Transport of Multiple Enzymes through Semipermeable Membrane and Effect of Surfactants

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ABSTRACT

Surfactants are known to influence functions of many proteins in membranes, cells and tissues. For the three metabolically important enzymes (namely, glutamate dehydrogenase (GDH), lactate dehydrogenase (LDH), and malate dehydrogenase (MDH)), relative activities could vary more than 35 % with 1 ppm difference in anionic surfactant concentrations in solution. For the four anionic surfactants used in this study, there seemed to be a pH dependence on how hydrophilicity would affect single cellar protein transfer across a semipermeable membrane in solution. For the three enzymes with all the surfactant used in this study at pH 6.95, the amount of normalized mass transferred of individual enzyme across a semipermeable membrane was consistently in the same descending order: LDH, GDH, and MDH. For multiple enzymes without surfactant, pH would affect the permeability of the smallest enzyme (MDH) more than the largest enzyme (GDH) between pH range of 6.5 to 7.4. At pH 6.95, 0.1 ppm of hydrophobic surfactant in solution with GDH and MDH would nearly double the net mass transfer of GDH but revealed no significant enhancement to MDH.

Keywords: surfactant, multiple enzyme transport, dehydrogenase, permeability, membrane

1. Introduction

Surfactants are known to influence functions of many proteins in membranes, cells and tissues. Because most studies to elucidate the effects of surfactants on membrane-bound proteins and cells employed heterogeneous or complex systems, it is difficult to extrapolate the results of such studies to delineate the effects of surfactants on a single protein. We therefore systematically investigated how anionic surfactants of different hydrophilicities affected three metabolically important enzymes (namely, glutamate dehydrogenase (GDH), lactate dehydrogenase (LDH), and malate dehydrogenase (MDH)) of various molecular masses at a pH range relevant to body functions (6.5-7.4). Activity of enzyme protein of larger molecular mass (GDH) in solution showed less variation compared to those with smaller molecular masses (LDH and MDH), with changes in pH, hydrophilicity, and surfactant concentration. For LDH and MDH, relative activities could vary more than 35 % with 1 ppm difference in surfactant concentrations [1]. All three enzymes were more active in hydrophilic than in hydrophobic surfactants. LDH activity also showed time dependent decreases with different surfactant concentrations. Thus, our results suggest that, for results to be comprehensive, surfactant effects should be studied with a wide range of concentrations and also with time as another variable. The outcome of these studies prompted us to investigate the effects of surfactants on enzyme protein transport across a semipermeable membrane.

For the four anionic surfactants used in this study, there seemed to be a pH dependence on how hydrophilicity would affect single cellar protein transfer (across the membrane).
For the three enzymes with all the surfactant used in this study at pH 6.95, the amount of normalized mass transferred across a semipermeable membrane was consistently in the same descending order: LDH, GDH, and MDH.

This report concentrated on how the anionic and non-ionic surfactants would affect the permeabilities of these three enzymes simultaneously between pH 6.5 to 7.4, and how the phenomena can be realistically extrapolated to body functions.

2. Materials and Methods

Materials and experimental setup were similar to our previous report except that 0.1 ppm of surfactant was employed to the right half-cell with multiple enzymes in solution [2]. The surfactant concentration was chosen because the surfactant effect on enzymes was relatively mild, as determined in our previous study [1].

3. Results

As shown in Figure 1, surfactants can drastically affect the activities of enzyme, although their effect on larger molecular enzymes are less than those of smaller enzymes [1], particularly at lower concentrations. Figure 2 to 4 show how pH would affect multiple enzyme transport through a semipermeable membrane: It appeared that without surfactant, MDH (MM 70,000) was most affected by the pH variation compared to GDH (MM 2,200,000), although the pH effect was relatively mild. Figure 5 to 7 show how 0.1 ppm of hydrophilic, hydrophobic, and non-ionic surfactant affected MDH and GDH transport across the semipermeable membrane at pH 6.95. The non-ionic surfactant (Triton x-100) slightly enhanced the mass transfer of both MDH and GDH (Figure 3 and 7), and hydrophilic surfactant slightly suppressed the mass transport of GDH. However, mass transport of GDH was more than double with the hydrophobic surfactant and the total mass (normalized activity) transfer was more than double of MDH as well, while the same surfactant only mildly enhanced the mass transport of MDH at pH 6.95. The fact that the molecular mass of GDH is about 30 times of MDH, the mass transfer phenomena of these enzymes we observed were totally reversed of the conventional diffusional theory. This also implied that other molecular forces were more dominating than molecular diffusion in the system we studied.

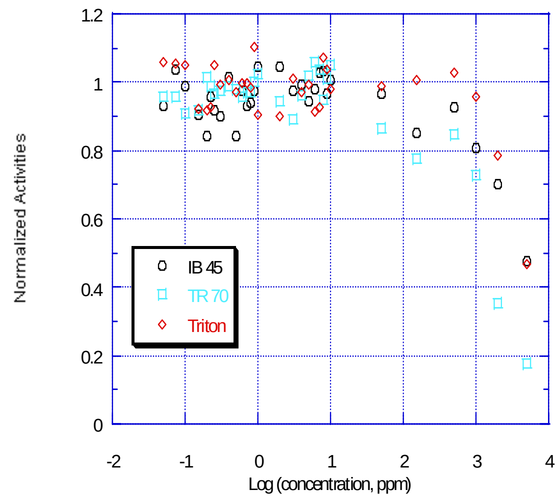

Figure 1: Effects of surfactants on GDH activities of pH 6.95.

