KOREAN J. FOOD SCI. TECHNOL.
Vol. 10, *No.* 4 (*1978*)

Studies on the Microbial Glucose Isomerase

Part 3. Enzymatic Characteristics of Glucose Isomerase from *Streptomyces spp.* K-14

Moon-Hi Han and Tai-Wha Chung

Applied Biochemistry Laboratory, Korea Institute of Science & Technology, Seoul

(Received September 19, 1978)

微生物의 葡萄糖 異性化 酵素에 關한 硏究

(第三報) *Streptomyces* spp. K-14에서 生産된 葡萄糖 異性化 酵素의 特性에 關하여

韓 文 熙 · 鄭 兌 和

韓國科學技術硏究所 應用生化學硏究室

(1978년 9월 19일 수리)

Abstract

Enzymatic characteristics of glucose isomerase from *Streptomyces* spp. K-14 were studied. The optimum pH and temperature of the enzyme reaction are pH 7.5~8.0 and 70°~75°C, respectively, in the presence of 5 mM $MgSO_4 \cdot 7H_2O$ and 2 mM $CoCl_2 \cdot 6H_2O$. The enzyme activity was activated by both Mg^{++} and $Co.^{++}$ Mg^{++} is required for the initial activation of the isomerization reaction, whereas Co^{++} was essential for the increased stability of the enzyme protein. Glucose concentration up to 60% did not affect the reaction velocity as well as the equilibrium conversion of the enzyme.

Glucose isomerase (D-xylose ketol-isomerase, EC No. 5.3.1.5) is an enzyme which catalyzes isomerization reaction from glucose to fructose. In practice, microbial glucose isomerase has been widely used for the production of high fructose corn syrup, one of the popular sugar substitutes developed in the last decade.

In the course of study of microbial glucose isomerase, we were able to isolate a strain of *Streptomyces* spp. K-14 (KFCC 35051) which demonstrated high productivity of the enzyme. Microbial characteristics of the strain and cultural conditions for the production of glucose isomerase were previously reported[1,2].

In this paper, the authors report the results of enzyme characteristics of glucose isomerase obtained from *Streptomyces* spp. K-14. A particular emphasis on the effect of divalent cations such as Mg^{++} and Co^{++} on the enzyme activity was made in this communication.

Materials and Methods

1. Enzyme preparations

Streptomyces spp. K-14 was cultivated in a 5 l jar fermentor (Marubishi Model M.J. 5, Japan) or a 30 l Microferm fermentor (New Brunswick Scientific, N.J., U.S.A.) containing the medium described previously[2]. Having the culture been incubated for 48~72 hours, cells were harvested by centrifugation at 7,000 rpm for 20 min (Sorval RC2—B) and washed twice with distilled water. The collected

cells were used as a whole cell enzyme preparation. Or else otherwise, cells were suspended in distilled water and sonicated in a sonicator (Blackstone Model BP5) at a frequency of 20 KC for 10 minutes. The sonicated cells were centrifuged at 12,000 rpm for 10 minutes and the resulting supernatant was used as a soluble glucose isomerase preparation. The wet cell preparation contains about 94% water and was either stored at −20°C or freeze dried until being used.

2. Enzyme assay

The procedure for assaying the glucose isomerase activity was same as described previously[1]. Enzyme preparations used were either whole cell or cell free extract. One unit of the enzyme activity was defined to be 1 mg fructose produced for 1hr at a given condition. Or else otherwsie mentioned, the enzyme activity was measured at 70°C, pH 7.5, and 0.1 M substrate concentration.

3. Determination of fructose and glucose

Fructose was determined by the Cysteine-carbazole method[3] for the forward reaction of glucose isomerase. Glucose was determined by the enzymatic method with the glucose oxidase-peroxidase system[4] for the backward reaction of the enzyme.

4. Protein determination

Protein was dermined by the method of Lowry et al.[5] by using bovine serum albumin as a standard.

5. Materials

Glucose and fructose were obtained from Wako Chem. Co., Japan, albumin, O-dianisidine. 2HCl, glucose, and peroxidase were purchased from the Sigma Chemical Co., U.S.A. Other Chemicals were reagent grades.

