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# Inhibition of human renin by concanavalin A

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Renin (EC 3.4.99.19) is an endopeptidase which splits a Leu—Leu bond in plasma renin substrate to yield a decapeptide, angiotensin I. We have reported two kinds of renin inhibitors, one is the deoxycholic acids [1, 2] and the other is the tetrapeptides which have a Leu—Leu bond [3]. Other natural and synthetic renin inhibitors have been also reported by several laboratories [4–11]. Among them pepstatin is the most potent inhibitor of human renin [12, 13].

In a previous paper, we reported that human renin binds to concanavalin A-sepharose column and is eluted with 0.2 M α-D-methylglucoside and α-D-methylmannoside [14]. In this short communication, we report that concanavalin A inhibited human renin competitively. The $K_i$ value for concanavalin A was 90 μg/ml (about 1.5 μM).

Concanavalin A was purchased from Boehlinger, Mannheim, West Germany. Ileu$^5$-angiotensin I was obtained from Protein Research Foundation, Osaka, Japan. Human renin (Lot No. 68/356: 0.1 Goldblatt unit/vial) was provided by the National Institute for Biological Standards and Control, London, England. Human renin substrate was partially purified from outdated blood bank plasma by the method reported previously [14]. This renin substrate preparation contained 963 ng angiotensin I equivalents/mg of protein. The renin substrate preparation contained angiotensinase activity, but not angiotensin I converting enzyme activity. The renin–renin substrate reaction was carried out by the modified method of Boucher *et al.* as described in Ref. 14. The reaction mixtures in a total volume of 2.0 ml consisted of 0.1 ml of renin ($2.5 \times 10^{-3}$ Goldblatt unit), 0.5 ml of physiological saline containing varying amounts of renin substrate (1095–3650 ng angiotensin I equivalents), 0.5 ml of 0.4 M acetate buffer, pH 6.0, containing 12 mM $CaCl_2$ and $MnCl_2$, 0.9 ml of physiological saline containing concanavalin A (0 to 1.6 mg), and 1.0 ml of moist Dowex 50W-X2 ($NH_4^+$) resin (Dow Chemical Co., Midland, MI, U.S.A.). The reaction mixtures also contained 2 drops of 0.27 M diisopropylfluorophosphate (Sigma Chemical Co., St. Louis, MO, U.S.A.). The formed angiotensin I in the reaction mixtures was measured by a bioassay described previously [15]. Angiotensin I was used as the standard pressor substance. Two hundred ng of angiotensin I added in the reaction mixture without renin was almost completely recovered after a 1-hr incubation. The pressor substance in the samples was identified as angiotensin I by radioimmunoassay of angiotensin I [14]. The glass apparatus used for the experiments was all siliconized.

When various amounts of concanavalin A were added to the reaction mixtures, the dose-related inhibition curve was obtained. A Lineweaver–Burk plot and Dixon plot [16] gave competitive inhibition kinetics. From the Lineweaver–Burk plot, the $K_m$ value for human renin with partially purified human renin substrate was shown to be $0.58 \times 10^{-6}$ M which is consistent with the values reported by Favre and Vallotton [17] and Poulsen *et al.* [10]. The inhibitor constant of concanavalin A ($K_i$) obtained from the Dixon plot was 90 μg/ml (about $1.5 \times 10^{-6}$ M), as shown in Fig. 1. This value is similar to that of pepstatin

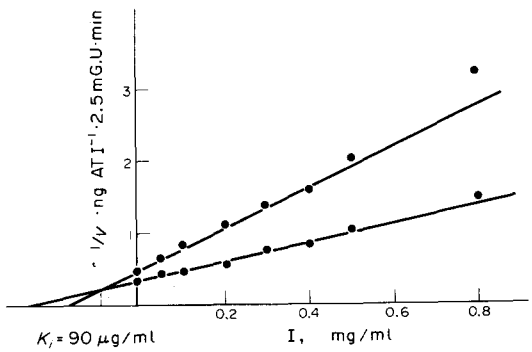


Fig. 1. Dixon plot of the relationship between the reciprocals of reaction velocity and various amounts of concanavalin A. The incubation mixtures contained 0.1 ml of human renin ($2.5 \times 10^{-3}$ Goldblatt unit), 0.5 ml of human renin substrate (2200 or 3650 ng angiotensin I equivalents), concanavalin A (0 to 1.6 mg) in 0.9 ml of physiological saline, 0.5 ml of 0.4 M acetate buffer, pH 6.0, containing 12 mM $CaCl_2$ and $MnCl_2$, and 1.0 ml of moist activated Dowex 50W-X2. The reaction mixtures also contained 2 drops of 0.27 M diisopropylfluorophosphate. The mixtures were incubated at 37° for 45 min.

($1.2 \times 10^{-6}$ M) [13] and of synthetic pro(phe$^5$phe$^6$)octapeptide ($1.3 \times 10^{-6}$ M) [10]. Although the $K_i$ value for pepstatin on human renin found by McKnown *et al.* [12] was extremely low ($1.3 \times 10^{-10}$ M), the substrate used by them was not human renin substrate but a tridecapeptide substrate coupled through the *N*-terminus to a polyglutamic acid [18]. After the mixture of 0.2 μg angiotensin I and 5 mg concanavalin A was incubated at 37° for 45 min in the presence of 3 mM $CaCl_2$ and $MnCl_2$, the pressor response of the angiotensin I in the mixture was unchanged in the rat. This indicates that concanavalin A neither modified the physiological pressor activity of angiotensin I, nor showed a hypotensive effect in the rat. Since concanavalin A binds to human renin substrate [14], the following experiments were done to determine whether the concanavalin A-renin substrate complex could be hydrolyzed by renin. In a previous paper [14] we have shown that renin substrate bound to concanavalin A was eluted completely with 0.05 M glucose. Thus, the renin–renin substrate reaction with 0.6 mg concanavalin A was carried out with and without 0.05 M glucose in the reaction mixture. After incubation, the amounts of angiotensin I formed with and without 0.05 M glucose were the same. Therefore, it seems that concanavalin A does not bind to the moiety of Leu–Leu bond in human renin substrate.

The results presented here demonstrate that concanavalin A inhibits the enzymic activity of human renin competitively, and these observations strongly suggest that human renin is a glycoprotein and contains concanavalin A binding carbohydrates around its active center.

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