[Frontiers in Bioscience 14, 3286-3291, January 1, 2009]

A peptide YY inhibits the human renin activity in a pH dependent manner

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1. ABSTRACT

Royal jelly (RJ) is known to possess several physiological and pharmacological properties. A dipeptide YY derived from RJ proteins is known to inhibit angiotensin converting enzyme (ACE) activity. Our previous study showed that the dipeptide YY inhibited the human renin activity at physiological pH. In this study, we investigated the pH dependency of the inhibitory effect of the dipeptide YY to the renin reaction with angiotensinogen. The renin activity was expressed at a wide pH range with two peaks around at 6.0 and 8.0. The dipeptide YY was found to inhibit the renin activity only at the acidic pH range lower than 8.0. The K_i was estimated 4.6 μM at pH 6.0 when the K_m of human renin was determined 0.07 μM using sheep angiotensinogen as the substrate. The K_m was 0.25 μM at pH 8.5. A stereo structure of the complex of human renin with the dipeptide YY was modeled to discuss its non inhibitory effect on the renin activity at the basic pH. It possibly owes to a local sift of YY space from the center of renin cleft into the N-domain side of renin molecule at basic pH range higher than 8.0.

2. INTRODUCTION

Renin, a key enzyme of renin-angiotensin (RA) system, catalyzes the reaction with its substrate angiotensinogen for producing angiotensin I (Ang I). Angiotensin converting enzyme (ACE) further processes the decapeptide into a potent vasoconstrictor angiotensin II as the final product of RA system; a contributory factor in the pathogenesis of hypertension (1). Inhibition of the renin activity has become an important target to control blood pressure and fluid volume balance along with other biological responses due to its unique substrate specificity and strategic role in the regulation of blood pressure. So, the most recent advances in antihypertensive therapies include drugs targeting the RA system. Development of a renin inhibitor is required for the treatment of hypertension and heart failure in which the RA system is involved, although suppression of this system with ACE inhibitors or angiotensin receptor blockers has proven to be clinically effective, producing a reduction in the blood pressure.

Royal jelly (RJ), an essential food of queen honeybee (*Apis mellifera*) and their larvae, has been reported to possess many pharmacological characteristics such as

antihypertensive activity, antitumoral activity, insulin-like activity, vasodilative and hypotensive activities, anti-inflammatory activity and wound-healing properties (2-6). Maruyama *et al*. (7) reported the inhibitory ability of peptide YY derived from proteolytically digests of RJ proteins (ProRJ) against the activity of ACE. Recently, we revealed that a dipeptide YY from ProRJ inhibited the activity of renin *in vitro* at physiological pH, which also lowered the systolic blood pressure (SBP) in spontaneously hypertensive rats (SHR) (8).

The pH dependent of human renin activity was first reported by Inagami and Murakami in 1977 (9). The pH dependence of the renin reaction showed two separate peaks using sheep angiotensinogen and one peak with a shoulder using human, rat, and pig angiotensinogens (10 and 11). Recently, the pH dependence of the human renin activity has been reported to have two peaks using the sheep angiotensinogen as the substrate, besides rat and mouse Ren1 renin had one peak with a shoulder (12). In this study, we investigated how the dipeptide YY inhibition of the human renin activity changed in a wide range of pH between 3.5 and 11 using sheep angiotensinogen.

3. MATERIALS AND METHODS

3.1. Isolation of dipeptide YY from proteolytically digested RJ protein fraction

The dipeptide YY was isolated from digested RJ protein according to method as described previously (8).

3.2. Preparation of partially purified recombinant sheep angiotensinogen and recombinant human renin

The Chinese hamster ovary (CHO) cells containing sheep angiotensinogen cDNA were obtained as described previously by Nagase *et al*. (13). The expression vector containing wild-type sheep angiotensinogen cDNA was transfected into CHO cells by a phosphate-mediated method (13). The cloned cells producing sheep angiotensinogen cDNA were routinely propagated in DMEM supplemented with 5% FBS, 0.1 mM non-essential amino acids, 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin and 2.3 mM MTX. Cells were incubated at 37°C with continuous supply of 5% CO2 and 99% humidity. Sheep angiotensinogen secreted in the medium was collected and purified by CM-Toyopearl column chromatography (14, 15).

The recombinant human renin used in this study was produced as prorenin; harboring the human prorenin cDNA in CHO cells and was followed by purification and activation of trypsin treatment under the same conditions as described previously (16).