Results

1. Effect of metal ions

Having been known that glucose isomerase is a metalloenzyme, the effect of metal on the enzyme was examined. The results are summarized in Table 1. It was found that 2 mM Mg^{++} and Co^{++} activated the enzyme activity significantly. Particularly, Mg^{++} was the most effective in the enzyme activation, The activation effect of Co^{++} and Mn^{++} were lower than that of Mg^{++}. Among other metal ions, 2 mM Ca^{++},

Table 1. Effect of metal ions on glucose isomeras activity

The reaction mixture contains 0.1 M D glucose, 50 ml phosphate buffer (pH 7.5 and 2 mM metal ions. The enzyme reactio was carried out at 70°C for 1 hour.

Salt	Fructose (mg/ml)	Rel. Act. (%)	Salt	Fructose (mg/ml)	Rel. Act (%)
None	13.1	100	$LiSO_4$	12.5	95
$MgCl_2$	20.4	155	$FeSO_4$	12.0	91
$MgSO_4$	20.0	153	$MnCl_2$	10.4	79
$CoCl_2$	18.6	142	$CaCl_2$	9.8	75
$CoSO_4$	18.4	140	$CaSO_4$	8.9	63
KCl	13.5	102	$ZnSO_4$	6.2	47
NaCl	13.0	99			

Mn^{++} and Zn^{++} markedly inhibited the enzyme activity, whereas Fe^{++}, Li^{++}, K^{+}, and Na^{+} at the same concentration had no effect.

In order to find the optimum concentration of Mg^{++} and Co^{++} for the maximum activation of glucose isomerase activity, effect of various concentration of the metal ions was examined (Fig. 1). The result demonstrated that the effect of Mg^{++} and Co^{++} on the enzyme activity is biphasic and the optimum concentration of Mg^{++} and Co^{++} are 6 mM and 2 mM, respectively.

Fig. 2 shows that the effect of Mg^{++} and Co^{++} concentration in the presence of either Co^{++} or Mg^{++} in fixed concentrations respectviely. It was demonstrated that the presence of both metal ions enhanced the

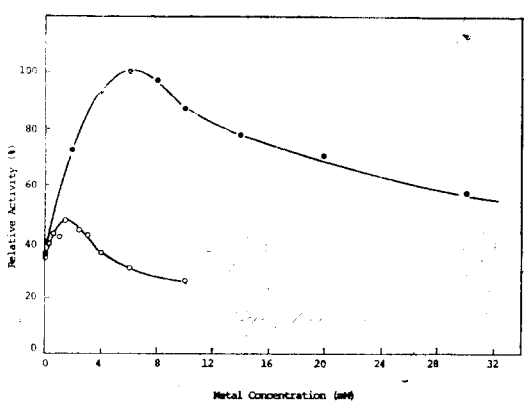

Fig. 1. Effect of Mg^{++}(•) and Co^{++}(○) concentrations on glucose isomerase activity. The assay conditions were described in Materials and Methods except 5 ml reaction volume.

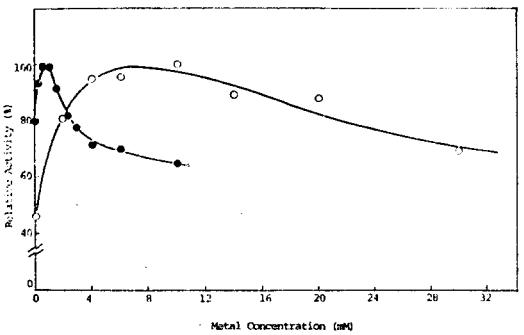

Fig. 2. Effect of Mg^{++}(•) and Co^{++}(○) concentrations on glucose isomerase activity in the presence of either Co^{++} or Mg^{++} in 2 mM and 5 mM respectively. The assay conditions were same as described in Fig. 1.

enzyme activity in addition to the activation effect of a single metal ion. The optimum concentration of Mg^{++} in the presence of 2 mM Co^{++} was 5~10 mM. while that of Co^{++} in the presence of 5 mM Mg^{++} was about 1.0 mM.

