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Immunological Investigation of the Distribution of Cytochromes Related to the Two Terminal Oxidases of *Escherichia coli* in Other Gram-Negative Bacteria

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Monospecific antibodies were raised against the two terminal oxidase complexes of the aerobic respiratory chain of *Escherichia coli*. These are the cytochrome *d* and cytochrome *o* complexes. The antibodies were used to check for the occurrence of cross-reactive antigens in membrane preparations from a variety of gram-negative bacteria by rocket immunoelectrophoresis and immunoblotting techniques. With these criteria, proteins closely related to the cytochrome *d* complex of *E. coli* appeared to be widely distributed. Among the strains containing cytochrome *d*-related material were *Serratia marcescens*, *Photobacterium phosphoreum*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, and *Azotobacter vinelandii*. The data suggest that the *d*-type terminal oxidase in many of these strains is associated in a complex with *b*-type and a_1-type cytochromes, as has been found to be the case in *E. coli*. *K. pneumoniae* and *S. typhimurium* were also shown to have material cross-reactive to the *E. coli* cytochrome *o* complex.

The aerobic respiratory chain of *Escherichia coli* is branched and contains two terminal oxidases, the cytochrome *d* complex and the cytochrome *o* complex (10). Both of these oxidases have been purified to homogeneity (25, 26, 31, 33, 37) and have been shown to carry out electrogenic reactions in reconstituted proteoliposomes (24, 28, 33). Electron flow through either oxidase generates a transmembrane voltage difference. Whereas the cytochrome aa_3-type terminal oxidases oxidize ferrocytochrome *c*, the oxidases of *E. coli* appear to directly oxidize ubiquinol in the bacterial membrane (25, 28). The cytochrome *o* complex predominates in the *E. coli* membrane when cells are grown with high aeration (31), whereas the cytochrome *d* complex, which has a higher affinity for oxygen (44), is induced when cells are grown under oxygen-limiting conditions (31; see also references 10, 20, and 41).

The cytochrome *d* complex has been shown to contain three cytochrome components, b_{558}, a_1, and *d*, but only two polypeptides by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (26, 27, 37). The larger of the two subunits (subunit I; molecular weight, 57,000) has been shown to contain the cytochrome b_{558} component of the complex (8). One question of interest which is addressed in this work is whether other gram-negative bacteria, many of which contain *d*-type cytochrome by spectroscopic criteria (20, 41), contain immunocross-reactive material to either or both of these subunits. This was determined by using antibodies raised specifically against either subunit I or subunit II (molecular weight, 43,000) of the cytochrome *d* complex of *E. coli*.

The cytochrome *o* complex of *E. coli* contains heme *b* (25, 31, 33) and copper (25) and is reported to have two (25) or four (31, 33) subunits by SDS-PAGE analysis. Antibodies raised against the native oxidase were used to check for cross-reactivity with preparations from various gram-negative bacteria. Several of these strains contain *o*-type terminal zoxidases by spectroscopic criteria, and it was interesting to investigate their immunological relationships.

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MATERIALS AND METHODS

Organisms and growth conditions. The bacterial strains used in this work are listed in Table 1. The local isolates were taken from stocks used for teaching purposes, and most were originally obtained from the American Type Culture Collection. Before use, the strains were checked by metabolic characterization tests. Most bacterial strains were grown in Penassay broth (50 ml or 100 ml; Difco Laboratories, Detroit, Mich.) in 250 ml Klett flasks. The flasks were shaken at 200 rpm at the recommended temperature for each strain, and the cells were harvested at stationary phase. *Photobacterium phosphoreum* required 3% NaCl in the medium. Several strains, *Rhodopseudomonas palustris*, *Rhodospirillum rubrum*, *Rhodospirillum fulvum*, and *Rhodopseudomonas sphaeroides*, were grown in Sistrom basal medium (47). *Rhodospirillum fulvum* was grown microaerophilically in the dark for 2.5 days in medium supplemented with 5 μg of para-aminobenzoic acid per ml. *Pseudomonas putida* (48), *Klebsiella aerogenes* (11), and *Paracoccus denitrificans* (12) were grown in media and conditions previously described. *Azotobacter vinelandii* (19) was grown microaerophilically in Winogradsky medium with sucrose, agar, and molybdenum. *E. coli* SHSP19 (45), which does not synthesize heme, was grown semianaerobically.