3.3. Preparation of wide range buffer

Each of Buffer used in this experiment contained 30 mM citric acid-30 mM potassium phosphate-30mM boric acid-30 mM barbital-43mM sulfuric acid, and each pH of buffer was adjusted to from 3.5 to 11.0 with 0.2 M NaOH (10 and 17).

3.4. Observation of the pH dependence of renin activity

The pH dependent activities of human renin with and without dipeptide YY were measured from pH 3.5 to 11.0 at 37ºC for 30 minutes in the citrate-borate buffer using 0.2 μM of the substrate sheep angiotensinogen. Ang I produced in the reaction was assayed by Ang I enzyme-linked immunosorbent assay (ELISA) (18).

3.5. Renin assay at pH 6.0 and 8.5 with and without dipeptide YY

In the presence of citrate-borate buffer at pHs 6.0 and 8.5, the recombinant human renin was incubated with 0.4, 0.2, 0.15, 0.1, 0.05 and 0.025 μM of recombinant sheep angiotensinogen for 30 minutes at 37ºC with and without 6 μM of the dipeptide YY. The renin activity was expressed as the amount of Ang I generated by this reaction for 1 h in the 1 ml of original renin preparation and was assayed by AngI-ELISA method (18). The kinetic parameters, K_m and K_i at pH 6.0 and 8.5 were estimated by using Lineweaver-Burk plot.

4. RESULTS

4.1. The pH dependence of human renin activity with and without dipeptide YY

As shown in Figure 1, the pH dependence of the renin activity without dipeptide YY was biphasic that had two peaks at pH 6.0 and around 8.0. The dipeptide YY inhibited the renin activity from at pH 3.5 to 7.5. The renin activity with the dipeptide YY was similar to that without the peptide between at pH 8.5 and 11. All data were indicated as means (n=4). Under the standard assay conditions, the standard error of mean at each of points was less than 5% of mean (n=4).

4.2. The Kinetic parameters of the inhibition of renin activity with and without dipeptide YY at pH 6.0 and 8.5

By using Lineweaver-Burk plots, the K_i of peptide YY at pH 6.0 was calculated to be 4.6 μM when the K_m of the substrate of renin was determined to be 0.070 μM, as shown in Figure 2 A and B. All data were indicated as means (n=4). Under the standard assay conditions, the standard error of mean at each of points was less than 5% of mean (n=4). The plots were also indicated the competitive inhibition of renin activity by the dipeptide YY.

At pH 8.5 the dipeptide could not inhibit the human renin activity at pH 8.5 when the K_m of the substrate of renin was estimated to be 0.25 μM, as shown in Figure 3 A and B. All data were indicated as means (n=4). Under the standard assay conditions, the standard error of mean at each of points was less than 5% of mean (n=4).

5. DISCUSSION

In this study, we confirmed that the pH dependency of the renin was similar to previous data (10 and 11). The renin activity had two optima pH at 6.0 and 8.0 using the substrate sheep angiotensinogen (Figure1). The renin activity with dipeptide YY at different pH from pH 3.5 to

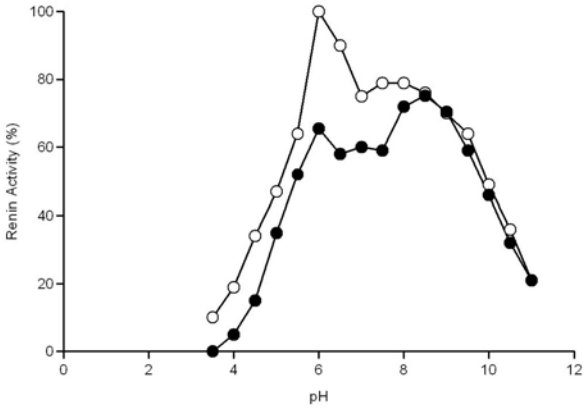

Figure 1. The pH dependence of human renin activity with and without dipeptide YY. The renin activity was measured with (●) and without (○) the dipeptide at pH range from 3.5 to 11.0 using 0.2 μM sheep angiotensinogen as the substrate. The renin activity was indicated by the percentages of that at pH 6.0 (0.25 nM Ang I/min).

