A C T A A L I M E N T A R I A P O L O N I C A

Vol. XIII (XXXVII), No. 4 1987

ANDRZEJ KRAKOWIAK

IMMOBILIZATION OF GLUCOSE ISOMERASE ON COLLAGEN BY THE METHOD OF MEMBRANE IMPREGNATION AND CHARACTERISTIC OF THE OBTAINED COMPLEX

Institute of Fermentation Industry, Warszawa

Key words: *Actinoplanes missouriensis*, collagen, glucose, isomerase, membrane impregnation method.

Conditions of immobilizing on collagen glucose isomerase isolated from *Actinoplanes missouriensis* cells by the method of membrane impregnation were optimized. The respective properties of immobilized and free enzyme were compared and glucose was isomerized in a continuous process in a single-stage reactor.

The previous publication [2] contains a detailed discussion of various techniques of immobilizing on collagen microorganism cells and isolated enzyme. The author subsequently performed experimental immobilization of microorganism cells containing glucose isomerase with the macromolecular complexation technique and characterized the obtained complex. The studies confirmed the advantages of this method; the results are given elsewhere [5].

In immobilizing the enzyme isolated from cells by membrane impregnation it was also necessary to determine the basic parameters of the process such as the times of impregnation and of the collagen membranes' contact with glutaraldehyde as well as optimum concentration of the latter. All these findings served to optimize parameters of enzyme immobilization ensuring on the one hand carrier polymerization, and on the other the formation of an enzyme-carrier complex that would permit enzyme elution during enzymatic catalysis. The available literature does not contain information about glucose isomerase immobilization on collagen in general, or about the membrane impregnation method in particular. This prevents comparisons with similar research by others authors. Older materials that are available — all in unpublished form — report immobilization with this method of other enzymes such as L-asparaginase, pectinase, urease, hespe-

ridinase [1, 6-8]. The authors of these reports assessed this immobilization method favourably.

The objective of the present research was to optimize parameters of immobilization with the membrane impregnation method of glucose isomerase isolated from *Actinoplanes missouriensis* cells, to characterize the obtained complex, and to determine its usefulness in continuous glucose isomerization in a single-stage reactor.

METHODS

1. Microorganism and carrier. The experiments were performed with the microorganism species *Actinoplanes missouriensis* obtained by a previously devised submerged culture technology [3, 4]. The carrier for the immobilized enzyme was collagen from ox tendons obtained from Eastern Research Laboratories, Philadelphia, Pa., U.S.A.

2. Isolation of glucose isomerase from *Actinoplanes missouriensis* cells. This was done by continuous disintegration of cells in a Cole-Parmer apparatus in which cells were ground between two rough metal surfaces. 30 g of *Actinoplanes missouriensis* cells, dried at room temperature, were added to 300 ml of distilled water pH 7.8 which was earlier treated with 0.01 M $MgSO_4 \times 7H_2O$ and 0.001 M $CoCl_2 \times 6H_2O$. The water suspension of cells was twice subjected to continuous disintegration. The ground microorganism cells were then separated from the enzymatic solution in a Sorval centrifuge. The obtained solution with glucose isomerase was used in enzyme immobilization experiments.

3. Preliminary collagen preparation and glucose isomerase immobilization. Collagen was homogenized in a water environment (pH 7.8) at 4-6°C. The comminuted collagen was then transfered to a suction flask, pressure below atmospheric was produced by a vacuum pump and air was removed from the collagen mass. This mass, already without air bubbles, was shaped into a membrane and dried at room temperature in an air flow. Impregnation was performed at 4 and 20°C by immersion of the membrane in the enzymatic solution obtained by the method described above. The times of impregnation were 2, 6, 10, 14, 18 and 22 h. The humid membrane containing the enzymatic protein was dehydrated in room-temperature air flow until air-dry mass was obtained. The binding of the enzyme to the carrier was done by immersing the dried membrane in a water solution of glutaraldehyde. Glutaraldehyde concentrations were 0.5, 1.0, 3.0 and 5.0% and times of contact with the membrane were 1, 2, 3, 4, 5 and 6 min. Following the prescribed time the membrane was removed from the glutaraldehyde solution and immediately transferred into running water where it was rinsed for 2 h in order to remove the excess glutaraldehyde.

