

メチルアミンによってチオールエステル結合が
開裂したウマ α_2 -マクログロブリンの
電気泳動及び分光学的解析

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**Electrophoretic and Spectroscopic Analyses of Equine α_2 -Macroglobulin
with Cleavage of the Thiol Ester Bonds by Methylamine**

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ABSTRACT Reaction of equine α_2 -macroglobulin (α_2 M) with methylamine caused generation of 3.7 mol of thiol groups per mole of the protein, and the second-order rate constant of the generation was calculated to be $3.5 \text{ M}^{-1}\text{S}^{-1}$. The inhibitory profile of caseinolytic activity of trypsin indicated that one molecule of equine α_2 M inhibited two molecules of trypsin, similar to human α_2 M. The methylamine-treated equine α_2 M, with complete cleavage of the thiol ester bonds, still inhibited the activity of trypsin, though human α_2 M lost its inhibitory activity by treatment with methylamine. These results indicate that the mode of inhibition of trypsin by equine α_2 M is substantially unperturbed by cleavage of the thiol ester bonds and that the intact thiol ester bonds per se are not essential for the ability of equine α_2 M to bind the enzyme. Ultraviolet absorption difference, intrinsic fluorescence, and circular dichroism spectra of the methylamine-treated equine α_2 M showed that this treatment caused only a small change in conformation of the protein. Reaction of the methylamine-treated protein with trypsin induced appreciable changes in the spectra, indicating a large change in conformation of the protein. These findings were consistent with the results obtained by electrophoresis: The band of methylamine-treated equine α_2 M showed indistinguishable mobility from that of the unmodified protein, indicating that no appreciable change in conformation occurred, and distinctly different mobility from that of the unmodified or methylamine-treated equine α_2 M when each had reacted with trypsin.

抄録 ウマ α_2 -マクログロブリンをメチルアミンで処理すると1分子当り, 3.7モルのSH基が生成した。この修飾ウマ α_2 -マクログロブリンは未修飾タンパクと同様なトリプシン阻害曲線を示し, さらに電気泳動や分光学的解析によっても未修飾タンパクと類似の立体構造を保持していることが確認された。これらの結果からウマ α_2 -マクログロブリンは, ヒトのそれと異なり, チオールエステルがタンパク分解酵素のトラップに直接関与しないことが示唆された。

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