"Interatomic Distances Supplement" (1965) *Chem. Soc., Spec. Pubi. No. 18.*
Kannan, K. K., Nostrand, B., Fridborg, K., Lövgren, S., Ohelsson, A., & Petef, M. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 51.
Klug-Roth, D., Fridovich, I., & Rabani, J. (1973) *J. Am. Chem. Soc. 95*, 2786-2790.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265-275.
McAdam, M. E., Fielden, E. M., Lavelle, F., Calabrese, L., Cocco, D., & Rotilio, G. (1977) *Biochem. J. 167*, 271-274.
McCord, J. M., & Fridovich, I. (1969) *J. Biol. Chem. 244*, 6049-6055.
Michelson, A. M., McCord, J. M., & Fridovich, I., Eds. (1977) *Superoxide and Superoxide Dismutases*, Academic Press, London.
Moss, T. H., & Fee, J. A. (1975) *Biochem. Biophys. Res. Commun. 66*, 799-808.
Rabani, J., Klug-Roth, D., & Lilie, J. (1973) *J. Phys. Chem. 77*, 1169-1175.
Reeke, G. N., Hartsuck, J. A., Ludwig, M. L., Quiocho, F. A., Steitz, T. A., & Lipscomb, W. N. (1967) *Proc. Natl. Acad. Sci. U.S.A. 58*, 2220.
Richardson, J. S., Thomas, K. A., & Richardson, D. C. (1975a) *Biochem. Biophys. Res. Commun. 63*, 986-992.
Richardson, J. S., Thomas, K. A., Rubin, B. H., & Richardson, D. C. (1975b) *Proc. Natl. Acad. Sci. U.S.A. 72*, 1349-1353.
Sadler, P. J., Bakka, A., & Beynon, P. J. (1978) *FEBS Lett. 94*, 315.
Sudmeier, J. L., & Bell, S. J. (1977) *J. Am. Chem. Soc. 99*, 4499-5000.
Suzuki, K. T., & Maitani, T. (1978) *Specialia 34*, 1449.

Resolution and Identification of Iron-Containing Antigens in Membrane Vesicles from *Escherichia coli*†

Peter Owen, Gregory J. Kaczorowski,‡ and H. Ronald Kaback*

ABSTRACT: Iron-containing antigens present in membrane vesicles prepared from *Escherichia coli* ML 308-225 were analyzed by crossed immunoelectrophoresis following growth of the organism in the presence of ^{59}Fe. Seven discrete antigens (or antigen complexes) are detected by autoradiography, and six contain primarily nonheme iron. Three immunoprecipitates are positively identified as NADH dehydrogenase (EC 1.6.99.3), NADPH dehydrogenase (EC 1.6.99.1), and glutamate dehydrogenase (EC 1.4.1.4) based on activity stains for these enzymes. Two other immunogens containing nonheme iron correspond to antigens no. 22 and 37 in the crossed immunoelectrophoresis reference pattern of Owen & Kaback [Owen, P., & Kaback, H. R. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3148]. In addition, the immunoprecipitate corresponding to Braun's lipoprotein contains tightly bound iron.

Iron-containing proteins are important components of the respiratory chain of microorganisms (Orme-Johnson, 1973; Haddock & Jones, 1977). However, resolution of the individual iron-containing redox proteins comprising the aerobic respiratory chain of *Escherichia coli*, for example, has not been achieved nor has a clear notion of the functional organization of the redox carriers emerged. Most of the cytochromes and iron–sulfur proteins involved in aerobic electron transfer are membranous and refractory to extensive purification (Hendler & Burgess, 1972, 1974). Moreover, analysis of membrane proteins by sodium dodecyl sulfate–polyacrylamide gel electrophoresis usually results in loss of intrinsic biological activity and also nonheme iron (Clegg & Skyrme, 1973).

