

## CHAPTER V

### CONCLUSION

The present study using *A. halophytica* has revealed the following findings:

1. *A. halophytica* contains an  $\text{Na}^+/\text{H}^+$  antiporter (ApNhaP) homologous to plants, mammals and some bacteria (*Pseudomonas* and *Synechocystis*) but not with *E. coli* antiporter
2. *A. halophytica*  $\text{Na}^+/\text{H}^+$  antiporter could complement the  $\text{Na}^+$ -sensitive *E. coli* mutant TO114 cells
3. ApNhaP but not SynNhaP could complement the  $\text{Na}^+$ -sensitive *E. coli* mutant TO114 cells at 30 ° C and alkaline pH
4. ApNhaP had virtually no activity of  $\text{Li}^+/\text{H}^+$  antiporter but showed high  $\text{Ca}^{2+}/\text{H}^+$  antiporter at alkaline pH
5. Replacement of a long C-terminal tail between ApNhaP and SynNhaP altered the ion specificity of antiporter
6. The effective condition for degradation of glycinebetaine in *A. halophytica* is hyposmotic stress (salt downshock) by the action of betaine-homocysteine methyltransferase (BHMT, EC 2.1.1.5)
7. BHMT was purified to homogeneity by Hydroxyapatite, Sepharose CL-6B and Sephadex G-200 column chromatography with a 11% overall yield and 24-fold purification
8. BHMT was found to have an octameric structure with a molecular mass 45 kDa
9. The apparent  $K_m$  for glycinebetaine and L-homocysteine were 4.3 mM

and 1.3 mM respectively.

10. BHMT showed optimum activity at 37°C and pH 7.5

11. BHMT was inactivated by dimethylglycine and choline, slightly inactivated by monomethylglycine and completely inactivated by betaine aldehyde

The finding that the Na<sup>+</sup>/H<sup>+</sup> antiporter of *A. halophytica* lacks the exchange activity between Li<sup>+</sup> and H<sup>+</sup> is interesting. More important finding is the results showing that the ion specificity is affected the C-terminal tail of the Na<sup>+</sup>/H<sup>+</sup> antiporter. This study therefore provides the foundation for further investigation on the structure and function relationship of the Na<sup>+</sup>/H<sup>+</sup> antiporter which may be useful for the better understanding of this antiporter in higher plants and mammalian cells.

On the other hand, the existence of BHMT which is the first ever reported in cyanobacteria suggests that glycinebetaine can be metabolized in certain cells under certain condition. This is in contrast to the previously reported role of glycinebetaine as an end product. *A. halophytica* thus appears to be a unique organism and can be used as a model system for further study the Na<sup>+</sup>/H<sup>+</sup> antiporter as well as for osmoregulation.