Immunological methods. The antibody preparations used in this work have all been previously described. Polyclonal antibodies against *E. coli* cytochrome *o* were raised against immunopurified cytochrome (31). Polyclonal antibodies were raised against subunit II of the cytochrome *d* complex, which had been excised from an SDS-polyacrylamide gel after electrophoresis (29). A monoclonal antibody (A14-5) which has been shown to react with subunit I of the cytochrome *d* complex was also used (32).

Harvested cells were washed and disrupted by sonication. Membranes were prepared and solubilized by using Zwittergent 3-12 as previously described (31). For SDS-PAGE immunoblotting (2), samples containing 60 μg of membrane

TABLE 1. Distribution of cross-reactive proteins to *E. coli* cytochromes *d* and *o* in various gram-negative bacteria

Bacterial strains	Source	Spectroscopic evidence for cytochrome *d*		Immunological evidence for cytochrome:	
		Reference[c]	This work	*d*	*o*
Escherichia coli K-12 (MR43L)	W. Shipp (46)	+ (9, 37, 42)	+	+	+
Serratia marcescens	LI[a]		ND[b]	+	−
Enterobacter aerogenes	LI	+ (38)	+	+	−
Pseudomonas putida	LI	+ (48, 49)	ND	−	−
Proteus vulgaris	LI	+ (39)	+	+	−
Pseudomonas fluorescens	LI		ND	−	−
Escherichia coli W191-6	(31)		+	+	+
Proteus mirabilis	LI		ND	+	−
Pseudomonas aeruginosa	LI	− (35)	ND	−	−
Rhodopseudomonas palustris	LI	− (20)	ND	−	−
Paracoccus denitrificans	LI	+ (12)	ND	−	−
Photobacterium phosphoreum	T. Baldwin	+ (54)	+	+	−
Escherichia coli SHSP19	J. Cronan		ND	+	−
Rhodospirillum rubrum	LI	− (20)	ND	−	−
Rhodospirillum fulvum	LI		ND	−	−
Rhodopseudomonas sphaeroides	LI	− (20)	ND	−	−
Arthrobacter pyridinolis	T. Krulwich	+ (40)	−	−	−
Acinetobacter HO1N	W. Finnerty	+ (5)	+	+	−
Klebsiella pneumoniae	R. Ugalde	+ (11)	−	+	+
Salmonella typhimurium	LI	+ (4)	+	+	+
Azotobacter vinelandii	R. Ugalde	+ (13, 14, 18, 19, 21, 22, 36)	+ −	+	−
Vitreoscilla sp.		− (20)	ND	−	−

[a] LI, University of Illinois local isolate.
[b] ND, Not determined.
[c] + and − denote the reported presence or absence, respectively, of cytochrome *d*.

protein each were used. Details are given elsewhere (29). For dot immunoblotting, samples of Zwittergent 3-12 solubilized membranes (50 μg of protein) were first mixed with SDS-PAGE sample buffer and then filtered through nitrocellulose labeled with ^{125}I-protein A and autoradiographed as described previously (30). Rocket immunoelectrophoresis was performed as before (31) with the Zwittergent-solubilized membranes (approximately 100 μg of protein).

