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

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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, I,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 (Km = 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.