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Isolation and Translation of Catalase mRNA of Bovine Liver

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Messenger ribonucleic acid (mRNA) directing the biosynthesis of catalase [EC 1.11.1.6] was purified from bovine liver. The procedure consists of: (1) isolation of catalase-synthesizing polysomes by immunoprecipitation, (2) extraction of RNA from the polysomes with phenol, and (3) isolation of catalase mRNA by oligo(dT)-cellulose chromatography. Purified catalase mRNA has a molecular weight of $4—5 \times 10^5$ determined by electrophoresis on polyacrylamide–agarose gel. The mRNA stimulated catalase synthesis in a cell-free system derived from wheat germ; *i.e.*, the incorporation of ^{14}C-amino acids into catalase increased proportionally to the amount of mRNA added in the cell-free system and with incubation time. On SDS–polyacrylamide gel the radioactivity peak of the cell-free products precipitated by anti-catalase antibody coincided with the migration of carrier catalase. In translating system the mRNA preparation directed the incorporation of ^{14}C-amino acids into catalase at least 79% as efficiently as into total proteins.

Keywords——catalase; messenger RNA; oligo(dT)-cellulose chromatography; wheat germ cell-free system; protein synthesis

The purification of globin mRNA from mammalian reticurocytes[2] is the first in isolating mRNA coding a specific protein in eukaryotic cells. It is convenient that the protein synthesized in reticulocytes is almost globins and the size of globin mRNA (9—10 S) differs distinctly from that of rRNA (5, 18 and 28 S) or tRNA (4 S). From the same reason immunoglobulin mRNA was next purified employing mouse plasmacytoma cells which synthesize mainly γ-globulin.[3] However, it is rather an exception that one type of cell produces a single species of protein. Catalase occupies only a few percent of the proteins synthesized in liver cells, so that the isolation of catalase-synthesizing polysomes should be the first essential step for purifying catalase mRNA. Higashi *et al.*[4,5] attempted to precipitate catalase-synthesizing polysomes based on the immunochemical reaction between nascent catalase peptides on the polysomes and anti-catalase antibodies. Later, Sakamoto and Higashi[6] reported the isolation of catalase mRNA from rat liver and its translation by a cell-free system. Schimke and his collaborators[7,8] also succeeded in isolating ovalbumin-synthesizing polysomes from chick oviduct by immunochemical procedures and purified the mRNA directing the synthesis of this protein. Following these papers, the isolation of many eukaryotic mRNAs coding specific proteins by such an immunochemical method has been reported.[9–16]

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In the present study, the authors have attempted to purify catalase mRNA from bovine liver and translate it in a cell-free system derived from wheat germ. The procedure for isolating catalase mRNA consists of: (1) separation of catalase-synthesizing polysomes by immunoprecipitation, (2) extraction of RNA from the polysomes with phenol, and (3) purification of catalase mRNA by oligo(dT)-cellulose chromatography. Sakamoto and Higashi[6] used nitrocellulose membrane filters to adsorb catalase mRNA at the third step of purification, since mRNAs of eukaryotes were reported to have a poly(A)-structure at the 3-'end.[17-20] Oligo(dT)-cellulose has been widely used to detect poly(A)-segment of eukaryotic mRNAs. Aviv *et al.*[21] used it for the first time in isolating globin mRNA.

As the cell-free system translating specific mRNAs purified from various sources, reticulocyte lysates or preparations from Krebs-II ascites tumor cells were most favorably used in early 1970 s. Ribosomes prepared from eggs of a brine shrimp, *Artemia salina*, and the cell sap of rat liver were employed when catalase mRNA of rat liver was translated.[6] The authors have chosen a cell-free system derived from wheat germ[22,23] for translating the catalase mRNA obtained from bovine liver, since recently, the system has been used most extensively.

This report deals with the isolation and the translation of catalase mRNA from bovine liver in the manner described above. The results indicate that the catalase mRNA has been isolated with a purity of more than 79% in terms of mRNA, and its molecular weight has been estimated to be $4—5\times10^5$.

Experimental

Materials——Bovine liver catalase (crystallized enzyme, specific activity: approximate 50000 units/mg) was obtained from Boehringer Mannheim., complete adjuvant (Freund) was from Iatron Laboratories and oligo(dT)-cellulose was from Collaborative Research Inc. ATP, GTP, creatine phosphate and creatine kinase [EC 2.7.3.2] were purchased from Sigma Chemical Co. ^{14}C-amino acid mixture (55 mCi/milliatom of carbon) was obtained from the Radiochemical Centre, Amersham. Protosol was obtained from New England Nuclear, and Scintilamine-OH, POPOP and DPO were from Dojindo Laboratories.

