プロナーゼから得たN－サクシニル－L－トリアラニンp－ニトロアニリド水解酵素のウマα_2マクログロブリンによる阻害

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Inhibition by Equine α_2-Macroglobulin of an N-Succinyl-L-Trialanine p-Nitroanilide-Hydrolyzing Protease Purified from Pronase

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ABSTRACT: The binding of an N-succinyl-L-trialanine p-nitroanilide-hydrolyzing protease (STA-protease) purified from pronase to equine α_2-macroglobulin (α_2M) was investigated in comparison with that of trypsin. The α_2M subunits (about 90000 daltons), which were electrophoretically detected in the reaction mixture of α_2M and trypsin, were undetectable in that of α_2M and STA-protease. The binding molar ratios of enzyme to α_2M were estimated from the inhibition curves of caseinolytic activity to be 1.5:1 for native and acetylated STA-protease and 2:1 for native and acetylated trypsin. The finding of greater incorporation of monodansylcadaverine into α_2M reacted with acetylated enzymes than into that reacted with the native enzymes suggests that free amino groups in the enzymes are involved at least partly in the formation of the α_2M-proteinase complexes. The numbers of thiol groups generated in α_2M bound to STA-protease and in α_2M bound to trypsin were both estimated to be approximately 4 mol per mol of α_2M by the use of thiol-directed fluorescent probes, though there were slight differences in the microenvironments of thiol groups generated in the two α_2M-proteinase complexes. The values of K_{cat}/K_m were one-half (α_2M-STA-protease complex) and one-sixth (α_2M-trypsin complex) of those of the uninhibited enzymes. These results suggest that STA-protease binds to α_2M both covalently and noncovalently, as does trypsin, and its hydrolytic activities towards casein and low-molecular-weight substrates

are inhibited to various extents.

抄録 プロナーゼから得たN－サクシニル－L－トリアラニンp－ニトロアニリド水解酵素（STA－protease）とウマα_2マクログロブリン（α_2M）との結合についてトリプシンの場合と比較検討した。電気泳動分析によってSTA－protease とα_2Mの複合体はトリプシンのそれと異なる泳動パターンを示したが、チオール基の生成、複合体形成へのアミノ基の関与、動力学的定数の変化など両酵素に著明な差は認められなかった。

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