Binding of Enzymes to Microbial Cell

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In the previous papers^{1,2)} the author reported that heat treatment of glucose isomerase-containing cells of *Streptomyces* sp. at 60~85°C inactivates enzymes participating in the autolysis of cells and that glucose isomerase is not dissolved out of the cells even under favorable conditions for autolysis. On the basis of this finding the author investigated the binding of glucoamylase to microbial cells and found that many kinds of enzymes can be crosslinked with various microbial cells by bifunctional reagent such as tolylene diisocyanate, glutaraldehyde, etc. According to this method, glucoamylase can be bound to cells of *Streptomyces* which contain glucose isomerase.

Binding of glucoamylase to cells of *Streptomyces* sp. YT-No. 5 which is the producer of glucose isomerase is shown below as an example of a general preparation method for cell-bound enzymes.

Streptomyces sp. YT-No. 5 was grown on the reciprocal shaker in the medium (pH 7.0) containing 1% polypeptone, 1% glucose, 0.3% K₂HPO₄ and 0.1% MgSO₄·7H₂O in distilled water at 30°C. After 24 hr cultivation, cells were collected by filtration, washed with distilled water and suspended in distilled water (cell concentration 100mg/ml as dry substance). This cell suspension was heated at 65°C for 20 min. To 10 ml of the cell suspension, 2 ml of 20% of tolylene diisocyanate-water emulsion was added, and this mixture

was shaken at 30°C. After 30 min, the cell materials were filtered and washed with distilled water.

Aspergillus niger or *Rhizopus oryzae* was cultured in a medium containing 1% polypeptone, 2% sucrose, 0.3% KH₂PO₄, 0.1% MgSO₄·7H₂O in distilled water for 48 hr at 30°C on the reciprocal shaker. The preparation of tolylene diisocyanate treated-cells was carried out in the same way as described above.

The tolylene diisocyanate-treated cells were put into 10 ml of *Aspergillus niger* glucoamylase solution (54 units/ml) and shaken for 3 hr at 30°C. The cells were filtered, washed with distilled water and used for the enzyme assay.

The assay of glucoamylase was carried out in a water bath at 50°C in a mixture (20 ml, pH 4.5) of 2% soluble starch, 0.05 M acetate buffer and an adequate amount of the enzyme.

One unit was defined as the amount of enzyme which produces 1 mg of glucose per ml per hr. For the assay of cell-bound enzyme, the reaction was carried out by shaking.

Protein was determined by the method of Lowry *et al.*³⁾ by using bovine albumin as the standard.

Table I shows crosslinking of glucoamylase from *Aspergillus niger* with cells of *Streptomyces* sp. YT-No. 5, *Rhizopus oryzae* and *Aspergillus niger* by tolylene diisocyanate. The actual activity of the cell-bound glucoamylase was 52~82% of the calculated cell-bound enzyme activity (c) which was determined from the enzyme activity added (a) and the activity remaining after the washing (b). The specific activity of the cell-bound enzyme was expressed as the actual enzyme units per mg of cells. Among the cells tested, the specific activity of the *Streptomyces* cell-bound glucoamylase was the highest.

Table II shows the binding of various enzymes to cells of *Streptomyces* sp. YT-No. 5. Each preparation was carried out in the same way as described above except for the use of β -amylase, invertase, glucose isomerase, trypsin and glucose oxidase-catalase instead of glucoamylase. The losses in the enzyme activity by

TABLE I. BINDING OF GLUCOAMYLASE FROM *Aspergillus niger*
TO VARIOUS MICROBIAL CELLS

Microorganism	Activity (units)				Ratio assayed/ calculated bound	Specific activity (units/mg cell)
	Added ^{a)}	Washing ^{b)}	Calculated bound ^{c)}	Assayed		
<i>Streptomyces</i> sp.	1626.0	1488.0	138.0	86.7	62.8	1.73
<i>Aspergillus niger</i>	1626.0	1467.6	158.4	66.0	41.7	1.32
<i>Rhizopus oryzae</i>	1626.0	1573.5	52.5	42.9	81.7	0.86

^{a)} Original glucoamylase was 360.0 units/mg protein.