The washed membrane with immobilized enzyme was dried at room temperature.

4. Determination of the degree of collagen membrane polymerization. In order to determine the stability of the carrier, the dry collagen membrane was cut into 5 × 5 mm squares and immersed for 70 h in water heated to 80°C. Samples of the membrane were taken at 10-h intervals and the degree of their dissolution was determined visually through comparisons with analogous membrane samples which were not incubated in water.

5. Parameters characterizing the properties of glucose isomerase were determined according to the method given in [5].

6. Enzymatic reactor. Glucose isomerization was a continuous process in a single-stage glass reactor described in [5]; the parameters of the process are also given in that publication.

7. The isomerized substrate was a 1 M solution of commercial glucose prepared according to the method given in [5].

8. The degree of glucose isomerization to fructose was calculated according to the formula given in [5].

9. Glucose isomerase activity was determined according to the method given in [5].

RESULTS AND DISCUSSION

The time of contact of the enzyme with the collagen membrane had a significant effect, unlike the temperature of impregnation (Fig. 1). The temperature of 4°C retarded the saturation of the membrane with the

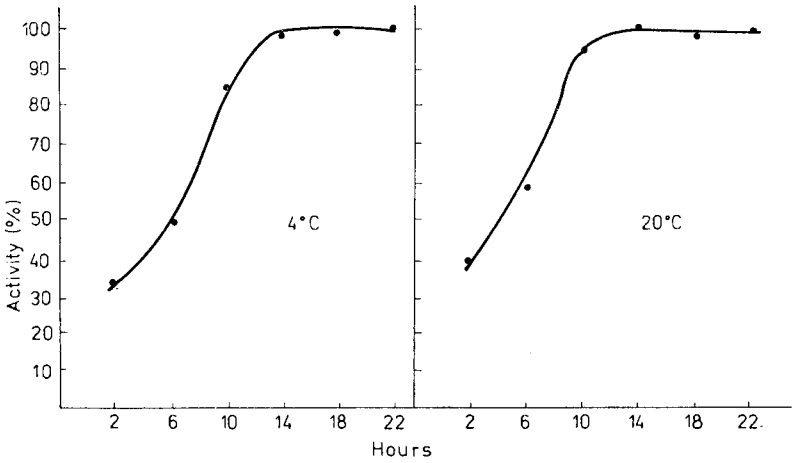

Fig. 1. Effect of temperature and time on degree of collagen membrane impregnation glucose isomerase

enzyme by about 10% in comparison with an analogous process at 20°C, this being probably due to slower swelling of collagen in lower temperatures. Regardless of the temperature of the process, the membrane was fully saturated with the enzyme after 12-14 h.

As shown in Fig. 2, the lowest inactivation of the enzyme occurred when glutaraldehyde concentration was 0.5%; it amounted to a mere 6% of initial activity in the 6th minute. At 5% glutaraldehyde concentration

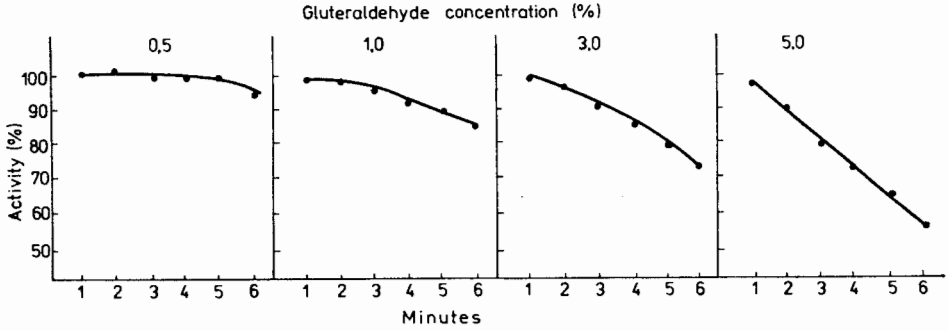

Fig. 2. Effect of glutaraldehyde concentration and time of the reagent's contact with the collagen membrane on glucose isomerase activity