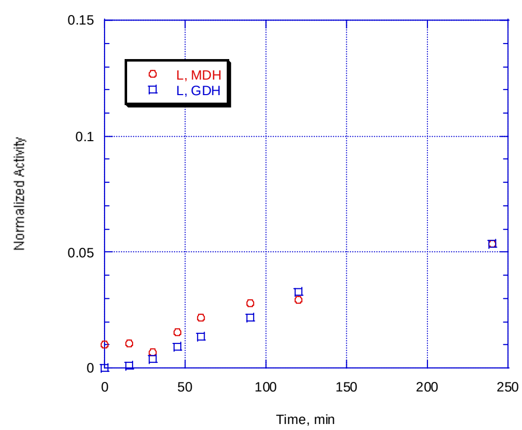

Figure 2: Enzyme Activities of GDH and MDH (after permeation through the semipermeable membrane) in the left half-cell at pH 6.5 and absence of surfactant.

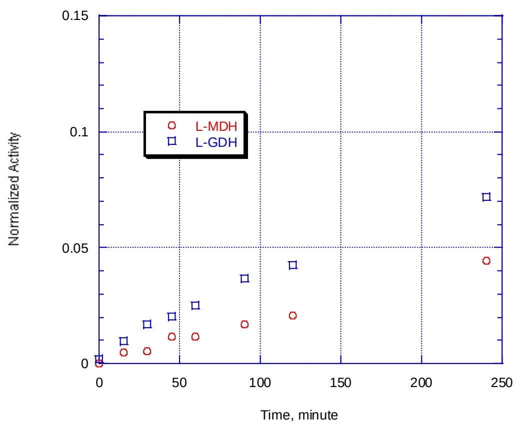

Figure 3: Enzyme Activities of GDH and MDH in the left half-cell at pH 6.95 and absence of surfactant.

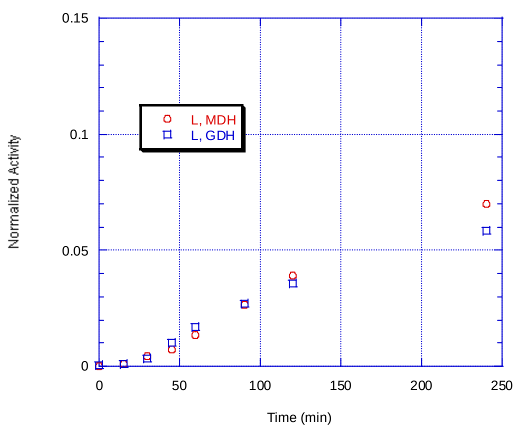

Figure 4: Enzyme Activities of GDH and MDH in the left half-cell at pH 7.4 and absence of surfactant.

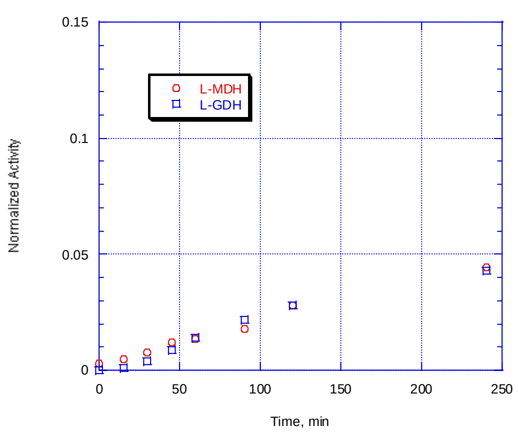

Figure 5: Enzyme Activities of GDH and MDH in the left half-cell at pH 6.95 and 0.1 ppm of IB 45 hydrophilic surfactant.

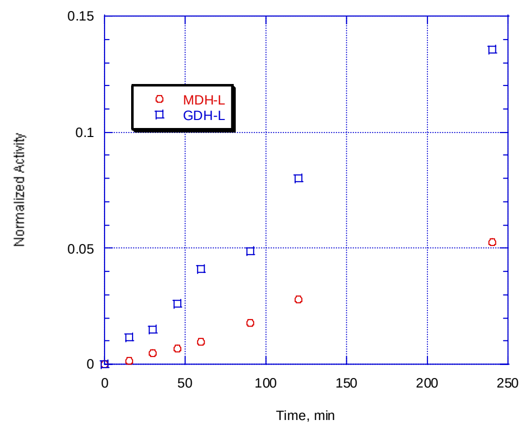

Figure 6: Enzyme Activities of GDH and MDH in the left half-cell at pH 6.95 and 0.1 ppm of TR 70 hydrophobic surfactant.

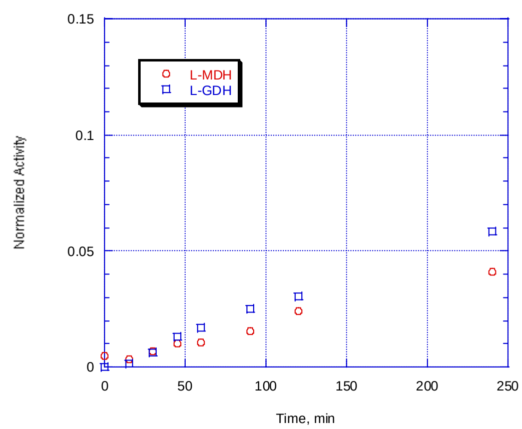

Figure 7: Enzyme Activities of GDH and MDH in the left half-cell at pH 6.95 and 0.1 ppm of Triton x-100 non-ionic surfactant.

Acknowledgements

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References

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