figure of 4000 units/g given for rat skeletal muscle[6]. Recalculation of the total phosphorylase content from data on enzyme activity in the cardiac tissue extracts, presented in two of the publications just cited[8,10], yields values of 408 and 675 Cori units/g for dog ventricular muscle and whole rat heart, respectively, in excellent agreement with values obtained in the present and other experiments performed in this laboratory. It would be surprising indeed to find cardiac muscle to be richer in phosphorylase than skeletal muscle, since it is comparatively poorly equipped with other glycolytic enzymes. Moreover (see ref. 11), its glycogenolytic power *in vivo* does not compare to that of skeletal muscle.

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1 M. KLARWEIN, W. LAMPRECHT AND E. LOHMANN, *Z. Physiol. Chem.*, 328 (1962) 41.
2 A. WOLLENBERGER, O. RISTAU AND G. SCHOFFA, *Pflügers Arch.*, 270, (1960) 399.
3 N. HAUGAARD, M. E. HESS, J. SHANFIELD, G. INESI AND W. R. KUKOVETZ, *J. Pharmacol. Exptl. Therap.*, 131 (1961) 137.
4 G. T. CORI AND B. ILLINGWORTH, *Biochim. Biophys. Acta*, 21 (1956) 105.
5 C. H. FISKE AND Y. SUBBAROW, *J. Biol. Chem.*, 66 (1925) 375.
6 S. E. MAYER AND N. C. MORAN, *J. Pharmacol. Exptl. Therap.*, 129 (1960) 271.
7 H. J. HOHORST, F. H. KREUTZ AND T. BÜCHER, *Biochem. Z.*, 332 (1959) 18.
8 B. E. WAHLER AND A. WOLLENBERGER, *Biochem. Z.*, 329 (1958) 508.
9 D. H. BROWN AND C. F. CORI, in P. D. BOYER, H. LARDY AND K. MYRBÄCK, *The Enzymes*, Vol. 5, Academic Press, New York and London, 1961, 2nd Ed., p. 207.
10 W. R. KUKOVETZ, M. E. HESS, J. SHANFIELD AND N. HAUGAARD, *J. Pharmacol. Exptl. Therap.*, 127 (1959) 122.
11 A. WOLLENBERGER, *Acta Biol. Med. Germ.*, Suppl. I, (1961) 135.

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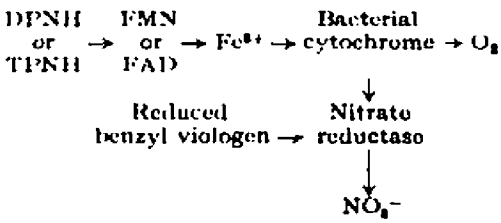
PN 10025

The purification of nitrate reductase of *Achromobacter fischeri*

The purification and properties of the nitrate reductase (cytochrome: nitrate oxidoreductase, EC 1.9.6.1) of *Achromobacter fischeri* have been described in an earlier communication[1]. Evidence was presented to show that the sequence of electron transport for nitrate reduction in *A. fischeri* is as follows:



The purified enzyme so obtained was strongly colored and showed absorption bands at 550 mμ, 520 mμ and 419 mμ in the reduced state. Although a certain amount of indirect evidence (inhibitor studies) was obtained which indicated that the terminal nitrate reductase from *A. fischeri* does not contain iron porphyrin, it was

not certain whether the observed changes characteristic of a cytochrome-like component were due to nitrate reductase or to an associated impurity.

The present communication describes a purification procedure by which the specific activity of the enzyme was increased 2.5-fold to a value of 210 μmoles NO_2 formed/mg protein/h. The absorption bands that were observed earlier have now been shown to be due to cytochrome impurities.

