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ABSTRACT

Two different buffers containing salt were determined to bring sialidase in 100,000 *g* supernatant, while some detergents like cholic acid, taurocholic acid and Triton X-100 were tried as solubilizing agents. Acetate buffer could be solubilized sialidase activity of about 5 %, this was increased to 25 % with the addition of 0.15 *M* NaCl. Fifteen percent of the sialidase activity was recovered with the addition 1 % Triton X-100, and 40 % when 0.15 *M* NaCl and 1 % Triton X-100 were added. In the phosphate buffer system about 20 % of the sialidase activity was found in the supernatant, and was increased to 30 and 35 % with the addition of 0.15 *M* NaCl and 1 % Triton X-100, respectively. The sialidase activity found in the supernatant was increased of approximately 45 % with addition of both 0.15 *M* NaCl and 1 % Triton X-100. Cholic acid, taurocholic acid and Triton X-100 showed the same effect in solubilizing the sialidase. The amount of detergent required in solubilization of sialidase from membrane preparation could be reduce from 1 to 0.5 % with addition of 0.25 *M* sucrose in the solubilization buffer. From the result above, phosphate buffer showed specific effect on sialidase solubilization from horse liver, and this solubilization effect increase when salt was added. But detergent showed no specific effect in solubilizing sialidase.

INTRODUCTION

Purification of membrane-bound enzymes is faced difficulties because of its low stability and showing a small of yield. The common solubilization agent used for this purpose is detergent and protease, however high ionic charge of solution was also reported showing the same effect [1]. Sialidase (EC 3.2.1.18) hydrolyzed sialic acid from glycolipid and oligosaccharide. In eukaryotic cells, sialidase was found as cytosolic enzyme [2], as well as in almost sub cellular components, i.e. intra-lysosomes and lysosomes [3], nuclear membrane [4], and Golgi membrane [5]. In this report we showed that high ionic charge buffer showing the best solubilization effect on sialidase from horse liver, a membrane-bound enzyme, among some different solubilization buffers used.

MATERIALS AND METHODS

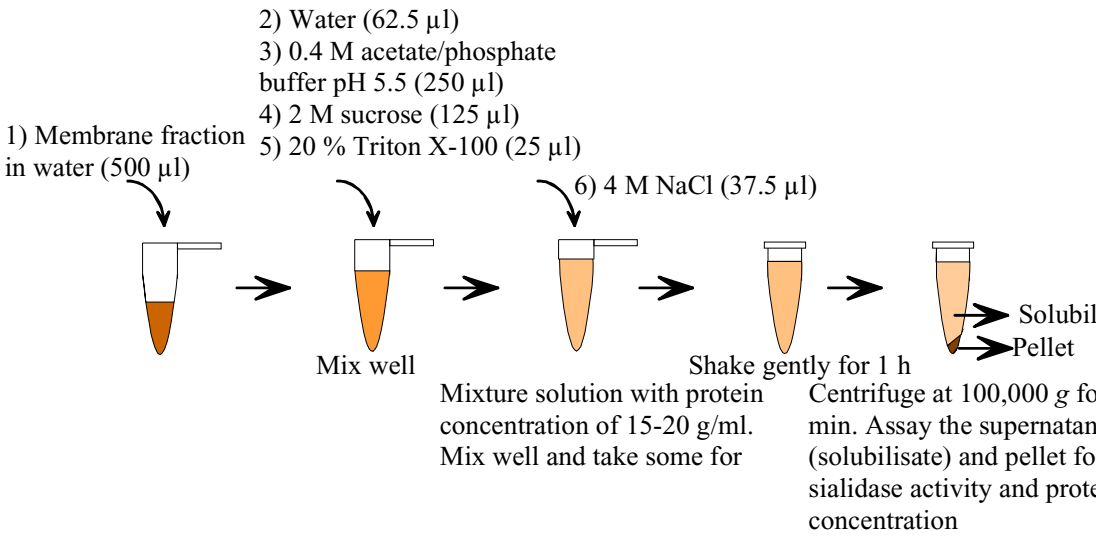
Membrane Preparation

The homogenate of 65 g frozen horse liver was centrifuged at 100,000 g for 60 minutes following homogenization in 240 mL water with ultra turrax (3 x 1 minute) and homogenization for 10 strokes in Potter Elvehjem apparatus. The membrane was then collected and suspended in water by concentration of protein of about 20 mg/mL.