The use of crossed immunoelectrophoresis (CIE;[1] Johansson & Hjerten, 1974; Owen & Salton, 1975, 1977; McLaughlin & Meerovitch, 1975; Smyth et al., 1976, 1978; Alexander & Kenny, 1977, 1978) represents an important innovation in the analysis of microbial membrane components. In a recent study (Owen & Kaback, 1978), a high-resolution CIE reference pattern comprised of over 50 discrete antigens was established for *E. coli* membrane vesicles, and many of the immunogens were identified by zymogram techniques that rely on retention of intrinsic biological activity (Owen & Kaback, 1979a). Furthermore, quantitative immunoadsorption was utilized to study the topological distribution of antigens across the plane of the membrane (Owen & Kaback, 1978, 1979b), and it was demonstrated that vesicles prepared in the manner described (Kaback, 1971; Short et al., 1975) retain essentially the same polarity and configuration as the membrane of the intact cell. In view of the obvious advantages of CIE, we have undertaken an immunological investigation of the iron-containing antigens in membrane vesicles prepared from cells of *E. coli* grown aerobically on the nonfermentable carbon source succinate. In this paper, seven such antigens are clearly resolved and four are identified.

Experimental Procedures

Methods

Growth of Cells and Preparation of Membrane Vesicles. *E. coli* ML 308-225 ($i^-z^-y^+a^+$) was grown on minimal medium A (Davis & Mingioli, 1950) containing 1.0% disodium succinate (hexahydrate) and $^{59}FeSO_4$ (32.5 mCi/mg) at a final concentration of 1 mCi/L. Membrane vesicles were prepared as described (Kaback, 1971; Short et al., 1975), frozen in liquid nitrogen, and maintained at or below −70 °C. The prepara-

† From the Department of Microbiology, Trinity College, University of Dublin, Ireland (P.O.), and The Laboratory of Membrane Biochemistry, Roche Institute of Molecular Biology, Nutley, New Jersey 07110 (G.J.K. and H.R.K.). *Received September 10, 1979.* This research was supported in part by a grant to P.O. from the Medical Research Council of Ireland.
‡ G.J.K. is a postdoctoral fellow of the Helen Hay Whitney Foundation.

[1] Abbrevations used: CIE, crossed immunoelectrophoresis; EDTA, ethylenediaminetetraacetic acid.

0006-2960/80/0419-0596$01.00/0

tions contained a specific activity of 863 dpm/μg of protein.

Preparations of Detergent Extracts of Membrane Vesicles. For analysis by CIE, vesicles were sedimented by centrifugation (48000*g* for 1 h at 4 °C) and resuspended in 50 mM Tris-HCl (pH 8.6) containing 4% Triton X-100 (v/v) and 5 mM ethylenediaminetetraacetic acid (EDTA) to a concentration of 15–20 mg of protein per mL. The samples were incubated for 1 h at 25 °C and centrifuged at 17000*g* for 45 min at 4 °C. After aspiration of the supernatant, the sediment was extracted again in the same manner. Extracts were stored in 50–100-μL aliquots at −70 °C and thawed only once immediately prior to CIE.

CIE against Antivesicle Immunoglobulins. Antiserum to membrane vesicles prepared from *E. coli* ML 308-225 was raised in rabbits and immunoglobulins were fractionated and concentrated to 150 mg/mL as described (Owen & Kaback, 1978, 1979a). Methods for performing CIE in the presence of Triton X-100 and for washing, drying, and staining immunoplates with Coomassie brillant blue have been described elsewhere (Owen & Salton, 1977; Smyth et al., 1978; Owen & Kaback, 1979a).

Enzyme Staining Techniques (Zymograms) and Autoradiography. Methods described by Owen & Salton (1977) and Smyth et al. (1978) were used to detect immunoprecipitates exhibiting NADH dehydrogenase (EC 1.6.99.3) and glutamate dehydrogenase (EC 1.4.1.4) activities, respectively. Detection of NADPH dehydrogenase activity (EC 1.6.99.1) was accomplished by substituting NADPH (at a final concentration of 250 μM for NADH in the NADH dehydrogenase assay (Owen & Salton, 1977). Autoradiograms were obtained by exposing dried immunoplates to Kodak X-Omat H film for 3–90 days depending on the activity of the preparation.