The results suggest that glucose isomerase activity from *Streptomyces* spp. K-14 is enhanced by both Mg^{++} and Co^{++}, although the effects of these two metal ions on the enzyme activity appear different as shown is the following experiments.

2. Effect of pH

As shown in the pH-activity profile of glucose isomerase (Fig. 3), the optimum pH of the enzyme appears to be about 7.5~8.0 regardless of the presence of metal ions.

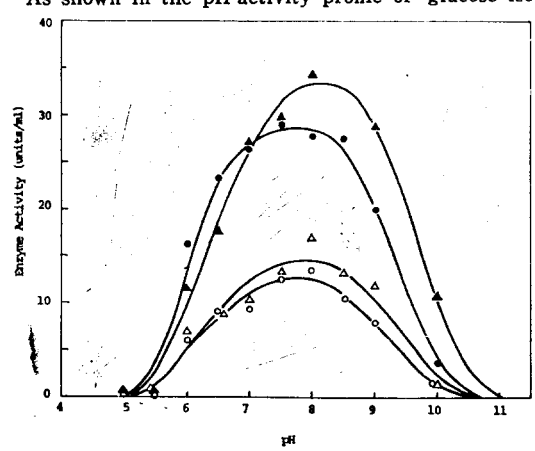

Fig. 3. Effect of pH on glucose isomerase activity in the presence of Mg^{++}(•), Co^{++}(▵), were counted after incubation of the treated both Mg^{++} and Co^{++}(▲), and no cations(○). The concentrations of Co^{++} and Mg^{++} were 2 mM and 5 mM respectively. 50 mM acetate buffer (pH 5-5.5), 50 mM phosphate buffer(pH 6-8), and 50 mM phosphate-NaOH buffer (pH 8-10) were used.

It is observed that the initial enzyme activity was markedly activated by Mg^{++} in all pH regions, whereas Co^{++} activated slightly the enzyme activity only in alkaline pH irrespective of the presence of $Mg.^{++}$

3. pH stability

Fig. 4 demonstrates the pH stability of glucose isomerase obtained from *Streptomyces* spp. K-14 with and without Mg^{++} and $Co.^{++}$ It is found that the enzyme is unstable in the acidic region and thus detected no activity at pH 5.0 after 12 hours incubation at 70°C, On the contrary to the metal effect on the initial activity, Mg^{++} demonstrated no effect on the stabilization of the enzyme whereas Co^{++} showed significant stabilization of the enzyme against acid inactivation resulting in increased enzyme activities at the given conditions.

4. Effect of temperature

Fig. 5 demonstrates viable cell counts and glucose isomerase activity after heat treatment of the culture broth, which contains 15 units of the enzyme, at various temperature from 40°C to 90°C for 10 min. It is noted that all cells are completely destroyed by the heat treatment at 70°C for 10 minutes, whereas glucose isomerase activity is stable against thermal

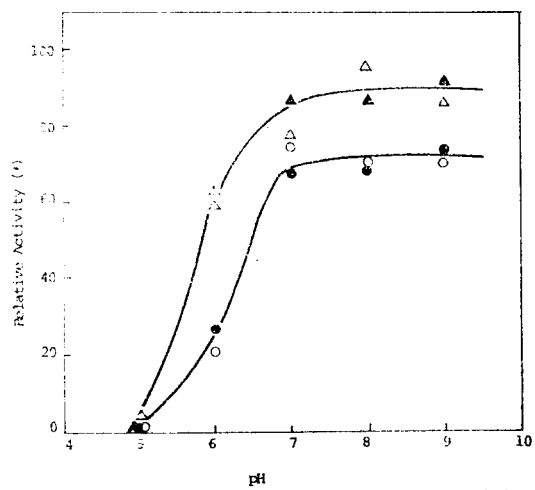

Fig. 4. pH stability on glucose isomerase activity in the presence of Mg^{++}(○), Co^{++}(▵), both Mg^{++} and Co^{++}(▲), and no cations (•). Enzyme activitywas measured after 12 hours incubation at 70°C. 2 mM Co^{++} and 5 mM Mg^{++} were used.