RESULTS AND DISCUSSION

Cytochrome *d* complex. A number of bacteria other than *E. coli* have been reported to contain cytochrome *d* (previously called cytochrome a_2) based on spectroscopic criteria (20, 41). In addition to the strains listed in Table 1, other strains have also been reported to have cytochrome *d*, including *Haemophilus parainfluenzae* (15), *Achromobacter* strain D (1), and *Pasteurella tularensis* (6). In most cases the presence of cytochrome a_1 in the membrane correlates with the presence of cytochrome *d* (20, 41). Very few studies have included biochemical characterization, although a study has been reported on the cytochrome *d* from *Photobacterium phosphoreum* (54). Often the amount of cytochrome *d* can be optimized by using selective growth conditions, usually limited-oxygen conditions. No attempt was made in the current work to optimize for cytochrome *d* production in all the bacterial strains which were examined. Figure 1 shows an SDS-PAGE immunoblot of membrane preparations with monoclonal antibodies directed against subunit I of the cytochrome *d* complex (32). Many of these strains contained a protein closely related to subunit I of the *E. coli* cytochrome *d* complex, which has previously been shown to be cytochrome b_{558} (8). Most strains with cytochrome *d* by

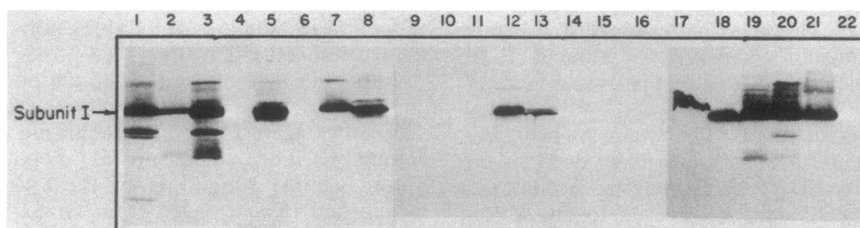

FIG. 1. SDS-PAGE immunoblotting of membrane preparations from various gram-negative bacteria with a monoclonal antibody preparation directed against subunit I of the cytochrome *d* terminal oxidase complex of *E. coli* K-12. Lanes: 1, *E. coli* K-12 (MR43L); 2, *Serratia marcescens*; 3, *Enterobacter aerogenes*; 4, *Pseudomonas putida*; 5, *Proteus vulgaris*; 6, *Pseudomonas fluorescens*; 7, *E. coli* W191-6; 8, *Proteus mirabilis*; 9, *Pseudomonas aeruginosa*; 10, *Rhodopseudomonas palustris*; 11, *Paracoccus dentrificans*; 12, *Photobacterium phosphoreum*; 13, *E. coli* SHSP19; 14, *Rhodospirillum rubrum*; 15, *Rhodospirillum fulvum*; 16, *Rhodopseudomonas sphaeroides*; 17, *Arthrobacter pyridinolis*; 18, *Acinetobacter* H01N; 19, *Klebsiella pneumoniae*; 20, *Salmonella typhimurium*; 21, *Azotobacter vinelandii*; 22, *Vitreoscilla* sp.

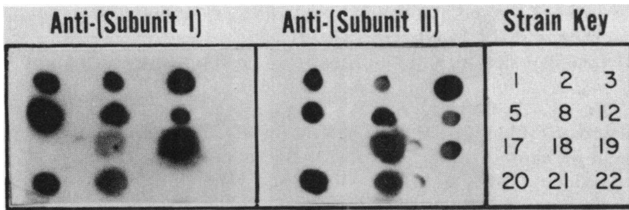

FIG. 2. Dot immunoblot of membrane preparations from various gram-negative bacteria with the monoclonal antibody directed against subunit I and a polyclonal antibody preparation directed against subunit II of the *E. coli* cytochrome *d* complex. The strain numbers given in the key correspond to the lane numbers given in the legend to Fig. 1.

spectroscopic criteria contained this subunit. It is reasonable to conclude that a *b*-type cytochrome is associated with cytochrome *d* in the other bacterial strains as it is in *E. coli*. The presence of the bands in Fig. 1 other than that identified as subunit I does not reflect a lack of specificity of the antibody, which was a monoclonal antibody. Bands below subunit I (e.g., lanes 1 and 3) are due to proteolysis and have been previously noted (32). Occasional smearing above subunit I (e.g., lanes 19 and 20) probably resulted from a tendency of this protein to aggregate, which has also been previously observed (37). Note that the sample from *Arthobacter pyridinolis* (Fig. 1, lane 17) ran anomalously, with most of the protein migrating as a single band. Blotting results were probably due to nonspecific trapping in this case. In all other cases, the membrane proteins were well resolved on the gel, as indicated by protein staining with Coomassie blue, and the blotting results were quite specific. The five strains reported to contain no cytochrome *d* by spectroscopic criteria (Table 1) also contained no cross-reactive material (Fig. 1).

