J. Biochem. **140**, 725–730 (2006)
doi:10.1093/jb/mvj201

Effects of Nucleotides on the Interaction of Renin with GlcNAc 2-Epimerase (Renin Binding Protein, RnBP)

Saori Takahashi[1,*], Kazuyuki Hori[1], Hironobu Ogasawara[1], Kazuyuki Hiwatashi[1] and Toshihiro Sugiyama[2]

[1]Akita Research Institute for Food and Brewing, 4-26, Sanuki, Arayamachi, Akita 010-1623; and [2]Department of Biochemistry, School of Medicine, Akita University, 1-1-1 Hondo, Akita 010-8543

Received August 6, 2006; accepted September 25, 2006

Renin binding protein (RnBP), a cellular renin inhibitor, was identified as an enzyme, GlcNAc 2-epimerase. Recombinant RnBP inhibited porcine renin activity in a dose dependent manner. However, the inhibition was neutralized by nucleotides, such as ATP, dATP, dGTP, dCTP or dTTP. Moreover, ATP inhibited the formation of hetero-complex of renin with RnBP, called high molecular weight (HMW) renin. On the other hand, *N*-ethylmaleimide (NEM), a SH-alkylating reagent inhibited the GlcNAc 2-epimerase activity concomitant with the decaying of the dimer to the monomer of the enzyme. The inhibition was modulated in the presence of ATP. These results indicate that nucleotides stabilize the dimeric form RnBP (GlcNAc 2-epimerase) and inhibited the formation of the renin-RnBP hetero complex, HMW renin.

Key words: ATP, binding protein, epimerase, GlcNAc, nucleotides, renin.

Abbreviations: AHOX, *N*-acyl hexosamine oxidase; GlcNAc, *N*-acetylglucosamine; HMW, high molecular weight; HTIB, 3-hydroxy 2,4,6-triiodobenzoic acid; NEM, *N*-ethylmaleimide; RnBP, renin binding protein.

Renin [EC 3.4.23.15] is a key enzyme of controlling the activity of renin-angiotensin-aldosterone system (*1*). Renin binding protein (RnBP) is a protein that inhibits renin activity with the formation of a renin-RnBP hetero complex called high molecular weight (HMW) renin (*2–5*). The nucleotide sequences of porcine, human, and rat RnBP cDNAs were determined and the amino acid sequences consisted of 402, 417 and 419 amino acid residues, respectively (*6*, *7*). The co-expression of renin and RnBP cDNAs in AtT-20 cells showed that RnBP regulates active renin secretion from the transferred cells (*8*). The human and rat RnBP genes were also isolated from genomic DNA libraries (*9–11*). Both genes span about 10 kilobase pairs and consisted of 11 exons separated by 10 introns. The human RnBP gene was found to located in human chromosome X by means of PCR of hybrid DNAs from human and hamster somatic cells (*9*), and it has been mapped in the distal Xq28 chromosome band (*12*, *13*). In addition, a polymorphism in intron 6 of the RnBP gene was shown to be associated with a 40% increase in the renin/prorenin ratio in Caucasian men (*10*). Moreover, the expression of RnBP mRNA was determined in rats with RNase protection analysis (*14*). The RnBP mRNA was detected in the kidney, brain, lung, adrenal gland, heart, spleen, ovary and testes. The expression of renin and RnBP mRNA in the kidneys of rat with two-kidney one-clip hypertension has been investigated (*14*, *15*). The renin mRNA level of the clipped kidney is increased significantly. On the other hand, the RnBP mRNA levels of the kidneys remain unchanged.

The cDNA cloning of porcine kidney *N*-acetylglucosamine (GlcNAc) 2-epimerase [EC 5.3.1.8] (*16*) and the expression and characterization of human RnBP (*17*, *18*) showed that RnBP was identical to GlcNAc 2-epimerase. The enzyme catalyzes the conversion between GlcNAc and *N*-acetylmannosamine (ManNAc). The human GlcNAc 2-epimerase specifically inhibited by *N*-ethylmaleimide (NEM), monoiodoacetic acid, 5,5′-dithiobis (2-nitorobenzoic acid), and the active site residue of the enzyme was identified as cysteine 380 by site-directed mutagenesis (*19*). In our previous studies it was demonstrated that nucleotides, such as ATP, dATP, ddATP, ADP and GTP, enhance human, rat and porcine GlcNAc 2-epimerase activity (*20*). Moreover, the nucleotide binding domain and the amino acid residues conferring nucleotides were also determined (*21*, *22*). However the function of nucleotides on the interaction of renin with RnBP had not been understood. In the present study, the effects of several nucleotides on the interaction of renin with RnBP (GlcNAc 2-epimerase) were investigated. Our results demonstrated that nucleotides, such as ATP, inhibited the formation of HMW renin to stabilize the dimeric form of RnBP (GlcNAc 2-epimerase).

