The Nitrate Respiration Complex of *Escherichia coli*: Subunit Composition

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The presence of nitrate in the growth medium of *Escherichia coli* causes the production of a multi-enzyme system capable of mediating electron transport between the existing respiratory chain and nitrate, resulting in its reduction to nitrite [for a review see Payne (1973)]. The point of interaction with the NADH-oxygen respiratory chain is probably at the level of ubiquinone (Enoch & Lester, 1974). This nitrate respiration system consists minimally of nitrate reductase (EC 1.7.99.4) and a b-type cytochrome (Ruiz-Herrera & DeMoss, 1969). These components are located in the membrane fraction of *E. coli* and their activity is regulated by the concentration of oxygen in the growth medium (Showe & DeMoss, 1968). The present communication describes the identification of the protein components of this nitrate respiration system by three independent methods.

The experiment illustrated in Fig. 1 indicates the relative electrophoretic mobilities in sodium dodecyl sulphate–polyacrylamide gels of inner-membrane subunits of *E. coli* whose synthesis (or incorporation into the membrane) is induced by nitrate (the term 'induced' is used throughout to include both classical induction and de-repression). Several similar experiments have been performed with different pairs of growth conditions whose common feature, between one experiment and the next, is the presence of nitrate in one-half of each pair and its absence from the other. Inspection of the data

![Mobility relative to Bromophenol Blue marker dye](image)

**Fig. 1. Nitrate-induced protein subunits of the inner membrane of E. coli**

Two minimal-medium anaerobic cultures of *E. coli* K12 were grown to equal cell densities in mid-exponential phase. One contained [14C]valine, glycerol and fumarate and the other [3H]valine, glycerol, fumarate and nitrate. Cells were harvested and mixed; an inner-membrane fraction was prepared by the method of Osborn *et al.* (1972). A sample was subjected to electrophoresis in a 7.5% polyacrylamide gel containing sodium dodecyl sulphate. The gel was sliced and counted for radioactivity. Peaks in the above ratio plot indicate nitrate-induced components. Methods were as described by Clegg & Skyrme (1973). The 3H/14C ratio of the unfractionated membrane preparation was 13.
Fig. 2. Subunits of the nitrate respiration complex

Profiles of sodium dodecyl sulphate–polyacrylamide-gel-electrophoretic separations (7.5 % (w/v) polyacrylamide gels) are shown. (a) Immunoprecipitate quantified as $^3$H c.p.m.; the starting material was the inner-membrane preparation described in the legend to Fig. 1. No $^{14}$C counts were detectable in this gel. (b) Haem-containing nitrate reductase fractions from the preparative procedure; densitometric scan of a Coomassie-Blue-stained gel. Methods were as in Fig. 1. The mobility and $E_{546}$ are given relative to Bromophenol Blue and the nitrate reductase $\alpha$ peak respectively.

plotted as in Fig. 1, from all these experiments allows selection of those features common to them all and therefore most likely to be due specifically to nitrate rather than non-specifically to, for example, catabolite repression, anaerobiosis or intracellular oxido-reduction potential. These common features are indicated by numbered arrows in Fig. 1. Bands 1 and 4 have the same relative mobilities as the subunits $\alpha$ and $\beta_1 + \beta_2$ of purified nitrate reductase (Clegg, 1975). Fig. 2(a) shows the relative mobilities of subunits precipitated from a Triton X-100-dispersed inner-membrane preparation of *E. coli* by antiserum against purified nitrate reductase (R. A. Clegg, unpublished work). Again the nitrate reductase subunits are present and again other nitrate-induced components occur, having the same relative mobilities as those in Fig. 1. One of these (no. 7,
apparent mol.wt 24000) occurs, with components 4 and 1, in molar proportions 1:1:1. Spectrophotometric analysis of a larger-scale immunoprecipitate indicated the presence in it of cytochrome b (which must be cytochrome b$^{3+}$ because of the nitrate-specific nature of all immunoprecipitated components) in quantities relative to the precipitated nitrate reductase activity such that only band 7 is sufficiently concentrated in the immunoprecipitate to be the haem-bearing polypeptide. This identification assumes that not more than one protohaem is associated with a single polypeptide. Enoch & Lester (1974) have reported the preparation of a haem-containing nitrate reductase from E. coli containing, in addition to bands probably corresponding to α and β, a subunit of apparent mol.wt. 19000 which is lost from preparations devoid of haem. This is probably the same component as band 7 described here.

The preparative procedure yielding the haem-free nitrate reductase whose properties are summarized in the following report (Clegg, 1975) also gives rise to variable quantities of a haem-containing nitrate reductase which was discarded from early preparations as being impure. Its subunit composition, however, shown in Fig. 2(b) is identical with that of the immunoprecipitate: the enzymic properties of this preparation are under investigation.

Kemp et al. (1975) have presented data suggesting that the haem polypeptide of cytochrome b$^{3+}$ is not synthesized by a haem-requiring E. coli mutant growing anaerobically with nitrate under haem deficiency. The work described here establishes a situation where their suggestion can be subjected to direct experimental test and where the assembly of the nitrate respiration complex both in wild-type and chlorate-resistant E. coli mutants can be studied at a molecular level.

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The Size of Nitrate Reductase in Escherichia coli

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The presence of nitrate in the anaerobic growth medium of Escherichia coli K12 causes the expression of a respiratory nitrate reductase activity not found in aerobically grown E. coli [for a review see Payne (1973)].

Nitrate reductase (EC 1.7.99.4), assayed by the use of reduced Viologen dyes as reductants, has been purified to various degrees from E. coli, Klebsiella aerogenes and other micro-organisms. Some of the preparations from E. coli [those of Enoch & Lester (1974) and Taniguchi & Itagaki (1960)] contain a b-type cytochrome, although this is not necessary for catalytic activity assayed as above. There is disagreement over the size of the metalloprotein catalysing reduced Viologen–nitrate oxidoreductase activity (Taniguchi & Itagaki, 1960; Showe & DeMoss, 1968; Forget, 1974; MacGregor et al., 1974). The cytochrome-free enzyme of E. coli is reported to contain two subunits of mol.wt. 142000 and 58000 (MacGregor et al., 1974) or 155000 and 63000 (Enoch &