Immunoblotting after SDS-PAGE with anti-subunit II of the cytochrome *d* complex showed clear evidence of subunit II in close relatives of *E. coli*, including *Enterobacter aerogenes*, *Klebsiella pneumoniae*, and *Salmonella typhimurium*. However, subunit II does not transfer efficiently from the polyacrylamide gel to the nitrocellulose, and this could result in false negative results. In order to avoid this step, dot immunoblotting was used. Fig. 2 shows that all those strains which had a protein which cross-reacted to subunit I also had material cross-reactive to subunit II. *A. pyridinolis* did not appear to contain either subunit, at least not when grown under the conditions we used in these experiments. *Vitreoscilla* sp. was not reported to contain cytochrome *d*, and this is consistent with the data in Fig. 2; thus, *Vitreoscilla* sp. served as a negative control.

Immunoprecipitation studies were also performed with antibodies against the native cytochrome *d* complex from *E. coli*. Only in the case of *E. coli* was 100% of the cytochrome *d* precipitated by this antibody (not shown). In most other cases, very little cytochrome *d* was precipitated from a Zwittergent 3-12 solution after the addition of the antibody preparation. These findings suggest significant divergence between the *E. coli* enzyme and the cytochrome *d* present in the other strains.

Another experiment was performed to examine the membranes of *E. coli* strain SHSP19 (45). This strain does not synthesize heme in the absence of δ-amino levulinic acid. It has been reported that membranes prepared from this strain must contain cytochrome apoproteins because respiratory oxidase activity can be reconstituted by the addition of hematin and ATP (43). The immunoblotting results (Fig. 1) showed that the cytochrome *d* complex was present in the membrane, even though no heme was present.

Cytochrome *o* complex. Immunocross-reactivity data with cytochrome *o* are less complete than those obtained with cytochrome *d* because the antibody preparation against cytochrome *o* did not immunoblot well. Experiments were thus limited to rocket immunoelectrophoresis, which requires immunoprecipitation. As was seen with the anti-cytochrome *d*, it is likely that some strains may contain cross-reactive material which would be manifest in immunoblotting experiments but which will not be apparent by immunoprecipitation. The data (Fig. 3) show that cytochrome *o* similar to that found in *E. coli* is in close relatives, including *E. aerogenes*, *K. pneumoniae*, and *S. typhimurium*. In each of these cases the major immunoprecipitin arc stained for the presence of heme, confirming that the cross-reactive component is a cytochrome. The minor arcs which were observed in some cases did not contain heme. All other strains failed to show any heme-staining rocket immunoprecipitin arc, suggesting that there were no proteins strongly cross-reactive to cytochrome *o*. This would indicate that the

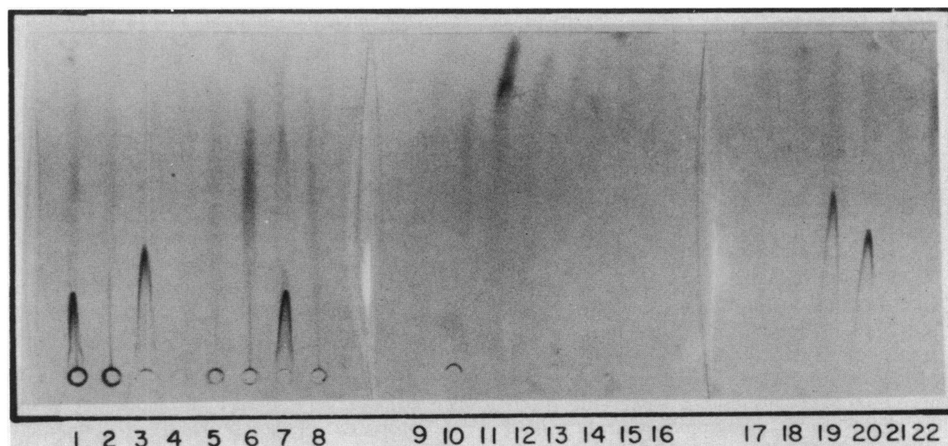

FIG. 3. Rocket immunoelectrophoresis of membrane preparations from various gram-negative bacteria with an antibody preparation which is monospecific against the *E. coli* cytochrome *o*. Lane numbers are as in legend to Fig. 1.

cytochrome *o* species reported in many of these organisms are not very closely related to the *E. coli* enzyme.

