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Extraction of Glucose Isomerase from *Streptomyces flavogriseus*†

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Cationic detergent (cetyltrimethylammonium bromide or cetylpyridinium chloride) treatment extracted almost the same amount of glucose isomerase from cells of *Streptomyces flavogriseus* as mechanical disruption (sonic oscillation or abrasive grinding). The specific activity of the enzyme extracted with cationic detergents was approximately 20% higher than that liberated by mechanical disruption.

Microbial enzymes are usually extracted by mechanical disruption (sonic oscillation, abrasive grinding, homogenization, or treatment with a French press) of the cell wall. The commonest method used in laboratories for the extraction of glucose isomerase is sonic disruption (2, 7, 10, 11). Abrasive grinding is also employed for the enzyme extraction (6, 12). However, mechanical disruption of the cell wall is time consuming and expensive and is not suited to large-scale production of enzymes.

Glucose isomerase was easily liberated by autolysis. Cationic detergents such as cetylpyridinium chloride, octadecyltrimethylammonium chloride, or dimethylbenzylalkylammonium chloride are reported to be utilized in the autolysis of cell suspensions containing glucose isomerase (4, 8, 9; W. P. Cotter, N. E. Nolan, and J. C. Chen [Standard Brands Inc.], Canadian patent 1,004,613, February, 1977). In addition to detergents, lysozyme, toluene, or a combination of lysozyme and toluene is used for the disintegration of the cell walls of enzyme-producing organisms (T. Hirota, T. Hishida, A. Kamata, I. Nakazawa, and H. Takamizawa [Mitsubishi Chemical Industries Co., Ltd.], Japanese patent 77,114,088, September, 1977; T. Sipos, German patent 2,061,371, January, 1971; M. Suekane, M. Kanno, and S. Hasegawa, U.S. patent 3,826,714, July, 1974). These findings provide a way for the economical extraction of glucose isomerase.

In this study, we used various chemicals to extract glucose isomerase from cells of *Streptomyces flavogriseus* and compared the yield and specific activity of the enzyme with those obtained by sonic disruption, abrasive grinding, or homogenization.

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A strain of *S. flavogriseus* was isolated from soil and used for this study. The organism was grown at 30°C for 48 h on a medium containing 1.0% straw hemicellulose, 2.5% corn steep liquor, and 0.1% $MgSO_4 \cdot 7H_2O$. The pH of the medium was adjusted to 7.0. Straw hemicellulose was prepared by the method of Chen et al. (1), and corn steep liquor was provided by Clinton Corn Processing Co. (Clinton, Iowa).

After the incubation period, the medium was centrifuged out, and the cell mass (mycelium) was washed twice with distilled water. The cell mass was resuspended in 0.05 M sodium phosphate buffer (pH 7.0). The resulting cell suspension contained 0.36% dry cells per ml and was used throughout the experiment. One of each of the chemicals shown in Table 1 was added to the cell suspension, and the mixture was incubated for 1 to 22 h at 37°C with or without shaking. Shaking decreased the extraction time somewhat, but did not affect the maximum enzyme level. A 20-ml amount of the cell suspension was mixed with 25 g of glass beads (Glasperlen, 0.11 to 0.12 mm, VWR Scientific Co.). The mixture was homogenized with a homogenizer (Brownwill model MSK) at 4°C for 1 min. A portion of the cell suspension was treated with a sonic oscillator (Biosonik IV, VWR Scientific Co.) at 4°C for 10 min. Another portion of the cell suspension was ground with sand at 4°C for 30 min. All treatments were followed by centrifugation at 12,000 × *g* and 4°C for 10 min to remove cell debris. The supernatant was used for the determination of glucose isomerase activity by a modified method of Dische and Borenfreund (3).

