiling the mixture for 5 min. Tube numbers 3 to 5 were separated again by hexanol column (28).

×—× G6P (for succinate as substrate), ×--× G6P (for NADH as substrate), ●—● phosphorus compound (for succinate as substrate), ●---● phosphorus compound (for NADH as substrate).

mixture when phosphorylation reaction is over. More than 90% of ^{32}P esterified was incorporated into the glucose 6-phosphate and ATP fractions which were the end products of oxidative phosphorylation in our systems. Most of the remainder was incorporated into the ADP fraction. Other phosphorus compounds containing ^{32}P were not found in the reaction mixture. These observations indicate that the data on oxidative phosphorylation in the present work do not include an error from side reactions.

Effect of inhibitors and uncouplers on oxidation and phosphorylation in small particles

Three representative strains from the group (ii) and (iii) described above were examined. As shown in Table 2, effect of various inhibitors and uncouplers on oxidation and phosphorylation in the SP of *M. lysodeikticus* is quite similar to that in the SP of *Brev. helvolum* but different from that in the SP of *E. coli*. Cyanide and N_3^- inhibited succinate oxidation in the SPs

Table 3. Effect of cyanide on oxidation and phosphorylation in small particle.

The reaction mixtures were the same as given in Table 1. SP contained 0.1–0.3 mg protein for NADH oxidation and 1.0–3.0 mg protein for succinate oxidation. The reaction was started by adding the substrate after 3 min of preincubation. The activities of oxidation and phosphorylation are expressed as a relative activity (%).

Bacteria	CN ⁻ -added (mM)	NADH		Succinate	
		ΔO	P/O	ΔO	P/O
<i>Ach. cycloclastes</i>	0	100	0.19	100	0.17
	0.1	14	0.57	11	0.49
	1.0	0	—	0	—
<i>E. coli</i>	0	100	0.20	100	0.13
	0.1	74	0.07	57	0.19
	1.0	3	—	3	—
<i>A. aerogenes</i>	0	100	0.13	100	0.22
	0.1	59	0.03	59	0.19
	1.0	0	—	0	—
<i>M. lysodeikticus</i>	0	100	0.24	100	0.81
	0.1	51	0.13	0	—
	1.0	7	—	0	—
<i>Brev. helvolum</i>	0	100	0.18	100	0.85
	0.1	61	0.08	0	—
	1.0	0	—	0	—

of the former two bacteria very strongly, while they inhibited succinate oxidation in the SP of the latter one only slightly. Oligomycin and tributyltin chloride lowered P:O ratio with NADH and succinate in *M. lysodeikticus* slightly while they inhibited phosphorylation in *E. coli* entirely.

Effect of cyanide was further studied in five strains from group (ii) and (iii). In SPs of strains from group (ii), 0.1 mM of cyanide inhibited NADH and succinate oxidation at a similar rate, while in SPs of strains from group (iii) cyanide inhibited succinate oxidation more strongly than NADH oxidation (Table 3).

Cytochromes in small particles

Cytochrome components in SPs of 11 bacteria were qualitatively examined. As shown in Table 4, *Ps. aeruginosa*, *Az. vinelandii*, *Ach. cycloclastes*, *M. lysodeikticus*, *M. luteus*, *B. megaterium*, and *B. subtilis* showed difference spectra in the α -band region similar to those of yeast and mammalian mitochondria. *Ps. aeruginosa*, *Az. vinelandii*, and *Ach. cycloclastes* lacked the peak of cytochrome a_3 in the Soret region. In other words, these three strains contain cytochrome a but not cytochrome a_3 . *E. coli*, *A. aerogenes*, *S. mar-*

Table 4. Cytochromes in small particles of bacteria.

SPs were suspended in 50 mM Tris-HCl buffer (pH 7.4). They were reduced by sodium hydrosulfite and were oxidized by hydrogen peroxide. Figures in parentheses indicate indistinct shoulder.

Bacteria	Visible range (nm)				Soret range (nm)
	α		β		γ
<i>Ps. aeruginosa</i>	607	560 553	523 530		427
<i>Az. vinelandii</i>	605	560 550	522 528		428
<i>Ach. cycloclastes</i>	605	560 555	515 525 532		430
<i>E. coli</i>	630 600	560	531		427
<i>A. aerogenes</i>	632 598	560	531		428
<i>S. marcescens</i>	630 598	562 (555)	(526) 531		428
<i>M. luteus</i>	605	560 551	520		443 430
<i>M. lysodeikticus</i>	605	565 555	525		445 431
<i>Brev. helvolum</i>	633 596	560 550	530		445 430
<i>B. megaterium</i>	603	560 (555)	530		443 427
<i>B. subtilis</i>	608	562 (555)	533		445 428

cescens, and *Brev. helvolum* contained a mixture of cytochrome a_1 and a_2 in place of cytochrome a . The bacteria belonging to Enterobacteriaceae and probably *S. marcescens* lacked cytochrome c .

