

Studies on the Proteinase-A Inhibitor I_3^A from Yeast

Ignacio NÚÑEZ de CASTRO and Helmut HOLZER

Institut für Biochemie der Gesellschaft für Strahlen- und Umweltforschung und Biochemisches Institut der Universität Freiburg i. Br.

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Summary: The purification and some properties of the two inhibitors I_2^A and I_3^A of proteinase A from yeast have previously been described [Saheki et al. (1974) *Eur. J. Biochem.* 47, 325]. An improved method for the preparation of I_3^A which is less time-consuming and leads to higher yields is presented. Based on amino acid analysis, I_3^A contains 68 amino acids per molecule. The molecular weight was 7676. The inhibitor contained no proline, no arginine, no cysteine and no tryptophan, but did contain a large number of the polar amino acids glutamate + glutamine, aspartate + asparagine and lysine. Neither by dansylation nor by Edman degradation could an

N-terminal amino acid be detected. Changes in the circular dichroism upon transition from pH 6.9 to 3.0 suggest different tertiary structures at these pH values. Experiments on the kinetics of inhibition of proteinase A revealed an apparent K_i value of $5.5 \times 10^{-8} M$ for I_3^A and $1.6 \times 10^{-8} M$ for pepstatin. A "non-stoichiometric inhibition" of a "pseudo-irreversible" type is concluded from the kinetic data. A hydrophobic type of binding of I_3^A to yeast proteinase A is suggested from experiments demonstrating a large decrease in the percentage of inhibition caused by addition of 2M urea, 2M guanidine hydrochloride, 0.125% Triton or 0.125% cholic acid.

Untersuchungen über den Proteinase-A-Inhibitor I_3^A aus Hefe

Zusammenfassung: Reinigung und einige Eigenschaften der beiden Proteinase-A-Inhibitoren I_2^A und I_3^A aus Hefe wurden früher mitgeteilt [Saheki et al. (1974) *Eur. J. Biochem.* 47, 325]. Es wird eine verbesserte Methode für die Präparation von I_3^A beschrieben. Sie ist durch geringeren Zeitbedarf und höhere Ausbeute charakterisiert. Aus der Aminosäureanalyse von I_3^A errechnet man 68

Aminosäuren pro Molekül und ein Molekulargewicht von 7676. Der Inhibitor enthält folgende Aminosäuren nicht: Prolin, Arginin, Cystein, Tryptophan. Er ist durch einen hohen Anteil an den polaren Aminosäuren Glutaminsäure/Glutamin, Asparaginsäure/Asparagin und Lysin gekennzeichnet. Weder durch Dansylierung noch durch Edman-Abbau konnte eine *N*-terminale

Enzymes:

Yeast proteinase A (EC 3.4.23.8);
yeast proteinase B (EC 3.4.22.9) and carboxypeptidase Y (EC 3.4.12.?) ; not listed).

Abbreviations:

I_2^A and I_3^A represent the yeast proteinase-A-inhibiting activities which appear in SP-Sephadex chromatography as described by Saheki et al.^[11] in Fig. 1 as peaks 2 and 3, respectively.