

Two distinct classes of rat intestinal mucosal enzymes incorporating putrescine into protein.

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摘要

Abstract

Tissue-transglutaminase (t-TGase) is a family of calcium-dependent enzymes. A Ca²⁺-independent soluble enzyme, in addition to t-TGase, capable of incorporating polyamines into proteins was demonstrated in rat intestinal mucosa. The Ca²⁺-independent enzyme was stimulated 2- to 5-fold by Fe²⁺ and Co²⁺ ions but inhibited by Cu²⁺ and Zn²⁺ ions. The Ca²⁺-stimulated t-TGase activity was inhibited by divalent ions in the following order: Zn²⁺, Fe²⁺ > Co²⁺ > Cu²⁺. The opposite effects of EGTA, Fe²⁺ and Co²⁺ on these two enzyme activities indicate that they are two distinct classes of enzymes. Competition studies demonstrated differential preferences of the two enzymes for substrates. The Ca²⁺-dependent enzyme preferred putrescine, monodansylcadaverine > cadaverine, spermidine, spermine > 1,10-diaminodecane > triethylbutylamine. On the other hand, the Ca²⁺-independent enzyme preferred putrescine > cadaverine > spermine, 1,10-diaminodecane > spermidine > monodansylcadaverine > triethylbutylamine. Further studies with divalent ions excluded the possible association of this novel Ca²⁺-independent enzyme with diamine oxidase. Finally, the Ca²⁺-independent enzyme had a higher affinity for putrescine (K_m = 0.02 mM) than did Ca²⁺-dependent t-TGase (0.2 mM). As judged by gel filtration on HiPrep Sephacryl 200 column, the Ca²⁺-independent enzyme had a molecular weight of approximately 48 kDa, the intestinal Ca²⁺-dependent t-TGase was about 188 kDa while that of testicular t-TGase was about 96 kDa. In conclusion, the Ca²⁺-independent enzyme is stimulated by cobalt or ferric ions, and selectively incorporates aliphatic diamines or polyamines with symmetric amino groups. The observed Ca²⁺-independent enzyme activity is not related to diamine oxidase or its products. With a 10 times greater affinity for putrescine, the calcium-independent, 48-kDa intestinal enzyme may mediate polyamine function better than calcium dependent, 188-kDa intestinal tissue transglutaminase in the intestinal mucosa.