Short Communications

Spectrographic determination of molybdenum in the nitrate reductase from *Escherichia coli*

EGAMI *et al.*[1] have separated a nitrate reductase from *E. coli* cells and studied its properties. Some of the results on the solubilization and purification of this enzyme are presented by TANIGUCHI AND ITAGAKI elsewhere. The present authors have collaborated with them in the spectrographic analysis of the metals present in the nitrate reductase and the results obtained are briefly described in this communication.

Qualitative spectrographic analysis of the nitrate reductase samples showed the presence of molybdenum and iron. Molybdenum was determined spectrographically and iron colorimetrically.

For the spectrographic determination of molybdenum in silicate materials, several authors have found that calcium phosphate, alumina and sodium carbonate enhanced the line intensity of molybdenum[2,3], and vanadium and palladium were suitable as internal standards[4,5]. After examination of various substances as base material for the analysis of molybdenum in biological materials, we have found NaCl to be the best, with palladium and vanadium as suitable internal standards[6].

Standard samples of molybdenum containing 5, 10, 26, 52, 104 and 205 p.p.m. MoO_3 which corresponded to 3, 7, 18, 35, 69 and 137 p.p.m. Mo were prepared by thoroughly mixing pure MoO_3 and graphite powder in an agate mortar. These mixtures were then mixed with NaCl and graphite powder containing 50 p.p.m. V and 50 p.p.m. Pd in the ratio of 1:1:1. 8 mg of the standard sample was placed in the hole of the cathode (Fig. 1). A medium quartz spectrograph was used under the following experimental conditions: excitation, D.C. 180 V; cathode layer method; exposure, the current was held at 3, 5 and 7 A. for 30 sec respectively and finally at 9 A until the sample was burnt off; Fuji spectroscopic process plate.

The line pairs selected were Mo 3170.35-V 3185.40 Å and Mo 3170.35-Pd 3442.70 Å. Linearity holds for the former pair in the range 7–140 p.p.m. Mo and for the latter in the range of 35–140 p.p.m. Mo. Therefore vanadium was used as the internal standard and the molybdenum content was calculated by using the working curve shown in Fig. 2.

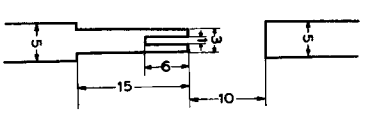

Fig. 1. Electrodes used for the spectroscopic analysis, with cathode below. The dimensions are mm.

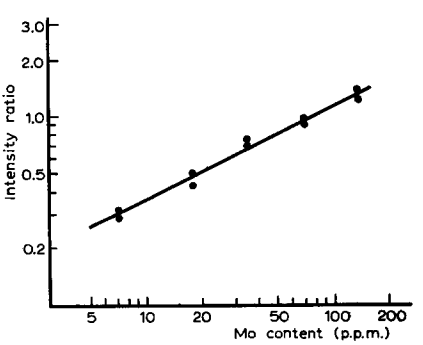

Fig. 2. Standard curve for the spectrographic analysis of molybdenum in the nitrate reductase.

The nitrate reductase separated from *E. coli* was decomposed by fuming HNO_3 and 30 % H_2O_2 and the residue was mixed with equal amount of graphite powder as described by TANIGUCHI AND ITAGAKI[7]. NaCl and the graphite powder containing 50 p.p.m. V and 50 p.p.m. Pd were then added in the ratio of 1:1:1.

The inactive protein which was separated at an intermediate step of the purification of the nitrate reductase was used as the control material. It was mixed with NaCl and graphite in the same way as the nitrate reductase sample, and other experimental conditions were also the same. The inactive protein contained 11 p.p.m. Mo and this value was used as a correction to the molybdenum content of the nitrate reductase. A dialysate of the nitrate reductase and the inactive protein also contained 11 p.p.m. molybdenum.

Two samples of the nitrate reductase were found to contain 87 p.p.m. and 108 p.p.m. Mo (corrected values). The reproducibility of the spectrographic analysis, measured by analysing repeatedly the inactive protein, was $\pm$ 11 % for a molybdenum content of about 11 p.p.m.

A step-sector method was also used to determine molybdenum in the nitrate reductase. In this case both the standard and the nitrate reductase samples were mixed with NaCl in the ratio of 1:1 and excited by the cathode-layer method. A sector with seven steps was placed in front of the slit of the spectrograph and the lengths of the molybdenum lines 3170.35 Å and 3193.97 Å were plotted against the molybdenum contents. A sample of the nitrate reductase was found to contain 96 $\pm$ 20 p.p.m. Mo (corrected value) by this method.

Thus the molybdenum contents of three samples of the nitrate reductase were 87, 96 and 108 p.p.m. which corresponds to almost one atom Mo for one molecule of the nitrate reductase.

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[1] F. EGAMI, M. ISHIMOTO AND S. TANIGUCHI, *Proceedings of the Symposium on Haematin Enzymes, Canberra*, 1959, in the press.
[2] R. O. SCOTT, *J. Soc. Chem. Ind.*, 64 (1945) 189.
[3] R. L. MITCHELL, *Commonwealth Bureau of Soil Science, Technical Communication*, 44 (1948) 88.
[4] L. H. AHRENS, *Quantitative Spectrochemical Analysis of Silicates*, Pergamon Press, London, 1954.
[5] T. M. MOROSHKINA AND V. K. PROKOFEV, *Zhur. Anal. Khim.*, 11 (1956) 714; C.A. 51 (1957) 7941f.
[6] C. IIDA, to be published.
[7] S. TANIGUCHI AND E. ITAGAKI, *Biochim. Biophys. Acta*, 44 (1960) 263.

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