MATERIALS AND METHODS

Materials—*N*-Acylhexosamine oxidase (AHOX), ManNAc, and 3-hydroxy 2,4,6-triiodobenzoic acid (HTIB) were obtained from Sigma (St. Louis). 4-Aminoantipyrine and horseradish peroxidase were from Nacalai Tesque (Kyoto) and Wako Pure Chemical (Osaka), respectively. Restriction enzymes, and dATP, dCTP, dGTP, and dTTP were obtained from Takara (Otsu, Shiga). Porcine kidney renin and recombinant porcine RnBP were purified as described previously (*3*, *20*).

Renin Activity—The renin activity was measured by the rate of formation of angiotensin I from porcine plasma angiotensinogen (*23*). After the incubation of renin with

*To whom correspondence should be addressed. Tel: +81-18-888-2000, Fax: +81-18-888-2008, E-mail: saori@arif.pref.akita.jp

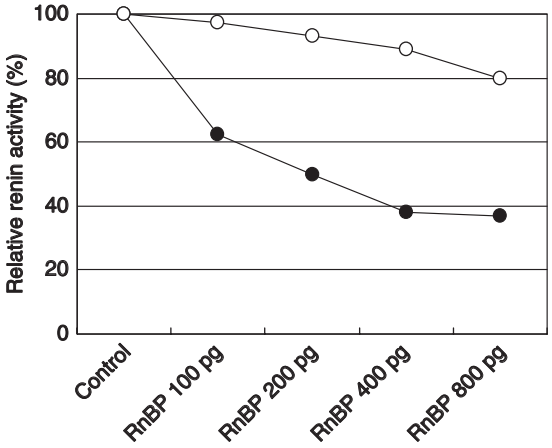

Fig. 1. **Inhibition of renin by RnBP in the presence or absence of ATP.** The reaction mixture containing 20 mM sodium-phosphate buffer, pH 6.5, 1 mM EDTA, 10 μM leupeptin, 0.1% BSA, 65 pg pure renin and RnBP in the absence (closed circles) or presence (open circles) of 5 mM ATP was incubated at 37°C for 5 min. Then, the residual renin activity was measured as described in the text.

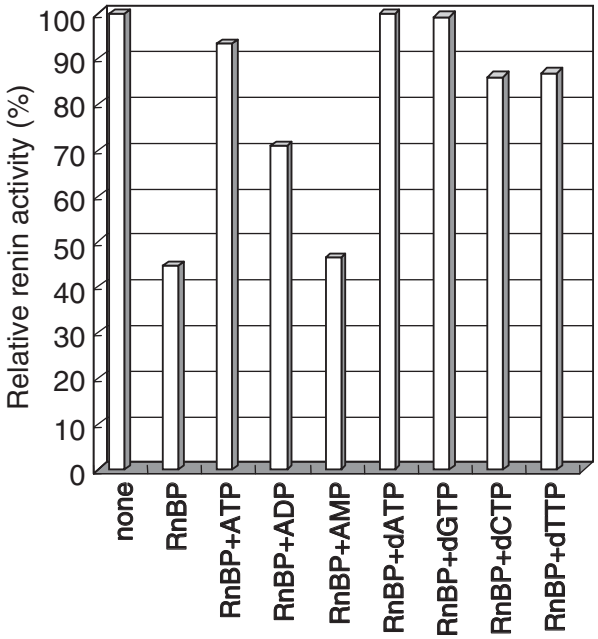

Fig. 2. **Effects of nucleotides on the renin inhibition by RnBP.** The purified renin (65 pg) was incubated with RnBP (800 pg) in the presence of 5 mM nucleotides. After the incubation at 37°C for 5 min, the residual renin activity was measured as described in the text.

partially purified angiotensinogen at 3°C for 60 min in 0.1 M sodium phosphate buffer, pH 6.5, containing 10 mM EDTA, 1 mM PMSF, angiotensin I produced was determined using a radioimmunoassay (*24*).

