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A Study of Yeast Catalase

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The enzyme catalase has been prepared in crystalline form from a number of animal sources and from the bacterium *Micrococcus lysodeikticus* (Herbert and Pinsent [1]); and a highly purified preparation from spinach leaf has recently been described (Galston, Bonnichsen and Arnon [2]). Little is known about the nature of catalase from sources other than the above due to the very low concentrations of the enzyme in most cells and tissues. Von Euler, Fink and Hellström [3] have demonstrated the presence of catalase activity in yeasts and have shown that the catalase and cytochrome-c contents vary in parallel.

The present paper describes the purification, and a study of the properties of catalase obtained from yeast cells.

EXPERIMENTAL

A. Estimation

Assays of catalase activity were made by the rapid titration method of Bonnichsen, Chance and Theorell[4], and monomolecular k values calculated from the titration data according to the equation:

$$k = \frac{1}{t} \log_{10} \frac{x_0}{x_t}$$

where x_0 is the titre at zero time and x_t is the titre t minutes after the reaction has begun. The Kat.f. value of von Euler and Josephson [5] was used to express the purity of samples where

$$\text{Kat.f.} = \frac{k}{\text{g enzyme used in test}}$$

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As solutions of the partially purified material appeared to be unstable and to lose a considerable amount of activity during dialysis, the k value was measured before dialysing the sample free of salt to estimate the dry weight, and protein determinations were made before and after dialysis to observe the dilution factor during this process. The dry weight per ml of the sample after dialysis for 24 hours against a total of 4 litres of distilled water was measured after drying a suitable volume on a tared watch-glass at 105° C for 12 hours. The protein determinations were carried out by adding appropriate amounts of the solution to 1 ml of 0.6 M ammonium sulphate, adding distilled water to 9.5 ml and 0.5 ml of 1.5 M trichloroacetic acid, then shaking and reading optical density at 350 mμ within a few minutes.

Observations were carried out on sufficient diluted, dialysed samples to give a standard curve for the sample. The weight of the preparation used in the Kat.f. determination was then calculated from the dry weight observation and the dialysis dilution factor.

B. Extraction

Preliminary experiments were carried out to find the most advantageous method of extracting catalase from yeast cells. Extracting air-dried or acetone-ether dried baker's yeast with two volumes of aqueous $M/15$ secondary sodium phosphate or $M/15$ sodium acetate gave extracts containing approximately 70 g of solids per kilogram of dried yeast, with Kat.f. values between 6 and 7. Preliminary extraction of the yeast with n-butanol did not appreciably affect this result. Autolysis under toluene and extraction with water according to Meyerhof [6] resulted in preparations with Kat.f. values between 7 and 8 but with no increase in total activity extracted per kilogram dry weight above that obtained from dried yeasts. From these figures it can be seen that the amount of extractable catalase in Swedish baker's yeast is very small, about 14 mg per kilogram of dried yeast if pure yeast catalase is assumed to have a Kat.f. of 36 000 as estimated below.

C. Purification of the Enzyme

20 kg of commercial dried yeast (supplied in this form by Svenska Jästbolaget) were stirred into 50 litres of $M/15$ sodium acetate and the mixture centrifuged at 2 000 r.p.m. for 1 hour after standing for 2 hours. 65 ml of $M/2$ basic lead acetate was added per litre of the cloudy yellow supernatant and the resultant suspension was centrifuged for 20 minutes at 2 000 r.p.m., the precipitate being discarded. 400 g of solid ammonium sulphate per litre were dissolved in the supernatant and the suspension left for 12 hours at 4° C and then centrifuged at 2 000 r.p.m. for 90 minutes. The supernatant was discarded and the precipitate dissolved in 450 ml water and 1/5 volume of 1.6 % calcium phosphate gel, prepared according to Keilin and Hartree [7], added. After standing at room temperature for 30 minutes the suspension was centrifuged and the precipitate discarded. The supernatant was made $M/15$ with respect to secondary sodium phosphate and cooled to 0° C. A $\frac{1}{2}$-volume of cold (−15° C) acetone was added slowly with vigorous stirring while the temperature of the mixture was lowered to −5° C and the precipitate discarded. The acetone concentration of the supernatant was then brought to 50 % while the temperature was lowered to −8° C. The precipitate was centrifuged down at −8° C, stirred with 200 ml of ice-cold distilled water, and dialysed against distilled water at 4° C for 12 hours. (No loss of activity was apparent during this dialysis if insoluble denatured material was left in suspension in the dialysis tube). The suspension was centrifuged clear