The cells suspended in 0.1% cetyltrimethylammonium bromide produced a maximum 3.02 U of enzyme per ml in 3 h (Table 1), and those in 0.1% cetylpyridinium chloride produced 2.92 U of enzyme per ml in 2 h. Similar levels of

TABLE 1. *Activity and specific activity of glucose isomerase extracted from cells of S. flavogriseus*

Treatment	Enzyme activity[a] (U/ml) at:				Sp act[b] (U/mg of protein) at:			
	1 h	2 h	3 h	22 h	1 h	2 h	3 h	22 h
Whole cells	2.65							
Chemical extraction								
Cetylpyridinium chloride (0.1%)	2.38	2.92	2.90	2.96	2.27	2.37	2.35	2.41
Cetyltrimethyl-ammonium bromide (0.1%)	2.14	2.62	3.02	3.00	1.90	2.12	2.42	2.36
Lysozyme (0.1%) + toluene (1.0%)	1.47	1.86	2.04	2.91	0.96	1.18	1.24	1.82
Toluene (1.0%)	0.96	1.63	1.98	2.87	0.94	1.12	1.27	1.79
Tween 80 (0.1%)	0.31	0.60	0.65	1.53	2.53	3.08	2.98	2.87
Dimethyl sulfoxide (0.1%)	0.42	0.52	0.67	1.45	3.62	3.25	3.58	3.21
Sodium lauryl sulfate (0.1%)	0.36	0.55	0.72	0.89	0.31	0.45	0.52	0.96
Dioctyl sodium sulfosuccinate (0.1%)	0.41	0.49	0.83	0.91	0.42	0.50	0.72	0.84
Mechanical disruption								
Sonic oscillation	2.97				1.94			
Abrasive grinding	2.87				1.91			
Homogenization	2.12				1.41			
Homogenization + cetyltrimethylammonium bromide (0.1%)	3.01				2.01			

[a] Amount of glucose isomerase liberated into the menstrum after incubation for the designated time period. One unit of enzyme activity was defined as the amount of enzyme that produced 1 μmol of D-fructose per min under the assay conditions described by Chen et al. (1).

[b] Specific activity of glucose isomerase was defined as 1 unit of enzyme activity per mg of protein. Protein was determined by the method of Lowry et al. (5).

enzyme were produced on cell-free extracts prepared by sonic disruption or abrasive grinding. The level of enzyme obtained from the supernatant of homogenized cells was significantly lower than those mentioned above, indicating that homogenization is not a very effective method of enzyme extraction. However, addition of 0.1% cetyltrimethylammonium bromide to the homogenized cells before centrifugation increased the enzyme yield to the same level. Whole cells without any treatment yielded a somewhat lower level of enzyme (2.65 U/ml) compared to those treated with cationic detergents, probably because the cell wall itself serves as a diffusion barrier. Anionic (sodium lauryl sulfate and dioctyl sodium sulfosuceinate) and neutral detergents (Tween 80) were ineffective; lysozyme and toluene were relatively less effective than cationic detergents (cetyltrimethylammonium bromide and cetylpyridinium chloride) in extracting the enzyme.

The specific activity of glucose isomerase extracted by different treatments varied (Table 1). The specific activity of the enzyme extracted with cationic detergents was higher than that liberated by mechanical disruption, but was the highest with dimethyl sulfoxide and Tween 80. However, because of the low yields, extraction with the latter two chemicals is not practical.

Takasaki and Kanbayashi (8) reported that the liberation of glucose isomerase was remarkably accelerated when a cationic detergent (cetylpyridinium chloride) was added to a cell suspension of *Streptomyces*. The yield of glucose isomerase from this treatment was almost complete compared with that by sonic disruption. Treatment of cell suspensions with lysozyme and toluene also proved to be a promising method for enzyme extraction (T. Hirota, T. Hishida, A. Kamata, I. Nakazawa, and H. Takamizawa [Mitsubishi Chemical Industries Co., Ltd.], Japanese patent 77,114,088, 1977; T. Sipos, German patent 2,061,371, January, 1971; M. Suekane, M. Kanno, and S. Hasegawa, U.S. patent 3,826,714, 1974). The results were similar to those obtained from this study; however, our study included detailed quantitative data on yields and release kinetics. Also, it quantitatively compared the effectiveness of alternate chemical treatments and mechanical disruption and such comparisons were not made in the previous work.

Not only is extraction of glucose isomerase by cationic detergents simple and inexpensive, but it yields the same level of enzyme as the sonic disruption and abrasive grinding methods. In addition, the specific activity of the enzyme extracted with cationic detergents was approximately 20% higher than that liberated by sonic disruption or abrasive grinding.

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