Note that most of the cytochrome *o* species which have been purified from organisms other than *E. coli* either function as cytochrome *c* oxidases or contain a *c*-type cytochrome as a tightly bound component. These include cytochrome *o* from *Rhodopseudomonas sphaeroides*, (50, 51), *R. capsulata* (16, 17), *R. palustris* (23), *P. aeruginosa* (29, 55), *A. vinelandii* (56, 57, 58), and *Methylophilus methylotrophus* (3). Hence, these may be quite distinct from the *E. coli* enzyme which neither contains a *c*-type cytochrome nor has cytochrome *c* oxidase activity (12, 29, 33). The well-characterized cytochrome *o* of *Vitreoscilla* sp. is a soluble enzyme of unknown function (52, 53) and is probably not closely related to the *E. coli* enzyme.

E. coli SHSP19 (heme deficient) was also examined by rocket immunoelectrophoresis for the presence of apo-cytochrome *o*. Surprisingly, no rocket was apparent, suggesting the absence of cytochrome *o* when the strain is not synthesizing heme. Others (43) have presented electrochemical evidence that apocytochrome *o* must be present in membranes of cells unable to synthesize heme. Further studies will be required to clarify this situation. Possibly detergent solubilization results in denaturation of the cytochrome in the absence of heme.

In summary, the data presented here clearly show that some of the close relatives of *E. coli* contain both of the terminal oxidases characterized in *E. coli* and that at least the cytochrome *d* complex is widely distributed among gram-negative bacteria. Furthermore, subunits I and II of the complex appear always to be present together, suggesting an association of the *b*-type cytochrome (i.e., subunit I) with cytochrome *d* in all the cases examined. Presumably, cytochrome a_1 is associated in a complex with cytochrome *d* in these other bacterial species as well. In many cases the published reduced-minus-oxidized difference spectra of membrane preparations from these bacteria show cytochrome a_1 along with cytochrome *d* (20, 41).

Finally, the immunoblotting data show that the apo-cytochrome *d* complex is synthesized and inserted into the membrane in the absence of heme biosynthesis. These findings complement the work of others and show that other apocytochromes are present in the membranes of heme-deficient *E. coli* (43).