the activity decrease in the 6th minute was already about 44% of initial activity. A very important factor in enzyme immobilization by the discussed method, in addition to binding of the enzyme to the carrier, was simultaneous polymerization of collagen. Given this fact, the full usefulness of the collagen carrier was determined by studying its ability to dissolve in a water medium following immobilization. The collagen membrane samples taken for analysis were kept in various concentrations of glutaraldehyde for various periods. The obtained results are presented in Table. As can be seen, the 0.5% concentration and times of 1-6 min did not suffice to polymerize the collagen membrane which dissolved in water completely. Thus, despite the fact that at this concentration the losses of activity were the lowest (Fig. 2) it could not be practically employed to promote isomerization in an enzymatic reactor. The carrier also failed to polymerize at glutaraldehyde concentration of 1% (Table) during 1-6 min. The obtained results (Table, Fig. 2) indicate that the optimal glutaraldehyde concentration is 3% and the briefest time of contact is 3 min. In these conditions an insoluble collagen membrane is produced, and the loss of glucose isomerase activity amounts to 5%.

The effect of temperature on the activity of immobilized and free glucose isomerase is illustrated in Fig. 3. The results show that the immobilized enzyme was most active at 72°C while the free enzyme — at 65°C.

T a b l e. Effect of glutaraldehyde concentration and time on the stability of collagen membrane

Stability of collagen during incubation in water heated to 80°C (h)	Glutaraldehyde concentration (%)																							
	0.5						1.0						3.0						5.0					
	time of contact (min)						time of contact (min)						time of contact (min)						time of contact (min)					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
10	−	−	−	+	+	+	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
20	−	−	−	−	−	−	+	+	+	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++
30	−	−	−	−	−	−	−	+	+	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++
40	−	−	−	−	−	−	−	+	+	+	+	+	+	++	++	++	++	++	++	++	++	++	++	++
50	−	−	−	−	−	−	−	−	−	+	+	+	+	++	++	++	++	++	++	++	++	++	++	++
60	−	−	−	−	−	−	−	−	−	−	+	+	+	+	++	++	++	++	++	++	++	++	++	++
70	−	−	−	−	−	−	−	−	−	−	−	−	−	+	++	++	++	++	++	++	++	++	++	++

− dissolved collagen
+ partly dissolved collagen
++ undissolved collagen

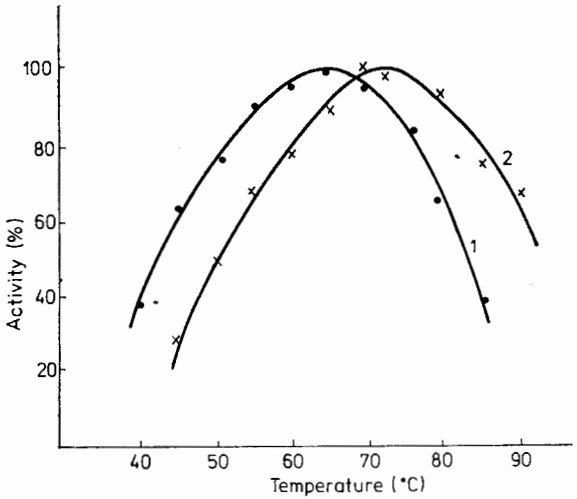

Fig. 3. Optimal temperature of activity of free and immobilized glucose isomerase; 1 — free isomerase, 2 — immobilized isomerase

From Fig. 4 showing the effect of pH of the medium on the activity of immobilized and free glucose isomerase we see that optimal activity of the former was at pH 7.8 and of the latter at pH 7.4.

