CO25946 : MAJOR BIOCHEMISTRY

KEY WORD : FERREDOXIN-NADP REDUCTASE/CHLAMYDOMONAS REINHARDTII/PARAQUAT RESISTANT

SUKUNTAROS TADAKITTISARN : FERREDOXIN-NADP REDUCTASE IN PARAQUAT RESISTANT CHLAMYDOMONAS REINHARDTII. THESIS ADVISOR : ASSO. PROF. SANHA PANICHAJAKUL, Ph.D. 126 pp. ISBN 974-581-655-8

Ferredoxin-NADP reductase was characterized and purified from the unicellular green alga Chlamydomonas reinhardtii wild type strain (137c) and paraquat resistant (PPQ-10/3) strains. The pattern of FNR synthesis was growth associative. The maximum growth of alga cells were established at 5x10 and $2.4x10^6$ cells/ml medium in 137c and PPQ-10/3 respectively, whereas the growth rate of PPQ-10/3 cell line was slower than the wild type. At the late log phase the maximum yield of FNR activity was observed to be about 0.021 and 0.031 units/10^7 cells in 137c and PPQ-10/3 respectively. The isozyme patterns of the crude enzyme in the two cell lines were not significantly different. Among 17 bands of FNR isozymes observed there were 2 major bands (Rf 0.20 and 0.28) illustrated by the polyacrylamide gel electrophoresis. The highly purified (Rf 0.28 band) was obtained by $(NH_4)_2So_4$ fractionation (0-70 % saturation), 1st DEAE-trisacryl, P 11 phosphocellulose, 2nd DEAE-trisacryl column chromatography and the non-denaturing polyacrylamide gel electrophoresis. The specific activity of FNR purified from the 2 cell lines was not significantly different (1.18 units/mg protein in 137c and 1.25 unit/mg protein for PPQ-10/3 strain). The pH optimum of the two cell lines was in the same range at 8.5-9.0 and also the optimum temperature was about 65-70°c in both cell lines. The low concentration of NaCl (60mM) stimulated the FNR-mediated diaphorase activity in the crude enzyme but not in the highly purified enzyme and of the enzyme obtained from the 2nd DEAE-trisacryl column were almost the same about 12.5±0.78 μM and 13.3±0.89 μM in 137c and PPQ-10/3 strain respectively. The molecular mass calculated by SDS-polyacrylamide gel electrophoresis in both strains were estimated to be about 33,000 dalton. The result suggested that the properties of FNR from the 137c and PPQ-10/3 were quite similar there was not significantly change in the major properties of enzyme both qualitative and quantitative through the function. It was concluded that the mechanism of paraquat resistant was not directly mediated by this redox enzyme in PS I system.