Extraction of Nonheme Iron and Total Iron. Extraction of nonheme iron and total iron was based on methods described by Brumby & Massey (1967) and performed after resolution of membrane antigens by CIE. For extraction of nonheme iron, pressed immunoplates were incubated individually for 10 min at 20 °C in 20 mL of 5% trichloroacetic acid (w/v), followed by addition of 25.5 mL of 1,10-phenanthroline (300 μg/mL). For extraction of total iron, pressed immunoplates were incubated individually for 10 min at 20 °C in 20 mL of 50% glacial acetic acid (v/v) and 1.25% thioglycolic acid (v/v), followed by addition of 14 mL of saturated sodium acetate (pH 6.0), 16 mL of water, and 50 mL of isoamyl alcohol containing 4,7-diphenyl-1,10-phenanthrolinesulfonate at 830 μg/mL. In both cases, incubation was continued with shaking for an additional 5 min after addition of iron chelators and the extracted immunoplates were then rinsed a total of 6 times in distilled water before pressing and air-drying. The length of time from initial acid treatment to air-drying did not exceed 45 min. Similar immunoplates, treated with appropriate volumes of distilled water, were run as controls.

Protein Determination. Protein was determined by using a modification (Dulley & Grieve, 1975) of the method of Lowry et al. (1951) that eliminates interference by Triton X-100. Bovine serum albumin was used as the standard.

Materials

$^{59}FeSO_4$ was purchased from New England Nuclear, and agarose and Triton X-100 were obtained from Miles Labs and Research Products International, respectively.

Results

Typical CIE profiles obtained for the first and second Triton X-100–EDTA extracts of ^{59}Fe-labeled *E. coli* ML 308-225 membrane vesicles are shown in Figure 1. The spectra of

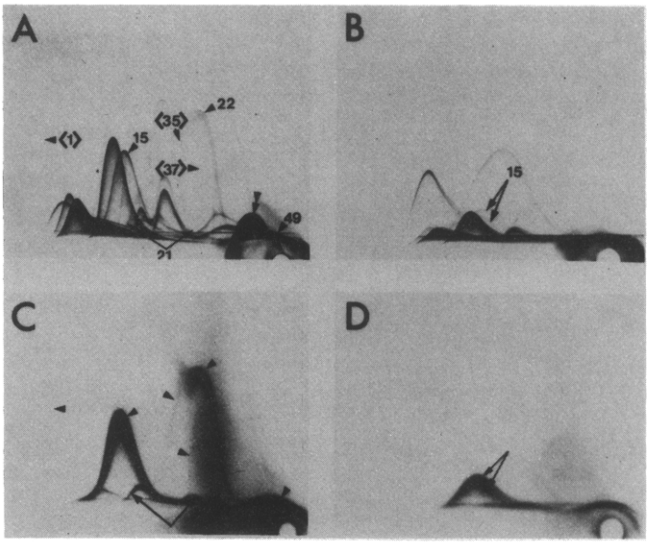

FIGURE 1: Resolution by CIE of the iron-containing antigens present in membrane vesicles prepared from *E. coli* ML 308-225. A first Triton X-100–EDTA extract (123 μg of protein; panel A) and a second Triton X-100–EDTA extract (57 μg of protein; panel B) of ^{59}Fe-labeled membrane vesicles were analyzed by CIE against antivesicle immunoglobulins (4.0 mg of protein per mL of gel). Panels C and D are the corresponding autoradiograms of gels displayed in panels A and B, respectively. Autoradiography was performed before the gels were stained with Coomassie brilliant blue. The salient radiolabeled immunoprecipitates in panels C and D are arrowed, and their identities in the full spectrum of immunoprecipitates are indicated in panels A and B. The double-headed arrow in panel A indicates antigen no. 47. Fences indicate that the immunoprecipitate in question is not readily observed in the protein-stained profile. Note the apparent splitting of immunoprecipitate no. 15 in panels B and D. The anode is to the left and top of the gels.

immunoprecipitates following staining with Coomassie brilliant blue (parts A and B of Figure 1) are qualitatively identical with those observed in earlier studies (Owen & Kaback, 1978, 1979a,b), and the reader is directed to these publications for a detailed analysis of the CIE profiles and for the numbering system used.