Solubilization

Two different buffers, sodium acetate buffer and sodium phosphate buffer, containing salt were determined to bring sialidase in 100,000 *g* supernatant, while some detergents like cholic acid, taurocholic acid and Triton X-100 were tried as solubilizing agents. Solubilization experiment was conducted as shown in Figure 1. A 500 µL homogenate in 1.5 mL eppendorf cap was added by water, 0.4 *M* acetate or phosphate buffer, 2 *M* sucrose, and 20 % detergent to bring an appropriate concentration for each (Figure 1.). After mixing well, some of 4 *M* NaCl

was added to bring at appropriate concentration. The mixture was then shaked gently at 4 ^{o}C, and was centrifuged using mini ultracentrifuge at 100,000 *g* for 15 min. The supernatant and the pellet were checked for protein and sialidase activity. Detergent-protein ratio was studied at beginning of this study to determine optimum concentration of protein of membrane fraction in solubilization mixture. Optimum concentration of detergent was studied using Triton X-100. Cholic acid and taurocholic acid were also tried beside Triton X-100.



Enzyme and Protein Assay

Sialidase as assayed as procedure described by Potier *et al*. [6] using MU-Neu5Ac of 0.1 mM in 0.07 M acetic acid buffer pH 4.3. After 60 min the reaction was stopped with 200 µl stop buffer (0.266 M glycine, 0.08 M Na_2CO_3, 0.12 M NaCl, pH 10.7). After centrifugation in an *Eppendorf* centrifuge, the clear supernatant was added to a black microtiter plate and the free methylumbelliferone was determined in a Dynatec spectrofluorimeter (with 5 voltage lamp) using excitation light at 365 nm and fluorescence emission at 450 nm. One unit of enzyme activity was defined as the amount of the enzyme releasing 1 µmole of methylumbelliferone from MU-Neu5Ac per min. The protein content was determined by Bradford method [7] using BioRad Kit.

RESULTS AND DISCUSSION

Optimum solubilization of protein using 1 % of Triton X-100 was achieved at concentration of membrane preparations of 4 mg protein/mL, with 75 % of total protein being solubilized. However, it gave no influence on solubilizing sialidase up to concentration of protein of 10 mg/mL, which gave approximately 6 % sialidase and protein of about 50 % in supernatant.

Triton X-100, cholic acid, and taurocholic acid with end concentration of 1 % could bring protein in supernatant of about 62, 83, and 78 %, respectively. However no different effect on sialidase detected, about 6 % of sialidase activity was found in supernatant. Sialidase activity found in supernatant decreased if detergent (Triton X-100) concentration added was more than 1 %, on the other hand protein may still increased. The high concentration of detergent may cause instability on the sialidase

Effect of different buffers containing solubilization agents was then studied on solubilizing sialidase. Acetate buffer of 0.1 *M* could be solubilized sialidase of about 5 %, this was increased to 25 % with the addition of 0.15 *M* NaCl. Fifteen percent of the sialidase activity was recovered with the addition 1 % Triton X-100, and 40 % when 0.15 *M* NaCl and 1 % Triton X-100 were added. In the phosphate buffer system about 20 % of the sialidase activity

was found in the supernatant, and was increased to 30 and 35 % with the addition of 0.15 *M* NaCl and 1 % Triton X-100, respectively. The sialidase activity found in the supernatant was increased of approximately 45 % with addition of both 0.15 *M* NaCl and 1 % Triton X-100. The amount of detergent required in solubilization of sialidase from membrane preparation could be reduce from 1 to 0.5 % with addition of 0.25 *M* sucrose in the solubilization buffer.

CONCLUSION

Phosphate buffer showed specific effect on sialidase solubilization from horse liver, and this solubilization effect increase when salt (sodium chloride) was added. But detergent showed no specific effect in solubilizing the sialidase.

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