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Regulation of Catalase Synthesis in *Escherichia coli* K12

I. Further Isolation of Catalase-less Mutants caused by Deficiency Apo-proteins

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Introduction

Catalase (EC 1. 11. 1. 6) is constituted of protoheme and apoprotein, therefore its synthesis is regulated by the synthesis of two components. Several studies have been reported as for the regulation of catalase synthesis in microbes.^(1,2,3,4,5,6,7) Săsarman et al. (1968) determined the loci of the gene that controls the catalase synthesis in *Escherichia coli*, but the catalase-less mutants he used were the hemin less-mutants⁽⁸⁾. All of the other catalase-less mutants already reported in some bacteria were heminless-mutants. We have isolated a catalase-less mutant, which is defective in apoprotein moiety, from a Hfr strain of *Escherichia coli*⁽¹⁰⁾. In this study, using *E. coli* K12 AB1157 which is a F⁻-strain and is applicable for genetic analysis, it was attempted to isolate the catalase-less mutants that are deficient in catalase apoprotein.

Materials and Methods

Organism ; *Escherichia coli* K12 AB1157, whose genetic markers are F⁻, gal⁻, his⁻, arg⁻, str⁺, xyl⁻, mtl⁻, thi⁻, thr⁻, ara⁻, leu⁻, pro⁻, lac⁻, was used in this study.

Isolation of Mutants ; The procedure for isolation of mutants was followed as described before⁽¹⁰⁾.

Assay of Catalase Activity ; (i) Titration with permanganate : The activity was assayed with intact cells as described before⁽¹⁰⁾. (ii) Spectrophotometry with Extracts : cells were disrupted with a Bronson Sonifier (150w) treating three times each for 30 seconds. Extracts were obtained after unbroken cells and cell debris were removed by centrifugation at 6,500 x g for 15min. Catalase activities were measured with Hitach Spectro Photometer Model 356 in quartz cuvettes of 10mm light path. The assay procedure for measurement of catalase was followed by referring the method of Beers and Sizer⁽¹¹⁾. Reference cuvette contained 2ml of cell extracts, 0.8ml of 0.02M phosphate buffer (pH 7.0) and 0.4ml of water. Sample cuvette contained 2ml of cell extracts, 0.8ml of the 0.02M phosphate buffer, 0.2ml of water and 0.2ml of 0.2M hydrogen peroxide. Reactions were started by the addition of hydrogen peroxide and followed at 240nm for 2min at room

temperature. Specific activities were calculated as the decrease of the optical density of hydrogen peroxide per min per mg nitrogen of the extracts.

Determination of Nitrogen ; The nitrogen contents of the intact cells were determined indirectly using a standard curve drawn between the optical density value at 660nm and micro Kjeldahl method. The nitrogen contents of the cell-free extracts were determined indirectly using a standard curve drawn between the amount of protein and nitrogen per ml of extracts, assayed by the buiret and micro-Kjeldahl method, respectively.

Measurement of Spectra ; The reduced cytochrome spectra and pyridine hemeochrome spectra were measured by the method described before⁽¹⁰⁾. Oxidized minus reduced cytochrome spectra were measured by recording the optical density of the reduced sample against oxidized sample. The cuvette of oxidized sample contained cells (6.75mg nitrogen per cuvette) and 0.02M phosphate buffer in total volume of 3 ml and cytochromes were oxidized by addition of two drops of H₂O₂. The cuvette of the reduced sample contained cells (6.75mg nitrogen per cuvette), 0.02M phosphate buffer and a few grains of Na₂S₂O₄ in total volume of 3 ml. Spectra were measured with a Hitachi two wavelength spectrophotometer Model 356.

Results and Discussion

After nitrosoguanidine treatment was repeated three times, 25 colonies that didn't form bubbles from hydrogen peroxide were isolated from about 5000 colonies on nutrient-agar plates. These colonies were transferred to agar slants, cells grown under the defined condition and subjected to duplicate quantitative assay of catalase activity with titration method. The result of this measurement showed that cells from 19 colonies had almost normal level of catalase activity, but cells from six colonies, designated by the symbols, cat 2, cat 7, cat 8, cat 11, cat 14, cat 17, showed distinctly low activity.

The measurement of catalase activity by cells from 6 colonies were repeated five times and the activity of parent cells was usually measured together with the mutants. The relative activities of the cells from 6 colonies were shown in Fig 1. The relative activities were calculated as the mean value of catalase activities of mutants obtained in five independent measurements divided by the mean value of parent's catalase activity. The mutant having the lowest activity was designated as cat 11 and the activity was about 10 percent relative to the activity of parent. The mutant having the highest activity was designated as cat 17, whose activity was about 70 percent relative to the activity of parent. To confirm these results, photo electric method were used to measure the enzyme activity with cell-free extracts. The results were shown in Fig 2.

