The membrane location of the $\beta$-subunit of nitrate reductase from *Escherichia coli*

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Nitrate reductase from *Escherichia coli* is a membrane-bound enzyme that consists of two subunits, $\alpha$ and $\beta$ ($M_r$, 150,000 and 59,000 respectively). After purification of the enzyme the $\beta$-subunit is often heterogeneous (Clegg, 1976; MacGregor, 1975) owing to the action of endogenous proteinases. Endogenous proteinases have also been shown to be responsible for the release of the enzyme from the membrane (MacGregor, 1975). DeMoss (1977) has shown that when the isolated enzyme is treated with trypsin it undergoes limited proteolysis to $\alpha$-subunit and a 43,000-mol.wt. fragment of the $\beta$-subunit (termed $\beta'$. This new homogeneous form of the enzyme ($\alpha\beta'$) is non-self-associating (unlike the $\alpha\beta$-form; see Clegg, 1976), and it appears that the removed fragment is responsible for the association properties of the solubilized enzyme. This fragment may be involved in binding of the enzyme to the membrane.

The effect of trypsin treatment on the structure and release of membrane-bound nitrate reductase was explored. E. coli strain EMG29 was grown on inorganic $^{35}$S-sulphate to label protein, and membrane vesicles were prepared (Giordano et al., 1978). $^{35}$S-labelled membrane vesicles (2.5 mg of protein) were suspended in the absence or in the presence of trypsin (2.5 mg) at 37°C for 1 h, then centrifuged at 250,000 g for 2 h at 4°C. The membrane pellets (unreleased enzyme) and supernatant fractions (released enzyme) were assayed for enzyme activity. Trypsin treatment of membrane vesicles did not release a significant quantity of nitrate reductase from the membrane (9.3% released, compared with 7.9% released in the absence of trypsin).

The nitrate reductase was solubilized from the membrane pellets by 2% (w/v) Triton X-100, and the detergent-released enzyme that remained membrane-bound after the trypsin treatment was also cleaved to the $\beta'$-fragment. The corresponding fractions obtained from the membrane vesicles not exposed to trypsin contained unmodified enzyme. Trypsin treatment therefore cleaves the $\beta$-subunit of the enzyme when membrane-bound, but this alone does not release the enzyme from the membrane.

The location of the $\alpha$-subunit has been reported by several authors (Boxer & Clegg, 1975; Graham & Boxer, 1978; MacGregor & Christopher, 1978), and it has been found to be located exclusively at the cytoplasmic face of the membrane. The location of the $\beta$-subunit has not been established. Since the $\beta$-subunit in the membrane-bound enzyme can be partially digested by trypsin treatment, we attempted to locate this subunit with respect to the cytoplasmic and periplasmic faces of the membrane.

Spheroplasts (right-side-out) and membrane vesicles (predominantly inside-out), from cells that were grown on inorganic $^{35}$S-sulphate to label protein, were incubated together in the absence or in the presence of trypsin (Fig. 1). The nitrate reductase immunoprecipitated from spheroplasts incubated in the absence or in the presence of trypsin consisted of $\alpha$- and $\beta$-subunits. The nitrate reductase from membrane vesicles incubated in the absence of trypsin consisted of $\alpha$- and $\beta$-subunits, whereas that from vesicles incubated in the presence of trypsin consisted of $\alpha$-subunit and $\beta'$-fragment. The $\beta'$-fragment has the same molecular weight ($M_r$, 43,000) as that obtained on trypsic digestion of native isolated nitrate reductase.

We therefore conclude that the $\beta$-subunit is accessible at the cytoplasmic face of the membrane.

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![Fig. 1. Dodecyl sulphate/polyacrylamide-gel electrophoresis of trypsin-treated membrane-bound nitrate reductase](image)

Spheroplasts (7.5 mg of protein) were mixed with membrane vesicles (2.4 mg of protein), then treated with trypsin (2.5 mg) or buffer. After 1 h at 37°C, the reaction was terminated and the preparations were separated. Nitrate reductase was recovered by immuno-precipitation. (a) Spheroplasts incubated in the presence (●) or in the absence (○) of trypsin; (b) membrane vesicles incubated in the presence (●) or in the absence (○) of trypsin.

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