Short Communication

Inhibition by Ovomacroglobulin of Pepsin and Rennin

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Ovomacroglobulin (abbreviated as OMG) is a high molecular weight glycoprotein in egg white which consists of four identical subunits (M_w about 180,000) linked in two pairs by disulfide bonds.¹⁾ The physicochemical properties and the mechanism of inhibition for protease are analogous to those of α_2 -macroglobulin.^{1~5)} OMG inhibits a wide variety of proteases: trypsin, chymotrypsin, subtilisin,

papain, thermolysin,^{2,4)} tadpole collagenase⁵⁾ and serratio 56K protease.⁵⁾ However, no inhibition by OMG of acidic proteases has been reported. We report here for the first time the inhibition by OMG of pepsin and rennin.

The inhibition by OMG of pepsin is shown in Fig. 1. Parallel experiments were done using hemoglobin and κ -casein as substrates. When hemoglobin was used as the substrate, the residual activity of pepsin decreased to 43% at the OMG/pepsin molar ratio of 1:1. On the other hand, when κ -casein was used as substrate, the residual activity was lowered almost to nothing. The active site of the protease trapped in OMG may be accessible to hemoglobin and not to κ -casein. The inhibitory activity of OMG for rennin was studied using κ -casein as the substrate. κ -Casein is a specific substrate for the primary action of rennin. After cleavage at Phe105-Met106 by rennin, *para*- κ -casein molecules associate with each other and form insoluble aggregates. Therefore, the rennin activity can be followed by measuring the aggregation of *para*- κ -casein.

Fig. 1. Inhibitory Activity by Ovomacroglobulin against Pepsin.

The activity of pepsin was measured with hemoglobin (●) and κ -casein (○) as substrates. To 125~500 μ l of 2.7×10^{-6} mol/l OMG were added 25 μ l of 2.8×10^{-5} mol/l pepsin solutions. The mixed solution was added to 2 ml of 0.1% hemoglobin solution in 0.1 M citric acid/ Na_2HPO_4 buffer, pH 2.2 or to 2.5 ml of 0.05% κ -casein solution in 0.067 M phosphate buffer, pH 5.4. The residual activity of pepsin was measured by the absorbance at 280 nm of the supernatant obtained by adding 2 ml of 10% trichloroacetate to the mixture after 5 min of reaction at 20°C.

Fig. 2. Inhibitory Activity by Ovomacroglobulin against Rennin.

Rennin activity was represented by the turbidity of κ -casein. ●, without OMG; □, OMG/rennin molar ratio of 0.5:1; △, 1:1; ▲, 2:1.

To 25~100 μ l of 2.7×10^{-6} mol/l OMG were added 50 μ l of 2.5×10^{-6} mol/l rennin solution. The mixed solution was added to 2.5 ml of 0.05% κ -casein solution in 0.067 M phosphate buffer, pH 5.4. Turbidity reading at 500 nm was continuously measured in a glass cell at 20°C.

Fig. 3. Polyacrylamide Gel Electrophoresis (lanes 1 and 2) and SDS-Polyacrylamide Gel Electrophoresis (lanes 3~5) of Various Ovomacroglobulin-Protease Mixtures. (A), OMG-pepsin mixture; (B), OMG-rennin mixture; lane 1, native OMG; lane 2, OMG-protease mixture (OMG/protease molar ratio of 1:1); lane 3, OMG in the presence of 0.9 M mercaptoethanol; lane 4, OMG-protease mixture in the presence of 0.9 M mercaptoethanol (OMG/protease molar ratio of 1:1); lane 5, protease.

Figure 2 shows the effects of OMG on the aggregation of κ -casein by rennin. Increases in the lag time of aggregation were observed as the concentration of OMG increased. These results indicate the inhibition of pepsin and rennin action by OMG.

Figure 3 shows the electrophoretic patterns of untreated, pepsin-treated, and rennin-treated OMGs. After the reaction with pepsin or rennin for 5 min at 20°C, polyacrylamide gel

electrophoresis (PAGE) was done in the absence and presence of SDS and mercaptoethanol. Electrophoresis patterns in the absence of SDS and mercaptoethanol showed the interaction of OMG with pepsin or rennin. On the other hand, SDS-PAGE showed that OMG was cleaved into two fragments whose molecules were smaller than the subunit, and that pepsin and rennin were released. The molecular weight of the smaller fragment was about 77,000, while that of another fragment was slightly larger. Although the specificity of rennin for its substrate is narrow, rennin hydrolyzed the OMG subunit and produced two fragments of $M_w = 88,000$ and 77,000. A similar observation was reported in the study of inhibition by OMG of other proteases.^{1,3)} This indicates that a flexible region sensitive to proteases exists in the middle of the OMG subunit. These results of the electrophoresis suggest that the inhibitory mechanism of OMG proposed for other proteases^{6,7)} may be applicable also to the case of pepsin and rennin, *i.e.*, that the cleavage of a peptide bond triggers a conformational change in OMG, trapping the protease molecules.

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