HUMAN PLASMA ANGIOTENSINOGEN: A REVIEW OF PURIFICATION PROCEDURES*

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Summary

The current status of the purification and characterization of human angiotensinogen is reviewed. One problem encountered in the past has been the copurification of a protein with similar properties. This protein has tentatively been designated alanine-protein. An efficient separation of angiotensinogen and alanine-protein was obtained on a zinc chelate column. Alanine-protein has been purified and its amino acid and carbohydrate composition determined. The COOH-terminal amino acid and the NH2-terminal amino acid were determined to be serine and alanine, respectively. Alanine-protein exhibited multiple forms on isoelectric focusing.

Introduction

Angiotensinogen is a plasma protein which serves as the substrate for the enzyme renin. Renin which is secreted by the kidney into the blood stream cleaves 1 Leu,Leu bond to release the decapeptide, angiotensin I. Angiotensin I is converted to an octapeptide, angiotensin II by the peptidyl dipeptide carboxyhydrolase, angiotensin converting enzyme. Angiotensin II is the principal biologically active component of this system. The principal actions of circulating angiotensin II are induction of a pressor response and stimulation of the production of aldosterone. Angiotensin II can be converted into another biologically active component, angiotensin III by removal of the amino terminal aspartic acid. Angiotensin III possesses less pressor activity than angiotensin II but is nearly equally potent in stimulating the production of aldosterone.

The present understanding of the factors which control the rate of production of the biologically active components of the renin angiotensin system are poorly understood. This is especially true for our understanding of the initial reaction of renin with angiotensinogen to produce angiotensin I. Many papers have appeared in the last decade suggesting the presence of activators or inhibitors of the renin catalyzed reaction. The majority of these claims have not been substantiated or refuted due in large part to the unavailability of pure human renin and pure human angiotensinogen. Recently two groups have reported the purification of human renin.1,2 This paper will review the work on the purification and characterization of human plasma angiotensinogen and will present new data on an isolated protein which tends to copurify with angiotensinogen.

In order to understand how the useful purification steps have evolved, it is pertinent to review some of the early studies on the purification of angiotensinogen from animal sources.

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The earliest attempts to concentrate angiotensinogen used fractional precipitation. By careful fractional precipitation of hog serum with ammonium sulfate, PLENTL et al. demonstrated in 1943 that angiotensinogen was an $\alpha_2$-globulin. GREEN and BUMPUS in 1954 introduced an acid denaturation step at pH 2.5 for the purification of hog angiotensinogen. In 1963 SKEGGS et al. succeeded in purifying hog angiotensinogen to apparent homogeneity and obtained a chemical and physical characterization of this material. They processed 3,7501 of hog plasma by means of a five step purification procedure in order to obtain gram quantities of three different angiotensinogens. The purification steps utilized included ammonium sulfate fractionation, acid denaturation at pH 2.5, batch fractionation on DEAE cellulose, column chromatography on DEAE cellulose utilizing a gradient in pH and a solvent extraction step. Since none of these three preparations appeared homogeneous on starch gel electrophoresis, each fraction was further purified by means of countercurrent distribution. Thus, five different preparations of angiotensinogen were obtained, three of which appeared homogeneous on starch gel electrophoresis, each fraction was further purified by means of countercurrent distribution. Thus, five different preparations of angiotensinogen were obtained, three of which appeared homogeneous on starch gel electrophoresis, each fraction was further characterized. These three preparations had a specific angiotensin content of 25.7, 12.2 and 14.5 Goldblatt units/mg protein. The only NH$_2$-terminal amino acid found in each of the preparations was aspartic acid. After treatment with renin, one other NH$_2$-terminal amino acid was detected. This amino acid could have been either leucine, isoleucine, or valine. The molecular weights as determined in the analytical ultracentrifuge ranged from 55,600 to 57,200. The amino acid composition of the three preparations was essentially the same while some small but significant differences were found in the carbohydrate analysis. The total carbohydrate accounted for 4–7% of the molecule.

The work on the purification of human angiotensinogen can be divided into two parts; those procedures which were designed to produce a partially purified preparation of human angiotensinogen with low renin activity and those procedures which were designed to purify human angiotensinogen to homogeneity. The impetus for the former work was supplied by the need for a source of substrate with which to do kinetic studies and the need to develop a more accurate measurement of renin in plasma. A summary of the former work is given in Table 1 in chronological order. The first two reports applied the fractional precipitation methods which had been previously worked out for hog angiotensinogen. The preparation of HAAS et al. exhibited considerable renin activity. Although ammonium sulfate fractionation is capable of producing a considerable increase in specific angiotensin I content, it does not provide an adequate separation of renin and angiotensinogen. The partial acid denaturation step utilized by GOULD et al. should not be used in the purification of human angiotensinogen since it has recently been demonstrated that while animal angiotensinogens are stable at an acid pH, human angiotensinogen is destroyed below pH 4. ARAWAKA et al. proposed the use of Cohn fraction IV-4 as a source of concentrated human angiotensinogen. The advantage of this fraction is that it is commercially available. The disadvantages of this preparation are that it contains appreciable renin activity and its reported specific angiotensin content of 0.008 $\mu$g angiotensin II/mg protein is low. ROSENTHAL et al. obtained an appreciable increase in specific angiotensin content with a three step purification of human angiotensinogen from plasma. It was stated that no renin activity was detected in this preparation but the lower limit of detection was not given. TEWKSBURY et al. were the first to use chromatography on hydroxylapatite as a purification step for angiotensinogen. This has proved to be a very powerful fractionation step since angiotensinogen is more easily eluted from hydroxylapatite than most of the other proteins which behave similarly to angiotensinogen on ion exchange chromatography. Thus, this preparation does not contain albumin which is not removed by ion exchange chromatography. The first two ion exchange steps were batch elution steps carried out in Buchner funnels so as to increase the amount of material that could be processed with ease. The renin activity of this preparation was less than 40 pg angiotensin I/mg protein/hr. SKINNER et al. demonstrated that most of the renin activity in human plasma can be separated from angiotensinogen by ion exchange chromatography on DEAE-Sephadex. The starting material utilized was plasma from