and the supernatant made 0.02 *M* with respect to acetate buffer pH 5.7 and the solution made 40 % with respect to ethyl alcohol, the temperature being lowered to −12° C during the slow addition of the alcohol. The suspension was centrifuged at −12° C and the precipitate dissolved in 75 ml of cold distilled water. An equal quantity of 1.6 % calcium phosphate gel was added at 0° C and the mixture was centrifuged after 1 hour. The precipitate was washed four times, each with 1 litre of cold distilled water. The enzyme was eluted by decomposing the calcium phosphate precipitate with 25 ml of a cold aqueous solution which was 0.5 *M* with respect to potassium oxalate and 0.5 *M* with respect to potassium hydrogen phosphate, and centrifuging down the resulting calcium oxalate which was washed with a further 5 ml of the potassium oxalate solution and 15 ml water. 52 g/100 ml of solid ammonium sulphate was added to the combined supernatant and washings and the resultant precipitate was centrifuged down. This precipitate was dissolved in sufficient 0.1 *M* acetate buffer, pH 5.0, to give an optical density of 1 at 405 mμ after centrifuging clear, and 24.3 g of solid ammonium sulphate/100 ml added, and the resulting precipitate was centrifuged down and discarded. A further 9.6 g of ammonium sulphate/100 ml were then added and the precipitate centrifuged down and stored at −15° C.

The Kat.f. of samples purified in this way varied between 5 000 and 8 000, about a 1 000-fold purification being achieved by the above method with yields varying between 16 % and 24 %. Electrophoresis at pH 7.0 in the Tiselius apparatus showed the presence of three components all moving to the anode. Attempts to purify the enzyme further by electrophoresis were unsuccessful due to the instability of solutions of the partially purified enzyme. Attempts to crystalline the partially purified material with ammonium sulphate and alcohol at low temperature met with no success.

D. Properties of the Purified Enzyme Preparation

1. Stability. Yeast catalase appears to be very much less stable than most of the catalases so far studied in a purified state. Precipitation of yeast catalase preparations at pH 7.0 with 60 % alcohol at temperatures above −5° C results in almost complete disappearance of activity, whereas fractionation with alcohol at room temperature has been a common step in the preparation of most of the other catalases so far studied. The action of acetone on yeast catalase appears to be less severe, approximately 50 % loss in activity occurring when it is precipitated at 4° C with 50 % acetone. A clarified solution of the purified material at pH 7 lost about 20 % of its activity on standing at 4° C for 3 hours. At pH 4.5 this percentage of activity was lost within 15 minutes.

2. Absorption Spectrum. The absorption spectra of a purified preparation with a Kat.f. value of 8 000 and its cyanide complex are shown in Fig.1. The spectra were taken of solutions in *M*/15 phosphate buffer pH 6.8, and show all the absorption peaks characteristic of catalases and their cyanide complexes. The peak at 340 mμ in the spectrum of the cyanide complex was probably due to the presence of a flavoprotein impurity which is masked by the Soret band in the spectrum of the original preparation. The ratio E_{280}/E_{405} for this preparation was 4.17.

3. Nature of the Prosthetic Group. An aqueous solution of the purified preparation was treated with three times its volume of cold 1 % HCl in acetone. The resulting precipitate of denatured protein was removed by centrifugation and the acetone removed from the supernatant by a stream of air. A brown precipitate appeared in the remaining colourless aqueous layer and was separated by centrifuging and dissolved in a small amount of

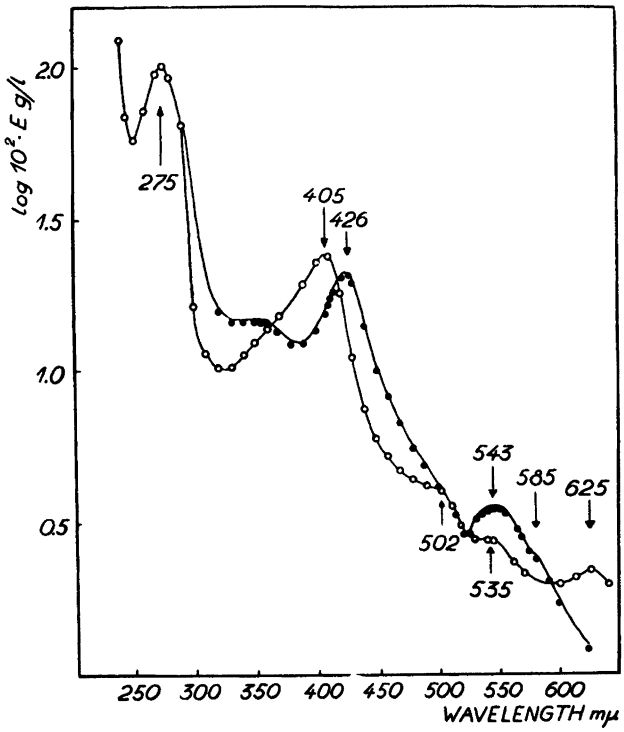

Fig. 1. The absorbtion spectra of purified yeast catalase and its cyanide complex. Yeast catalase: O——O——O *Yeast catalase cyanide:* ●——●——●

pyridine. After the addition of a quarter-volume of *N* NaOH and a few crystals of $Na_2S_2O_4$, a sharp absorption peak with a maximum at 556 mμ was observed with a Beckman spectrophotometer. This observation, and the fact that the aqueous layer after removal of acetone was colourless, indicates that the prosthetic group of yeast catalase is protohaematin and that no detectable bile pigment haematin is present.