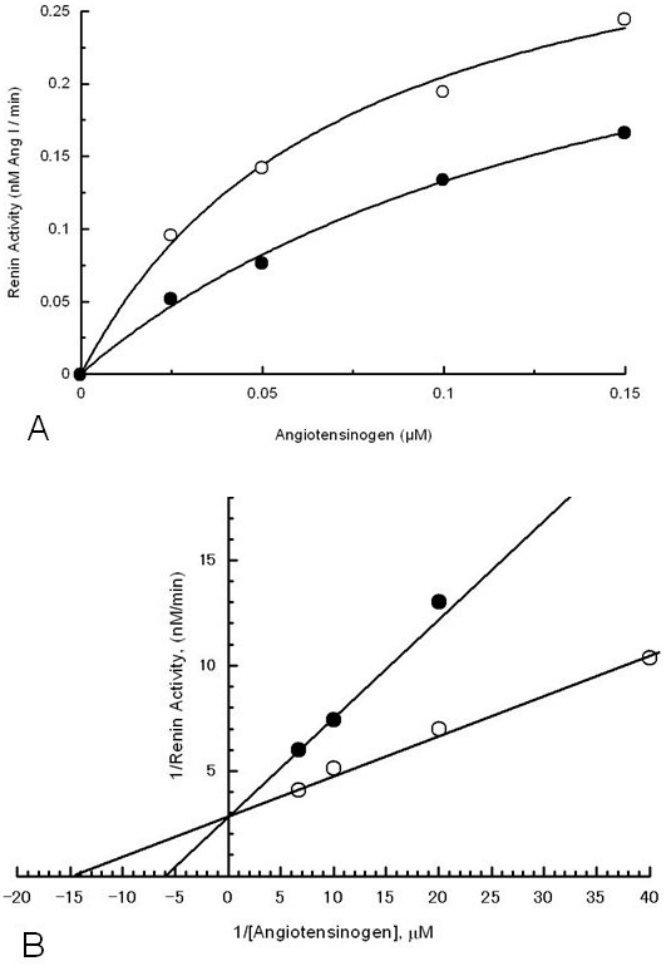

Figure 2. (A) Inhibition of renin activity by the dipeptide YY at pH 6.0. The renin activity was measured with (●) and without (○) the peptide at various concentrations of angiotensinogen. (B) Lineweaver-Burk Plots of the renin activities obtained in the Fig. (A) in the presence of dipeptide YY (●) and without the peptide (○) under the standard assay conditions.