GlcNAc 2-Epimerase Activity—The GlcNAc 2-epimerase activity was measured in a system of AHOX coupled with peroxidase (*25*). Samples (20 μl) were incubated at 37°C with 80 μl of 0.1 M Tris-HCl, pH 8.0, 10 mM $MgCl_2$, 50 mM ManNAc and 5 mM ATP. The reaction was terminated by adding 0.9 ml of ice-cold water. The diluted sample (20 μl) was incubated with 0.25 ml of 1 mM 4-aminoantipyrine, 0.5 unit/ml of AHOX, 5 units/ml of horseradish peroxidase in 0.1 M sodium phosphate buffer, pH 7.25, containing 0.1% NaN_3 and 0.25 ml of 2 mM HTIB in 0.1 M sodium phosphate buffer, pH 7.25, containing 0.1% NaN_3. After incubation at 37°C for 30 min, the absorbance at 515 nm was measured. One unit of the enzyme activity was defined as the amount of enzyme that produced 1 μmol of GlcNAc per min.

Effects of NEM on GlcNAc 2-Epimerase Activity—GlcNAc 2-epimerase (5 μg) was incubated at 25°C for 5 min with NEM (0–0.3 mM) in 20 mM sodium phosphate buffer, pH 7.0, 0.15 M NaCl, 0.02% NaN_3 in the presence or absence of 5 mM ATP in a total volume of 0.1 ml. Then the sample was mixed with 0.1 ml of 20 mM sodium phosphate buffer, pH 7.0, 0.1% 2-mercaptoethanol. After incubation at 4°C for 5 min, the residual GlcNAc 2-epimerase activity was measured.

Gel Filtration Chromatography—Samples (0.2 ml) were separated using Superdex 75 HR10/30 (Amersham Bioscience) in 20 mM sodium phosphate buffer, pH 7.0, containing 0.15 M NaCl and 0.02% NaN_3 at a flow rate of 1.0 ml/min and fractionated in 1-ml fractions. Bovine serum albumin (BSA, 67,000), ovalbumin (Oval, 45,000), and soybean trypsin inhibitor (SBTI, 20,100) were used as molecular weight standards.

Protein Determination—The concentration of protein was determined using a Bio-Rad protein assay kit (*26*) with bovine serum albumin as a standard. The purified porcine renin concentration was estimated with a coefficient of 9.1 (280 nm) for a 1% solution with a 1 cm path-length (*27*).

Effects of Nucleotides and NEM on the Hydrolysis of GlcNAc 2-Epimerase by Thermolysin—One microgram of NEM treated or intact RnBP was incubated with thermolysin in Tris-HCl, pH 7.5, containing 10 mM $MgCl_2$ in the presence or absence of 5 mM ATP. After incubation at 37°C for the indicated times, thermolysin was inactivated by adding 2 μl of 0.1 M *o*-phenanthroline. The sample was subjected to SDS-PAGE. After the electrophoresis, the protein was stained with Coomassie Brillinant Blue R-250.

RESULTS

Effects of ATP on Renin Inhibition by RnBP—As shown in Fig. 1, RnBP inhibited the renin activity in a dose-dependent manner in the absence of ATP (closed circles in Fig. 1). However, the renin inhibition was neutralized by the addition of ATP (open circles in Fig. 1). On the other hand, ATP had no effects on the renin activity in the absence of RnBP (data not shown). These results clearly indicate that ATP interrupts the interaction of renin with RnBP, GlcNAc 2-epimerase. The following experiments were performed to clarify the effects of nucleotides on the formation of RnBP homodimer and renin-RnBP heterodimer.

Modulation of Renin Inhibition of RnBP by Nucleotides—The effects of other nucleotides on renin inhibition by RnBP are also shown in Fig. 2. Deoxy nucleotides, such as dATP, dGTP, dCTP and dTTP, cancelled the inhibition of renin by RnBP. ADP had little effect on the cancellation of renin inhibition by RnBP. However, AMP had no effects of renin inhibition by RnBP. In connection

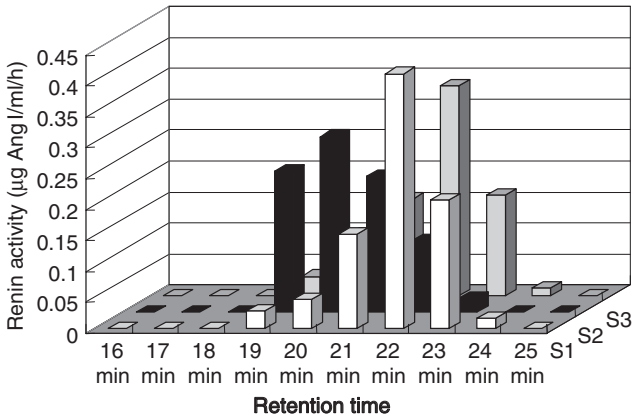

Fig. 3. **Effects of ATP on the formation of the renin-RnBP hetro-complex, HMW-renin.** Samples used were: S1, purified renin (0.1 μg); S2, reaction mixture of renin (0.1 μg) with RnBP (5.0 μg); S3, reaction mixture of renin (0.1 μg) with RnBP (5.0 μg) in the presence of 5 mM ATP.