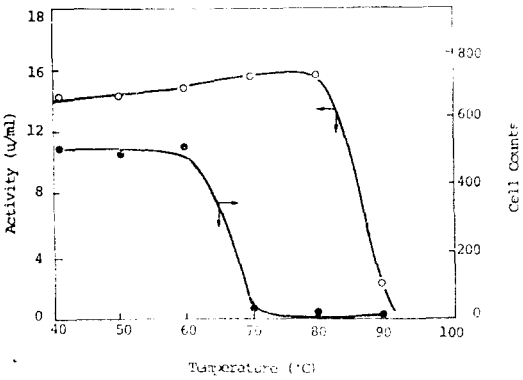

Fig. 5. Effect of temperature on viable cells(•) and glucose isomerase activity(o).
10 ml of culture broth containing 15 units of the enzyme per ml of broth was treated at the given temperature for 10 min. Viable cells were counted after incubation of the treated culture broth at 30°C for 48 hours on a nutrient agar plate.

inactivation up to temperature 80°C. However, the enzyme activity is sharply dropped by the heat treatment at 90°C for 10 minutes.

5. Thermal stabiltiy

In order to examine thermal stability of glucose isomerase from *Streptomyces* spp. K-14 for a longer time which is practically required for the process of glucose isomerization. It was found that the enzyme activity was retained more than 90% and 70% at 60° and 65°C, respectively, for 70 hours in the absence of divalent cations.

The thermal inactivation of the enzyme was markedly prevented by the addition of 2 mM Co^{++}. However, the addition of Mg^{++} did not alter the thermal stability of the enzyme (Fig. 6, 7). In most cases, the presence of 2 mM Co^{++} enhanced the thermal stability of the enzyme 4-5 times more than those without Co^{++} at pH 7.5. The half life of the enzyme inactivation was prolonged to 200 hours from 50 hours at 70°C by addition of 2 mM Co^{++} irrespective of the presence of Mg^{++}.

The results suggest that glucose isomerase from *Streptomyces* spp. K-14 is relatively stable for the period of 50-60 hours at temperature range of 60° and 70°C without and with Co^{++}, respectively.

6. Effect of substrate concentration

Fig. 8 shows kinetic patterns of glucose isomeriza-

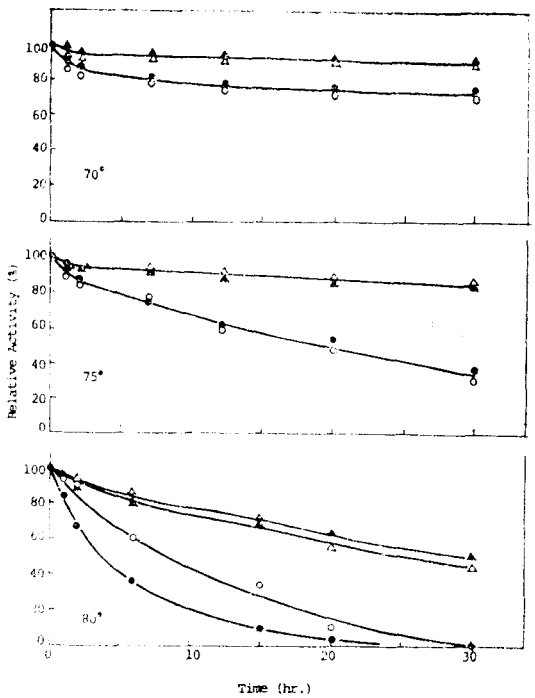

Fig. 6. Effect of temperature on glucose isomerase activity in the presence of Mg^{++}(o), Co^{++} (▵) both Mg^{++} and Co^{++}(▲), and no cations(•)◎ 2 mM Co^{++} and 5 mM Mg^{++} were used for the experiments.

