# Differential Effects of Surfactants on Enzyme Activity and Transport across a Semipermeable Cell

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## ABSTRACT

This study systematically investigated how anionic surfactants of various hydrophilicities affected the activities of three metabolically important enzymes — glutamate dehydrogenase (GDH), lactate dehydrogenase (LDH), and malate dehydrogenase (MDH) — of different molecular masses at a pH range important to body functions (6.5-7.4). We also investigated the time course of the surfactant concentration dependent effects on LDH. To observe how surfactants would affect the cellar protein transport, a separation cell with a semipermeable membrane was used to simulate a passive mass transport phenomenon between cells, with various concentration combinations of enzymes and surfactants.

***Keywords***: surfactants, dehydrogenase, membrane, separation, permeability

## 1. INTRODUCTION

Surfactants can drastically affect the functions of many proteins, thereby exerting their effects on functions of membranes, cells, and organs. Numerous studies have purportedly elucidated the effects of surfactants on cells or membrane-bound proteins employing heterogeneous or complex systems that contain many macromolecular structures and/or organelles. Consequently, it is difficult to extrapolate the results of such studies to delineate the effects of surfactants on single proteins. Thus, predicting how surfactants affect cellar protein transport with existing knowledge is nearly impossible.

We systematically investigated how anionic surfactants of various hydrophilicities affected the activities of three metabolically important enzymes — glutamate dehydrogenase (GDH), lactate dehydrogenase (LDH), and malate dehydrogenase (MDH) — of different molecular masses at a pH range that is important to body functions (6.5-7.4). We also investigated the time course of the surfactant concentration dependent effects on LDH. As a "standard" for comparison with results obtained using the anionic surfactants, we conducted similar studies with the well characterized non-ionic surfactant, Triton X-100. To observe how surfactants would affect the cellar protein transport, a separation cell with a semipermeable membrane was used to simulate a passive mass transport phenomenon between cells, with various concentration combinations of enzymes and surfactants.

## 2. MATERIALS AND METHODS

### 2.1 Enzyme proteins

Three well-characterized enzyme proteins were used in this study. They were: L-glutamate dehydrogenase (EC 1.4.1.3; G-2626; Sigma Chemical Co., St. Louis, MO; from bovine liver, MW 2,200,000), L-lactate dehydrogenase (EC 1.1.1.27; L-2518; Sigma Chemical Co.; from rabbit muscle, MW = 135,000), and L-malate dehydrogenase (EC 1.1.1.37; M-2634; Sigma Chemical Co.; from porcine heart, MW = 70,000). These molecules were chosen as representative of proteins with small (MDH), medium (LDH), and larger (GDH) molecular weight. The concentrations used for GDH, LDH, and MDH were $1.42 \times 10^{-7}$ M, $2.86 \times 10^{-8}$ M, and $1.82 \times 10^{-7}$ M, respectively. These enzyme concentrations were chosen because each enzyme concentration was in the range noted in mammalian cells and they were chosen to facilitate easy and reliable detection under our experimental conditions.

### 2.2. Surfactants

The surfactant mixture were formulated and supplied by Cytec Industries, Inc. (West Paterson, NJ) except Triton X-100 (tert-octylphenoxypolyethoxyethanol; T 9284; Sigma). IB-45 contained: 45% (w/w) sodium diisobutyl sulfosuccinate and 55% (w/w) water. MA-80-I contained: 80% (w/w) sodium dihexyl sulfosuccinate, 5% (w/w) isopropanol, and 15% (w/w) water. OT-75 contained: 75% (w/w) sodium dioctyl sulfosuccinate, 6% (w/w) ethanol, and 19% (w/w) water. TR-70 contained: 70% (w/w) sodium bis(tridecyl) sulfosuccinate, 20% (w/w) ethanol, and 10% (w/w) water. The surfactant mixture concentrations used in the experiments ranged from 0.05 to 5000 parts per million (ppm) by volume.

### 2.3. Procedures

For each enzyme studied, all experiments were conducted with the same enzyme concentrations. For experiments with surfactants only, appropriate amount of enzyme was inoculated into the desired pH buffer solution, surfactant was then added into the enzyme solution, and enzyme activity was assayed immediately. For kinetic time course experiments with surfactants, after enzyme and surfactant addition, the solution was assayed for enzyme activity periodically in a four-hour span. All experiments were conducted in 10 mM potassium phosphate buffer solution at pH 6.5, 6.95, or 7.4. These pH values were chosen because of their physiological importance in cell functions. Adjustment of pH was made by addition of monobasic or dibasic potassium phosphate. All chemicals used were laboratory or analytical grade. Water used was double distilled and deionized.

### 2.4. Separation cell

As shown in Figure 1, individual enzyme and surfactant were injected into one side (right) of a solution in a U-tube separation cell that had a membrane (1 μ thick of polycarbonate, Osmonics Laboratory Products) that separated two half-cells. The protein and surfactant in solution would then permeate to the other side (left) of the cell through the membrane. Other than the protein and surfactant, the half-cells were filled with 100 ml of 10 mM phosphate buffer solution that was well mixed. Two samples of 1 $cm^3$ each were taken periodically from both half-cells and placed in vials on an ice bath for immediate assays. Duration of experimentation was four hours. Experiments were conducted at 21-22 ˚C at pH 6.5, 6.95, and 7.4 that are important to mammalian cell functions.

