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Glucose-6-phosphate dehydrogenase adsorbed on collodion membranes*

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SUMMARY

The adsorption to and desorption from a collodion membrane of yeast glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate:NADP oxidoreductase, EC 1.1.1.49) is described. Of the enzyme adsorbed onto the matrix, that which adsorbed initially (1.75 μg per 0.59 cm^2 of membrane) retained about 50 percent of its activity. A four-fold increase in enzyme adsorption led to an increase in activity corresponding to only 5 percent of the original activity of the adsorbed enzyme. The adsorbed enzyme could be desorbed from the matrix, a desorption that had proven to be highly dependent on the presence of the specific substrates for this enzyme, *i.e.*, NADP and glucose 6-phosphate.

Collodion membranes have proven to be a useful matrix for the immobilization of enzymes[1-3]. In this communication we describe the adsorption to and desorption from a collodion membrane of yeast glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate:NADP oxidoreductase, EC 1.1.1.49). Even though the membrane had a high capacity for adsorbing glucose-6-phosphate dehydrogenase, the enzymic activity on the membrane reached a maximum at an early stage in the adsorption process. In contrast to other enzymes adsorbed on collodion membranes, adsorbed glucose-6-phosphate dehydrogenase was highly unstable.

The adsorption pattern of the enzyme on a collodion membrane was determined by immersing membrane discs of 0.59 cm^2 and 200 μm thick into a 1 ml solution consisting of 1 mM NADP, 0.1 M Tris buffer (pH 8.1, Sigma Chemical Corp.), and 14 μg glucose-6-phosphate dehydrogenase (*Torula* yeast, type X, Sigma). This adsorption solution was stirred constantly. At specified times, aliquots of the adsorption solution were assayed for enzyme activity in a mixture consisting of 1 mM NADP, 1 mM glucose 6-phosphate and 0.1 M Tris buffer (pH 8.1), by following the rate of NADPH production at 340 nm in a Zeiss PMQII spectrophotometer.

*Abbreviation used: PMBS, *p*-chloromercuriphenyl sulfonic acid.

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Fig. 1 shows that under three different conditions, most of the enzyme activity was exhausted from the solution during a 4-h period. At 2°, with or without NADP, the enzyme activities disappeared from solution at about the same rates, while at 27° (with NADP) it disappeared significantly faster.

In contrast, the enzyme activities associated with the corresponding "enzyme–membranes" (*i.e.* enzyme adsorbed on the collodion membrane) did not increase proportionally (Fig. 2). Of the enzyme adsorbed to the membrane, that which adsorbed initially retained a high percentage of its activity; *e.g.*, 50% of the enzyme which adsorbed within the first 15 min (1.75 μg), was active. Only 5% of the enzyme adsorbed between 0.5 h and 4 h (7 μg) was active even when adsorption was carried out at 2° in the presence of NADP.