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LITERATURE CITED

1. **Arima, K., and T. Oka.** 1965. Cyanide resistance in *Achromobacter*. I. Induced formation of cytochrome a_2 and its role in cyanide-resistant respiration. J. Bacteriol. **90:**734–743.
2. **Burnette, W. N.** 1981. Western blotting: Electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodination protein A. Anal. Biochem. **112:**195–203.
3. **Carver, M. A., and C. W. Jones.** 1983. The terminal respiratory chain of the methylotrophic bacterium *Methylophilus methylotrophus*. FEBS Lett. **155:**187–191.
4. **Drabikowska, A. K.** 1970. Electron Transport System of *Salmonella typhimurium* Cells. Acta Biochim. Pol. **17:**89–98.
5. **Ensley, B. D., Jr., and W. R. Finnerty.** 1980. Influences of growth substrates and oxygen on the electron transport system in *Acinetobacter* sp. HO1-N. J. Bacteriol. **142:**859–868.
6. **Fellman, J. H., and R. C. Mills.** 1960. Succinoxidase system of *Pasteurella tularensis*. J. Bacteriol. **79:**800–806.
7. **Gounaris, A. D., and L. P. Hager.** 1961. A resolution of the *Escherichia coli* pyruvate dehydrogenase complex. J. Biol. Chem. **236:**1013–1018.
8. **Green, G. N., R. G. Kranz, R. M. Lorence, and R. B. Gennis.** 1984. Identification of subunit I as the cytochrome b_{558} component of the cytochrome *d* terminal oxidase complex of *Escherichia coli*. J. Biol. Chem. **259:**7994–7997.
9. **Haddock, B. A.** 1973. The reconstitution of oxidase activity in membranes derived from a 5-amino-laevulinic acid-requiring mutant of *Escherichia coli*. Biochem. J. **136:**877–884.
10. **Haddock, B. A., and C. W. Jones.** 1977. Bacterial Respiration. Bacteriol. Rev. **41:**47–99.
11. **Harrison, D. E. F.** 1972. A Study of the effect of growth conditions on chemostat-grown *Klebsiella aerogenes* and kinetic changes of a 500-nm absorption band. Biochim. Biophys. Acta **275:**83–92.
12. **Henry, M. F., and P. M. Vignais.** 1979. Induction by cyanide of cytochrome *d* in the plasma membrane of *Paracoccus dinitrificans*. FEBS Lett. **100:**41–46.
13. **Hoffman, P. S., R. M. Irwin, L. A. Carreira, T. V. Morgan, B. D. Ensley, and D. V. DerVartanian.** 1980. Studies of photochemical action spectra on *N,N,N′,N′*-tetramethyl-p-phenylenediamine oxidase-negative mutants of *Azotobacter vinelandii*. Eur. J. Biochem. **105:**177–185.
14. **Hoffman, P. S., T. V. Morgan, and D. V. DerVartanian.** 1979. Respiratory-chain characteristics of mutants of *Azotobacter vinelandii* negative to tetramethyl-p-phenylenediamine oxidase. Eur. J. Biochem. **100:**19–27.
15. **Holländer, R., and W. Mannheim.** 1975. Characterization of hemophilic and related bacteria by their respiratory quinones and cytochromes. Int. J. Syst. Bacteriol. **25:**102–107.
16. **Hudig, H., and G. Drews.** 1982. Characterization of a *b*-type cytochrome *c* oxidase of *Rhodopseudomonas capsulata*. FEBS Lett. **146:**389–392.
17. **Hudig, H., and G. Drews.** 1982. Isolation of a *b*-type cytochrome oxidase from membranes of the phototrophic bacterium *Rhodopseudomonas capsulata*. Z. Naturforsch Teil C **37:** 193–198.
18. **Jones, C. W., and E. R. Redfearn.** 1967. Preparation of red and green electron transport particles from *Azotobacter vinelandii*. Biochim. Biophys. Acta **143:**354–362.
19. **Jones, C. W., and E. R. Redfearn.** 1967. The cytochrome system of *Azotobacter vinelandii*. Biochim. Biophys. Acta **143:**340–353.
20. **Jurtshuk, P., Jr., T. J. Mueller, and W. C. Acord.** 1975. Bacterial terminal oxidases. Crit. Rev. Microbiol. **3:**399–468.
21. **Kauffman, H. F., and B. F. van Gelder.** 1973. The respiratory chain of *Azotobacter vinelandii*. I. Spectral properties of cytochrome *d*. Biochim. Biophys. Acta **305:**260–267.
22. **Kauffman, H. F., and B. F. van Gelder.** 1973. The respiratory chain of *Azotobacter vinelandii*. II. The effect of cyanide on cytochrome *d*. Biochim. Biophys. Acta **314:**276–362.
23. **King, M.-T., and G. Drews.** 1976. Isolation and partial characterization of the cytochrome oxidase from *Rhodopseudomonas palustris*. Eur. J. Biochem. **68:**5–12.
24. **Kita, K., M. Kasahara, and Y. Anraku.** 1982. Formation of a membrane potential by reconstituted liposomes made with cytochrome b_{562}-*o* complex, a terminal oxidase of *Escherichia coli* K-12. J. Biol. Chem. **257:**7933–7935.
25. **Kita, K., K. Konishi, and Y. Anraku.** 1984. Terminal oxidases of *Escherichia coli* aerobic respiratory chain. I. Purification and properties of cytochrome b_{562}-*o* complex from cells in the early exponential phase of aerobic growth. J. Biol. Chem. **259:** 3368–3374.
26. **Kita, K., K. Konishi, and Y. Anraku.** 1984. Terminal oxidase of

Escherichia coli aerobic respiratory chain. II. Purification and properties of cytochrome b_{558}-*d* complex from cells grown with limited oxygen and evidence of branched electron-carrying systems. J. Biol. Chem. **259**:3375–3381.