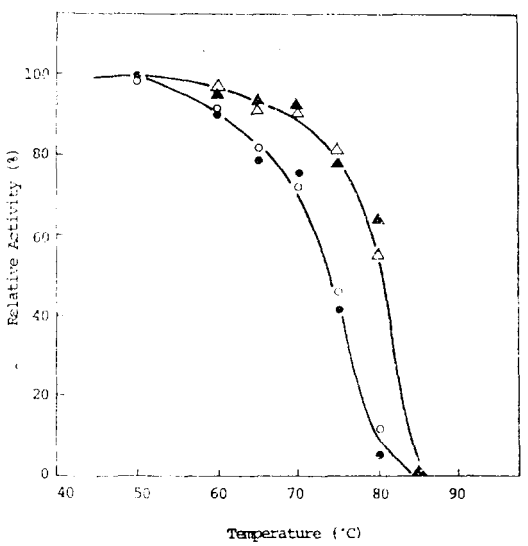

Fig. 7. Thermal stability of glucose isomerase activity in the presence of Mg^{++}(o), Co^{++} (▵), both Mg^{++} and Co^{++}(▵), and no cations(•) after 20 hours incubation at the given temperature.

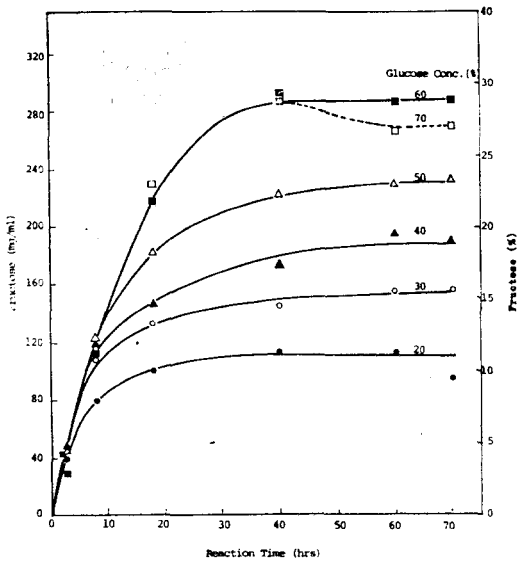

Fig. 8. Effect of glucose concentration on the glucose isomerization. 50 ml of reaction solution was incubated in 50 mM phosphate buffer pH 7.5 containing 10 mM of $MgCl_2$, 1 mM of $CoCl_2$ and 500 units of the enzyme at 65°C. After the indicated reaction time aliquats were taken and assayed fructose contents and pH of the reaction solution was adjusted to pH 7.5 by the addition of NaOH solution. •-•, 20% glucose; o-o, 30% glucose; ▲-▲, 40% glucose; △-△, 50% glucose; ■-■, 60% glucose; □-□, 70% glucose.

tion in the presence of varing concentratios of glucose. It demonstrates that the equilibrium of the isomerization process reached in about 40 hours in the presence of 500 mg glucose and 10 μ of the enzyme/ml of reaction volume at 65°C and pH 7.5. The equilibrium conversion was 44%. The equilibrium conversion was not affected by a high concentration of substrate up to 900 mg/ml (60%). However, at the glucose concentration in 70%, the extent of isomerization was decreased, markedly. It is, thus, demonstrated that 29 mg glucose can be converted by a unit of the enzyme in 40 hour process at the given conditions.

Discussion

Glucose isomerase is known to be a metalloenzyme which requires Mg^{++}, Co^{++}, or Mn^{++} depending on source of the enzyme (Table 2). Glucose isomerase from *Streptomyces.* spp. K-14 is also activated by Mg^{++} and Co^{++}. However, the activation meachanism of these two metal ions appears to be different since Mg^{++} activates the enzyme activity by increasing reaction velocity whereas Co^{++} activation is resulted by preventing inactivation of the enzyme. It is evident that Mg^{++} can activate the enzyme by increasing reaction rate of the isomerization process, while Co^{++} is essential for the conformational stability against thermal inactivation of the enzyme. In particular, Co^{++} is required for the prolonged reaction at temperature above 70°C.