Preparation of Anti-Catalase Antiserum and Purification of Antibody——Rabbits were immunized with bovine liver catalase according to Higashi *et al.*[24] Each rabbit, weighing 2—3 kg, was intramuscularly injected twice with an emulsion of bovine liver catalase and complete adjuvant at a 2-week interval. Antisera were collected from immunized rabbits 2 weeks after the second injection. The antisera were treated at 56° for 30 min and anti-catalase antibody was purified according to Levy *et al.*[25] The immunoglobulin fraction was obtained by 33% ammonium sulfate precipitation and DEAE-cellulose chromatography. The concentration of the immunoglobulin solution used in following experiments was 16 mg/ml.

Immunochemical Characterization of Anti-Catalase IgG——Agar diffusion was performed as described by Ouchterlony.[26] A solution of 1% agar, 0.9% NaCl and 10 mM phosphate buffer (pH 7.4) was boiled and poured into a petri dish. Samples were placed in each well and the agar plate was kept standing at 4° for a few days. Quantitative precipitin reaction of bovine liver catalase was performed as described by Shibata *et al.*[27]

Preparation of Polysomes——Polysomes were prepared from bovine liver by a modification of the procedure of Clemens *et al.*[28] Bovine liver was purchased from a slaughter house and processed at 0—4° as rapidly as possible. The liver, perfused, and minced, was homogenized in 2 volumes of Medium A (0.25 M

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sucrose, 20 mM Tris-HCl, pH 7.6, 4 mM magnesium acetate, 6 mM 2-mercaptoethanol, and 200 μg/ml heparin) by a waring blender and Tornado (Braun). Post-mitochondrial supernatants were obtained by centrifugation at 12000×*g* for 15 min. Triton X-100 was then added to give a final concentration of 2% and the magnesium concentration was raised to 40 mM with an appropriate volume of 0.4 M magnesium acetate. After standing at 0° for 45 min, the mixture was centrifuged at 5000×*g* for 10 min. The pellet was suspended in Medium A containing 20 mM magnesium acetate and recentrifuged at 5000×*g* for 10 min. The pellet of polysomes thus obtained was suspended in Medium B (20 mM Tris–HCl, pH 7.6, 100 mM KCl, 40 mM NaCl, 4 mM magnesium acetate, 6 mM 2-mercaptoethanol and 200 μg/ml heparin) and was kept standing at 0° for 10 min. After large aggregates were removed by centrifugation, the resulting polysome suspension was used immediately.

Direct Immunoprecipitation of Catalase-Synthesizing Polysomes——Polysomes synthesizing catalase were immunochemically isolated by the direct method according to Higashi *et al.*[4,5]. The polysome suspension was incubated with anti-catalase IgG at 0° for 90 min, followed by a further 60 min incubation at 0° with carrier catalase added. The incubation mixture consisted of (in 1.0 ml): 100 A_{260} units of polysomes, 0.5 ml of antibody solution and 50 μg of carrier catalase. Catalase-synthesizing polysome: anti-catalase IgG: carrier catalase complex was sedimented by centrifugation at 2000×*g* for 15 min and washed three times with Medium B.

Isolation of Catalase mRNA——RNA was extracted from the immunoprecipitates described above by a modification of the procedure of Jost *et al.*[16] The extraction was carried out at 4°. The immunoprecipitates were dissolved in Medium C (0.1 M Tris–HCl, pH 9.0, 0.025 M EDTA, and 0.5% SDS) and 1 volume of redistilled phenol was added. The mixture was shaken for 5 min. Chloroform (1 volume of the original sample) was added and the extraction continued for another 5 min. After centrifugation at 2000×*g* for 10 min, the phenol phase was reextracted with 1 volume of Medium C. The combined water phase was treated twice with 1 volume of cold chloroform. RNA was precipitated with 2 volumes of ethanol in the presence of 1% sodium acetate (pH 5.0) at −20° overnight. The RNA was separated by centrifugation at 10000×*g* for 20 min, washed three times with 70% ethanol by centrifugation and dissolved in a small amount of water.