The pattern of the relative activities shown in Fig 2 were as same as that shown in Fig 1. As evident from Fig 1 and Fig 2 the genes controlling catalase in *Escherichia coli* K12 AB1157 were damaged to the different extent by the nitrosoguanidine treatment. It might be that cat 11 and cat 14 lost the most important site of genes controlling catalase, while cat 2 and cat 17 lost the most minor site of the genes or lost more easily reversible site of the genes. No colony that completely lost catalase activity was isolated. In this

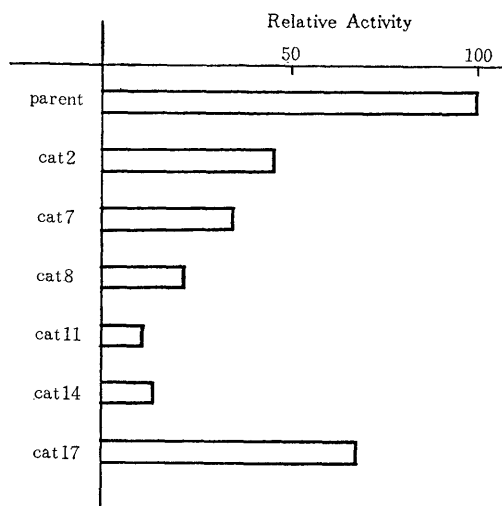


Fig 1. Relative activity of catalase of intact cells measured with titration by KMnO_4

The assay mixture and the assay procedure was described before⁽¹⁰⁾. The measurement of catalase activity was done five times. The value in this figure are the mean of five relative activities. The relative activities are calculated by the catalase activities in mutants divided by that of the parent.

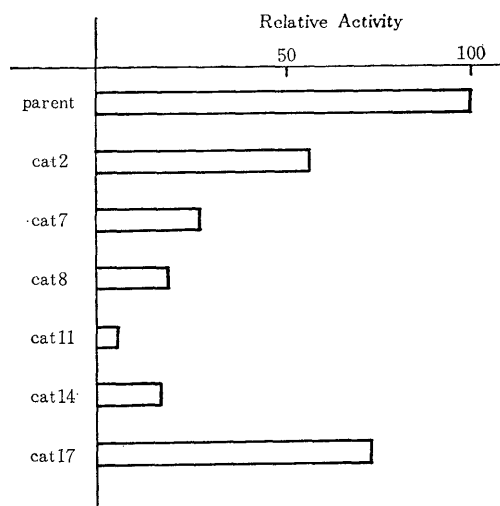


Fig 2. Relative activity of catalase in extracts measured with spectrophotometric method

The procedure to obtain cell-free extracts and assay of the activity are described in "Materials and Method". The relative activities in this figure are the means of four determinations.

respect, the genes controlling catalase might be difficult to be damaged by nitrosoguanidine treatment. From Fig 1 and Fig 2, it was shown that the result of the measurements of catalase activity with intact cell were almost the same as that of the measurement with cell-free extracts.

The curves in Fig 3 are reduced cytochrome spectra of parent and catalase-less mutants. The peaks and shoulders of the spectra are due to the presence of cytochrome b_1 (559, 530, 428nm), cytochrome d(635nm), and cytochrome a_1 (595nm). The peak at 500nm of the spectra of cells from parent, cat 2, cat 11, cat 14, cat 17 colonies are probably due to the presence of porphyrin because the peak disappeared in the oxidized-minus reduced cytochrome spectra (Fig 4).

The known catalase-less mutants except one mutant⁽¹⁰⁾ so far reported lacked cytochrome components but the mutants reported in this study have the normal quantity of cytochrome as shown in Fig 3 and Fig 4, although the quantity of cytochrome in cat 7 was about 60% of that of the parent. The mutant cat 11 has almost the same quantity of cytochromes as that of the parent but has only 10% of catalase activity of the parent. To examine the ability of the present catalase-less mutants to synthesize heme, the pyridine

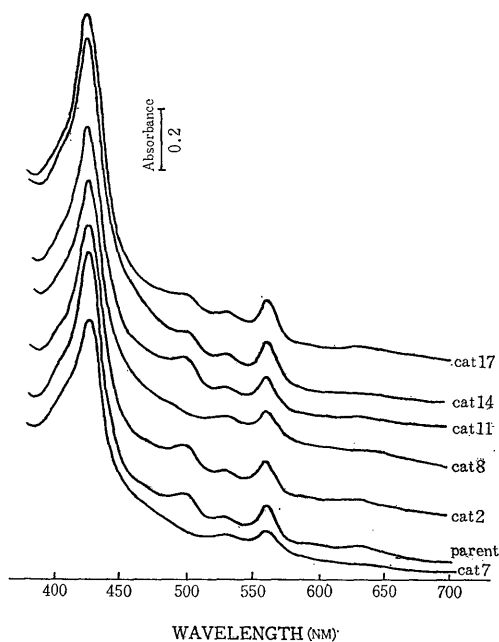


Fig 3. Reduced cytochrome spectra with intact cells

Each cuvette contained 6.7mg nitrogen of intact cells. Cytochromes were reduced with $\text{Na}_2\text{S}_2\text{O}_4$ and spectra were measured as described before⁽¹⁰⁾.