Co^{++} can bind anionic site such as COO^- of the enzyme protein and thus brings about strengthening conformational integrity of the enzyme. This explains the diminished effect of Co^{++} for the enzyme stability in the acidic pH region below 5.0 where carboxylic group become protonated.

The optimum condition of glucose isomerase obtained from *Streptomyces* spp. K-14 is summarized and compared with the enzyme from other sources in Table 2. Glucose isomerase from *Streptomyces* spp. K-14 can be used in practice for the industrial production of high fructose corn syrup. The reaction condition for the glucose isomerization with the enzyme is recommended to be pH 7.5~8.0 and temp 70~75°C in the presence of 5 mM $MgSO_4 \cdot 7H_2O$ and 2 mM $CoCl_2 \cdot 6H_2O$.

The substrate concentration can be brought up to 60% without impairing the reaction velocity as well as equilibrium conversion. The extent of equilibrium conversion at the substrate concentration above 70% was markedly decreased. The theoretical equilibrium conversion was estimated to be 52~56%[24]. In practice, however, the actual conversion value was slightly lower than that of the theoretical value at the high substrate concentration. It appeared that the enzyme was inactivated by the rapidly liberating hydrogen ions during the isomerization process. It was actually observed that pH of the reaction mixture dropped from 7.5 to 6.5~6.0 in the initial 3 hour period when pH was not adjusted promptly. It is thus recommended that a coninuous pH adjustment is essential for the proper maintenance of the enzyme

Table 2. Properties of glucose isomerase from various microbial sources

Microorganisms	Form of Enzyme Preparation	Metal Ions Required	Optimal pH	Optimal Temp (°C)	Kmf (M)	Conversion Yield (%)	Ref.
Pseudomonas hydrophila	Whole cell	Mg, Mn, As	8.5	42	0.5	33	6
Aerobacter cloacae	Partially purified	Mn, Mg, Co	7.6	50	—	45~48	7
Aerobacter aerogenes	Whole cell	As	6.8	39	—	30	8
Lactobacillus brevis	Partially purified	Mn, Co	6.5	60	0.92	60	3
Escherichia intermedia	Whole cell	Mg, Co	7.0	50	—	45	10
Brevibacterium pentosoaminoacidicum	Partially purified	Co	8.0~8.5	70~75	0.5	47~52	11
	Whole cell	Co	8.3	90	—	50	
Arthrobacter spp. (ATCC 21748)	Whole cell	Mg	7.5	80~90	—	54	12
Bacillus megaterium	Crude extract	Mg	7.7	35	—	55	13
Bacillus coagulans	Purified	Mg	7.0~7.5	40	0.09	50	14
Bacillus stearothermophilus	Whole cell	Mg, Co	7.2~7.8	45	—	50	15
Streptomyces albus	Whole cell	Mg, Co	7.0	68	—	50	16
	Purified	Mg, Co	8.0~8.5	80	0.16	52	17
Streptomyces phaeochromogenes	Whole cell	Mg, Co	6.7~7.5	60	—	50	18
	Whole cell	Mg, Co	8.0~8.5	80	0.24	57	19
	Partially purified	Mg, Co	9.3~9.5	80	0.30	53	20
	Partially purified	Mg, Co	8.0	80	0.25	50~54	21
Streptomyces flavovirens	Whole cell	Mg, Co	8.5	85	0.15	—	22
Streptomyces spp. (ATCC 21175)	Whole cell	Mg, Co	7.0	70	—	50	23
Streptomyces spp. (ATCC 21176)							
Streptomyces spp. K-14 (KFCC 35051)	Whole cell	Mg, Co	7.5~8.0	70~75	0.3*	52~56*	present study *24
	Sonicated						

activity at a high substrate concentration applied.

The results presented in this communication suggest that glucose isomerase from *Streptomyces* spp. K-14 is just as good as any other enzyme sources. Industrial source of the enzyme which is being used at present are as follows: *Streptomyces* spp. (ATCC 21175), *Streptomyces phaeochromogenes*, *Bacillus megaterium*, and *Arthrobacter* spp.