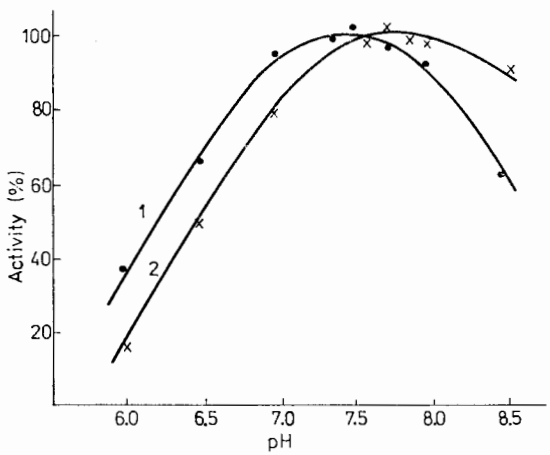

Fig. 4. Optimal pH of activity of free and immobilized glucose isomerase; 1 — free isomerase, 2 — immobilized isomerase

Thermostability of both enzyme forms is illustrated in Fig. 5. The curves show that immobilized glucose isomerase was more resistant to elevated temperatures than the free enzyme. At 90°C the immobilized enzyme lost about 30% of its initial activity, while the free enzyme lost as much as 65% of this activity.

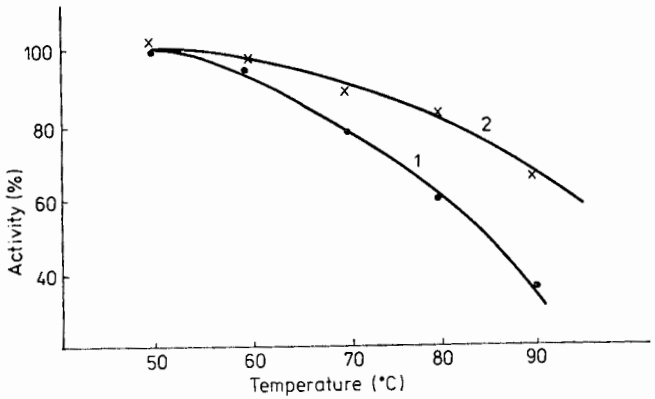

Fig. 5. Thermostability of free and immobilized glucose isomerase; 1 — free isomerase, 2 — immobilized isomerase

The stability of the immobilized enzyme was studied by using it to isomerize glucose in an enzymatic reactor. The results of this experiment are illustrated in Fig. 6. During 360 h of the process enzymatic activity dropped by about 22%, while the degree of glucose isomerization was on average 21.5%. These results confirm the stable activity of immobilized glucose isomerase.

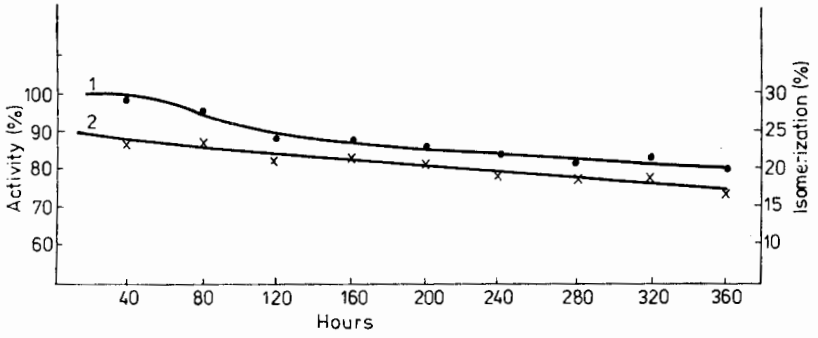

Fig. 6. Continuous glucose isomerization in a reactor; 1 — activity, 2 — isomerization

The results of this research may be compared with those reported by the author in [5] despite different immobilization techniques (membrane impregnation vs. macromolecular complexation) and different methods of preparing the material for immobilization (enzyme isolated from cells vs. cells containing the enzyme) that were employed in the two investigations. It was demonstrated beyond doubt that regardless of the immobilization technique, it is always necessary to experimentally determine the optimal glutaraldehyde concentration. In the case of membrane impregnation this concentration was 3% but in the case of macromolecular complexation comparable effects were obtained already at 0.5% glutaraldehyde con-

centration [5]. Optimum temperatures and pH were practically the same in both immobilization methods for free enzyme and for the enzyme in free cells. Greater differences were apparent during determinations of thermal stability of free preparations and those immobilized with both methods. When the enzyme was immobilized by macromolecular complexation its activity at 90°C decreased by about 20% in comparison to the initial activity; the figure for the enzyme in free cells was about 50% [5]. The enzyme immobilized by membrane impregnation lost about 30% of its initial activity in this temperature; this same loss in the free enzyme was as much as 65%.