Autoradiography of dried immunoplates reveals that at least seven discrete immunoprecipitates contain bound iron (compare parts A and C of Figure 1). Additional iron-containing antigens were not resolved by manipulation of antigen loading over the range of 9–246 μg of protein or by analysis of the second Triton X-100 extract (Figure 1D). Six of the immunoprecipitates appear to contain the metal in the form of nonheme iron primarily, as judged by autoradiograms of immunoplates subjected to treatment with reagents that remove either nonheme iron or total iron (Figure 2). In contrast, immunoprecipitate no. 49 apparently contains ^{59}Fe in a form that has the extraction characteristics of heme iron. In all cases, the intensity and resolution of the immunoprecipitin profiles were unaffected by extraction, as demonstrated by subsequent staining with Coomassie brillant blue (data not shown). Thus, the reduction in peak intensity in the autoradiograms following extraction of nonheme or total iron is due solely to labilization of the isotope and not to dissociation or extraction of antigen–antibody complexes. In a practical context, it is relevant that procedures used routinely to stain CIE immunoplates for protein (Weeke, 1973) also cause labilization of ^{59}Fe (compare parts A and D of Figure 2). Therefore, it is essential that autoradiograms of the type described here are performed on dried but unstained immunoplates.

Comparison of autoradiograms and corresponding zymograms and/or protein-stained precipitin profiles demonstrates

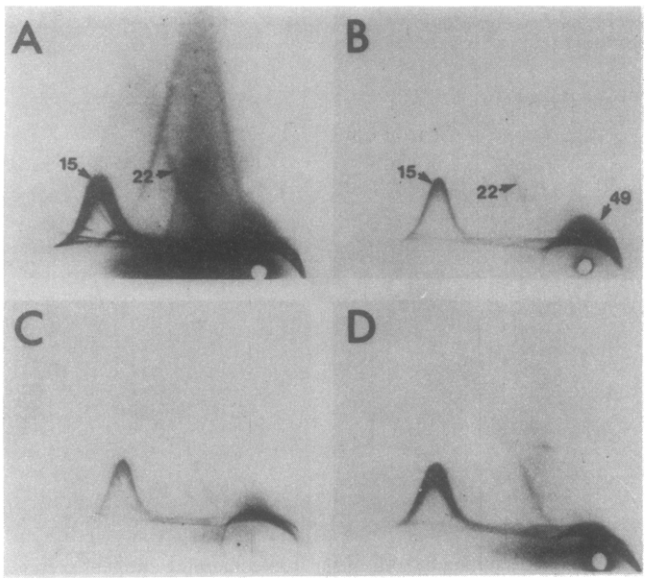

FIGURE 2: Extraction of ^{59}Fe from radiolabeled antigens. In all instances a Triton X-100–EDTA extract (95 μg of protein) of ^{59}Fe-labeled membrane vesicles was analyzed by CIE against antivesicle immunoglobulins (4.0 mg of protein per mL of gel). Individual immunoplates were then either treated with distilled water (panel A) or extracted for nonheme iron (panel B) or total iron (panel C) as detailed under Experimental Procedures. A separate immunoplate was treated with distilled water as in panel A and subsequently stained for protein in the usual way (panel D). Autoradiograms of all four gels are presented. The anode is to the left and top of all gels.