요 약

Streptomyces spp. K-14에서 생성되는 포도당 이성화 효소의 특성에 대해서 연구하였다. 효소 반응의 최적 pH와 온도는 5 mM $MgSO_4 \cdot 7H_2O$와 2 mM $CoCl_2 \cdot 6H_2O$의 존재 아래에 각각 7.5~8.0 그리고 70°~75°C로 나타났다. 이러한 이성화 효소는 Mg^{++}과 Co^{++}두 양이온에 의하여 활성화 되었는데 Mg^{++}는 이성화 반응의 초기 활성화에 소요 되었으며 Co^{++}는 효소 단백질을 안정화 하는데 소요 되었다. 포도당 농도 60%까지는 효소 반응속도나 효소의 이성화율에 영향을 끼치지 않았다.

Acknowledgment

We are grateful to Mr. Hong-soo Lee for his enzyme analysis, and to Mr. Sae-hun Chung for the production of glucose isomerase throughout the experiments.

References

1. Chung, T. W., Kim, H. W. and Han, M.H.: *Korean J. Appl. Microbiol. Bioeng.*, **4**, 138 (1976).
2. Chung, T. W. and Han, M. H.: *Korean J. Appl. Microbiol. Bioeng.*, **4**, 145 (1976).
3. Dische, Z. and Borenfreund, E.: *J. Biol. Chem.*, **192**, 583 (1951).
4. Bergmeyer, H. U.: *Methods of Enzymatic Analysis*,

Vol. 3, Academic Press, New York, p. 1204 (1974).
5. Lowry, O. H., Rosebrough, N.J., Farr, A.C., and Randall, R. J.: *J. Biol. Chem.*, **193**, 265 (1951).
6. Marshall, R. O. and Kooi, E. R.: *Science.* **125**, 648 (1957).
7. Sato, T. and Tsumura, N.: *Agr. Biol. Chem.*, **29**, 1123 (1965).
8. Natake, M. and Toshimura, S.: *Agr. Biol. Chem.*, **27**, 342 (1963).
9. Yamanaka, K.: *J. Agr. Chem. Soc., Japan*, **37**, 231 (1963).
10. Natake, M.: *Agr. Biol. Chem.*, **30**, 887 (1966).
11. Ichimura, M., Hirose, Y., Katsuya, N. and Yamada, K.: *J. Agr. Chem. Soc., Japan*, **39**, 291 (1965).
12. Lloyd, N. E., Lewis, L. T., Logan, R. M., and Patel, D. N.: *U.S. Patent*, 3,817,832 (1974).
13. Takasaki, Y. and Tanabe, O.: *J. Agr. Chem. Soc. Japan*, **36**, 1013 (1962).
14. Yoshimura, S., Danno, G., and Natake, M.: *Agr. Biol. Chem.*, **30**, 1015 (1966).
15. Suekane, M., Kanno, M., and Hasegawa, S.: *U.S. Patent*, 3,826,714 (1974).
16. Takasaki, T.: *Agr. Biol. Chem.*, **30**, 1247 (1966).
17. Takasaki, Y., Kosugi, Y., and Kamibayashi, A.: *Report of Fermentation Research Institute*, **41**, 37 (1971).
18. Suzuki, S. and Tsumura, N.: *Japan Agr. Res. Quarterly*, **6**, 245 (1972).
19. Ryu, D. Y., Chung S. H., and Katoh, K.: *Biotech. Bioeng.*, **14**, 159 (1977).
20. Tsumura, N. and Sato, T.: *Agr. Biol. Chem.*, **29**, 1129 (1965).
21. Strandberg, G. W. and Smiley, K. L.: *Appl. Microbiol.*, **21**, 588 (1971).
22. Vaheri, M. and Kauppinen, V.: *Process Biochem.*, **12**, 5 (1977).
23. Cotter, W. P., Lloyd, N. E., and Hinman, C.W.: *U.S. Patent*, 3,623,953 (1971).
24. Han, M. H., Chung, T. W., and Park, Y. H.: *KIST Report* 493, 944-5 (1977).