Catalase mRNA was isolated from this fraction of RNA by oligo(dT)-cellulose chromatography.[21,29] The RNA, 30—50 A_{260} units/ml of 0.01 M Tris–HCl (pH 7.4) containing 0.5 M KCl, was applied to a column oligo(dT)-cellulose, previously equilibrated with the same buffer. The column was eluted sequentially with 0.01 M Tris–HCl (pH 7.4) containing 0.5 M KCl, 0.01 M Tris-HCl (pH 7.4) containing 0.1 M KCl, and water. Chromatography on oligo(dT)-cellulose was performed at room temperature.

Polyacrylamide-Agarose Gel Electrophoresis of RNA——Electrophoresis of RNA was performed with 1.8% polyacrylamide–0.5% agarose gel by a modification of the procedure of Dingsman *et al.*[30] RNA preparations before and after oligo(dT)-cellulose chromatography were precipitated with ethanol and dissolved in a small amount of buffer (40 mM Tris–HCl, pH 7.4, 20 mM sodium acetate, 2 mM EDTA, 10% sucrose and 0.1% bromphenol blue). The solution was loaded on a gel and electrophoresis was performed at 4° for 4 hr at 3 mA/gel (0.6×8 cm). After electrophoresis, the gels were stained overnight with 1% methylene blue in 15% acetic acid, followed by washing with 7.5% acetic acid. The stained gels were scanned at 610 nm with a recording spectrophotometer (Densitomaster-8, Atago).

Translation of Catalase mRNA by Wheat Germ Cell-Free System——Wheat germ S-30 was prepared according to the methods of Roberts *et al.*[23] and Green *et al.*[29] with minor modifications. Being allowed to swell for 1 min, raw wheat germ was gently homogenized in 4 volumes of Medium D (20 mM Tris–HCl, pH 7.4, 120 mM KCl, 3.5 mM magnesium acetate, 2 mM $CaCl_2$ and 6 mM 2-mercaptoethanol) with a mortar and a pestle. The homogenate was centrifuged at 30000×*g* for 10 min and the supernatant fluid was carefully aspirated, avoiding the top fatty layer. The supernatant fraction was brought to 1 mM ATP, 0.1 mM GTP, 8 mM creatine phosphate and 40 μg/ml creatine kinase, and incubated at 30° for 15 min. The mixture was then passed through a column of Sephadex G-25 previously equilibrated with Medium D, and the first peak fraction was used as wheat germ S-30. Protein concentration of the S-30 fraction was measured by Lowry's method.[31] One ml of the reaction mixture for translating catalase mRNA contained the following components: 5 mg of S-30 protein, 0.1—1.0 A_{260} unit of mRNA purified by oligo(dT)-cellulose chromatography once, 20 mM Tris–HCl (pH 7.4), 120 mM KCl, 5 mM magnesium acetate, 1 mM ATP, 0.2 mM GTP, 8 mM creatine phosphate, 50 μg of creatine kinase, 2 mM 2-mercaptoethanol, 40 μM amino acids mixture and 1 μCi of ^{14}C-amino acid mixture. Incubation was performed at 30° for 120 min unless otherwise indicated.

Measurement of Radioactivity incorporated in Cell-Free Products——Being diluted 5 times with medium D after incubation, the cell-free mixture was centrifuged at 105000×*g* for 1 hr to separate the supernatant. In measuring the total protein synthesis, 2 ml of the supernatant was incubated with 1 volume of 10% trichloroacetic acid overnight, and the mixture was centrifuged at 1500×*g* for 10 min. The pellet was washed

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3 times with 5% trichloroacetic acid, dried and incubated with 0.2 ml of Scintilamine-OH at 60° for 20 min. Ten ml of toluene scintillation cocktail (1 liter of toluene, 0.1 g of POPOP and 4 g of DPO) were added and the radioactivity was measured. To measure the catalase synthesis, on the other hand, 3 ml of the supernatant was first incubated with 0.5 ml of anti-catalase antibody at 37° for 1 hr, and then at 4° overnight with 50 μg of carrier catalase added. The mixture was centrifuged at 1500×*g* for 10 min and the precipitate was washed 3 times with physiological saline. The radioactivity was measured as described above.

