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Anomalous behaviour of catalase on agarose gel*

Anomalies have been observed in the elution patterns for catalase (E.C. 1.11.1.6) chromatographed on agarose gel.

Experimental

The catalase used was crystalline material from beef liver (batches CTR-8EA, CTR-9AA, Worthington Biochemical Corp.).

The agarose used was Bio-gel A-0.5 m (Bio-Rad Laboratories, batches 4781 and 5206), which was packed in glass columns 0.9 cm (I.D.) × 50 cm. Sephadex G-200 (Pharmacia Ltd.) was used for chromatography on dextran gels. The buffers were prepared from citric acid and sodium citrate of analytical-reagent grade (Anachemia Ltd.) in doubly distilled water. Chromatographic fractions were collected on a drop-counting apparatus at flow-rates of 10–15 ml/h.

Catalase activity was assayed by the procedure of BEERS AND SIZER[1] using a Beckman DB spectrophotometer and recorder. Protein content was measured using the conventional method of FOLIN AND LOWRY.

Results and discussion

Catalase was purified by chromatography on Sephadex G-200 equilibrated with citrate buffer at pH 6.0, $I = 0.2\ M$. A single active peak of specific activity 160 000 units per mg was eluted that contained 10% of the applied protein and 80% of the applied activity, and had an elution volume (V_e) such that a molecular weight of 260,000 was calculated (*cf.* ref. 2).

The purified catalase was applied to columns of agarose equilibrated with the citrate buffer system used in this work ($I = 0.2\ M$, pH = 6.0). A representative elution pattern of the enzyme, which was recovered in yields ranging from 25 to 35%, is shown in Fig. 1a. A single major peak with $V_e = V_0$ was obtained from each buffer used, though there was suggestion of the presence of enzyme of low molecular weight in "trailing" of the peak in a few experiments. It is apparent that the enzyme purified on Sephadex G-200 is eluted anomalously from agarose.

Experiments with agarose columns such as those described above were repeated with buffer containing dithiothreitol ($10^{-2}\ M$). This reducing agent was added to eliminate oxidized species[3,4] and obviate oxidation during chromotography. A single, symmetrical, catalytically active peak was obtained (Fig. 1b) in 65% yield, with V_e such that a molecular weight of 160,000 was estimated[5]. Chymotrypsin (Worthington, three times crystallized) when chromatographed on the same column eluted with a V_e value such that the molecular weight calculated[5] was 25,000; bovine γ-globulin (Sigma) was chromatographed within a broad elution peak of the expected[5,6] weights (150,000–170,000). The rapidly-eluting ($V_e = V_0$) material of Fig. 1 must have arisen from oxidative dimerization of the enzyme[3,4].

Even in the reducing medium used in this work, catalase behaved anomalously on agarose, eluting with a low apparent molecular weight of 160,000. This is com-

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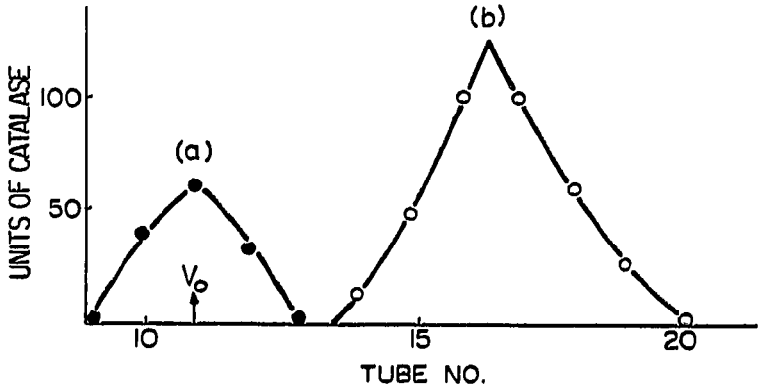

Fig. 1. Elution of purified catalase on agarose (a) in oxidizing atmosphere; (b) in presence of dithiothreitol. Elution medium: citrate buffer, pH 6.0, $I = 0.2$ M. V_0 estimated with Dextran Blue 2000 (Pharmacia).

parable with that obtained during chromatography on agarose equilibrated with buffers of alkaline pH (ref. 6).

An explanation of the delayed elution of catalase, even in the presence of dithiothreitol, may follow from the nature of the agarose gel-filtration packing. The hydrogen bonding on which the gel depends for structural rigidity may so affect the hydroxyl groups of the carbohydrate that they present, to catalase, a pseudo-substrate to which it is bound with sufficient strength to arrest its elution. This explanation is analogous to that offered for the retention of lysozyme on dextran gels[7]. The binding of catalase to the agarose may require such deformation of the enzyme that it becomes extremely susceptible to oxidation so causing the low yields reported above for experiments in an oxidizing medium.

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