# A CATALATIC NUCLEOPROTEIN OF BAKER'S YEAST

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## 1. Introduction

Some years ago we reported the existence in *Saccharomyces cerevisiae* of an atypical catalase, which we named catalase A; upon purification, this proved to be a heme-containing tetramer with a lower molecular weight (180 000) than usual for catalases of any species [1]. Catalase A did not cross-react immunologically [1] with the typical catalase (mol. wt 240 000) of baker's yeast whose purification and properties we also described and which we named catalase T [2]. During the purification of these isofunctional enzymes of high specific activity, we noted the presence in a variety of yeast strains of a curious protein, containing non-heme iron and RNA, which had significant catalatic activity and which cross-reacted immunologically with catalase A; these findings were mentioned in abstracts [3,4] but details have never been published due to our having left this field of research before being able to complete the experimental work.

Recently, Ruis et al. published a series of interesting studies of yeast catalase; using a modification of our purification protocol, they have confirmed and extended knowledge of the two isofunctional and non-cross-reacting catalases [5–7]. They have recently observed a surprising similarity in peptide fingerprints of catalases A and T and raised the question of a possible developmental or evolutionary relation between them (Ruis, personal communication). We have thus decided to publish the available information about this unusual protein.

## 2. Materials and methods

The purification procedure for CNP was based on that described for catalases T and A [1,2] and made use of commercial, dried baker's yeast obtained from Standard Brands Inc. A CNP with identical properties was also isolated by this procedure from laboratory wild-type strains FL-100 (from F. Lacroute, Strasbourg) and D585-11c and D-587-4b (from F. Sherman, Rochester).

Pancreatic ribonuclease was purchased from Calbiochem and treated to inactive contaminating DNAase by the method in [8]. Sartorius membrane filter dialysis sacs were obtained from BDH Chemicals. The nucleic acid moiety of CNP was isolated after deproteination by the phenol method [9]. Antibody to CNP was prepared by methods described [10]. All other materials, assays and procedures were as described for catalases A and T [1,2].

## 3. Results

A typical purification is shown in table 1. The first ammonium sulfate precipitate was dissolved as described [2] and brought to 45% saturation with the same salt. After centrifugation the supernatant contained catalases A and T. The precipitate contained the CNP whose specific catalatic activity is lower than that of the two catalases, which accounts for the

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Table 1
Purification of CNP of baker's yeast

| Fraction | Volume (ml) | Total protein (mg) | Total activity (kat.f. × mg) | Specific activity (kat.f.) | Purification (fold) | Recovery (%) |
|---|---|---|---|---|---|---|
| Crude extract[a] | 62.0 | 2702.58 | 2 589 071 | 958 | 1 | 100 |
| Ethanol and chloroform treatment | 52.0 | 963.56 | 2 085 143 | 2164 | 2.3 | 80.50 |
| First $(NH_4)_2SO_4$ ppt 40–65% | 7.5 | 158.33 | 1 044 028 | 6594 | 6.9 | 40.30 |
| Second $(NH_4)_2SO_4$ ppt 0–45% | 1.5 | 16.14 | 34 765 | 2154[b] | 2.3[b] | 1.34 |
| Sephadex G-75, Hydroxylapatite 0.1 M $PO_4$, pH 7 | 1.5 | 3.84 | 13 566 | 3533 | 3.7 | 0.52 |

[a] Prepared from 30 mg dry yeast extracted in 100 ml of 0.03 M veronal–HCl buffer, pH 8.2
[b] Most of the catalase activity, in the form of catalases T and A of high specific activity, is in the supernatant of this step, which accounts for this apparently negative purification

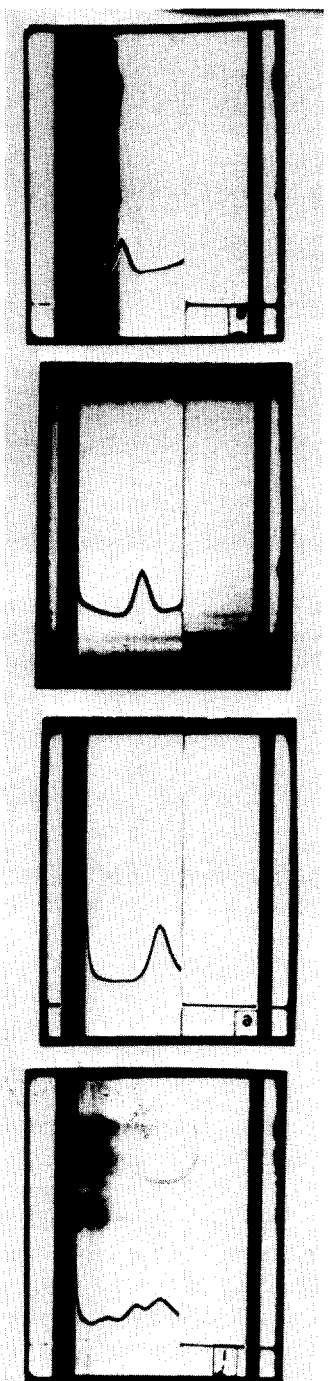
anomalously low degree of purification. The precipitate was then dissolved in 0.1 M phosphate buffer, pH 8.0, chromatographed on a Sephadex G-75 column as described [2] and eluted with distilled water. The fractions with catalatic activity were pooled and brought to 50% saturation with ammonium sulfate. The precipitate was taken up in distilled water and chromatographed on a column of hydroxylapatite which had been pre-equilibrated with 0.1 M phosphate buffer, pH 6.0 and eluted with 0.1 M phosphate buffer at pH 7.0. The enzymatically active fractions of the eluate were pooled, concentrated by vacuum dialysis and stored frozen until required; this material contained the CNP in apparently homogeneous form.

