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**Purification and characterization of angiotensin-II-generating enzymes from
porcine renal tissue**

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Summary

The object of this work was the purification, characterization and identification of angiotensin-II-generating enzymes from porcine renal tissue. Three different angiotensin-II-generating enzymes were purified using protein liquid chromatography techniques. During the course of purification angiotensin-II-generating enzyme activity was measured and characterized using a novel mass spectrometry based assay system (MES-assay system) for the detection of proteolytic enzyme activities.

One active fraction, here named Fraction I was purified using a combination of anion exchange, hydroxyapatite, lectin affinity and chymostatin-antipain-inhibitor affinity chromatography. Protein identification experiments using peptide mass fingerprint identified this enzyme as Cathepsin G. Cathepsin G was detected in kidney for the first time. The second active fraction, here named Fraction II was purified using a combination of anion exchange, hydroxyapatite, and lentil lectin sugar affinity and size exclusion chromatography. Protein identification experiments identified this enzyme as Angiotensin converting enzyme. The successful purification of ACE is a proof of principle for the chosen purification strategy and the novel MES-assay system.

The third angiotensin-II-generating enzyme fraction, here named Fraction III was purified using a combination of anion exchange, wheat germ lectin sugar affinity and aprotinin-inhibitor affinity chromatography. The identification of this enzyme did not succeed. Experiments in order to characterize this enzyme fraction were carried out. The molecular weight of the enzyme fraction was approximately 160 kDa. A unique feature of Fraction III was its substrate specificity. Besides its angiotensin-II-generating activity it cleaved the L-kininogen analogue Lys-Bradykinin-Ser-Val-Gln-Val-Ser to form Des-Arg¹⁰-Kallidin. The proteolytic activity of Fraction III does not coincide with the known proteases of the Kinintensin system. Further research efforts should be made to reveal the identity of this enzyme.

Zusammenfassung

Das Thema dieser Arbeit war die Reinigung, Charakterisierung und Identifizierung von Angiotensin- II generierenden Enzymen aus der Schweineniere. Drei verschiedene Angiotensin- II generierende Enzyme wurden mit Hilfe der Flüssigchromatographie aufgereinigt. Die Angiotensin-II generierende Enzymaktivität wurde während der Aufreinigung mittels eines neuen Massenspektrometrie basierten Nachweissystems (MES-System) detektiert und charakterisiert.

Eine aktive Fraktion, hier Fraktion I genannt, wurde mit einer Kombination von Anionenaustausch-, Hydroxyapatit-, Lectin- und Chymostatin-Antipain-Affinitätschromatographie gereinigt. Mit Hilfe von Proteinidentifizierungsexperimenten wurde das Angiotensin-II generierende Enzym dieser Fraktion als Cathepsin G identifiziert. Cathepsin G wurde das erste Mal in der Niere nachgewiesen. Eine zweite aktive Fraktion, hier Fraktion II genannt, wurde mit einer Kombination von Anionenaustausch-, Hydroxyapatit-, Lectin- und Größenausschlusschromatographie gereinigt. Mit Hilfe von Proteinidentifizierungsexperimenten wurde das Angiotensin-II generierende Enzym dieser Fraktion als Angiotensin Converting Enzym identifiziert. Die erfolgreiche Aufreinigung ein Beweis für das Funktionieren der gewählten Aufreinigungsstrategie und des neuartigen MES- Nachweissystems. Eine dritte aktive Fraktion, hier Fraktion III genannt, wurde mittel einer Kombination aus Anionenaustausch-, Lectin- und Aprotinin- Affinitätschromatographie aufgereinigt. Die Identifizierung des Angiotensin-II generierenden Enzyms dieser Fraktion gelang nicht. Experimente zur Charakterisierung dieser Enzymfraktion wurden durchgeführt. Das Molekulargewicht betrug ca. 160 kDa. Ein besonderes Merkmal dieser Enzymfraktion war die Substratspezifität. Neben der Angiotensin-II generierende Aktivität spaltete es das L-Kininogen Analogon Lys-Bradikin-Ser-Val-Gln-Ser Substrat proteolytisch zu Des-Arg¹⁰-Kallidin. Weitere Anstrengungen müssen unternommen werden, um die Identität dieses Enzyms zu klären.

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Curriculum vitae

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