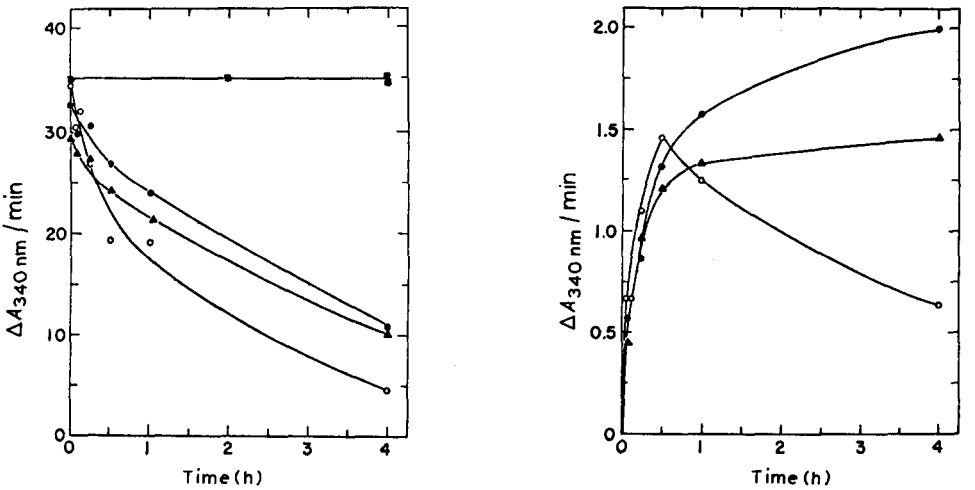

Fig. 1. Time course of uptake of glucose-6-phosphate dehydrogenase activity from an adsorption solution by a collodion membrane. The adsorption mixtures consisted of approximately 14 μg of enzyme dissolved in 1 ml Tris buffer, 0.1 M (pH 8.1) and a collodion membrane (0.59 cm^2). Two adsorption mixtures were 1 mM in NADP; one (○—○) was incubated at 27° and the other (●—●) was incubated at 2°. The adsorption mixture without NADP (▲—▲) was incubated at 2°. Separate adsorption mixtures were used to obtain each of the given points. (■—■): controls for the activities of the three adsorption mixtures (without membranes). At the specified times, 5-μl aliquots of the adsorption solutions were assayed.

Fig. 2. Enzyme activity associated with enzyme–membranes obtained under different adsorption conditions. Adsorption conditions and symbols are given in the legend to Fig. 1. At the specified times, the membranes were removed from the adsorption solutions and were assayed in a 3-ml quartz cuvette (10 x 10 mm) containing 1 ml of 0.01 M NADP, 0.01 M glucose 6-phosphate and 0.3 M Tris buffer (pH 8.1). The cuvette was placed in a 27° water bath, and the solution was agitated by means of a magnetic stirrer. The reaction was initiated by dropping the enzyme–membrane, which had been blotted between two sheets of absorbent paper, into the stirred solution. At 1-min intervals, a quartz block (8 x 9 mm) was inserted into the cuvette and the absorbance was read at 340 nm. Thus, it was possible to read the high absorbances obtained and to carry out the entire procedure in the same reaction vessel.

The reaction was carried out for 10 min, at which time the membrane was removed and the block was placed into the solution. The absorbance was recorded at 1-min intervals for an additional 5 min in order to measure the enzyme activity desorbed from the enzyme–membrane during the 10 min assay. At most, 2.5% of the substrates were consumed during the assay.

Under the three differing conditions of the experiment, a rapid increase in enzyme–membrane activity occurred during the first 30 min of adsorption. In the experiments carried out at 2°, the increase in activity of the enzyme–membrane taking place in the interval starting at 30 min and up to 4 h was markedly slower; the one conducted in the presence of NADP showed a slightly faster rate of increase in enzyme–membrane activity. At 27° even in the presence of NADP, the activity of the enzyme–membrane decreased by 55% during the same 3.5-h period. The inactivation of the enzyme–membrane at 27° reflects the temperature instability of the adsorbed enzyme; native glucose-6-phosphate dehydrogenase was virtually 100% stable under the experimental conditions used (Fig. 1).

To eliminate the possibility that the disappearance of enzymic activity from the adsorption solution might be due to the enzyme adsorbing to the membrane, inactivating there and desorbing back into the solution, we determined the amounts of protein and the amounts of activity removed from solution during the adsorption process. There was a good correlation between those values (Table I).

The pattern of inactivation of glucose-6-phosphate dehydrogenase appears to be related to the course of adsorption of proteins to the collodion matrix. Previous studies[1,3] indicated that enzymes adsorbed on collodion membranes coat the pores of the matrix with a monomolecular layer of protein. The adsorption advances along sharp boundaries starting with the outer surfaces of the membrane and saturating infinitesimally thin, consecutive layers until eventually the whole membrane is saturated with enzyme. This adsorption pattern leads to the formation of layers of sharp boundaries, the thicknesses of which depend on the time of adsorption, the amount and concentration of the enzyme in the adsorption solution and the capacity of the membranes for the protein in question[1,3]. We obtained essentially the same pattern of adsorption with glucose-6-phosphate dehydrogenase.

Accordingly, the membrane enzymic activity observed during the first 15 min of absorption could be due to enzyme adsorbed to the outer surface of the membrane. Perhaps, here, fewer bonds are involved in the association of the enzyme and the matrix than are involved in the association of enzyme molecules adsorbed on inner parts of the membrane. The inactivation observed with glucose-6-phosphate dehydrogenase is not common to enzymes adsorbed on collodion membranes; *e.g.*, papain (EC 3.4.4.10)[1] and alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1)[3] retained 80–100% of their activity when adsorbed on the membrane regardless of the amount of enzyme adsorbed.