SDS-Polyacrylamide Gel Electrophoresis of Cell-Free Products——Electrophoresis was performed by a modification of the procedure of Fairbanks *et al.*[32] Five ml of the diluted supernatant described above, was incubated with 0.1 ml of anti-catalase antibody at 37° for 1 hr, and then at 4° overnight with 10 μg of carrier catalase added. The precipitate, separated and washed, was dissolved in 50 μl of solubilizing solution (1% SDS, 1 mM EDTA, 10 mM Tris–HCl, pH 7.4, 1% 2-mercaptoethanol, 10% sucrose and 0.1% bromphenol blue) and boiled for 3 min. The boiled sample was subjected to electrophoresis in 7.5% polyacrylamide containing 0.1% SDS at 5 mA/gel (0.6×8 cm) for 4 hr. The gel was stained with a solution containing 0.25% coomassie brilliant blue R-250, 45% methanol and 10% acetic acid, and destained with a solution containing 5% methanol and 7.5% acetic acid. Melting of the gel and measurement of the radioactivity were performed according to Zähringer *et al.*[33] The gel was sliced in 1 mm thickness with a razor. Each gel slice was incubated in 0.1 ml of 33% hydroperoxide at 50° overnight and subsequently for another 1 hr with 0.7 ml of Protozol added. Melted gel slice was dissolved in 10 ml of toluene scintillation cocktail and measured for radioactivity.

Results

Agar Diffusion of Anti-Catalase IgG

The specificity of anti-catalase antibody was examined by Ouchterlony method (Fig. 1). A distinct precipitin line was observed with bovine liver catalase. Rat liver catalase gave a feeble line which fused with the one produced by bovine catalase, and a spur was also seen between these two kinds of liver catalase. The findings indicate that bovine liver catalase has both a common antigen determinant(s) with, and also a different one(s) from those of rat liver catalase. The precipitin line for bovine hemolysate completely fused with the one for bovine liver catalase and no spur was detectable. The anti-catalase antibody gave no visible precipitin line against either bovine or rat serum. Therefore, bovine erythrocyte catalase is immunologically indistinguishable from bovine liver catalase and no antigenic materials reacting with anti-catalase antibody occurs in the serum.

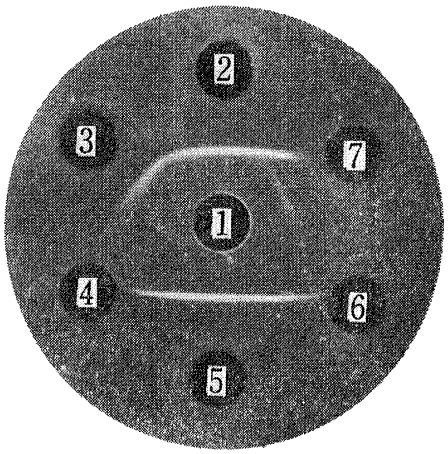

Fig. 1. Agar Diffusion of Anti-catalase Immunoglobulin

Each well contained as follows: 1; anti-bovine liver catalase, 2,5; bovine liver catalase, 3; bovine hemolysate, 4; bovine serum, 6; rat serum, and 7; rat liver catalase.

Immunoprecipitation of Bovine Liver Catalase by Antibody

A standard precipitin curve for bovine liver catalase is shown in Fig. 2. One-tenth ml of antibody solution can completely precipitate up to approximately 20 μg of catalase.

Immunoprecipitation of Catalase-Synthesizing Polysomes

Conditions for precipitating catalase-synthesizing polysomes were investigated (Fig. 3 and 4). In the first experiment, increasing amounts of polysomes were incubated with constant amounts of antibody and carrier catalase (Fig. 3). One-tenth ml of antibody solution and 5 μg of carrier catalase was found to precipitate catalase-synthesizing polysomes as much as those involved in 20 A_{260} units of total polysomes most efficiently. The catalase-synthesizing polysomes were estimated to be about 2.5% of liver polysomes.

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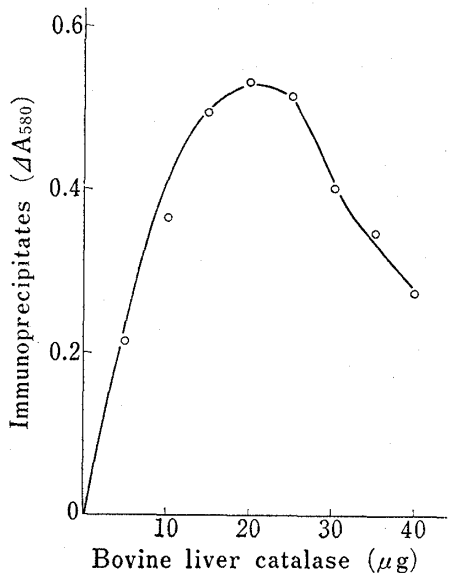

Fig. 2. Precipitin Curve of Bovine Liver Catalase

Increasing amounts of bovine liver catalase were incubated with 0.1 ml of anti-catalase immunoglobulin solution. Immunoprecipitates were assayed by the BSP method.