27. **Koland, J. G., M. J. Miller, and R. B. Gennis.** 1984. Potentiometric analysis of the purified cytochrome *d* terminal oxidase complex from *Escherichia coli*. Biochemistry **23**:1051–1056.
28. **Koland, J. G., M. J. Miller, and R. B. Gennis.** 1984. Reconstitution of the membrane-bound, ubiquinone-dependent pyruvate oxidase respiratory chain of *Escherichia coli* with the cytochrome *d* terminal oxidase. Biochemistry **23**:445–453.
29. **Kranz, R. G., C. A. Barassi, M. J. Miller, G. N. Green, and R. B. Gennis.** 1983. Immunological characterization of an *Escherichia coli* strain which is lacking cytochrome *d*. J. Bacteriol. **156**:115–121.
30. **Kranz, R. G., and R. B. Gennis.** 1982. A quantitative radioimmunological screening method for specific gene products. Anal. Biochem. **127**:247–257.
31. **Kranz, R. G., and R. B. Gennis.** 1983. Immunological characterization of the cytochrome *o* terminal oxidase from *Escherichia coli*. J. Biol. Chem. **258**:10614–10621.
32. **Kranz, R. G., and R. B. Gennis.** 1984. Characterization of the cytochrome *d* terminal oxidase complex of *Escherichia coli* using polyclonal and monoclonal antibodies. J. Biol. Chem. **259**:7998–8003.
33. **Matsushita, K., L. Patel, R. B. Gennis, and H. R. Kaback.** 1983. Reconstitution of active transport in proteoliposomes containing cytochrome *o* oxidase and *lac* carrier protein purified from *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **80**:4489–4893.
34. **Matsushita, K., E. Shinagawa, O. Adachi, and M. Ameyama.** 1982. *o*-Type cytochrome oxidase in the membrane of aerobically grown *Pseudomonas aeruginosa*. FEBS Lett. **139**:255–258.
35. **Matsushita, K., M. Yamada, E. Shinagawa, O. Adachi, and M. Ameyama.** 1983. Membrane-bound respiratory chain of *Pseudomonas aeruginosa* grown aerobically. A KCN-insensitive alternate oxidase chain and its energetics. J. Biochem. (Tokyo) **93**:1137–1144.
36. **McInerney, M. J., K. S. Holmes, and D. V. DerVartanian.** 1982. Effect of O_2 limitation on growth and respiration of the wild type and an ascorbate-tetramethyl-*p*-phenylenediamine-oxidase-negative mutant strain of *Azotobacter vinelandii*. J. Bioenerg. Biomembr. **14**:451–456.
37. **Miller, M. J., and R. B. Gennis.** 1983. The purification and characterization of the cytochrome *d* terminal oxidase complex of the *Escherichia coli* aerobic respiratory chain. J. Biol. Chem. **258**:9159–9165.
38. **Moss, F.** 1956. Adaptation of the cytochromes of *Aerobacter aerogenes* in response to environmental oxygen tension. Aust. J. Exp. Biol. Med. Sci. **34**:395–406.
39. **Moyed, H. S., and D. J. O'Kane.** 1956. Enzymes and coenzymes of the pyruvate oxidase of *Proteus*. J. Biol. Chem. **218**:831.
40. **Pelliccione, N., B. Jaffin, M. E. Sobel, and T. A. Krulwich.** 1979. Induction of the phosphoenolpyruvate: hexose phosphotransferase system associated with relative anaerobiosis in an obligate aerobe. Eur. J. Biochem. **95**:69–75.
41. **Poole, R. K.** 1983. Bacterial cytochrome oxidase. A structural and functionally diverse group of electron-transfer proteins. Biochim. Biophys. Acta. **726**:205–243.