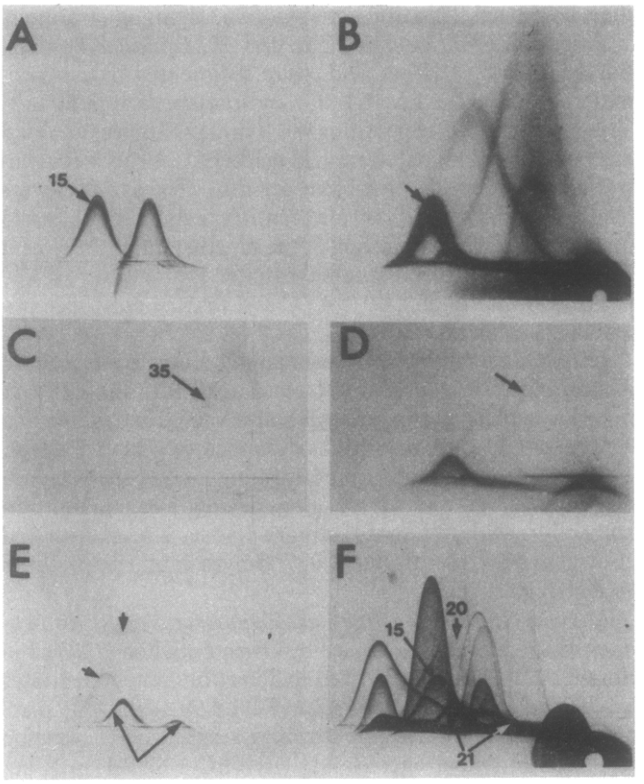

FIGURE 3: Identification of ^{59}Fe-labeled antigens. Triton X-100–EDTA extracts (95 μg of protein, panel A; 57 μg of protein, panel C) of ^{59}Fe-labeled membrane vesicles were analyzed by CIE against antivesicle immunoglobulins (4.0 mg of protein per mL of gel). Gels were subsequently stained for NADH dehydrogenase (panel A) or for glutamate dehydrogenase (panel C) as detailed under Experimental Procedures. Autoradiograms of these gels are shown in panels B and D, respectively. A similar Triton X-100–EDTA extract (80 μg of protein) of an unlabeled preparation of membrane vesicles was analyzed in an identical fashion against antivesicle immunoglobulins (4.0 mg of protein per mL of gel). One gel was stained for NADPH dehydrogenase activity (panel E), and the other was stained for NADPH dehydrogenase activity and further counterstained with Coomassie brilliant blue (panel F). The ^{59}Fe-labeled immunoprecipitates arrowed in autoradiograms B and D are identified by number in the corresponding zymograms (panels A and C, respectively). The identities of the main immunoprecipitates exhibiting NADPH dehydrogenase activity (panel E) are identified by number following counterstaining (panel F). The anode is to the left and top of all the gels.

clearly that the major iron-containing immunogen corresponds to the more acidic of the two NADH dehydrogenases detected in ML 308-225 membrane vesicles (parts A and C of Figure 1 and parts A and B of Figure 3) and that one of the minor antigens corresponds to glutamate dehydrogenase (antigen no. 35; parts C and D of Figure 3). Interestingly, on occasion, immunoprecipitate no. 15 (NADH dehydrogenase) appears as a doublet (Figure 1B). In these instances, both components of the doublet contain ^{59}Fe (Figure 1D).

Four of the five remaining iron-containing immunoprecipitates correspond to antigens no. 21, 22, 37, and 49 (parts A and B of Figure 1). Antigen no. 49 corresponds, in part at least, to Braun's lipoprotein (Owen & Kaback, 1979a). In addition, we have now been able to identify antigen no. 21. CIE of a Triton X-100–EDTA extract of unlabeled membrane vesicles, followed by zymogram staining, demonstrates that this immumoprecipitate (no. 21) exhibits NADPH dehydrogenase activity (parts E and F of Figure 3). The enzymatic activity is heterogeneous, and two antigenically related species contain both catalytic activity (parts E and F of Figure 3) and nonheme iron (Figure 1C and 2B). Two additional antigenically unrelated antigens also exhibit NADPH dehydrogenase activity (Figure 3E). One is the nonheme iron-containing NADH dehydrogenase (antigen no. 15), and the other probably corresponds to a minor immunogen (no. 20) in the CIE reference pattern (Owen & Kaback, 1978).

