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Two-dimensional electrophoretic analysis of transglutaminase reaction with histidine-rich protein

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A shift in isoelectric point (pI) of histidine-rich protein (HRP) from neutral to extremely basic (pI >9.5) is a major event occurring during cornification of epidermis. However, the molecular basis for the pI shift remains unclear. The aim of this study was to demonstrate a pI shift caused by transglutaminase (TG) reaction with HRP. Two forms of TGs, 81 kDa (pI 5.5) and 55 kDa (pI 7.5) were partially purified from newborn rat epidermis first by ammonium sulfate fractionation, followed by sucrose density gradient isoelectric focusing, then by gel filtration chromatography on a Sephacryl S-300 column. HRPs of 49 Da and 47 kDa were obtained from the same tissue source by gel filtration and ion exchange chromatography on a Mono-S column of FPLC system. The 81 kDa and 55 kDa TGs catalyzed [^{14}C]putrescine incorporation into HRP at 29% and 41%, respectively, of the maximum rate attained with casein as substrate. The pI shift of HRPs was analyzed by two-dimensional electrophoresis after 10 h incubation of 1.5 mg each HRP with 0.05 mU each TG in 0.33 ml aliquots of 0.1M Tris-HCl (pH 8.3) containing 5 mM spermidine and 5 mM $CaCl_2$. Spermidine conjugation caused the pI shift of 49 kDa HRP with pI 6.3-7.3 to pI 6.8-7.8, and of 47 kDa HRP with pI 8.5-9.5 to pI >9.5. These results demonstrate that rat HRPs can serve as substrate for TGs purified from rat epidermis, and the reaction causes a basic pI shift. This property of TGs suggests yet another function of theses enzymes during the cornification process of epidermis through changing pI of HRP in addition to cross-linking of the envelope proteins.