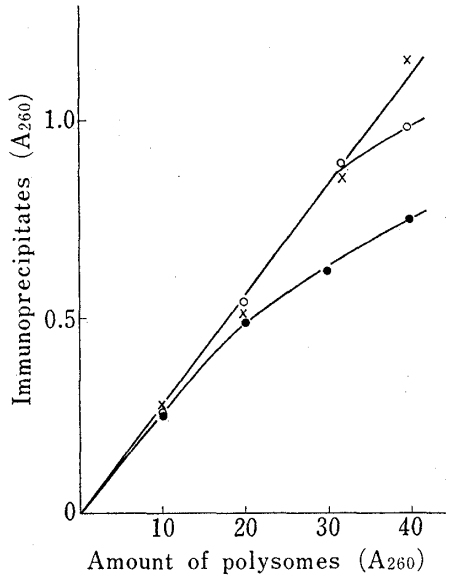

Fig. 3. Direct Immunoprecipitation of Catalase-synthesizing Polysomes

Increasing amounts of polysomes were incubated with anti-catalase for 90 minutes at 0°, followed by a further 60 min incubation with carrier catalase added. Immunoprecipitates were dissolved in 0.1 N NaOH and assayed at 260 nm. ●: anti-catalase 0.1 ml, carrier catalase 5 μg; ○: anti-catalase 0.3 ml, carrier catalase 15 μg; ×: anti-catalase 1.0 ml, carrier catalase 50 μg.

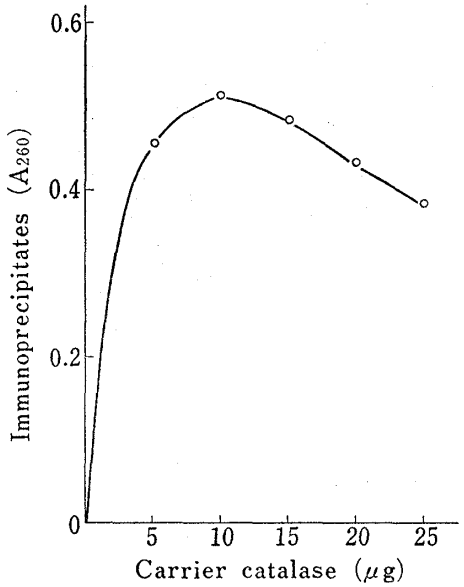

Fig. 4. Amount of Carrier Catalase Necessary for Complete Precipitation of catalase-synthesizing Polysomes

Twenty A_{260} units of polysomes were incubated with 0.1 ml anti-catalase for 90 min at 0°, followed by a further 60 min incubation with increasing amounts of carrier catalase added. Immunoprecipitates were dissolved in 0.1 N NaOH and assayed at 260 nm.

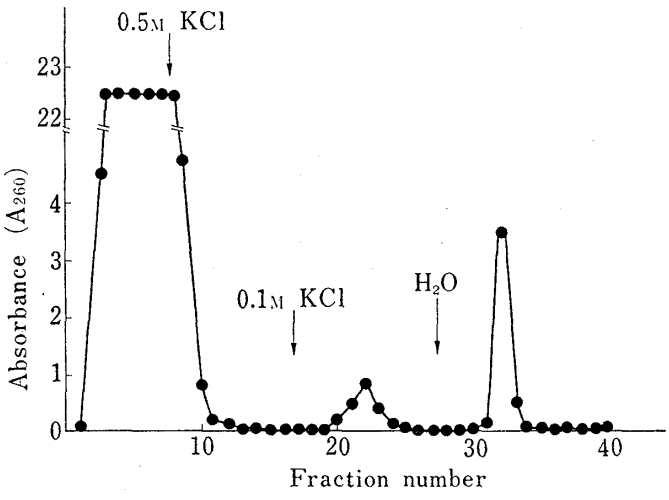

Fig. 5. Purification of Catalase mRNA by Oligo(dT)-cellulose Chromatography

510 A_{260} units of RNA extracted from catalase-synthesizing polysomes were applied on a column (1×4 cm). Elution with 0.5 M KCl solution, 0.1 M KCl solution, and water is indicated by arrows. Eluate of 0.5 M KCl solution was fractionated in 3 ml, and that of 0.1 M KCl solution and water was in 1 ml. The flow rate was 10—20 ml/hr.