Finally, the most acidic iron-containing antigen (Figure 1C) cannot be detected as a protein-staining immunoprecipitate under normal loading conditions. Possibly, this immunogen corresponds to antigen no. 1 which is observed only at extremely high antibody concentrations (Owen & Kaback, 1978).

Discussion

It is apparent from the data presented here that CIE provides an interesting and useful technique for the analysis of certain iron-containing components in membrane vesicles from *E. coli*. Of the many vesicular antigens previously resolved by CIE (Owen & Kaback, 1978, 1979a), seven are now shown to contain bound iron. With the exception of antigen no. 49, which will be discussed later, all seem to contain primarily nonheme iron. However, the possibility cannot be excluded that residual ^{59}Fe associated with some immunoprecipitates (e.g., no. 15 and part of no. 22) following extraction with trichloroacetic acid represents small amounts of heme iron (compare Figures 2A–C). It is also relevant that only ~37% of the radioactivity is recovered in immunoplates following CIE, and it remains to be established whether the ^{59}Fe that is not amenable to analysis is in the form of specifically or nonspecifically bound isotope.

The major iron-containing immunogen in membrane vesicles from *E. coli* ML 308-225 is clearly antigen no. 15 which exhibits NADH and, to a lesser extent, NADPH dehydrogenase activity. As shown previously (Owen & Kaback, 1979a), this immunogen partitions almost exclusively with the membrane fraction of the cell in contrast to another antigenically unrelated NADH dehydrogenase (antigen no. 19/27)

that partitions equally between the membrane and the cytoplasm. Moreover, antigen no. 19/27 does not contain bound iron and can be further differentiated from antigen no. 15 by its inability to oxidize NADPH. NADH dehydrogenase from *E. coli* has been studied by several workers (Bragg, 1965; Bragg & Hou, 1967; Gutman et al., 1968; Hendler, 1971; Hendler & Burgess, 1974; Dancey & Shapiro, 1976; Dancey et al., 1976), but in no case has clear and unequivocal evidence been presented either for two distinct enzymes or for the presence of bound iron. Indeed, some of these studies on partially purified enzyme preparations are confusing and often contradictory. In retrospect, this may be due, in part at least, to the use of preparations containing differing proportions of the two immunologically distinct enzymes reported here.

The observations that antigen no. 15 contains iron and that it is expressed almost exclusively on the inner surface of the plasma membrane (Owen & Kaback, 1978, 1979a,b) suggest that it is the major NADH dehydrogenase associated with the respiratory chain of *E. coli*. Whether the ^{59}Fe is associated with the catalytic moiety alone and/or associated iron–sulfur proteins (and possibly cytochromes) is a debatable point [cf., for example, the multiplicity of iron–sulfur centers in the mitochondrial NADH–ubiquinone reductase (Hatefi & Stiggall, 1976)]. There is compelling evidence to suggest that some immunoprecipitates in CIE profiles contain complexes that may reflect in vivo association of membrane components (Owen & Smyth, 1977). The finding that antigen no. 15 is occasionally resolved as a doublet is not at variance with such a possibility.

Detection of an iron-containing antigen (no. 21) that exhibits NADPH (but not NADH) dehydrogenase activity is interesting since, to our knowledge, an enzyme exhibiting these properties has not been documented in *E. coli*, although it has been demonstrated in membranes from *Azotobacter vinelandii* (Ackrell et al., 1972). On the other hand, *E. coli* does have a well characterized NADPH-sulfite reductase (EC 1.8.1.2). Significantly, this complex hemoflavoprotein catalyzes the NADPH-dependent reduction of a variety of electron acceptors in addition to sulfite (Siegel et al., 1973, 1974). Thus, it is feasible that the activity detected for antigen no. 21 is a manifestation of the NADPH "diaphorase" activity associated with the flavoprotein subunits of the sulfite reductase complex (Hatefi & Stiggall, 1976).