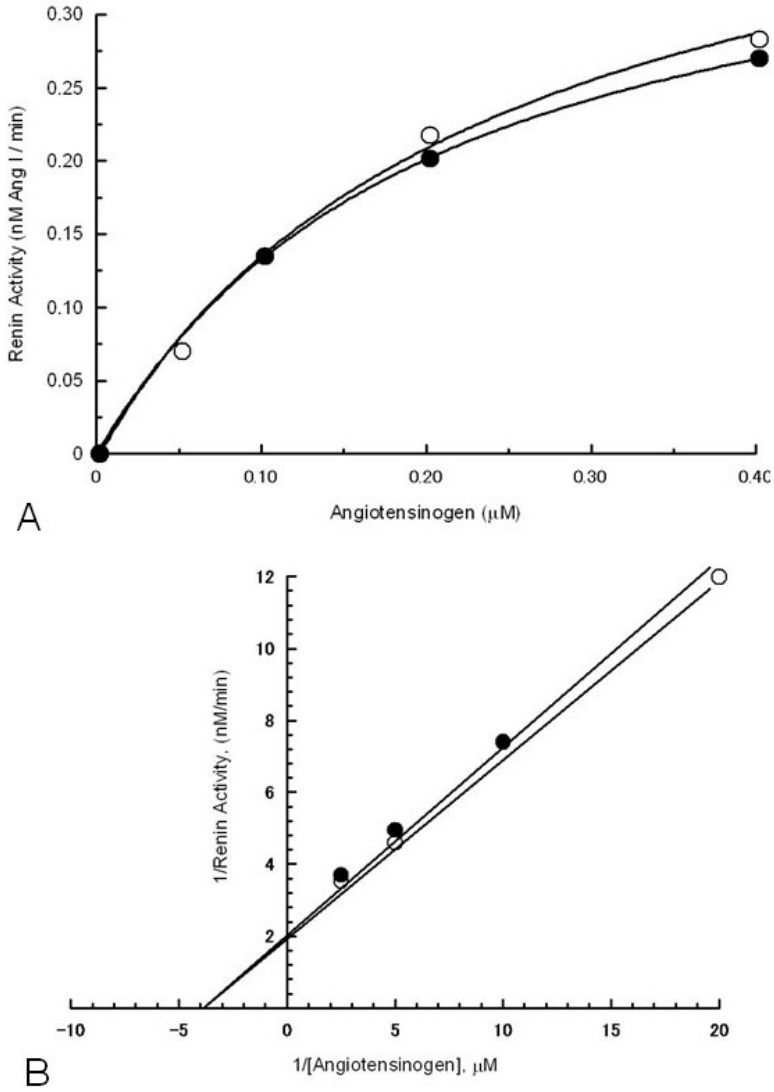

Figure 3. (A) Inhibition of renin activity by the dipeptide YY at pH 8.5. The renin activity was measured with (●) and without (○) peptide at various concentrations of angiotensinogen (μM). (B) Lineweaver-Burk Plots of the renin activities obtained in the Fig. (A) in the presence of dipeptide YY (●) and without peptide (○) under the standard assay conditions.

11.0 was investigated, as shown in Figure 1. The dipeptide YY inhibited the renin activity at pH 3.5 to 8.0 and a little inhibition has been indicated at basic pH 8.5 to 11. This is the first evidential report that a renin inhibitor effects on the renin activity in a pH dependent manner.

By using Lineweaver-Burk plot, the K_i value of the dipeptide YY at pH 6.0 was determined to be 4.6 μM when the K_m of the substrate sheep angiotensinogen was calculated to be 0.070 μM (Figure 2 A and B). At pH 8.5 the K_m of the human renin activity was 0.25 μM (Figure 3 A and B) that is higher than that at pH 6.0. These data suggest that ionic bonds in the whole enzyme-substrate reaction contribute to keeping the higher affinity of renin with angiotensinogen. This property probably causes to the reason why the dipeptide has no effect on the renin activity in the basic pH ranges (Figure 1). To understand this property in detail from stereo-structural aspect, we predicted a stereo structure of complex of human renin molecule with the dipeptide YY. As shown in Figure 4, the atomic coordinates of the complex were visualized by software MolFeat (FiatLux Co.). The dipeptide YY localizes in the center space of cleft in renin molecule to inhibit the renin reaction with angiotensinogen. In this model, configuration of R82 was partially modified in the original structure of renin molecule (PDB code: 1RNE). The dihedral angle in the R82, χ1, was only rotated from -96º to -20º using software MIFit (Rigaku Co.). This rotation was within acceptable modification to related neighbor atoms. Other atomic coordinates of the complex were kept same as those