^{b)} Activity remaining in washing (unbound enzyme activity).

^{c)} Determined from units "Added" minus "Washing."

TABLE II. BINDING OF VARIOUS KINDS OF ENZYMES TO CELLS OF *Streptomyces* sp.

Enzyme	Activity (units)				Ratio assayed/ calculated bound	Specific activity (units/mg cell)
	Added	Washing	Calculated bound	Assayed		
β -Amylase ^{a)}	513.5	322.2	191.3	82.7	43.2	0.83
Invertase ^{b)}	568.2	467.2	101.0	66.1	65.4	0.66
Glucose isomerase ^{c)}	1170	600	570	468	82.1	4.68
Trypsin ^{d)}	5676	1568	4108	1159	28.2	11.6
Glucose oxidase-catalase ^{e)}	614.0	196.7	417.3	271.8	65.1	27.2

^{a)} One units of β -amylase (from wheat) was defined as the enzyme amount which produces 1 mg of maltose per min at 30°C in a reaction mixture (20 ml, pH 5.5) containing 2% soluble starch, 0.05 M acetate buffer and the enzyme. The specific activity of the original β -amylase used was 54.0 units/mg protein.

^{b)} One unit of invertase (from *Candida utilis*) was defined as the enzyme amount which produces 1 mg of glucose per min at 30°C in a reaction mixture (20 ml, pH 4.5) containing 0.1 M sucrose, 0.05 M acetate buffer and the enzyme. The specific activity of the original invertase was 60.4 units/mg protein.

^{c)} Assay of glucose isomerase (from *Streptomyces*) was carried out according to the method previously reported by the author.^{4,5)} The specific activity of the original glucose isomerase was 97.5 units/mg protein.

^{d)} Assay of trypsin was carried out according to the method of Northrop⁶⁾ and Anson.⁷⁾ The specific activity of the original trypsin was 540.6 units/mg protein.

^{e)} One unit of glucose oxidase-catalase system (Glucostat of Worthington Biochemical Corporation) was defined as the enzyme amount which diminishes 1 mg of glucose per hr at 30°C in a reaction mixture (10 ml, pH 7.0) containing 0.1 M glucose, 0.05 M phosphate buffer and the enzyme. The specific activity of the original enzyme was 67.5 units/mg protein.

binding these enzymes on the cells of *Streptomyces* were 56.8% (β -amylase), 34.6% (invertase), 17.9% (glucose isomerase), 71.8% (trypsin) and 34.9% (glucose oxidase-catalase).

The characteristic of this enzyme immobilization method is the use of microbial cells as the enzyme carrier. This method can be applied to the process using two (or more) enzymes. If one of them is an intracellular enzyme and the other an extracellular enzyme, the extracellular enzyme can be bound to the cells which contain the intracellular enzyme. For example, glucoamylase can be bound to the cells of *Streptomyces* sp. which contain glucose isomerase, so that by using this enzyme preparation the direct production of fructose is possible from dextrin or starch.

The enzymatic properties of the cell-bound glucoamy-

lase will be reported in a forthcoming paper.

REFERENCES

- 1) Y. Takasaki, "Fermentation Advances," ed. by D. Perlman, Academic Press Inc., New York, London, 1969, p. 561.
- 2) Y. Takasaki, *Report of Fermentation Research Institute*, **37**, 31 (1970).
- 3) O. H. Lowry, N. J. Rosenbrough, A. L. Farr and R. L. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
- 4) Y. Takasaki, *Agr. Biol. Chem.*, **30**, 1247 (1966).
- 5) Y. Takasaki, *ibid.*, **33**, 1527 (1969).
- 6) J. H. Northrop, *J. Gen. Physiol.*, **16**, 41 (1932).
- 7) M. L. Anson, *ibid.*, **22**, 79 (1938).