The specific catalatic activity of the CNP, measured as kat.f., varied from 2000–4000, about 10% that of authentic catalases. By way of comparison, we found that purified yeast lactate dehydrogenase, a heme-flavoprotein, had a catalatic activity with kat.f. 450, which was more than 100 times that of hemoglobin. Generally, purified non-heme proteins are devoid of catalatic activity.

The protein migrated as an homogeneous boundary in the analytical ultracentrifuge as shown in fig.1 (second from bottom), where it is compared to preparations of catalases T (top) and A (second from top). In order to rule out the possibility that these were not distinct species of protein, the three catalatic proteins were mixed together and then subjected to analytical ultracentrifugation, with the results shown in fig.1 (bottom); three distinct peaks separated, each comparable in rate of sedimentation, as measured by distance from the meniscus, to the isolated proteins shown above in the same figure. The sedimentation constant ($s_{20,w}$) of the CNP was 4.1 S. Assuming the same values for diffusion constant and partial specific volume as [1,2], we estimate its mass to be 90 000 daltons.

Its absorption spectrum was like that of a nucleoprotein, with an $A_{260/280\,nm}$ ratio of about 1.5. There was no absorption in the visible but there was a very small peak always present at 410 nm. The absorption spectrum remained the same after dialysis. Pyridine hemochromogen tests were uniformly negative when performed on 3–4 mg samples of CNP; the test would have detected 20 μg heme. Iron was found to be present to the extent of 0.25%, corresponding to mol. wt 22 000 assuming 1 iron atom/molecule.

Electrophoresis on polyacrylamide gel showed a single homogenous band with the protein stain. When the run was repeated after the protein had been incubated with 1% sodium dodecyl sulfate for 2 h, a single band appeared, which migrated much more rapidly toward the anode than did the native protein.



The molecular weight of this subunit was estimated to be 23 000 by the method in [11]. We do not know whether this subunit has a polynucleotide moiety or whether it is protein alone.

When the native CNP was incubated with pancreatic ribonuclease in a dialysis bag, the $A_{260\,nm}$ of the material within the bag remained practically constant during prolonged dialysis, as shown in fig.2A. The protein-free polynucleotide moiety of the CNP was relatively stable to prolonged dialysis as shown in fig.2B; the polynucleotide moiety was sensitive to ribonuclease as shown by the relatively rapid loss of material absorbing at 260 nm during exposure to the nuclease in the dialysis bag (fig.2C). We conclude that the protein contains a polyribonucleotide moiety and that this is protected against ribonuclease action in the native CNP. The RNA moiety isolated by the phenol method was subjected to analytical centrifugation; a single symmetrical peak was noted ($s_{20,w}$ 2.7 S, which corresponds to mol. wt 13 000) but this had a marked tendency to flatten and broaden during the run, suggesting the presence of an inhomogenous population of RNA molecules.

CNP was not as good an antigen as were catalases A and T but nevertheless gave rise to antibody preparations which reacted in immunodiffusion tests with homologous antigen as well as showing cross-reaction to catalase A but not T. CNP did not cross-react with antiserum to catalase T, nor did catalase A, as reported [1,5]. No cross-reaction between CNP and T was found in studies involving immunoprecipitation coupled with enzyme assay, microimmunodiffusion as well as standard Ouchterlony procedure. On the other hand, there was always unambiguous immuno-

Fig.1. Sedimentation patterns of purified catalase T, A and CNP of baker's yeast. Catalase T (top); Catalase A (second from top); CNP (second from bottom); a mixture of the three purified proteins (bottom). Protein concentrations were 3.5, 5 and 7 mg/ml for T, A and CNP, respectively. In the mixture, final protein concentration was 7.75 mg/ml, consisting of 1.75 mg/ml catalase T, 2.5 mg/ml catalase A and 3.5 mg/ml CNP, i.e., half the concentration at which each was run individually. Bar angles were 45°, 60°, 60° and 60°. Photographs were all taken at 48 min after reaching speed of 48 000 rev/min and the direction of sedimentation was from right to left. Ultracentrifugation experiments were performed using a $5 \times 10^{-3}$ M phosphate buffer, pH 7.0.