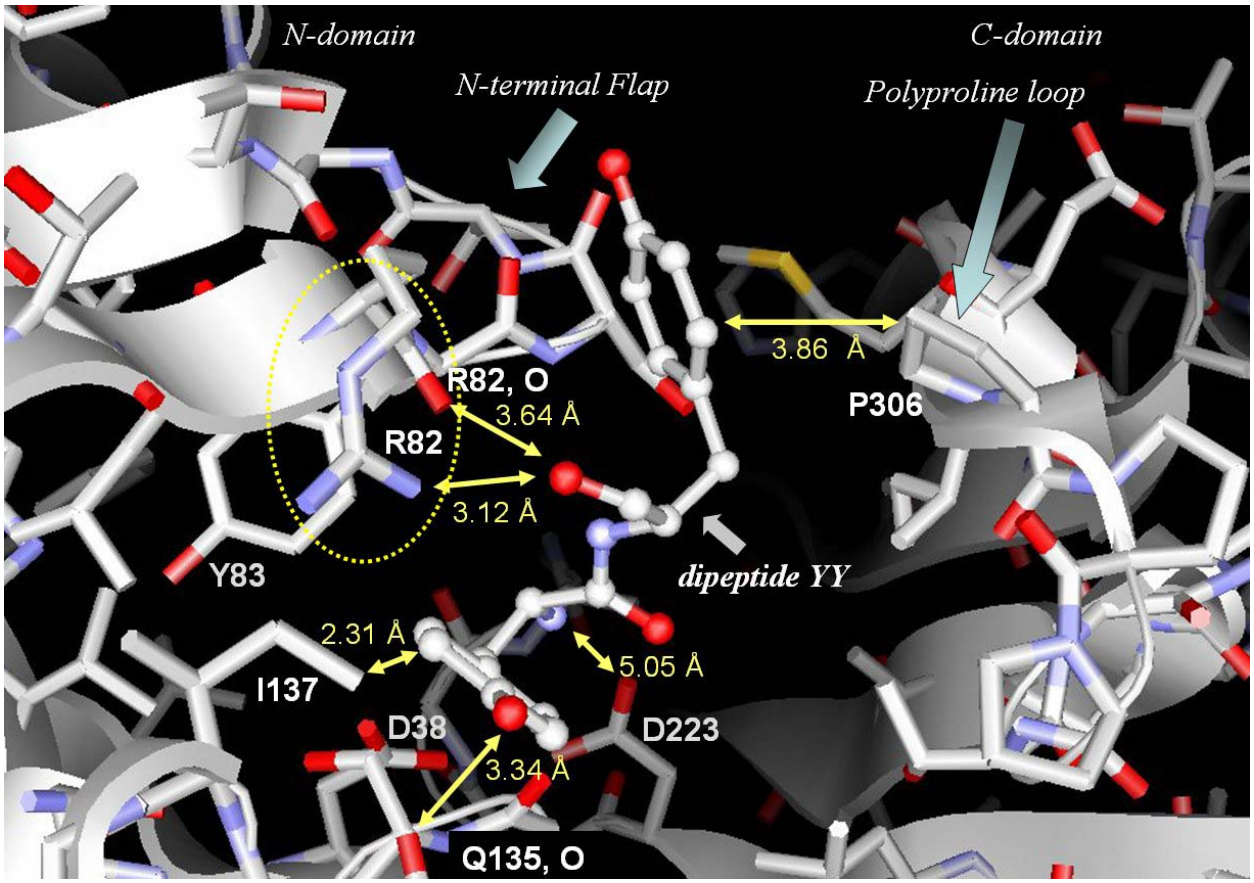

Figure 4. Possible stereo structure model of human renin molecule complexed with dipeptide YY. Atomic coordinates of renin molecule and dipeptide YY were extracted from those of renin and YY region in the structures of a complex of human renin with inhibitor (PDB code: 1RNE) superimposed with another complex of mouse renin with peptide inhibitor including YY sequence (PDB code: 1SMR). And then, the dihedral angle in the R82, χ_1, was rotated from -96º to -20º using software MIFit (Rigaku Co.). The side chain of R82 after modified is circled with dots in this figure. The model structure is visualized by software MolFeat, and atomic-atomic distances are also calculated by the software. The renin molecule and dipeptide YY are indicated by stick-model with ribbon and ball & stick model, respectively.

in the predicted model as previously reported (8). As shown in Figure 4, some important ionic bonds were possibly indicated between peptide YY and N-domain of renin molecule and also hydrophobic bond can be shown between the peptide and renin molecule. At basic pH ranges, amino groups, e.g. amino group of dipeptide YY and R82 should be deprotonated to break those ionic bonds. Also the hydrogen bond, e.g. the hydroxide group of the YY with oxygen of Q135, was possibly weaken with increase of pH from 8.0, and then the YY can make strong the hydrophobic bond with I137 in the N-domain. Overall the dipeptide YY probably shifts closer space to N-domain side of renin molecule at basic pH to open the cleft space for angiotensinogen.

According to our previous study, the dipeptide YY inhibited the human renin activity at pH 7.0 and had dual inhibitory action against human renin and ACE by decreasing SBP significantly in SHR (7 and 8). Therefore, the dipeptide YY can be effective to inhibit the renin activity on the tissue and circulation not only in the physiological condition but also in local inflammatory condition.

6. ACKNOWLEDGEMENTS

This study was supported in part by a Grant-in-aid for Scientific Research (1907165) from the Ministry of Education, Science and Culture of Japan, and AHM NN is the recipient of a Postdoctoral Fellowship for Foreign Researchers (2007/2009: P07165) from Japan Society for the Promotion of Science.

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Abbreviations: RJ: royal jelly; ACE: angiotensin converting enzyme; RA system: renin-angiotensin system; Ang I: angiotensin I; Ang II: angiotensin II; ARBs: angiotensin receptor blockers; BP: blood pressure; ProRJ: protease digests of RJ proteins; CHO cells: chinese hamster ovary cells; SBP: systolic blood pressure; SHR: spontaneously hypertensive rats; ELISA: enzyme-linked immunosorbant assay

Key Words: Renin, pH dependence of renin reaction, Royal jelly derived dipeptide, YY

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