Table 1. **Effects of NEM on GlcNAc 2-epimerase activity.**

NEM concentration (mM)	–ATP	+ATP
0	100	100
0.1	38.6	89.9
0.2	17.7	70.5
0.3	10.4	63.9

with the results, dATP enhanced porcine GlcNAc 2-epimerase activity 1.35-fold in comparison with ATP. But the effects of ADP and AMP on the enzyme activities were 58% and 12% in comparison with ATP (*20*).

Stabilization of Dimeric Form of RnBP by ATP—It is well known that RnBP binds renin and forms a hetero complex, so-called HMW renin (*2–4*). The purified porcine renin showed a single peak with an apparent molecular weight of 40,000 (Fig. 3, S1). When renin was incubated with RnBP, the molecular weight of renin was shifted to about 65,000 (Fig. 3, S2). The molecular weight of renin remained of 40,000 in the presence of ATP, even after the incubation of renin with RnBP (Fig. 3, S3). Thus, ATP inhibited the renin-RnBP heterodimer formation.

Effects of NEM on GlcNAc 2-Epimerase—In our previous studies, we showed that sulfhydryl-oxidizing and -alkylating reagents dissociated the dimeric form of RnBP to monomer (*5*). Moreover, these reagents specifically inactivated the GlcNAc 2-epimerase activity. Thus, we have studied the effects of NEM, a sulfhydryl-alkylating reagent, on the dimeric form of RnBP (GlcNAc 2-epimerase) and its enzymatic activity. As shown in Table 1, NEM strongly inhibited the GlcNAc 2-epimerase activity in the absence of ATP. The inhibition was modulated in the presence of 5 mM ATP. High concentration of NEM (5 mM) completely inhibited the GlcNAc 2-epimerase activity even in the presence of ATP. The effects of NEM and ATP on the dimeric form of GlcNAc 2-epimerase are also shown in Fig. 4. As already pointed out (*18*, *20*), native recombinant GlcNAc 2-epimerases (RnBPs) existed as a dimer with an apparent molecular weight of 65,000 (Fig. 4, S1). When GlcNAc 2-epimerase was incubated with NEM, the

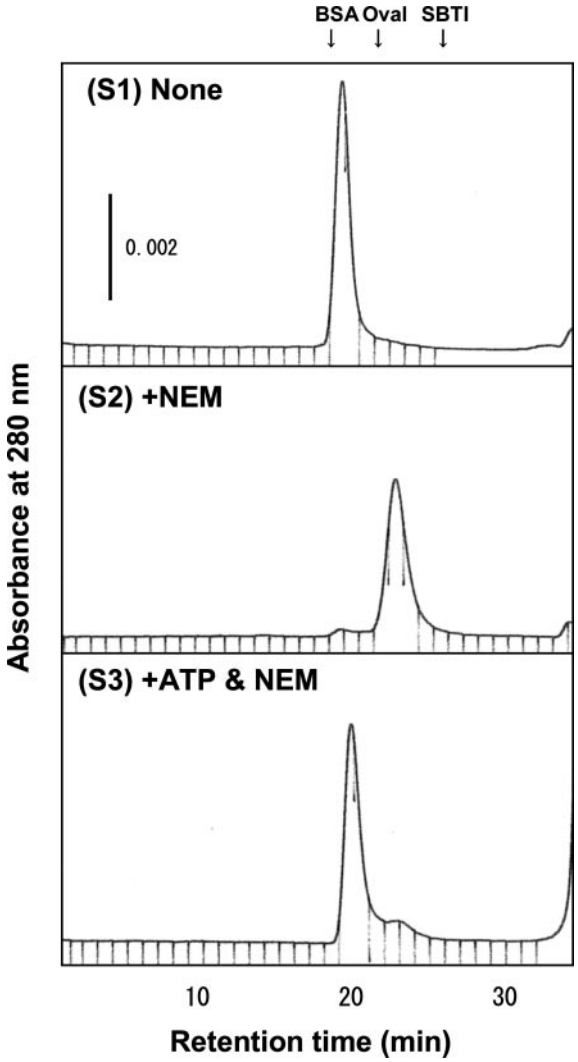

Fig. 4. **Gel filtration of intact or NEM-treated RnBP (GlcNAc 2-epimerase).** Samples used were: (S1), intact RnBP (12.5 μg); (S2), 0.3 mM NEM treated RnBP (12.5 μg); (S3), 0.3 mM NEM treated RnBP (12.5 μg) in the presence of 5 mM ATP.

