CHAPTER IV

DISCUSSION

1. Tracer study with radioactive choline

In higher plants and some bacteria the biosynthetic pathway of glycine betaine has been reported to occur by a two-step oxidation of choline, via the intermediate betaine aldehyde (Lerma et al. 1988,1991). By radiotracer experiments using [^{14}C] choline it was found that the radioactivity was incorporated into betaine aldehyde and glycine betaine. The results indicated that the same pathway of glycine betaine synthesis also occurs in cyanobacteria. The efficiency of the separation of quaternary ammonium compounds eluted from the ion-exchange column was tested by thin-layer chromatography. Table 2 shows the conversion of [^{14}C] choline to [^{14}C] betaine aldehyde and [^{14}C] glycine betaine. The efficiency of the cation exchange column to elute quaternary ammonium compounds accounted for 98.6% recovery.

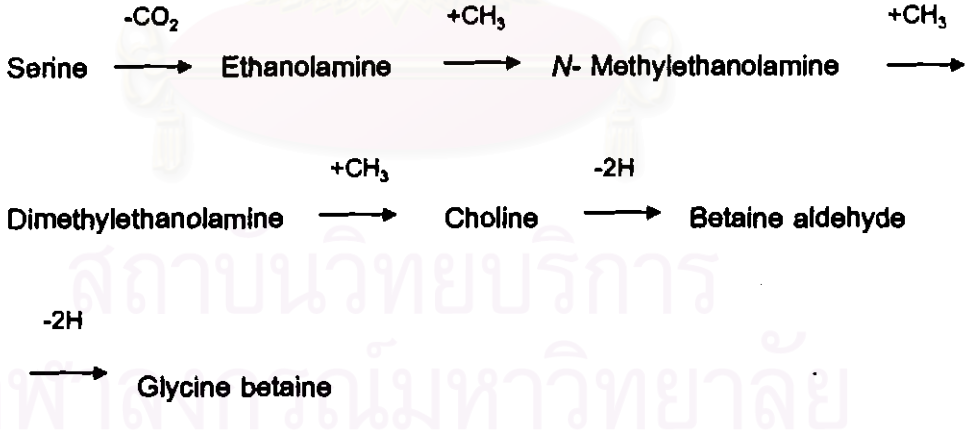
2. Time course and product of choline oxidation

A. halophytica is able to increase the intracellular level of glycine betaine through *de novo* synthesis in defined medium. When [^{14}C] choline was added to a suspension of *A. halophytica* containing 0.5 M or 2.0 M NaCl its conversion to [^{14}C] betaine aldehyde and [^{14}C] glycine betaine was followed by scintillation counter. The increased formation of betaine aldehyde occurred in the first 30 min suggesting that it originated from choline. The formation of glycine betaine was slightly observed in the first 30 min. The radioactive glycine betaine was formed rather slowly after 30 min in control cells but was more rapidly formed in stressed cells. The increase in the amount of glycine betaine up to 180 min corresponded well with the increase in the amount of

betaine aldehyde suggesting the conversion of betaine aldehyde to glycine betaine. Our data confirmed that glycine betaine is an osmoprotective compound in *A. halophytica* and that choline and betaine aldehyde serve as its precursors. Overall results indicated that glycine betaine was synthesized from choline via betaine aldehyde. The results presented in this study firmly establish that the choline-glycine betaine pathway enables *A. halophytica* to cope efficiently with high-osmlarity growth conditions.

3. Formation of [^{14}C] glycine betaine from various precursors

Hanson and Scott (1980) reported in wilted barley, that the patterns of ^{14}C-labeled products were qualitatively and quantitatively consistent with *de novo* synthesis of this glycine betaine from serine via ethanolamine, choline, and betaine aldehyde and that water stress might increase the activities of all steps in this pathway except the last.



In our work, a dose of 1μCi of each of [^{14}C]-labeled precursors was supplied to *A. halophytica* under control and stress conditions, and the incorporation of [^{14}C] into glycine betaine was followed. The formation of [^{14}C] glycine betaine from [^{14}C] choline, [^{14}C] ethanolamine or [^{14}C] glycine was about 3 to 5 folds greater in stressed cells than in control cells. This indicates that all 3 precursors could be used to

synthesize glycine betaine in *A. halophytica* and that higher formation of glycine betaine occurred in stressed cells than in control cells. These results agree with those in barley leaves (Hanson and Scott 1980). Furthermore, our findings showing the faster rate of conversion of glycine to glycine betaine than that of choline or ethanolamine might suggest that glycine could undergo direct methylation to from monomethyl glycine, dimethyl gylcine and finally glycine betaine, respectively. The conversion of choline, ethanolamine or glycine to glycine betaine in *A. halophytica* appears to serve a physiological function in addition to its role in adaptation to extreme osmolarities.

4. [^{14}C] glycine betaine biosynthesis and glycine betaine accumulation after various periods of stress

Although *A. halophytica* has been shown to adapt to a broad range of salt concentration from 0.1 to 3.0 M NaCl (Reed et al. 1984), the rate of cell growth decreased when the cells were transferred to media that contained NaCl at higher concentrations (2M NaCl). The properties of glycine betaine in protecting several enzymes against salt inhibition have been demonstrated by Pollard and Wyn Jones (1979) and Manetas et al. (1986). Glycine betaine is thought to be a major osmoticum in this cyanobacterium (Mackay et al. 1984; Reed et al. 1984, 1986), although ions such as K^+ and Na^+ are also accumulated. Since glycine betaine is a molecule that contains nitrogen, the accumulation of glycine betaine in *A. halophytica* may also be regulated by the level of available nitrogen (nitrate) in the culture medium. Nitrate appears to be utilized as a nitrogen source for glycine betaine biosynthesis. Previously we provided evidence showing that nitrate could increase intracellular glycine betaine in *A. halophytica* under salt stress condition whereas this increase was not observed under non-stress condition (Wutipraditkul et al. 1999). In the present study we examined the effect of salt stress on glycine betaine accumulation, and biosynthesis. In salt stress

condition the uptake of [^{14}C] choline, and [^{14}C] glycine betaine biosynthesis occurred at a higher rate than control condition. This suggests that in stress condition, the increased accumulation of glycine betaine is a result of increased uptake of [^{14}C] choline inside the cells to increase the biosynthesis of [^{14}C] glycine betaine for survival and growth of *A. halophytica*.

5. Disruption and centrifugal fractionation of cells

Overall results in the present study concerning the biosynthetic pathway of glycine betaine suggest that the formation of glycine betaine occurs through the two-step oxidation of choline. It is therefore essential that the evidence showing the existence of enzymes is provided. *A. halophytica* exhibited the activities of choline dehydrogenase (which catalyzes the oxidation of choline to betaine aldehyde) and betaine aldehyde dehydrogenase (which catalyzes the oxidation of betaine aldehyde to glycine betaine). The presence of choline dehydrogenase and betaine aldehyde dehydrogenase activities (Incharoensakdi and Kum-arb 1998) from cell grown under normal condition and under salt stress supported the notion that glycine betaine is synthesized by a two-step oxidation of choline via betaine aldehyde. Choline dehydrogenase was mainly present in membrane fraction whereas betaine aldehyde dehydrogenase was found mainly in cytoplasmic fraction. Formation of these enzymes was also regulated by the NaCl concentration of the medium. These results suggested that NaCl concentration of the medium could induce the biosynthesis of both enzymes. In fact, the increased salinity of the medium was shown to stimulate the activity of betaine aldehyde dehydrogenase in *A. halophytica* (Incharoensakdi and Kum-arb 1998).