ERYTHROCYTE SPECTRIN IS AN E2 UBIQUITIN CONJUGATING ENZYME

STEVEN R. GOODMAN, DAVID G. KAKHNIASHVILI, JOSÉ SANGERMAN, TANUJA CHAUDHARY, WARREN E. ZIMMER, F. ALADAR BENCSATH and IAN JARDINE
USA Comprehensive Sickle Cell Center, University of South Alabama College of Medicine, Mobile, AL. and Thermoquest, Finnigan, San Jose, CA, USA

We have previously demonstrated that control erythrocyte spectrin contains a DTT sensitive posttranslational modification which migrates slower than unmodified á-spectrin on SDS PAGE (-DTT) but is not observed in the presence of reducing agent or in significant amounts in sickle cell RBCs [1]. In the current study we demonstrate that this á´-Spectrin reacted with anti-ubiquitin antibodies on western blots (-DTT). Anti-ubiquitin antibody was also used to immunoprecipitate á´-spectrin under stringent conditions. We purified RBC spectrin by standard low ionic strength extraction (in the absence of reducing agent) and gel filtration chromatography. Immunodot assays demonstrated that the purified spectrin heterodimer fractions reacted with á-spectrin and ubiquitin antibodies. Upon treating the purified spectrin with DTT, followed by rechromatography, the ubiquitin reactive protein (– 8.5KD) was released from spectrin. Edman degradation and protein sequencing and LC/MS/MS mass spectrometry proved that the ubiquitin reactive protein had 100% identity with authentic ubiquitin. *In vitro* studies have demonstrated that ^{125}I ubiquitin in the presence of purified E1 ubiquitin activating enzyme and ATP is transferred to á-spectrin forming a DTT sensitive thioester bond. This E2 ^{125}I-ubiquitin - á-spectrin adduct then performs an intramolecular transfer of the ^{125}I-ubiquitin to an á-spectrin target site forming a DTT insensitive ubiquitin-á-spectrin conjugate. Computer analysis of the á-spectrin sequence indicated a segment within spectrin repeat 20 that is a likely candidate for E2 activity. Cys 2051 is surrounded by sequence which has 70% identity to the active site consensus sequence critical for previously studied E2 enzymes. A second region of interest surrounds Cys 2080 which conforms to the cleft structure surrounding the active site residues of E3 ubiquitin ligating enzymes. Finally, there is a cluster of lysine residues within spectrin repeat 21 (2179K - K2189) that is a likely target site for ubiquitin. In sickle cells, of all densities, both the E2 adduct and ubiquitin-spectrin conjugate are diminished by 80-90%. As ubiquitination is responsible for the turnover of many proteins during erythropoiesis, an inhibition of the ubiquitin-proteasome system in developing sickle cells could be extremely important to the accumulation of oxidative damage to spectrin and its associated proteins.

REFERENCE

1. Monteiro, C.A., Gibson, X.A., Shartava, A. and Goodman, S.R. Preliminary characterization of a structural defect in homozygous sickled cell alpha spectrin. **Am. J. Hematol.** 58 (1998) 200-205.