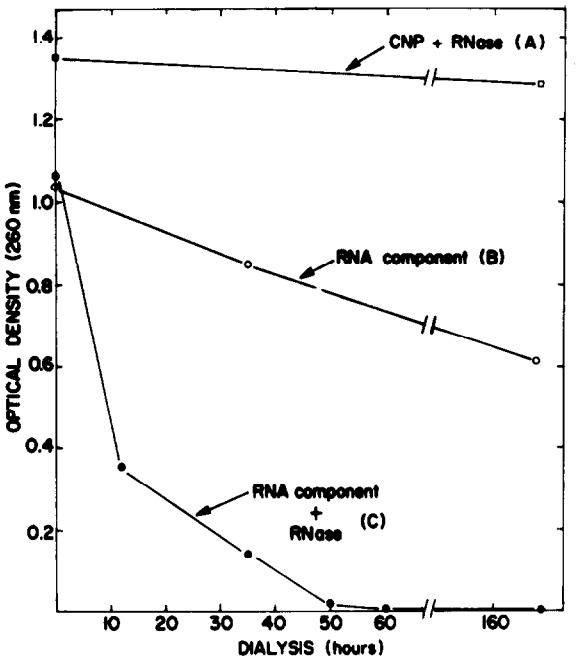



Fig.2. Dialysis of CNP and RNA moiety of CNP in the presence and absence of pancreatic RNAase. (a) One mg/ml CNP preincubated with 50 μg/ml of boiled pancreatic ribonuclease for 2 h at 37°C was continuously dialyzed against several changes of distilled water. (b) Purified RNA moiety was dialyzed as in (a) without pre-treatment by RNAase. (c) Purified RNA moiety of CNP was preincubated with boiled RNAase and subsequently dialyzed as in (a).

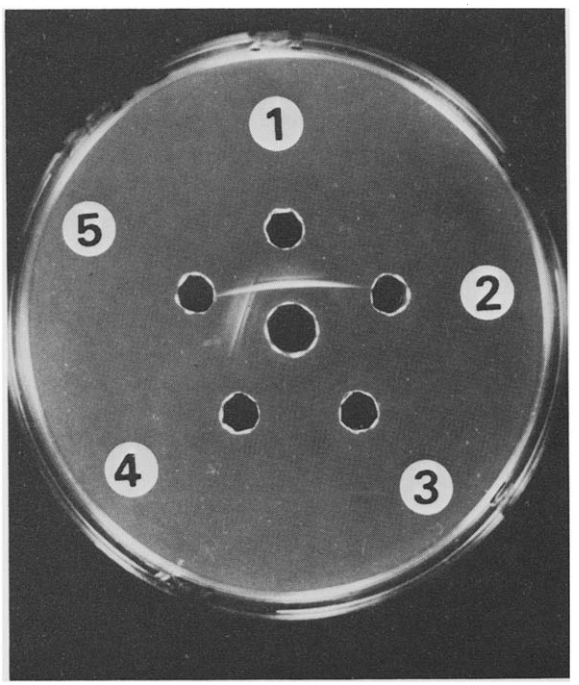



Fig.3. Immunodiffusion study of purified catalatic proteins against antibody to catalase A on double diffusion agar plate. Centre well: antibody to catalase A. Antigens: (1) catalase A, (2) catalase T, (3) rabbit serum control, (4) crystalline beef liver catalase, (5) CNP.

logical cross-reaction between CNP and catalase A, as shown in fig.3, in which spurring indicates non-identity between the two antigens.

## 4. Discussion

We have purified the CNP from a number of wild-type strains as well as from a mutant (CM9 Lys-1), isolated by virtue of inability of its colonies rapidly to decompose $H_2O_2$; this mutant did not contain detectable amounts of catalases A and T. This, together with the homogeneity of the CNP in ultracentrifuge and electrophoresis, the negative pyridine hemochromogen test and the antigenic non-identity shown in immunodiffusion, rules out the possibility that the catalatic activity of CNP is due to contamination by catalase A. It is not impossible that CNP is a breakdown product of catalase A with adventitiously bound RNA; this possibility is the more real since catalase A was found [5] to be localized in the cell vacuole, which has a hydrolytic as well as a storage function. On the other hand, there is also the possibility that CNP is a stage in the synthesis of catalase A and that the RNA moiety is in some way a consequence of this. It may be that the CNP is responsible for the low basal catalatic activity reported in a mutant yeast unable to make heme as a result of loss of ability to synthesize the precursor ∂-amino levulinic acid [12]. We hope that other workers in this field will be able to elucidate the relationship of CNP and catalase A as well as the function they both serve in the aerobic yeast cell.

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