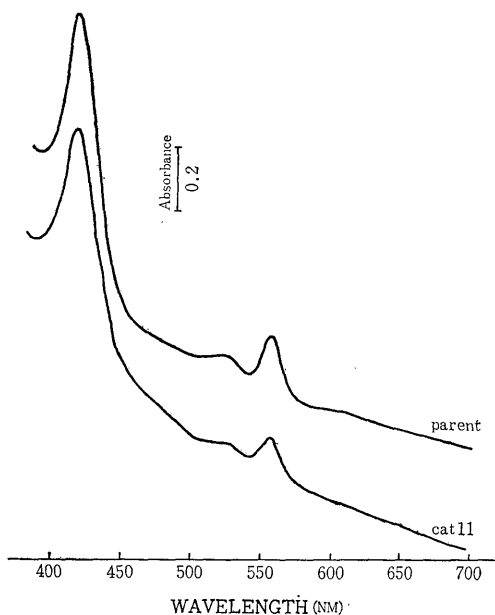


Fig 5. Pyridine hemochrome spectra

Each cuvette contained 3.1mg nitrogen of cell-free extracts. Procedures for measurement were described before⁽¹⁰⁾.

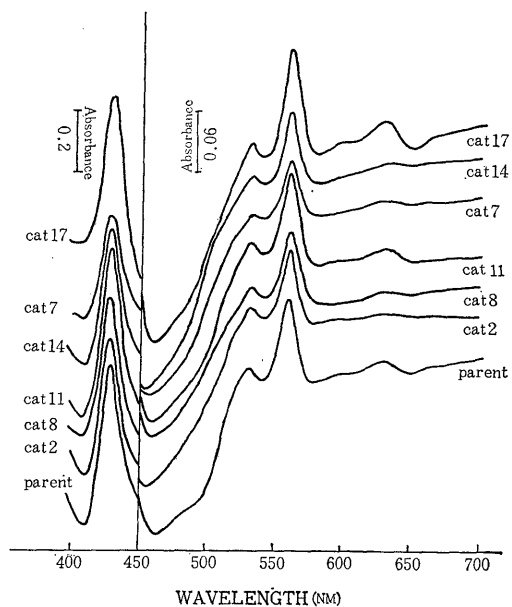


Fig 4. Oxidized minus reduced cytochrome spectra with intact cells

Each cuvette contained 6.75mg nitrogen of intact cell. Cytochrome were reduced with $\text{Na}_2\text{S}_2\text{O}_4$ and oxidized with 2 drops of 3% H_2O_2 . Procedures for measurement are described in "Materials and Methods"

hemochrome spectra of the mutant, cat 11, was measured (Fig 5). The protoheme contents of parent and cat 11 were calculated from difference in absorbancy between at 557nm (peak) and at 570nm, divided by protein-nitrogen per ml of the cell-free extracts in optical cuvettes. The result of such calculation showed that protoheme content of cat 11 was about 70% of that of the parent.

Although the ability of the present catalase-less mutants to synthesize protoheme fell down a little from that of the parent, the contents of cytochrom in

the mutants was almost the same with that of the parent. These facts suggest that the decrease of catalase activities in the present mutants was caused by the deficiency in catalase-apoprotein but not by the deficiency in heme-prosthetic group. The mutant having no catalase activity was not isolated through this and previous work and the mutant with the most diminished catalase activity still had 10% of the catalase activity of the parent. It is possible that a catalase isozyme occupying normally about 10% of the total activity is present in *Escherichia coli*. Although the mutant isolated in the previous study required casamino acids for growth, all of the mutants isolated in this work required only those amino acids that are required by the parent. Probably, all of the present mutants were caused by one point mutation. By the present method for isolating the catalase-less mutants, no mutant that completely lost the ability to synthesize heme has not been isolated.

Summary

Six mutants of *Escherichia coli* K12 AB1157 were isolated, the catalase activity of which was about from 10% to 60% of the activity of the wild type. Four mutants showed normal composition and contents of the cytochromes. Two mutants showed normal cytochrome composition, although having slightly less amounts of cytochromes than those of wild type. Measurement of the pyridine hemochrome spectra with the mutant cells having the lowest catalase activity, 10% of the parent, showed that protoheme content of the mutant was about 70% of the parent's level. These results suggest that the defects in catalase activity of mutants are not due to heme deficiency, but due to some defect in synthesis of catalase apoprotein, caused by mutation of a *cat* gene as described previously. All of the catalase-less mutants isolated in this work were most probably caused by one point mutation, so that the two mutants with about 10% of the catalase activity of parent may be useful for the analysis of the catalase gene in *Escherichia coli*.

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