The purification of the enzyme was carried out as follows: Nitrate reductase was extracted from the water-lysed cells as described earlier[1]. The enzyme was precipitated by adjusting the pH to 4.5 with 0.2 M acetic acid and the resulting precipitate collected by centrifugation. The precipitate was redissolved in about one-third of the original volume of 0.05 M potassium phosphate (pH 6.2) and protamine sulfate solution (pH 5.0) added to remove most of the nucleic acids. The precipitate was centrifuged off and discarded. The enzyme was then adsorbed on calcium phosphate gel at pH 6.0, eluted by 0.4 M potassium phosphate (pH 8.4), and the combined eluates brought to 0.65 saturation with solid ammonium sulfate. The pH of the solution was adjusted to 7.0 and the precipitate collected by centrifugation. The precipitate was dissolved in 4 ml of 0.05 M potassium phosphate (pH 6.4) and dialyzed overnight against buffer of the same composition. The dialyzed enzyme solution was then passed through a 20 × 1.5 cm column of the cation-exchange resin Amberlite IRC-50 (NH_4^+-form) which was previously treated according to MARGOLIASH[2]. The enzyme was adsorbed on the column and was eluted by washing the column with 0.25 M potassium phosphate (pH 7.5). The active fractions were combined and dialyzed immediately against 0.01 M Tris (pH 7.4), with 2 to 3 changes of the buffer. The dialyzed enzyme was chromatographed on a 20 × 1 cm column of DEAE-cellulose by continuous gradient elution with Tris (pH 7.4), with gradual increase in buffer strength over the range 0.01–0.5 M. The active fractions were pooled, dialyzed against 0.1 M potassium phosphate (pH 7.0) and precipitated with saturated ammonium sulfate solution, neutralized to pH 7.0. At this stage, the ratio of light absorption at 280 mμ to that at 260 mμ was 1.35 suggesting the presence of other non-protein ultraviolet-absorbing material, but it is not known whether this constitutes a contaminant or forms part of the enzyme. The enzyme was relatively unstable after adsorption on Amberlite and generally lost about 20% of its activity on storage at 4° for 24 h.

The purified enzyme had no activity towards DPNH or TPNH, with O_2, dyestuff or ferricytochrome *c* as acceptor. Treatment of the enzyme with Nuchar completely inactivated the enzyme and the activity of the Nuchar-treated enzyme could not be restored by the addition of FMN, FAD or boiled bacterial extract.

The absorption spectrum of the enzyme as isolated is shown in Fig. 1. The purified enzyme preparation had no specific absorption peak except that of protein in the vicinity of 280 mμ and the absorption decreased gradually over the entire near ultraviolet and visible region with increasing wavelength indicating none of the characteristics of a haem enzyme.

The spectrum of the purified enzyme reduced with dithionite did not show any change indicative of a flavin band in the enzyme. The enzyme showed no stimulation in activity by any of the following (singly or in combination): Mn^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, molybdenum (as molybdenum trioxide or ammonium molybdate), FMN or FAD.

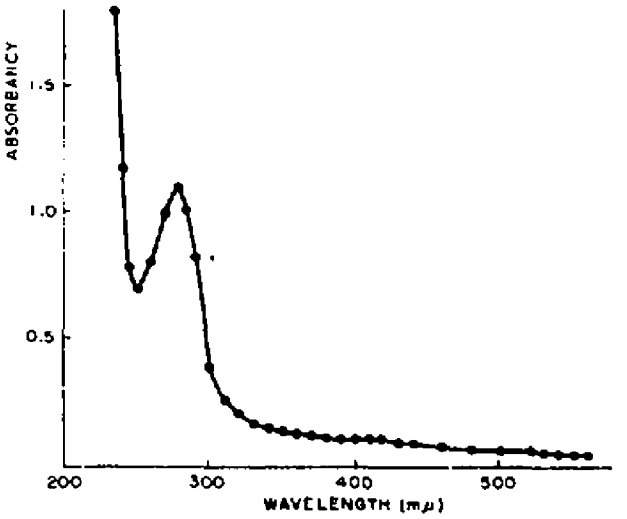


Fig. 1. Absorption spectrum of purified nitrate reductase from *A. fischeri*.

Ultracentrifugal studies indicated the presence of at least two components, a slow moving fraction with an $s_{20,w}$ value of about 1.5 and a faster-moving component with an $s_{20,w}$ value of about 4.5. Though it was not determined which of the two components represents the enzyme, these results indicate that nitrate reductase from *A. fischeri* is a much smaller molecule than the enzyme from *Escherichia coli*[3] which is reported to have a $s_{20,w}$ value of 25 corresponding to a molecular weight of about 1 000 000.

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[1] J. C. SADANA AND W. D. MCELROY, *Arch. Biochem. Biophys.*, 67 (1957) 16.
[2] E. MARGOLIASH, *Biochem. J.*, 56 (1954) 529.
[3] S. TANIGUCHI AND E. ITAGAKI, *Biochim. Biophys. Acta*, 44 (1960) 263.

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γ, δ-Dioxovalerate aminotransferase activity in *Rhodopseudomonas spheroides*

It was previously reported[1] that L-α-alanine-γ,δ-dioxovalerate aminotransferase (transaminase) activity is present in *Rhodopseudomonas spheroides* extracts, and that in contrast to an enzyme detected in mammalian tissues[2–4], L-α-alanine and β-alanine are from 5–7 times more effective than L-glutamic acid. Transamination