In the next experiment, varying amounts of carrier catalase were incubated with 0.1 ml of antibody solution and 20 A_{260} units of polysomes (Fig. 4). With 10 μg of carrier catalase precipitation was found to be maximum. Therefore, polysomes, antibody solution and carrier catalase were mixed at a ratio of 20 A_{260} units: 0.1 ml: 10 μg in isolating catalase-synthesizing polysomes.

Isolation of Catalase mRNA

The RNA extracted from catalase-synthesizing polysomes as described under Materials and Methods was chromatographed on oligo(dT)-cellulose. Fig. 5 shows an elution profile from an oligo(dT)-cellulose column. The column was washed with 0.5 M KCl and 0.1 M KCl solution, and the mRNA was finally eluted with water. This mRNA fraction was rechromatographed on the same column to obtain a preparation for electrophoresis, with a recovery of 50% (data not shown).

Electrophoretic Analysis of Catalase mRNA Preparation

Catalase mRNA purified by oligo(dT)-cellulose chromatography was analysed by electrophoresis on polyacrylamide–agarose gel (Fig. 6). After rechromatography, the preparation showed a single peak. As demonstrated in the figure, the catalase mRNA was estimated to have a molecular weight of $4—5\times10^5$. This value is in accordance with the calculated molecular weight of catalase mRNA (about 5×10^5) based on the size of a catalase subunit (about 6.2×10^4).

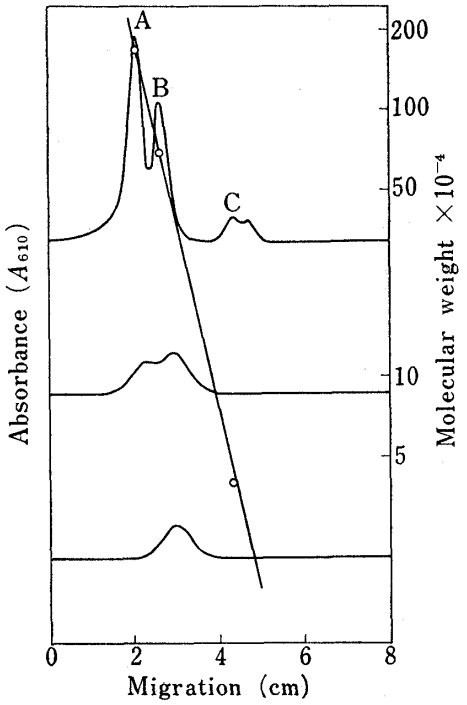

Fig. 6. Electrophoresis of RNA Preparations on Polyacrylamideagarose Gels

Gels were stained with methylene blue after electrophoresis and scanned at 610 nm. The upper curve represents 40 μg of RNAs before oligo(dT)-cellulose chromatography, the middle curve 15 μg of catalase mRNA after the first chromatography, and the lower curve 10 μg of catalase mRNA purified by rechromatography on oligo(dT)-cellulose. Peak A was taken as 28 S rRNA (m.w.$=1.7\times10^6$), B, as 18 S rRNA (m.w.$=7\times10^5$) and C, as 5 S rRNA (m.w.$=4\times10^4$).

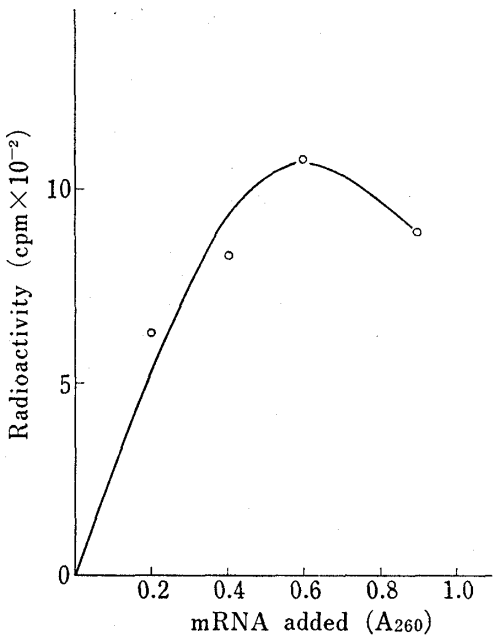

Fig. 7. Effect of Added mRNA on Catalase Synthesis

Increasing amounts of catalase mRNA were added to 1.0 ml of reaction mixture. Incubation was carried out at 30° for 120 min and the incorporation of [^{14}C]-amino acids into catalase was measured.

