# HEPATIC MICROSOMAL MEMBRANE: ACTIVATION OF GLUCOSE-6-PHOSPHATASE

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## 1. Introduction

Liver glucose-6-phosphatase is a constitutive enzyme firmly bound to endoplasmic reticulum [1]. Membrane localization and orientation of the enzyme may be visualized to play a role in the vectorial release of glucose from liver and regulation of blood glucose. Formation and distribution of the enzyme in developing hepatocytes has been used to study biogenesis and differentiation of the membrane [2]. Like other membrane-bound enzymes the activity is increased, but becomes very labile when solubilized with detergents [1]. Similar increase in the activity also occurs when the pH is increased by 1 M $NH_4OH$ [3] or $Al_2O_3$ [4] and the enzyme becomes more stable. We have shown [5] that pyridoxal-5′-phosphate (PLP) inactivates the enzyme, by competing with the substrate and by reaction at the active site. This has offered us an opportunity to investigate the mechanism of activation by $NH_4OH$ which seems to be the method of choice as recently shown by Arion et al. [6]. The rationale of the present experimental approach are: [1] glucose-6-phosphatase activity expressed in the absence of $NH_4OH$ is due to exposed enzyme sites which are accessible to the substrate or its competitor, and [2] the activity increment which results after $NH_4OH$ treatment is due to enzyme sites which are not accessible to substrate or its competitor in the absence of any dispersing agent. It follows from such a model that if one partially inactivates the enzyme by reaction with PLP and then treats with $NH_4OH$ (raising the pH), percent activation (ratio of enzymatic activity with and without $NH_4OH$ x 100) of the inactivated sample would be higher than that of the control or unreacted sample. If reaction conditions for inactivation are such that there is no significant non-specific reaction of PLP with the membranous components then percent activation would increase with increasing degree of inactivation.

## 2. Experimental

Lyophilized microsomes [4, 5] were prepared from a 10% homogenate (0.25 M sucrose) of liver from normally-fed rat (200 g body weight). Inactivation by PLP was as described [5], and incubation was carried out for 15 min at 30°C. The control consisted of mixing PLP and 50-fold molar excess lysine before adding microsomes. The residue obtained after washing the reaction mixture four times was resuspended in ice-cold 0.25 M sucrose. The suspension was made 0.1 M in $NH_4OH$ by slowly adding 1 M $NH_4OH$ and allowed to stand at 0°C for 22 hr. An equivalent volume of water was added to the control sample. Glucose-6-phosphatase activity was measured at 30°C in 0.1 M acetate (pH 6.5) and 0.04 M glucose-6-phosphate, as described and is expressed as μmoles of inorganic phosphorous liberated per 10 min/mg protein.



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Table 1
Activation of glucose-6-phosphatase after inactivation by pyridoxal-5'-phosphate (PLP).

| | Enzyme activity* Without $NH_4OH$ | With $NH_4OH$ | Percent increase (after $NH_4OH$ treatment) |
|---|---|---|---|
| Control | 1.165 ± 0.037 | 1.435 ± 0.068 | 23.2 |
| Inactivation with 10 mM PLP | 1.09 (8.5)** | 1.40 | 28.4 |
| Inactivation with 18.5 mM PLP | 0.71 (39.1) | 0.97 | 36.6 |
| Inactivation with 25 mM PLP | 055 (52.8) | 0.84 | 52.7 |
| Inactivation with 35 mM PLP | 0.44 (62.3) | 0.61 | 38.6 |

*Micromoles of phosphorous liberated per 10 min/mg protein at 30°C.
**Numbers in parentheses represent percent inactivation.

## 3. Results and discussion

Results of $NH_4OH$ activation at four different levels of inactivation by PLP are shown in table 1. On the average, enzymatic activity of the control was increased after $NH_4OH$ treatment by 23% (20–25.4) which is similar to the reported values [9]. When the microsomal preparation was slightly inactivated (8.5%) by reacting with PLP the percent activation was increased to 28. When percent inactivation was 40 and 50 (by PLP), the percent activation (by $NH_4OH$) was increased to 36.6 and 52.7, respectively. The activity increase (the difference between with and without $NH_4OH$) appears to be constant at the three different levels of inactivation. It is also significant that at greater degrees of inactivation, namely 62%, the percent activation by $NH_4OH$ was 38.6 which is lower than that observed at 50% inactivation. These results suggest that at higher concentrations of PLP there could be some inactivation of unexposed or latent enzyme molecules which may be due either to reaction at those enzymic sites or to reaction at non-specific sites of the membrane components.

In order to study the effect of $NH_4OH$ on the sedimentability of glucose-6-phosphatase, distribution of the enzyme activity was measured in the supernatant and residue fractions after the microsomal suspension was treated with $NH_4OH$ and centrifuged at 105 000*g* for 1 hr. About 90% of the enzymatic activity (table 2) appeared in the supernatant after $NH_4OH$ whereas less than 10% appeared in the control. Electron microscopy has shown that integrity of the microsomal membrane structure was retained after $NH_4OH$ treatment [7] but it was not so after detergent treatment. Potassium cyanate reacts faster with $NH_4OH$ treated microsomes suggesting that the treatment has increased reactible amino groups (our unpublished experiments). By weakening the binding forces dependent on cationic amino groups in the membrane [8] mild alkali treatment may not only expose more enzymic sites but also decrease the density of membrane vesicles.

Other interpretations of these observations are

Table 2
Effect of $NH_4OH$ on the sedimentability of glucose-6-phosphatase.*

| Treatment | Protein (mg) | Glucose-6-phosphatase activity |
|---|---|---|
| Control | 65.0 | 39.8 |
| Supernatant (105 000*g*) | 15.3 (23.5)** | 2.4 (6) |
| Residue | 49.7 (76.4) | 37.4 (94) |
| $NH_4OH$ | 73.0 | 72.0 |
| Supernatant (105 000*g*) | 67.3 (92) | 61.3 (85.1) |
| Residue | 5.7 (7.8) | 10.7 (14.9) |

*Microsomal suspension in 0.25 M sucrose was treated with 1 M $NH_4OH$ (to make it 0.1 M) or water and left to stand at 0°C for 22 hr. The resulting mixture was centrifuged at 105 000*g* for 1 hr at 0°C.
**Numbers in parentheses represent the percent.

also possible. For example, reaction with PLP may not hinder $NH_4OH$ dependent conformational changes, but it would seem unlikely that it would lead to a higher percent of activation than that elicited with the unreacted control. Also, removal of ribosomes from membranes may not be the cause of activation [7]. Experiments using conventional membrane dispersing agents, like detergents, have not yet been successful since washing the microsomes after reaction with PLP further increases the lability of glucose-6-phosphatase activity. Whether the inaccessibility of some glucose-6-phosphatase enzymes sites is a consequence of in vitro homogenization or in vivo orientation of the enzyme is not presently clear. However, activation studies with microsomes from glucocorticoid treated animals support the latter [9]. The idea that inaccessibility of several glucose-6-phosphatase sites may be due to their being buried in the membrane matrix is in keeping with the present concepts of membrane as a fluid mosaic structure [10].

In summary, these data are consistent with the concept that liver endoplasmic reticulum contains two kinds of glucose-6-phosphatase active sites. We have shown that the readily accessible sites can be inactivated by PLP and that subsequent $NH_4OH$ treatment can then expose the less accessible sites.

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