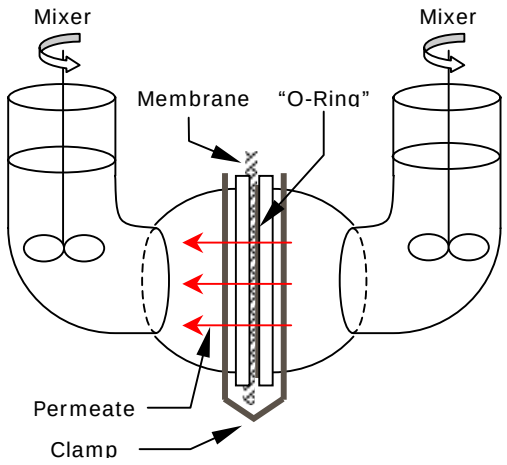


Figure 1: The separation cell with a 1 micron semipermeable polycarbonate membrane.

### 2.5. Analysis

Solution samples were analyzed with an Agilent 8453 Photodiode Array UV/VIS Spectrophotometer with an eight-cell multi-sample module at a room temperature which varied from 21-23°C. Sample solutions were prepared and placed on sample rack surrounded by chopped ice; temperature of the sample solutions was at constantly 10°C. The procedures for determining enzymatic activities were accurate and well established [1]; thus, they will not be discussed further here. Kinetics results (concentrations or activities) were calculated by the Biochemical Analysis UV-VIS Software from Agilent. Each sample was collected twice and analyzed in duplicate: thus, all data points reported in this study were the arithmetic mean of four measurements. In general, each measurement was within ±10% of the mean. As samples were measured by the eight-cell module, for each kinetic run, six samples would be measured with two internal standards. The internal standards were enzyme solutions with no surfactant added. Activities of the enzymes of the samples were calculated as normalized activities by comparing them with the average of the internal standards for each kinetic run.

## 3. RESULTS

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 change more than 35% with 1 ppm difference in surfactant concentrations. All three enzymes were more active in hydrophilic than in hydrophobic surfactant. LDH activity also showed time dependent decreases with different surfactant concentrations. These results suggest that, for results to be comprehensive, surfactant effects should be investigated with a wide range of concentrations and with time as a variable. When mass balances of enzymes are calculated based on their activities, caution must be exercised to ensure that the relationship of mass and activity are linear and no other factors in the system can alter enzymatic activity.

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

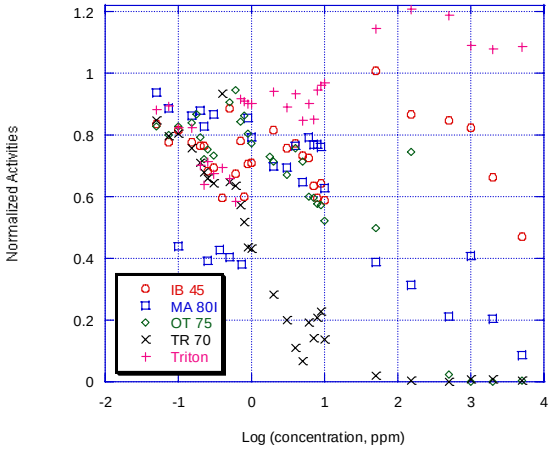


Figure 2. Effects of surfactants on LDH activities at pH 6.95

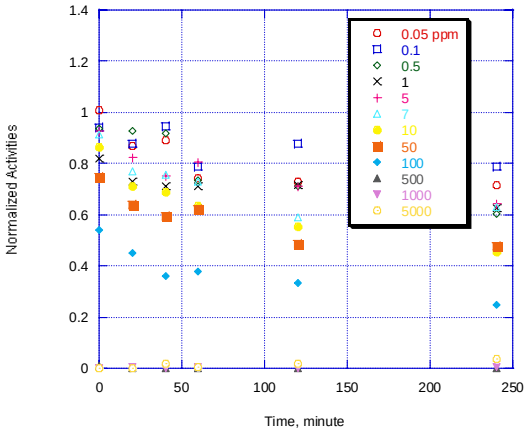


Figure 3: LDH activities with hydrophobic surfactant (IB-45) at various concentrations (ppm) at pH 6.95

## REFERENCES

[1] Clark JB, Lai JCK (1989) Glycolytic, Tricarboxylic Acid Cycle, and Related Enzymes in Brain. In: Boulton A A, Baker G B, Butterworth, eds. *NeuroMethods*. Clifton: Humana, Vol. 11, pp. 233-281.

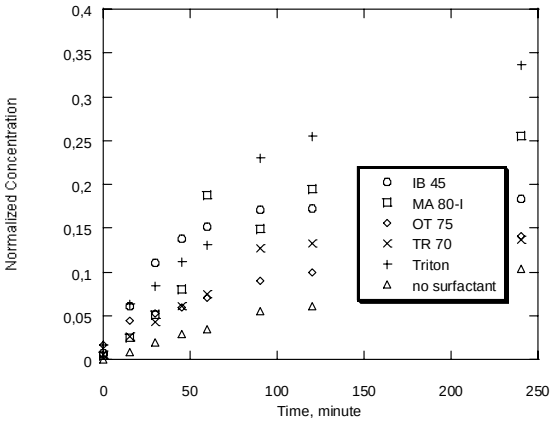


Figure 4: Permeation of LDH in solutions with various surfactants at 0.1 ppm at pH 6.95