The usefulness of both methods of immobilization was tested in practice by performing prolonged continuous isomerization of glucose in an enzymatic reactor. After 400 h [5] and 360 h of uninterrupted catalysis the losses of glucose isomerase were small (22.8 and 22%, respectively) and in this respect the differences between the two methods of immobilization were practically none. The obtained results prove that both methods are equally applicable in continuous isomerization processes. The method of preparing the material to be immobilized (i.e. either enzyme isolated from cells or cells containing glucose isomerase) is also of no consequence [5].

CONCLUSIONS

1. Membrane impregnation as a technique of immobilization on collagen ensures the binding of glucose isomerase to the carrier.
2. The concentration of glutaraldehyde and the time of its contact with the collagen membrane affected the activity of the enzyme and the degree of carrier polymerization.
3. Immobilization of glucose isomerase on collagen changed the properties of the enzyme as regards optimum temperature and pH and also its thermostability.

LITERATURE

1. Balaji S.: Preparation and characterization of collagen immobilized hesperidinase, non published.
2. Krakowiak A.: Przemysł Spożywczy 1981 (5-6), 25.
3. Krakowiak A.: Prace Inst. Lab. Bad. Przem. Spoż., 1981, 35, 39.
4. Krakowiak A.: Prace Inst. Lab. Bad. Przem. Spoż., 1981, 35, 51.
5. Krakowiak A.: Acta Alimentaria Polonica 1987, **13 (XXXVII)** (4), 359.
6. Olanoff L. S., Bernath F. R., Venkatasubramanian K.: Reduction on canine serum asparagine levels by L-asparaginase immobilized on collagen, non published.
7. Sheiman M. I.: Properties application of free and immobilized pectic enzymes, non published.

8. Zweibel S. R.: Some studies of urease immobilized on collagen, non published.

Manuscript received: February, 1987
Author address: 02-532 Warszawa, Rakowiecka 36

A. Krakowiak

UNIERUCHAMIANIE IZOMERAZY GLUKOZOWEJ NA KOLAGENIE METODĄ IMPREGNACJI BŁONOWEJ I CHARAKTERYSTYKA OTRZYMANEGO KOMPLEKSU

Instytut Przemysłu Fermentacyjnego, Warszawa

Streszczenie

Optymalizowano warunki unieruchamiania metodą impregnacji błonowej wyizolowaną z komórek izomerazę glukozową. Określano właściwości unieruchomionego enzymu w stosunku do analogicznego wolnego oraz prowadzono izomeryzację glukozy w reaktorze jednostadiowym w układzie ciągłym. Stwierdzono, że temperatura środowiska i czas kontaktu izomerazy glukozowej z nośnikiem miały wpływ na proces impregnacji błony kolagenowej enzymu i wynosiły odpowiednio 20°C i 12 h (rys. 1) Optymalne stężenie aldehydu glutarowego niezbędne do unieruchomienia izomerazy glukozowej powinno wynosić 3%, zaś czas konieczny do spolimeryzowania nośnika 3 min (rys. 2, tabela). Unieruchomiona izomeraza glukozowa wykazywała optimum działania w temp. 72°C zaś wolna w temp. 65°C (rys. 3). Optymalne pH działania unieruchomionej izomerazy glukozowej występowało przy 7,8 zaś wolnej przy 7,4 (rys. 4). Po 1 h inkubacji w temp. 90°C unieruchomiona izomeraza glukozowa traciła ok. 30% swojej pierwotnej aktywności zaś enzym wolny inaktywował się w analogicznej temperaturze w ok. 65% (rys. 5). Podczas 360 h prowadzenia procesu katalizy w reaktorze enzymatycznym następowało obniżenie aktywności enzymatycznej o ok. 22% w stosunku do początkowej zaś stopień izomeryzacji glukozy wynosił średnio 21,5% (rys. 6).