Immunochemical Localization of Nitrate Reductase in *Escherichia coli*

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The membrane-bound nitrate reductase from *Escherichia coli* functions as the terminal enzyme of the respiratory chain of the organism when it is grown anaerobically in the presence of nitrate. The enzyme consists of two types of polypeptide chain, α and β, of approx. mol.wts. 155,000 and 65,000 respectively (Enoch & Lester, 1975). In conjunction with cytochrome *b* 

it constitutes a proton-translocating segment of the respiratory chain which catalyses the transfer of reducing equivalents from the quinone pool to nitrate, the terminal electron acceptor (Garland et al., 1975). Therefore the establishment of the location of this enzyme with respect to the cytoplasmic and periplasmic faces of the cell membrane is an important step in the elucidation of the mechanism of respiratory-driven proton translocation. Previous work to establish the location of the enzyme by using lactoperoxidase-catalysed radioiodination, diazobenzene-[35S]sulphonate and dansylcadaverine labelling (Boxer & Clegg, 1975; MacGregor & Christopher, 1978) has demonstrated that the enzyme is present at the cytoplasmic face of the membrane. Here we report the result of similar studies by using fluorescently labelled specific antibodies.

Nitrate reductase used for raising antisera in rabbits was purified by a modification of the procedure of Enoch & Lester (1975). In contrast with the findings of these authors, but in agreement with De Moss (1977), the preparation did not contain any cytochrome. The enzyme had a specific activity of 67 pmol of nitrate/min per mg of protein, measured by using reduced Benzyl Viologen as electron donor, and sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the preparation revealed only α- and β-subunits.

The immunoglobulin fraction of the antiserum obtained gave one precipitin line on double diffusion analysis of Triton X-100-solubilized membrane fractions, which showed complete identity with that of the purified enzyme. This immunoglobulin fraction cross-reacted with both α- and β-subunits as demonstrated by two-dimensional immunoelectrophoresis (Converse & Papermaster, 1975). The independence of the precipitin arcs obtained for the subunits shows that they do not share common determinants and are therefore distinct polypeptide chains.

Spheroplasts (periplasmic face) and sonicated membrane vesicles (periplasmic and cytoplasmic faces) were exposed to the immunoglobulin fraction and binding was detected by using an indirect method employing fluorescein isothiocyanate-coupled goat anti-(rabbit immunoglobulin).

We could not demonstrate any antigenic determinants on the surface of spheroplasts (Table 1), but the membrane vesicles strongly reacted with anti-(nitrate reductase) anti-
Table 1. Immunological location of E. coli nitrate reductase

Antigens were injected subcutaneously in Freund's complete adjuvant into separate rabbits previously bled to obtain pre-immune serum. The immunoglobulins from each antiserum were then purified by (NH₄)₂SO₄ precipitation and DEAE-cellulose chromatography. Spheroplasts, each giving identical results, were prepared by the method of Garland et al. (1975) or Osborn et al. (1972) and their integrity was monitored by β-galactosidase assay. Sonicated membrane vesicles were prepared from spheroplasts (Boxer & Clegg, 1975). Nucleopore membrane filters (0.4 μm pore size for spheroplasts, 0.1 μm for vesicles) were used for washing osmotically sensitive spheroplasts. This decreased breakage and facilitated observation in the fluorescence microscope. Spheroplasts or membrane vesicles (50 μl of approx. 0.1 mg of protein/ml) were collected on to the appropriate filter and washed with 10 ml of iso-osmotic buffer. Specific rabbit immunoglobulins (100 μl of approx. 3 mg of protein/ml in iso-osmotic buffer) were placed on the filter, incubated with fluorescein-coupled goat anti-(rabbit immunoglobulin). After extensive washing the filter was viewed in a phase-contrast microscope to confirm recovery of material and then examined with a fluorescence microscope. Staining was scored — if no fluorescence and + if fluorescence was observed. No staining was detectable by using only the fluorescein-coupled goat immunoglobulins.

<table>
<thead>
<tr>
<th>Immunoglobulin fraction</th>
<th>Spheroplasts</th>
<th>Membrane vesicles</th>
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</thead>
<tbody>
<tr>
<td>Anti-nitrate reductase</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>Anti-α subunit</td>
<td>—</td>
<td>+</td>
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<tr>
<td>Pre-immune</td>
<td>—</td>
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body. These results were reinforced by the experiments using immunoglobulin specific for only the α-subunit (Table 1). Homogeneous α-subunit was prepared by gel filtration, on Sepharose CL-6B, of nitrate reductase in the presence of sodium dodecyl sulphate. The immunoglobulin fraction of the antiserum raised produced a precipitin arc with native nitrate reductase and did not cross-react with β-subunit of the enzyme. These results support the conclusions of previous work (Boxer & Clegg, 1975; MacGregor & Christopher, 1978) by demonstrating that the α-subunit is located at the cytoplasmic face of the membrane and by failing to locate either subunit at the periplasmic face. Our results do not allow us to conclude whether or not the β-subunit is present at the cytoplasmic face.

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Effects of Activation Agents on Uridine Diphosphate Glucuronosyltransferase Activity towards Acceptor Substrates of Different Octanol/Buffer Partition Coefficients

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Illing & Benford (1976) have shown that the activity in rat liver homogenate for a series of acceptor substrates of UDP-glucuronosyltransferase (EC 2.4.1.17) could be related to their octanol/buffer partition coefficients (a measure of the lipid solubility of these