Binding of Competitive Inhibitors to S-Chymotrypsin in the Alkaline pH Region. Competitive Inhibition Kinetics and Proton-Uptake Measurements

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The binding of the three competitive inhibitors benzyl alcohol, tryptophol, and N-acetyl-D-tryptophanamide to ?- and ?-chymotrypsins was studied over the pH range 7 to 11 by competitive inhibition kinetics using N-furyl-acryloyl-L-tryptophan methyl ester as substrate. The results indicate that the binding of these inhibitors to ?-chymotrypsin exhibits a pH dependence significantly different from the pH dependence obtained with ?-chymotrypsin. Analysis of K, vs. pH profiles for the interaction of benzyl alcohol, tryptophol, and N-acetyl-D-tryptophanamide with ?-chymotrypsin indicates that the pKa of an ionizing group of the enzyme (9.2, 9.5, and 9.2, respectively) is shifted to a pKa of 10.0, 10.1, and 9.8, respectively, in the enzyme-inhibitor complex. This behavior differs from that of ?-chymotrypsin, where, in agreement with previous reports, the binding of the three inhibitors was found to be strictly dependent on the ionization of a group in the enzyme with a pKa of 9.0 that appare