Characterization of purified rat testicular transglutaminase and age-dependent changs of the enzyme activities

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Abstract

The Ca2+-dependent tissue transglutaminase is widely distributed in various tissues and has been reported to participate in many cellular growth and differentiation processes. In the past decade, tissue transglutaminase is also identified as a G protein, G h, for intercellular signaling. To further characterize testicular transglutaminase, the rat testicular transglutaminasewas purified by ammonium sulfate precipitation, DEAE ion-exchange, heparin–agarose, and GTP–agarose affinity chromatographies. This purification protocol resulted in a 8400-fold enrichment of the enzyme with a reproducible 15% yield. The purified enzyme showed as a single band of 78 kDa on SDS-polyacrylamide gel. Western blot analysis using anti-liver tissue transglutaminase monoclonal antibody also recognized the enzyme, indicating it is a t-TGase in nature. The Km values of purified testicular transglutaminase for putrescine and N,N-dimethylcasein were determined to be 35 and 17┐M, respectively. Its transglutaminasecross-linking activity was strongly inhibited by EGTA, GTP, polyamines, and cystamine, as well as moderately by ATP and NaCl. The enzyme exhibited a magnesium-dependent GTP-hydrolyzing capacity, but its GTP-binding activity did not require magnesium. Furthermore, the enzyme activity was found to be closely related with the first wave of spermatogenesis. Thus, testicular transglutaminase is speculated to participate in the event of spermatogenesis. In conclusion, the purified testicular transglutaminase displays property of either the tissue-type transglutaminase, or the GTP-binding and hydrolyzing characteristics. The activity of testicular transglutaminase is age-dependent, greatly stimulated during the first wave of spermatogenesis.