Thesis Title Study on Cytosol and Membrane-bound Proteases in β°-Thalassemia / Hb E Red cell

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ABSTRACT

Patients with β°-thalassemia/Hb E disease have a wide range of anemia can be divided into two groups, one group with hemoglobin levels below 7.7 g/dl and the other above. Patients with low hemoglobin level have more severe anemia. Erythrocyte cytosol protease in patients with β°-thalassemia/Hb E disease had been previously studied by Promboon (Birth Defects 1988; 23(5A): 249-56)and Macotpet (Birth Defects 1988; 23(5A): 257-61). Assay condition were changed to make for complete dissolution of globin substrate and optimized for linear kinetics during 20 min at 30°C The results showed that erythrocyte cytosol protease activity of patients with β°-thalassemia/Hb E were significantly higher than control ($p<0.05$). The protease activities in the group with milder anemia were not different from severe group. This is in contrast to the results by Promboon and Macotpet, who found that protease activity in mild group was higher than severe group, but is consistent with the study of Hamakubo (Contemporary Theme in Biochemistry 1986 ; 6 : 624-5). Protease activity was not changed when red cell membrane was present in the assay solution.

The effects of freeze-thaw on erythrocyte lysate protease activity were also investigated. The lysate was frozen for 15 min in a mixture of dryice and acetone (-78.5°C) and was completely thawed in a water bath at 37°C , then immediately

stored on ice until used. The protease activities in cytosol fraction after freeze-thawing , were not different from untreated activity. However, following freeze-thawing of the cytosol plus membreane preparation, there was an enhancement of activity. There were no differences in protease activities of such preparations between control subjects and patients with severe anemia. However, activities in the patients with mild anemia could be devided into two groups : one group with the same activity as control and the other group with higher activity. These results suggested that membrene-bound protease may be a contributing factor in reducing the severity of anemia in some patients with β°-thalassemia/Hb E disease

Red cell membrane protein analysis by polyacrylamide gel electrophoresis in fresh blood of β°-thalassemia/Hb E and normal showed no significant difference. However, after storage at 4 C for 3 to 5 days , there were many degradative changes in all samples, especially of band 4.1 a. The degradative changes were more abvious in severe cases than mild cases. Defect of band 4.1 a in thalassemic red cell may be a contributing factor in reducing red cell survival.