These bacteria were classified by their cytochrome components into the following 4 groups; (i) a group containing cytochromes a , b , and c , (ii) a group containing cytochromes a and a_3 , b , and c , (iii) a group containing cytochromes a_1 and a_2 , and b , and (iv) a group containing cytochromes a_1 and a_2 , b , and c . No relation was found between these 4 groups and the ones classified by properties of oxidative phosphorylation as described above.

Quinones in bacteria

Quinones in the whole cells of the bacteria examined were determined (Table 5). Quinones in SP were qualitatively equal to those in the whole cells.

As has been reported (25), only benzoquinones were detected in *Ach. cycloclastes*, only naphthoquinones in *M. lysodeikticus* and *Brev. helvolum*, and both quinones in *E. coli* and *A. aerogenes*, though the side-chain length of the quinones in some bacteria was found to differ from those described in the previous reports (12, 32). In their mass spectra, main molecular peak was observed at m/e 716 in *M. lysodeikticus* and *E. coli*, and at m/e 784 in *Brev. helvolum*. A lower peak was observed at m/e 784 in the former bacteria and at m/e 852 in the latter bacterium.

According to the results in Table 5, bacterial groups classified by their

Table 5. Quinones in bacteria.

Bacteria	Quinones	Content (mg/g protein)
<i>Ach. cycloclastes</i>	Q ₁₀	0.314
<i>E. coli</i>	Q ₈	0.252
	K ₂ (C ₄₀), K ₂ (C ₄₅)	0.049
<i>A. aerogenes</i>	Q ₉	0.075 (mg/g dry wt.) ^a
	Naphthoquinone ^b	—
<i>M. lysodeikticus</i>	K ₂ (C ₄₀), K ₂ (C ₄₅)	0.527
<i>Brev. helvolum</i>	K ₂ (C ₄₅), K ₂ (C ₅₀)	0.532

^a This datum was presented by H. KAWAJI (The Institute of Applied Microbiology, University of Tokyo, Tokyo).

^b D.H.L. BISHOP, K.P. PANDYA and K.R. KING (38).

quinones seem to agree with the groups classified by the properties of oxidation and phosphorylation, and their sensitivity to cyanide.

Phosphorylation observed with malate and fumarate oxidation

As has been reported in the particulate fractions of *M. phlei* (33), phosphorylation coupled to oxidation of malate was also observed in the SPs of *M. lysodeikticus* and *Brev. helvolum*. In the SPs of *Ach. cycloclastes* and *E. coli*, malate was not oxidized (Table 6). The P : O ratio with malate was lower than that with succinate, though the former in *M. phlei* was reported to be equal to the latter (33). Fumarate was not oxidized in SPs of bacteria examined except *M. lysodeikticus*.

DISCUSSION

Oxidative phosphorylation in particulate fractions of eleven bacteria observed without adding any soluble factor is described here. Bacterial particles capable of oxidizing both NADH and succinate were classified into the following three groups; (i) the ones whose phosphorylation is scarcely coupled to the oxidation of both substrates, (ii) the ones whose P : O ratio with both substrates are nearly equal, and (iii) the ones whose P : O ratio with succinate is higher than that with NADH. This classification may not always be applicable to intact cell systems since the P : O ratio of about 3 was reported in intact cell systems of *E. coli* and *A. aerogenes* (22, 23), and since the lack of one or two phosphorylation sites by sonic disruption was reported in mitochondrial systems (34), etc.

As shown in Fig. 1, P : O ratio with NADH in *M. lysodeikticus* system became lower as the particles became smaller in size, while P : O ratio with succinate was almost constant. This might show that the "site I" is very fragile to cell disruption and that the "site II" or the "site II" and "site

Table 6. Phosphorylation observed with malate and fumarate oxidation.

The reaction mixtures were the same as given in Table 1 except that 10 μ moles malate or 10 μ moles fumarate was used as a substrate. SP contained 0.1–0.3 mg protein for NADH oxidation and 1.0–3.0 mg protein for succinate, fumarate, and malate oxidation.

Bacteria	Substrates	ΔO natom	ΔP nmole	P/O	QO ₂ ^a
<i>Ach. cycloclastes</i>	NADH	470	56	0.12	330
	Succinate	500	140	0.28	40
	Fumarate	0	0	—	0
	Malate	0	0	—	0
<i>E. coli</i>	NADH	770	104	0.14	320
	Succinate	460	32	0.07	50
	Fumarate	0	0	—	0
	Malate	0	0	—	0
<i>M. lysodeikticus</i>	NADH	1100	684	0.63	1175
	Succinate	750	708	0.97	87
	Fumarate	765	307	0.41	34
	Malate	950	535	0.56	638
<i>Brev. helvolum</i>	NADH	640	147	0.23	1140
	Succinate	450	447	0.99	44
	Fumarate	trace	0	—	trace
	Malate	520	206	0.54	210

^a natom/min/mg protein.