TABLE I

ENZYME ACTIVITY AND PROTEIN CONTENT OF AN ADSORPTION SOLUTION BEFORE AND AFTER A 4-h INCUBATION AT 2° WITH A COLLODION MEMBRANE [a]

The adsorption solution consisted of the specified amount of glucose-6-phosphate dehydrogenase in 1 ml 0.05 M phosphate buffer, pH 8.1.

Sample	*Enzyme activity* ($A_{340\ nm} \cdot min^{-1} \cdot ml^{-1}$)	*Protein*[4] (*μg*)
Before adsorption	218	78
After adsorption [b]	47 ± 6	21 ± 3

[a] A disc of 0.59 cm^2.
[b] Results are averages obtained in three experiments.

Attempts were made to protect glucose-6-phosphate dehydrogenase from inactivation while it was being adsorbed on the membrane. No significant protection was given by Mg^{2+}., dithioerythritol, phosphate, glucose, albumin or by pretreatment of the membrane with EDTA. In fact, less enzyme was adsorbed to the membrane when albumin was present in the adsorption solution because it competed for binding sites on the collodion.

Preliminary experiments showed that the thiol reagent *p*-chloromercuriphenyl sulfonic acid (PMBS) inactivated *Torula* yeast glucose-6-phosphate dehydrogenase and that NADP protected the enzyme from PMBS. Since during adsorption the enzyme was inactivated even in the presence of NADP or dithioerythritol, inactivation on adsorption did not appear to involve the oxidation of an essential thiol.

The adsorption of the enzyme to the membrane can be partially reversed by placing the enzyme–membrane in solutions devoid of enzyme. Because we have observed that enzyme adsorption is dependent upon both the presence of NADP and the concentration of Tris (0.05 M being optimal), we studied the effects of the three components of the assay mixture on desorption of the enzyme (Table II).

The desorption pattern was highly dependent upon the components of the solution. About 48% of the activity of the enzyme–membrane desorbed in 10 min in the presence of both substrates (Table II, line 3), whereas in the presence of one substrate, NADP, 40% desorbed. At both conditions, however, virtually no activity was lost during desorption (Table II, compare Column D, lines 2, 3 and 4).

In the absence of both substrates, (Table II, line 1) on the other hand, only 10% of the activity of the enzyme–membrane desorbed (Column A), and the total activity of this enzyme–membrane (line 1, Column D) was about 20% lower than that of the control and of the desorption experiments with one or two substrates present (lines 2 and 3, Column D). Thus, during desorption in the absence of substrates, significant enzyme

TABLE II

DESORPTION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE ASSOCIATED WITH COLLODION MEMBRANES, AS AFFECTED BY Tris, NADP AND GLUCOSE 6-PHOSPHATE

The adsorption solution consisted of 1 ml Tris buffer, 0.1 M (pH 8.1) and 14 μg crystalline enzyme. The membrane was incubated in this solution for 15 min at 2°. Results are expressed in $A_{340\ nm} \cdot min^{-1}$.

Components of desorption solution [a]	*A Activity desorbed* [b]	*B Residual activity of membrane* [c]	*C Activity desorbed during assay (B) of membrane* [d]	*D Total activity associated with membrane (A + B)*
Tris	0.087	0.747	0.278	0.834
Tris + NADP	0.445	0.673	0.227	1.118
Tris + NADP + glucose-6-phosphate	0.510	0.556	0.125	1.066
No treatment		0.967	0.458	0.967

[a] The desorption solution consisted of 3 ml Tris buffer, 0.1 M (pH 8.1), with or without 1 mM NADP and 1 mM glucose 6-phosphate.
[b] Each enzyme–membrane was stirred for 10 min at 2° in the desorption solution, after which time the membrane was removed and the solution was assayed.
[c] The enzyme–membrane was assayed for 10 min at 27° as described in the legend of Fig. 2.
[d] Activity found in assay solution (see footnote c) after removal of the enzyme–membrane.

inactivation occurred. Such an inactivation is not due to a high dilution of desorbed enzyme because controls showed that similar amounts of the soluble enzyme are stable under the same conditions.

The data in Table II suggest that the substrates facilitate desorption. Possibly local changes are induced which allow more rapid desorption of the enzyme.

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