Translation of Catalase mRNA

Catalase mRNA preparation after the first oligo(dT)-cellulose chromatography was translated in wheat germ S-30 cell-free system. Increasing amounts of the mRNA preparation were added in the system and the incorporation of ^{14}C-amino acids into catalase was measured (Fig. 7). The incorporated radioactivities increased with the amount of added mRNA up to 0.6 A_{260} unit.

Time course of catalase biosynthesis was studied using 0.6 A_{260} unit of mRNA (Fig. 8). The synthesis increased with incubation time and reached a plateau in 60 min.

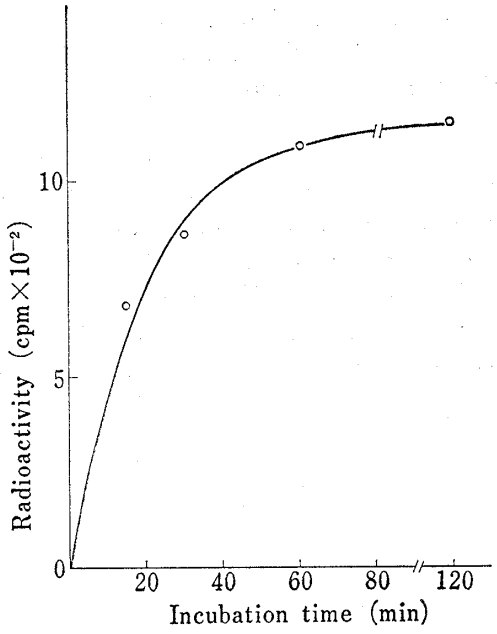

Fig. 8. Time Course of Incorrporation of [^{14}C]-Amino Acids into Catalase directed by Catalase mRNA

0.6 A_{260} unit of catalase mRNA was added to 1.0 ml of reaction mixture. Incubation was carried out at 30° for indicated time, and the incorporation of [^{14}C]-amino acids into catalase was measured.

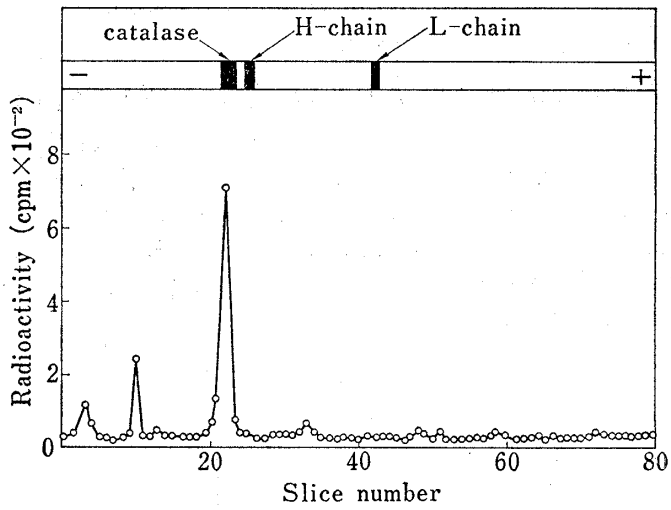

Fig. 9. Electrophoretic Analysis on SDS-polyacrylamide Gel of Cell-free Products precipitated by Anti-catalase

The upper represents a gel stained with coomassie brilliant blue R-250 after electrophoresis. Migration of catalase subunit, H-chain, and L-chain is indicated by arrows. The lower represents the radioactivity in each gel slice.

Electrophoretic Analysis of the Cell-Free Products

The cell-free products precipitated with anti-catalase were analysed by electrophoresis on SDS-polyacrylamide gel (Fig. 9). The peak of radioactivity coincided with the migration of carrier catalase, proving that catalase was synthesized in the cell-free system with the purified mRNA added.