III'' are relatively stable. However, phosphorylative properties of bacterial particles in group (iii), where P : O ratio in succinate oxidation is higher than that in NADH oxidation, cannot be explained by the fragility of phosphorylation sites. A hypothesis is proposed that NADH is oxidized by nonphosphorylative oxidation systems in particulate fractions similar to those reported in soluble fractions of *E. coli* and *M. phlei* systems (32, 35–37). If NADH is oxidized partly through nonphosphorylative by-passes and partly through cytochrome systems, while succinate is mainly oxidized through cytochrome systems, higher P : O ratio with succinate than that with NADH will be observed.

It is of interest that the inhibition of NADH oxidation by cyanide was different in its efficiency from that of succinate oxidation, as shown in Table 3. In SP of *Ach. cycloclastes*, cyanide equally inhibited NADH oxidation and succinate oxidation and rather higher P : O ratio was observed under the inhibition. In *E. coli* and *A. aerogenes* systems, succinate oxidation systems were a little more sensitive to cyanide than NADH oxidation systems, and P : O ratio with NADH decreased by inhibition contrary to a slight increase

in P : O ratio with succinate by the inhibition. In *M. lysodeikticus* and *Brev. helvolum* systems, cyanide inhibited NADH oxidation by about 50% and P : O ratio was reduced, whereas succinate oxidation was completely inhibited by the same concentration of cyanide. If NADH is oxidized through the same terminal oxidation systems as those of succinate oxidation, cyanide will inhibit the oxidation of both substrates equally. Oxidation and phosphorylation in SP of *Ach. cycloclastes* agree with this supposition. If NADH is oxidized partly through nonphosphorylative by-passes other than cytochrome systems and succinate is oxidized through cytochrome systems, P : O ratio with NADH will be reduced by cyanide while P : O ratio with succinate will be unchanged. Oxidation and phosphorylation in SPs of *E. coli*, *A. aerogenes*, *M. lysodeikticus*, and *Brev. helvolum* agree with this supposition.

Next problem to solve is which respiratory components are related to these oxidative and phosphorylative properties. As shown in Table 4, cytochromes in bacteria were widely varied. The most remarkable differences were found in *a*-type and *c*-type cytochromes. In some bacteria, cytochrome *a* was replaced by cytochrome *a*₁ and *a*₂, and in some bacteria cytochrome *c* was not detected. However, no correlation was observed between properties of oxidative phosphorylation and cytochrome components contained, except that relatively low P : O ratio was observed in SPs which lacked cytochrome *c*.

BISHOP *et al.* and other investigators found benzoquinones alone in gram-negative bacteria, naphthoquinones alone in gram-positive bacteria, and both quinones in *E. coli*, *Proteus vulgaris*, *A. aerogenes*, and *S. marcescens* (38-43). KASHKET reported that, in *E. coli*, succinate oxidase particles contain ubiquinone alone, whereas NADH oxidase particles contain naphthoquinone alone (44). In our experiments, Tables 3 and 5 suggested that sensitivity of bacterial oxidation system to cyanide is in accord with quinones present in the systems. From these results, we propose the following hypothesis:

NADH is oxidized through benzoquinones and/or naphthoquinones and finally by cytochrome systems. Succinate is oxidized through benzoquinones and then by cytochrome systems or directly through cytochrome systems. From naphthoquinones but not from benzoquinones, electrons flow partly to oxygen directly without coupling to phosphorylation. Cyanide inhibits the cytochrome oxidase but not nonphosphorylative NADH oxidation. Therefore, in the SP of *Ach. cycloclastes* in which electrons from NADH and succinate flow through benzoquinones and then through cytochrome systems, cyanide equally inhibits NADH and succinate oxidation and P : O ratio with both substrates is nearly equal. In the SPs of *M. lysodeikticus* and *Brev. helvolum*, electrons from NADH flow through naphthoquinones and then through cytochrome and/or nonphosphorylative oxidation systems, while electrons from succinate flow through cytochrome systems. Cyanide inhibits succinate oxidation completely, but inhibits NADH oxidation partly. P : O ratio with succinate is higher than that with NADH. In *E. coli* and *A. aerogenes*, which contain both benzoquinones and naphthoquinones, mixed type was observed.

In the present experiments, malate oxidation was detected only in the bacterial systems which contained naphthoquinone alone. It is possible to consider that higher P : O ratio with succinate in these bacteria was from this oxidation system, but such a possibility was denied since the P : O ratio with malate was lower than that with succinate.

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