molecular weight of the enzyme shifted to a lower molecular weight region with an apparent molecular weight of 40,000. On the other hand, NEM treatment of GlcNAc 2-epimerase in the presence of ATP had no effects on the molecular weight of the enzyme. The GlcNAc 2-epimerase activities of the eluate in Fig. 4 are also shown in Fig. 5. Native and NEM treated GlcNAc 2-epimerases in the presence of ATP showed the activity in the dimeric position (Figs. 5, S1 and S3). However, the enzyme activity was completely abolished by NEM in the absence of ATP (Fig. 5, S2).

Effects of NEM and ATP on the Hydrolysis of GlcNAc 2-Epimerase by Thermolysin—When GlcNAc 2-epimerase was incubated with thermolysin in the presence of ATP, no degradation product was seen, even after incubation for 20 min (Fig. 6, lanes 9–11). GlcNAc 2-epimerase was easily hydrolyzed by thermolysin in the presence of NEM (Fig. 6, lanes 5–7). On the other hand, the enzyme was not hydrolyzed by thermolysin in the presence of ATP, even

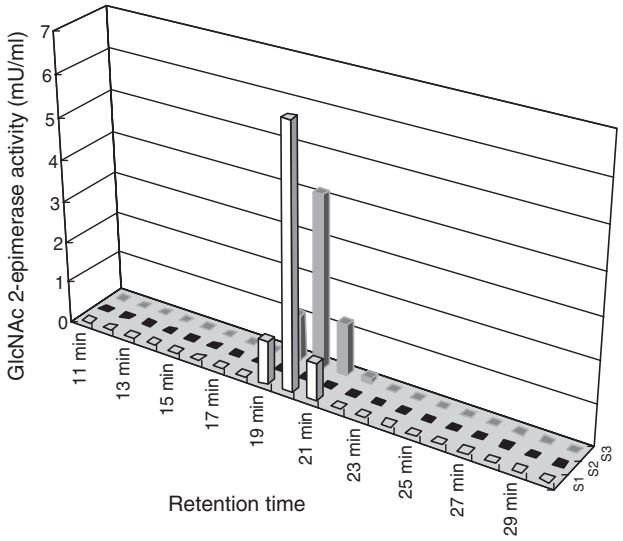

Fig. 5. **Effects of NEM on GlcNAc 2-epimerase activity.** GlcNAc 2-epimerase activity in Fig. 4 was measured as described in the text.

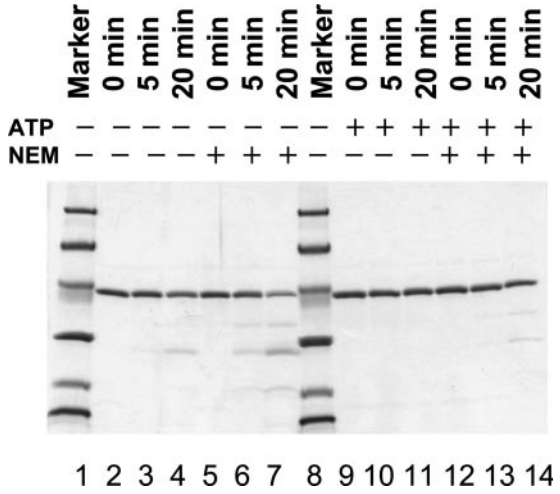

Fig. 6. **Effects of NEM and ATP on the hydrolysis of GlcNAc 2-epimerase by thermolysin.** Intact or NEM treated GlcNAc 2-epimerase was digested by thermolysin in the presence or absence of 5 mM ATP. After the SDS-PAGE, proteins were stained with Coomassie Brilliant Blue R-250.

in the presence of NEM (Fig. 6, lanes 12–14). These results suggest that the dimeric form of GlcNAc 2-epimerase is resistant to protease, but the monomeric form of the enzyme is sensitive to protease digestion.