42. **Poole, R. K., and B. A. Haddock.** 1975. Effects of sulfate-limited growth in continuous culture on the electron-transport chain and energy conservation in *Escherichia coli K-12*. Biochem. J. **152**:537–546.
43. **Reid, G. A., B. A. Haddock, and W. J. Ingledew.** 1981. Assembly of functional *b*-type cytochromes in membranes from a 5-aminolaevulinic acid-requiring mutant of *Escherichia coli*. FEBS Lett. **131**:346–350.
44. **Rice, C. W., and W. P. Hempfling.** 1978. Oxygen-limited continuous culture and respiratory energy conservation in *Escherichia coli*. J. Bacteriol. **134**:115–124.
45. **Sasarman, A., M. Surdeanu, and T. Horodniceanu.** 1968. Locus determining the synthesis of δ-aminolevulinic acid in *Escherichia coli* K-12. J. Bacteriol. **96**:1882–1884.
46. **Shipp, W. S.** 1972. Cytochromes of *Escherichia coli*. Arch. Biochem. Biophys. **150**:459–472.
47. **Sistrom, W. R.** 1962. The Kinetics of the synthesis of photopigments in *Rhodopseudomonas sphaeroides*. J. Gen. Microbiol. **23**:607–616.
48. **Sweet, W. J., and J. A. Peterson.** 1978. Changes in cytochrome content and electron transport patterns in *Pseudomonas putida* as a function of growth phase. J. Bacteriol. **133**:217–224.
49. **Sweet, W. J., and J. A. Peterson.** 1981. The respiratory system of *Pseudomonas putida*: participation of cytochromes in electron transport. Arch. Biochem. Biophys. **209**:256–265.
50. **Takamiya, K.** 1983. Properties of the cytochrome *c* oxidase activity of cytochrome b_{561} from photoanaerobically grown *Rhodopseudomonas sphaeroides*. Plant Cell Physiol. **24**:1457–1462.
51. **Takamiya, K., and H. Tanaka.** 1983. Isolation and purification of cytochrome b_{561} from a photosynthetic bacterium *Rhodopseudomonas sphaeroides*. Plant Cell Physiol. **24**:1449–1455.
52. **Tyree, B., and D. A. Webster.** 1978. Intermediates in the reaction of reduced cytochrome *o* (*Vitreoscilla*) with oxygen. J. Biol. Chem. **254**:176–179.
53. **Tyree, B., and D. A. Webster.** 1978. The binding of cyanide and carbon monoxide to cytochrome *o* purified from *Vitreoscilla*. Evidence for subunit interaction in the reduced protein. J. Biol. Chem. **253**:6988–6991.
54. **Watanabe, H., Y. Kamita, T. Nakamura, A. Takimoto, and T. Yamanaka.** 1979. The terminal oxidase of *Photobacterium phosphoreum*, a novel cytochrome. Biochim. Biophys. Acta **547**:70–78.
55. **Yang, T.** 1982. Tetramethyl-*p*-phenylenediamine oxidase of *Pseudomonas aeruginosa*. Eur. J. Biochem. **121**:335–341.
56. **Yang, T.-Y., and P. Jurtshuk, Jr.** 1978. Purification and characterization of cytochrome *o* from *Azotobacter vinelandii*. Biochim. Biophys. Acta. **502**:543–548.
57. **Yang, T. Y., and P. Jurtshuk, Jr.** 1978. Studies on the red oxidase (cytochrome *o*) of *Azotobacter vinelandii*. Biochem. Biophys. Res. Commun. **81**:1032–1039.
58. **Yang, T., D. O'Keefe, and B. Chance.** 1979. The oxidation-reduction potentials of cytochrome $o+c_4$ and cytochrome *o* purified from *Azotobacter vinelandii*. Biochem. J. **181**:763–766.