BIOCHEMICAL LETTERS

Methionine in rabbit α_1 -proteinase inhibitors

Rabbit plasma contains two chemically related forms of α_1 -proteinase inhibitor (α_1 -antitrypsin) designated according to their electrophoretic mobility F (fast) and S (slow). The two forms are immunologically indistinguishable and show similar amino acid compositions (Koj *et al.*, 1978) but they appear to be independently synthesized and catabolized (Regoecki *et al.*, 1980) and they differ in their abilities to inactivate serine proteinases (Koj & Regoecki, 1981). During preliminary characterization of rabbit α_1 -proteinase inhibitors, methionine was not found in the hydrolysates of either form (Koj *et al.*, 1978). This would be unique considering that α_1 -proteinase inhibitors of all mammalian species examined so far were found to contain at least 8 methionine residues per molecule of protein. Moreover, as shown by Travis and co-workers (cf. Matheson *et al.*, 1981), methionine is present in the active centre of the human inhibitor and its chemical or enzymic oxidation results in the loss or impairment of the biological activity of the protein. Therefore, during our recent studies on the kinetics and stoichiometry of inactivation of various proteinases by rabbit α_1 -proteinase inhibitor, we re-investigated the methionine content of this protein.

Approx. 7 methionine residues per protein molecule were found when 1 mg samples of the purified unfractionated inhibitor or separated F and S forms were hydrolysed in 6 M-HCl at 110°C for 24 or 48 h in tubes sealed under N_2 , followed by amino acid analysis in a JEOL analyser. No significant differences in methionine contents between the two forms of rabbit α_1 -proteinase inhibitor were observed. The value of 7 methionine residues may be an underestimate, since only traces of cysteine were found in the hydrolysate under the present conditions.

The cleavage of proteins at methionine residues by CNBr is a routine technique used in peptide mapping and sequence analysis. After incubation of 0.1 mg samples of rabbit α_1 -proteinase inhibitor with CNBr in formic acid (Schroeder *et al.*, 1969), followed by

polyacrylamide gel electrophoresis in the presence of 1% sodium dodecyl sulphate and 8 M-urea (Swank & Munkres, 1971), four principal and three to five poorly stained peptides were detected in the gel. This indicates that the cleavage of the inhibitor molecules occurred, though the digestion may not be complete. Comparison of the CNBr-cleavage products of α_1 -proteinase inhibitors F and S showed a considerable similarity in the peptide gel pattern between the two forms, except that the largest peptide present in the F form (M_r approx. 28000) was absent from the S form.

Finally, we found that exposure of rabbit α_1 -proteinase inhibitor to horse leucocyte myeloperoxidase and H_2O_2 under the conditions described by Matheson *et al.* (1981) resulted in a considerable decrease in trypsin inactivation rates, the F form being more affected. All these observations suggest that rabbit α_1 -proteinase inhibitors F and S do contain methionine and so they are not an exception among homologous proteins of other species. Moreover, it appears that methionine is important for the biological function of rabbit α_1 -proteinase inhibitors.

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