DISCUSSION

The RnBP was firstly isolated from porcine kidney as a complex of renin. The protein bound to renin to form HMW renin. Recently, RnBP was identified as the enzyme GlcNAc 2-epimerase by the cDNA cloning of the porcine enzyme (*16*) and the expression and characterization of human RnBP (*17*, *18*). Nucleotides, such as ATP, dATP and ADP, were essential for GlcNAc 2-epimerase activity. However, the effects of nucleotide on the interaction of renin with GlcNAc 2-epimerase had not been understood. In this report, we studied the effects of several nucleotides on GlcNAc 2-epimerase and renin. Our previous studies indicated that RnBP inhibited the renin activity in a dose dependent manner (*3*, *5*) (Fig. 1). Interestingly, the inhibition was modulated in the presence of ATP. Moreover, nucleotides, such as ADP, dATP, dGTP, dCTP and dTTP, were effective for the modulation of NEM treatment (Fig. 2). These nucleotides seem to stabilize GlcNAc 2-epimerase and to form active sites of the enzyme.

The purified RnBP from porcine kidney bound to renin to form a hetro-complex of renin with RnBP called HMW renin. The molecular weight of renin shifted to 65,000 when RnBP was incubated with renin (Fig. 3, S2). However, the shift of molecular weight was abolished by ATP. ATP had no direct effects on renin activity and the molecular weight of renin (data not shown). These results indicate that ATP directly affects the RnBP molecule to inhibit the interaction of renin with RnBP. The effects of NEM on GlcNAc 2-epimerase activity had already been studied (*19*). SH-reagents, including NEM, strongly inhibited GlcNAc 2-epimerase activity and the essential cysteine residue was identified as cysteine-380 of the human enzyme (*19*). In connection with the effects of NEM on RnBP, the recombinant porcine RnBP existed as a dimer and as dissociated monomers in the presence of SH-reagents. Interestingly, ATP protected the dissociation of the dimeric from of GlcNAc 2-epimerase, even in the presence of NEM (Fig. 4, S2). The enzyme activity also remained in the presence of ATP, even in the presence of NEM (Fig. 5, S3). Thus, ATP seems to stabilize dimeric form of GlcNAc 2-epimerase to form the catalytic domain of the enzyme. The stabilization effects of ATP on GlcNAc 2-epimerase are also shown in Fig. 6. The enzyme was quite stable in the presence of ATP, even in the presence NEM. No degradation product was seen on the gel (Fig. 6, lanes 9–14). However, the enzyme was easily degraded by thermolysin in the presence of NEM. These results clearly indicate that the dimeric form of the enzyme has a protease resistant configuration, but the NEM-treated monomer has an unstable configuration.

Taken together, nucleotides, such as ATP, ADP and dATP, stabilize the dimeric form of GlcNAc 2-epimerase (RnBP). In the absence of nucleotide(s), RnBP can easily bind to renin to form a hetero-complex renin called HMW renin (Fig. 7). On the other hand, the RnBP homodimer is hard to dissociate into monomers in the presence of nucleotide(s). Thus, the formation of the RnBP-renin hetro-complex is inhibited by nucleotide(s). The half-maximal concentration of ATP for porcine GlcNAc 2-epimerase was estimated to be 40 μM (*20*). The cytosolic ATP level in the normal cells was estimated to be about 1.0 mM and increased during apoptosis (*31*). Thus, the concentration of ATP used in this study seems to be physiological range in the cells.

In the present results, it is clearly shown that ATP regulates the formation of HMW renin to modulate renin activity *in vitro*. Further elaborate studies eg. *in vivo* and cell experiments will provide the physiological function of RnBP, GlcNAc 2-epimerase.