An antigen exhibiting glutamate dehydrogenase activity also appears to contain nonheme iron. However, the NADP-linked enzyme from *E. coli* is not reported to be a metalloprotein (Sakamoto et al., 1975) nor are similar enzymes from other bacterial sources (LeJohn et al., 1968; Winnacker & Barker, 1970; Johnson & Westlake, 1972; Coulton & Kapoor, 1973). However, a non pyridine nucleotide dependent, membrane-bound glutamate dehydrogenase has been described in *A. vinelandii* (Jurtshuk & McManus, 1974). Thus, it remains to be established whether the immunoprecipitate detected here represents (a) a novel form of the enzyme, (b) a complex between glutamate dehydrogenase and an iron–sulfur protein, or (c) an unrelated enzyme such as D-amino-acid oxidase which as a broad substrate specificity and contains nonheme iron (Olsiewski et al., 1980).

The observation that antigen no. 49 contains bound iron is intriguing, as it has been demonstrated previously by two independent techniques (Owen & Kaback, 1979a) that this immunoprecipitate corresponds to Braun's lipoprotein, the most abundant and best characterized protein in the *E. coli* envelope. The lipoprotein has a molecular weight of 7200, it has been fully sequenced, and it serves, in part at least, to connect the outer membrane to the peptidoglycan layer (Braun, 1975; DiRienzo et al., 1978). Since there are no reports of bound iron in purified preparations of the molecule, it seems likely that the observation reflects an in vivo association of Braun's lipoprotein with other iron-containing components. The outer membrane of *E. coli* is known to contain receptors for iron chelates, notably iron enterochelin (Braun, 1978). Furthermore, these receptors are known to retain their ability to bind ferric enterochelin following solubilization in Triton X-100–EDTA (Hollifield & Neilands, 1978), and there is ample evidence for an in vivo association of the lipoprotein with certain other molecules of the "porin" type [reviewed in DiRienzo et al. (1978)].

The identity of the remaining iron-containing antigens is uncertain. Obvious candidates are succinate dehydrogenase and other iron–sulfur proteins (and possibly cytochromes) of the electron transfer chain (Hendler & Burgess, 1972, 1974). However, despite numerous attempts, we have been unable to detect an immunoprecipitate exhibiting succinate dehydrogenase activity by zymogram techniques.

Finally, it is curious that none of the iron-containing immunoprecipitates correspond unequivocally to cytochromes. In this regard, it is noteworthy that CIE studies carried out with purified cytochrome b_{562} fail to yield any immunoprecipitate whatsoever. Thus, the cytochromes, like the *lac* carrier protein (Owen & Kaback, 1979a), may be poor antigens because they are extensively buried in the hydrophobic core of the membrane.

Acknowledgments

We are indebted to Dr. Robert B. Gennis of the University of Illinois at Urbana—Champaign for supplying a sample of purified cytochrome b_{562}.