Table I. Purity of Catalase mRNA Preparation based on the Specificity in Messenger Activity

Exp. No.	^{14}C in catalase (dpm/A_{260} mRNA)	^{14}C in total protein (dpm/A_{260} mRNA)	Purity (%)
1	4084	5042	81
2	5886	7813	75
3	4729	6072	78
4	5571	6686	83
Ave.	5068	6403	79

0.3—0.5 A_{260} unit of mRNA was added to cell-free system, and radioactivity incorporated into catalase and into total proteins were compared. Radioactivities were expressed as dpm per A_{260} unit of mRNA added. The purity was expressed as ([^{14}C] in catalase/[^{14}C] in total proteins)×100.

Purity of Catalase mRNA Preparation

In translation experiments, radioactivities incorporated into catalase precipitated with anti-catalase anti-body and into total proteins sedimented with trichloroacetic acid were compared (Table I). Seventy-nine percent of the radioactivity incorporated into the total proteins were found in the catalase, suggesting that catalase mRNA was more than 79% pure estimated from is messenger activity.

Discussion

For studying protein synthesis at molecular level, it is necessary to purify the factors concerning that process. Especially, the purification of mRNA coding a specific protein is undoubtedly indispensable to the study on its biosynthesis. A variety of methods for isolating specific mRNAs have been reported. When the immunochemical method is used, as in the case of catalase mRNA, the specificity of the immunochemical reaction deeply influences the purity of a final mRNA preparation obtained. If polysomes irrelevant to catalase synthesis co-precipitate in association with the immunochemical reaction, catalase mRNA to be obtained would have a lower purity. Some undesirable precipitation of polysomes other than catalase-synthesizing ones could occur because of the complexity of such a system as polysome–mRNA–nascent peptides. Higashi *et al.*[4,5] have recommended a pretreatment of antiserum at 56° for 30 min and the immunochemical reaction at 0° to eliminate nonspecific precipitation of polysomes. In addition, following procedures will be effective: (1) use of the antibody purified by affinity chromatography, (2) lowering polysome concentration and increasing ionic strength in the reaction mixture, (3) addition of a detergent at a suitable concentration, and (4) immunochemical precipitation of polysomes by the indirect method using a secondary antibody. In the present study, the author has prepared catalase mRNA with a purity of at least 79% based on its messenger activity, however, the possibilities described above for increasing the purity should be investigated.

The molecular weight of catalase mRNA of bovine liver is calculated to be about 5×10^5, assuming that bovine liver catalase has a molecular weight of 248000 and consists of 4 identical subunits.[34] As shown in Fig. 6, isolated catalase mRNA migrated at the position of molecular weight of $4—5\times10^5$ on the gel. However, the mRNA preparation did not show a distinct and sharp band, suggesting that some of the mRNA were degraded by RNase during isolation and their size became considerably smaller. This is another problem as important as that described above.

Table I shows 79% of the total proteins synthesized in a cell-free system was immunoprecipitated with anti-catalase antibody, and this protein migrated exactly with carrier catalase on electrophoresis (Fig. 9). If some catalase mRNA underwent degradation by RNase and its translation product was too small in size to be precipitable by anti-catalase, the value of 79% would be a minimum estimation for the purity of the catalase mRNA preparation. The purity of a specific mRNA is rather difficult to determine, and any assay is not sufficient by itself. In this paper the purity of the catalase mRNA preparation was examined both by electrophoresis and by translation in a cell-free system. Furthermore a purity test using the hybridization technique is in preparation.

The yield of catalase mRNA in the present study was about 0.001 A_{260} unit per g liver after rechromatography with oligo(dT)-cellulose, being not satisfactory. The low yield may be caused by the action of RNase during purification. Heparin added to media at a concentration of 200 μg/ml did not seem to produce a sufficient effect as an RNase inhibitor. It is also a serious problem how to inhibit RNase activity more effectively.

34) W.A. Schroeder, J.R. Shelton, J.B. Shelton, and B.M. Olson, *Biochim. Biophys. Acta*, **89**, 47 (1964).

As shown in Fig. 1, bovine and rat liver catalase immunologically cross-reacts with each other, confirming the previous result.[27] Amino acid compositions of both catalase have been reported not to resemble,[35–37] therefore, it is considered that there are the same or similar amino acid sequences in both molecules, with different ones on the other hand.

35) T. Higashi and Y. Shibata, *J. Biochem.*, **56**, 361 (1964).
36) G. Schnuchel, *H-S. Z. Physiol. Chem.*, **303**, 91 (1956).
37) W.A. Schroeder, A. Saha, W.D. Fenninger, and J.T. Cua, *Biochim. Biophys. Acta*, **58**, 611 (1962).