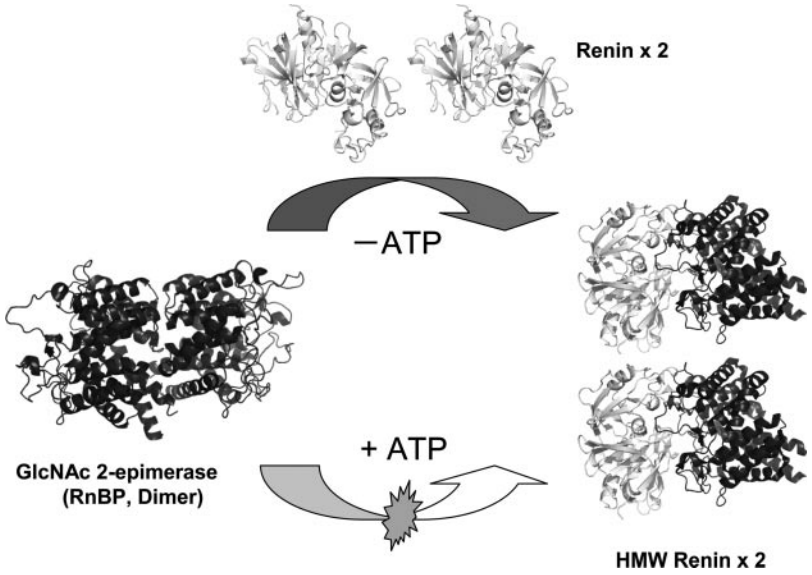

Fig. 7. **Effects of ATP on the interaction of renin with RnBP (GlcNAc 2-epimerase).** Three dimensional structure of human RnBP was predicted from porcine RnBP (PDB ID: 1FP3) using the protein homology–modeling server, SWISS-MODEL (*28*). Protein structure was drawing using the program PyMOL (*29*). Human renin data was from PDB ID: 2REN. Putative HMW-renin structure was provided by PachDock web server (*30*). RnBP can bind to renin to form HMW-renin in the absence of ATP. On the other hand, the RnBP homodimer is hard to dissociate into monomers in the presence of ATP.

This study was supported in part by a Grant-in-Aid for Scientific Research (No. 18580134) from the Japan Society for the Promotion of Science.

REFERENCES

1. Hackenthal, E., Paul, M., Ganten, D., and Taugner, R. (1990) Morphology, physiology, and molecular biology of renin secretion. *Physiol. Rev.* **70**, 1067–1116
2. Takahashi, S., Ohsawa, T., Miura, R., and Miyake, Y. (1983) Purification of high molecular weight (HMW) renin from porcine kidney and direct evidence that the HMW renin is a complex of renin with renin binding protein (RnBP). *J. Biohcem.* **93**, 265–274
3. Takahashi, S., Ohsawa, T., Miura, R., and Miyake, Y. (1983) Purification and characterization of renin binding protein (RnBP) from porcine kidney. *J. Biochem.* **93**, 1583–1594
4. Takahashi, S., Inoue, H., Fukui, K., and Miyake, Y. (1994) Structure and function of renin binding protein. *Kidney Int.* **46**, 1525–1527
5. Takahashi, S., Miura, R., and Miyake, Y. (1988) Characterization of hog kidney renin-binding protein: Interconversion between monomeric and dimeric forms. *Biochem. Int.* **16**, 1053–1060
6. Inoue, H., Fukui, K., Takahashi, S., and Miyake, Y. (1990) Molecular cloning and sequence analysis of a cDNA encoding a porcine renin-binding protein. *J. Biol. Chem.* **265**, 6556–6561
7. Inoue, H., Takahashi, S., Fukui, K., and Miyake, Y. (1991) Genetic and molecular properties of human and rat renin-binding protein with reference to the function of the leucine zipper motif. *J. Biochem.* **110**, 493–500
8. Inoue, H., Takahashi, S., and Miyake, Y. (1992) Modulation of active renin secretion by renin-binding protein (RnBP) in mouse pituitary AtT-20 cells transfected with human renin and RnBP cDNAs. *J. Biochem.* **111**, 407–412
9. Takahashi, S., Inoue, H., and Miyake, Y. (1992) The human gene for renin-binding protein. *J. Biol. Chem.* **267**, 13007–13013
10. Knöll, A., Schunkert, H., Reichwald, K., Jan Danser, A.H., Bauer, D., Platzer, M., Stein, G., and Rosenthal, A. (1997) Human renin binding protein: Complete genomic sequence and association of an intronic T/C polymorphism with the prorenin level in males. *Hum. Mol. Genet.* **6**, 1527–1534
11. Takahashi, S. (1997) Structure of the gene encoding rat renin binding protein. *Biosci. Biotechnol. Biochem.* **61**, 1323–1326
12. Van den Ouweland, A.M.V., Verdijk, M., Kiochis, P., Poustka, A., and Van Oost, B.A. (1994) The human renin-binding protein gene (RENBP) maps in Xq28. *Genomics* **21**, 279–281
13. Franada, S., Frattini, A., and Vezzoni, P. (1995) The human genes encoding renin-binding protein and host cell factor are closely linked in Xq28 and transcribed in the same direction. *Gene* **155**, 237–239
14. Tada, M., Takahashi, S., Miyano, M., and Miyake, Y. (1992) Tissue-specific regulation of renin-binding protein gene expression in rats. *J. Biochem.* **112**, 175–182
15. Leckie, B.J., Lacy, P.S., and Lidder, S. (2000) The expression of renin-binding protein and renin in the kidney of rats with two-kidney one-clip hypertension. *J. Hypertens.* **18**, 935–943
16. Maru, I., Ohta, Y., Murata, K., and Tsukada, Y. (1996) Molecular cloning and identification of *N*-acyl-D-glucosamine 2-epimerase from porcine kidney as a renin-binding protein. *J. Biol. Chem.* **271**, 16294–16299
17. Takahashi, S., Takahashi, K., Kaneko, T., Ogasawara, H., Shindo, S., and Kobayashi, M. (1998) Characterization of human renin binding protein (RnBP): Human RnBP has *N*-acetyl-D-glucosamine 2-epimerase activity in *Advances in Chitin Science*, Vol. 3 (Chen, R.H. and Chen, H.C., eds.) pp. 178–185, RITA Advertising, Taipei
18. Takahashi, S., Takahashi, K., Kaneko, T., Ogasawara, H., Shindo, S., and Kobayashi, M. (1999) Human renin-binding protein is the enzyme *N*-acetyl-D-glucosamine 2-epimerase. *J. Biochem.* **125**, 348–353
19. Takahashi, S., Takahashi, K., Kaneko, T., Ogasawara, H., Shindo, S., Saito, K., and Kobayashi, M. (1999) Identification of cysteine-380 as the essential residue for the human *N*-acetyl-D-glucosamine 2-epimerase (renin binding protein). *J. Biochem.* **126**, 639–642
20. Takahashi, S., Hori, K., Takahashi, K., Ogasawara, H., Tomatsu, M., and Saito, K. (2001) Effects of nucleotides on *N*-acetyl-D-glucosamine 2-epimerases (renin binding proteins): Comparative biochemical studies. *J. Biochem.* **130**, 815–821
21. Takahashi, S., Ogasawara, H., Takahashi, K., Hori, K., Saito, K., and Mori, K. (2002) Identification of a domain conferring nucleotide binding to the *N*-acetyl-D-glucosamine 2-epimerase (Renin binding protein). *J. Biochem.* **131**, 605–610
22. Takahashi, S., Ogasawara, H., Hiwatashi, K., Hata, K., Hori, K., Koizumi, Y., and Sugiyama, T. (2005) Amino acid residues conferring the nucleotide binding properties of