4. Iron Content. After digestion with sulphuric acid in the presence of H_2O_2, the preparation was washed into a 10 ml volumetric flask and 0.2 ml of 20 % sulphosalicylic acid added. Ammonia was then added slowly until the solution became yellow when it was made up to volume and the absorption at 424 mμ measured in a Beckman spectrophotometer. The sample with a Kat.f. value of 8 000 contained 0.021 % iron.

5. Inhibition Studies. The effects of potassium cyanide and sodium azide on the activity of yeast catalase are shown in Fig. 2. A NaN_3 concentration of $1.4. \times 10^{-6}$ *M* produces a 50 % inhibition of the enzyme activity, and the same percentage inhibition is produced by a 3.8×10^{-5} *M* concentration of KCN.

6. Electrophoretic Behaviour. Although it was not possible to purify the preparation any further by electrophoresis due to the large loss of activity that occurred during the electrophoresis and the preliminary dialysis, useful information concerning the composition of the preparation was obtained by observations on a sample with a Kat.f. value of

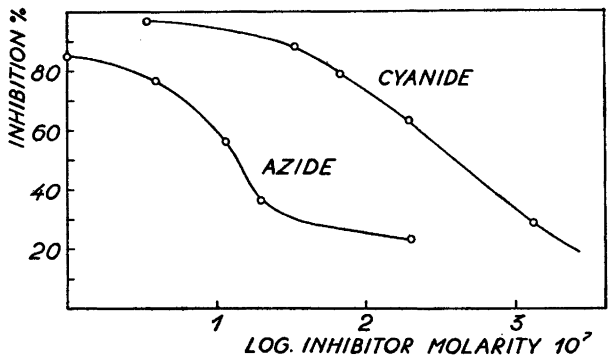

Fig. 2. The effect of potassium cyanide and sodium azide on the activity of purified yeast catalase.

7 200 and an iron content of 0.019 %. After 3 hours of electrophoresis at 25 mA, and 200 V of a 0.5 % solution in phosphate buffer, pH 7.05 of ionic strength 0.1, three distinct components A, B and C were observed all moving to the anode. A, the fastest moving component, was colourless, B had a deep yellow colour, and C a pale yellow colour. Samples were pipetted using a hollow needle from between the A and B boundaries, the B and C boundaries, and behind the C boundary in the anodic limb of the U-tube, and activity and iron determinations were carried out on each sample. The first two samples did not contain any detectable iron or enzyme activity, but the sample taken from behind the C boundary contained all the iron and the residual enzyme activity of the solution. It seems, therefore, that the component C was the partially inactivated catalase. From the areas enclosed under the peaks representing each boundary, and by assuming that the refractive index increments of the three components were the same, it was calculated that this preparation, with Kat.f. 7 200, contained 28 % of component A, 52 % of B, and 20 % of C.

DISCUSSION

Although the complete purification and crystallisation of yeast catalase has been so far prevented by its instability, it has been possible to characterise it sufficiently to show its similarity to other purified catalases. Assuming that the preparation with a Kat.f. value of 7 200, and an iron content of 0.019 %, was 20 % pure, as indicated by the electrophoresis data, pure yeast catalase would have a Kat.f. value of 36 000 and an iron content of 0.095 % as compared with the values of 65 000 and 0.095 % obtained by Bonnichsen [8] for horse erythrocyte catalase. If 0.095 % is accepted as the correct value for the iron content of pure yeast catalase, then the purest preparation with Kat.f. of 8 000 and iron content 0.021 % would have a purity of 23 %, and the Kat.f. of the pure catalase in this preparation would be 36 500. The value of E_{405}/E_{280} would then be 1.08 as compared with Bonnichsen's value of 1.26 for horse

erythrocyte catalase [8]. It appears, then, that the above facts are consistent with yeast catalase being a four haematin catalase with a Kat.f. value of 36 000.

The instability of yeast catalase is not so surprising in the light of the recent observation of Deutsch [9] who has shown the existence in erythrocyte catalase preparations of components with high Kat.f. values which rapidly decrease on dilution or standing to the values usually obtained. This instability may explain the low Kat.f. value estimated for yeast catalase in this work.

SUMMARY

A catalase from yeast has been purified over 1 000-fold and its properties studied. It has been shown that its properties are similar to those of catalases purified from other sources, except in its large degree of instability, and these properties are consistent with it being a four haematin catalase with a Kat.f. of 36 000.

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