References

Ackrell, B. A. C., Erikson, S. K., & Jones, C. W. (1972) *Eur. J. Biochem. 26*, 387.
Alexander, A. G., & Kenny, G. E. (1977) *Infect. Immun. 15*, 313.
Alexander, A. G., & Kenny, G. E. (1978) *Infect. Immun. 20*, 861.
Bragg, P. D. (1965) *Biochim. Biophys. Acta 96*, 263.
Bragg, P. D., & Hou, C. (1967) *Arch. Biochem. Biphys. 119*, 202.
Braun, V. (1975) *Biochim. Biophys. Acta 415*, 335.
Braun, V. (1978) *Symp. Soc. Gen. Microbiol. 28*, 111.
Brumby, P. E., & Massey, V. (1967) *Methods Enzymol. 10*, 463.
Clegg, R. A., & Skyrme, J. E. (1973) *Biochem. J. 136*, 1029.
Coulton, J. W., & Kapoor, M. (1973) *Can. J. Microbiol. 19*, 439.
Dancey, G. F., & Shapiro, B. M. (1976) *J. Biol. Chem. 251*, 5921.
Dancey, G. F., Levine, A. E., & Shapiro, B. M. (1976) *J. Biol. Chem. 251*, 5911.
Davis, G. D., & Mingioli, E. S. (1950) *J. Bacteriol. 60*, 17.
DiRienzo, J. M., Nakamure, K., & Inouye, M. (1978) *Annu. Rev. Biochem. 47*, 481.
Dulley, J. R., & Grieve, P. A. (1975) *Anal. Biochem. 64*, 136.
Gutman, M., Schejter, A., & Avi-Dor, Y. (1968) *Biochim. Biophys. Acta 162*, 506.
Haddock, B. A., & Jones, C. W. (1977) *Bacteriol. Rev. 41*, 47.
Hatefi, Y., & Stiggall, D. L. (1976) *Enzymes, 3rd Ed. 13(C)*, 175.
Hendler, R. W. (1971) *J. Cell Biol. 51*, 664.

Hendler, R. W., & Burgess, A. H. (1972) *J. Cell Biol. 55*, 266.
Hendler, R. W., & Burgess, A. H. (1974) *Biochim. Biophys. Acta 357*, 215.
Hollifield, W. C., Jr., & Neilands, J. B. (1978) *Biochemistry 17*, 1922.
Johansson, K.-E., & Hjerten, S. (1974) *J. Mol. Biol. 86*, 341.
Johnson, W. M., & Westlake, W. S. (1972) *Can. J. Microbiol. 18*, 881.
Jurtshuk, P., & McManus, L. (1974) *Biochim. Biophys. Acta 368*, 158.
Kaback, H. R. (1971) *Methods Enzymol. 22*, 99.
LeJohn, H. B., Suzuki, J., & Wright, J. A. (1968) *J. Biol. Chem. 243*, 118.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265.
McLaughlin, J., & Meerovitch, E. (1975) *Can. J. Microbiol. 21*, 1635.
Olsiewski, P., Kaczorowski, G. J., & Walsh, C. T. (1980) *J. Biol. Chem.* (in press).
Orme-Johnson, W. H. (1973) *Annu. Rev. Biochem. 42*, 159.
Owen, P., & Salton, M. R. J. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 3711.
Owen, P., & Salton, M. R. J. (1977) *J. Bacteriol. 132*, 974.
Owen, P., & Smyth, C. J. (1977) in *Immunochemistry of Enzymes and Their Antibodies* (Salton, M. R. J., Ed.) p 147, Wiley, New York.
Owen, P., & Kaback, H. R. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3148.
Owen, P., & Kaback, H. R. (1979a) *Biochemistry 18*, 1413.
Owen, P., & Kaback, H. R. (1979b) *Biochemistry 18*, 1423.
Sakamoto, N., Kotre, A. M., & Savageau, M. A. (1975) *J. Bacteriol. 124*, 775.
Short, S. A., Kaback, H. R., & Kohn, L. D. (1975) *J. Biol. Chem. 250*, 4291.
Siegel, L. M., Murphy, M. J., & Kamin, H. (1973) *J. Biol. Chem. 248*, 251.
Siegel, L. M., Davis, P. S., & Kamin, H. (1974) *J. Biol. Chem. 249*, 1572.
Smyth, C. J., Friedman-Kien, A. E., & Salton, M. R. J. (1976) *Infect. Immun. 13*, 1273.
Smyth, C. J., Siegel, J., Salton, M. R. J., & Owen, P. (1978) *J. Bacteriol. 133*, 306.
Weeke, B. (1973) *Scand. J. Immunol.* 2 (*Suppl. 1*), 15.
Winnacker, E. L., & Barker, H. A. (1970) *Biochim. Biophys. Acta 212*, 225.