N-acetyl-D-glucosamine 2-epimerase (renin binding protein). *Biomed. Res.* **26**, 117–121
23. Murakami, K., Takahashi, S., Suzuki, F., Hirose, S., and Inagami, T. (1980) Intermediate molecular weight renin and renin-binding protein(s) in the hog kidney. *Biomed. Res.* **1**, 392–399
24. Haber, E., Koerner, T., Page, L.B., Kliman, B., and Purnode, A. (1969) Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. *J. Clin. Endocrinol. Metab.* **29**, 1349–1355
25. Takahashi, S., Kumagai, M., Shindo, S., Saito, K., and Kawamura. Y. (2000) Renin inhibits *N*-acetyl-D-glucosamine 2-epimerase (renin-binding protein). *J. Biochem.* **128**, 951–956
26. Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254
27. Inagami, T. and Murakami, K. (1977) Pure renin: Isolation from porcine kidney and characterization. *J. Biol. Chem.* **252**, 2978–2983
28. Schwede, T., Kopp, J., Guex, N., and Peitsch, M.C. (2003) SWISS-MODEL: An automated protein homology-modeling server. *Nucleic Acids Res.* **31**, 3381–3385
29. DeLano, W.L. (2002) The PyMOL molecular graphics system. DeLano Scientific, San Carlos, CA, USA, http:/www.pymol.org/
30. Duhovny D., Nussinov R., and Wolfson H.J. (2002) Efficient unbound docking of rigid molecules in *Proceedings of the 2'nd Workshop on Algorithms Bioinformatics (WABI). Lecture Notes in Computer Science* (Gusfield *et al*., eds.) 2452, pp. 185–200, Springer Verlag, Rome, Italy
31. Zamaraeva M.V., Sarbirov R.Z., Maeno E., Ando-Akatsuka Y., Bessonava S.V., and Okada Y. (2005) Cells die with increased cytosolic ATP during apoptosis: A bioluminescence study with intracellular luciferase. *Cell Death Differ.* **12**, 1390–1397