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(Pro)renin receptor and prorenin: their plausible sites of interaction

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1. ABSTRACT

Before discovery of (pro)renin receptor, prorenin was regarded as a source of renin and probable diagnostic marker for diabetic nephropathy/Wilms' tumor. It is now considered that prorenin can perform renin activity by binding to (P)RR and binding mechanism of (pro)renin to (P)RR was indicated in many *in vitro* studies. Considering the physiological importance and pathological involvement of (P)RR, it is indeed a demand of time to determine the three dimensional structure of (P)RR to design (P)RR blocker(s) effective for (pro)renin. It may also facilitate to explain the incompatible data about the effective application of decoy peptide as (P)RR blocker. So far, studies have discussed the bindings of (pro)renin to (P)RR using peptides mimicking the structures of ligands (e.g., the decoy including "handle" region peptide, the "hinge" peptide etc). In this review, the binding mechanism of ligands has been highlighted from the structural aspect of (P)RR using several anti-(P)RR antibodies designed from the primary structure of (pro)renin receptor. Therefore, this review would give us a clue regarding the plausible binding region(s) for prorenin in the (P)RR.

2. INTRODUCTION

(Pro)renin receptor or (P)RR can bind both renin and prorenin (1-4). It consists of a single transmembrane domain with 350 amino acid residues (39 kDa) and a large unglycosylated amino terminal domain with short cytoplasmic tail (2). This protein shows sequence homology with CAPER (endoplasmic reticulum-localized type 1 transmembrane adaptor precursor) and ATPase, H^+-transporting lysosomal accessory protein 2 (ATP6ap2), a protein that associates with a vacuolar H^+-ATPase (5, 6). The catalytic activity of renin increased 4/5-fold after binding to (P)RR (2). Even a decade before, prorenin was only considered as proenzyme, the pre-active source of renin as well as a possible marker for diagnosing diabetic nephropathy (7) and Wilms' tumor (8) without having any physiological function of its own. However, discovery of (P)RR has set a new perception about the physiological role and pathological involvement of prorenin (9-17) as this molecule can also perform renin activity after binding to (P)RR by undergoing a change in its conformation rather completely releasing the 43-amino acid residues containing prosegment part (1-4). More interestingly, renin/prorenin

not only mediates their activity through an angiotensin-II dependent pathway but they also exert their pathophysiological effects via angiotensin-II independent pathways by stimulating signal transduction (1-4,18-20). The C-terminal region of (P)RR has a direct contact with a protein known as promyelocytic zinc-finger (PLZF) (21). Upon activation of (P)RR by renin binding, the PLZF is translocated to nucleus which in turn showed a negative feed back loop by repressing the expression of (P)RR (21). The direct pathological contribution of (P)RR includes elevated blood pressure (over-expressed in smooth muscle of transgenic rats) and increased heart rate (22). The association between (pro)renin receptor gene polymorphism and high blood pressure has been reported in Caucasian men (23) and Japanese men (24). Indirect effects of (P)RR includes retinopathy (11-13) and glomerulosclerosis as well as proteinurea (over expressed ubiquitously with no record of hypertension and diabetes) (25) by deteriorating diabetic nephropathy (an indirect role by activating prorenin non-proteolytically). Moreover, this indirect role of (P)RR has also been suggested for the initiation of cardiac fibrosis and glomerulosclerosis in stroke-prone spontaneously hypertensive rats (14,15), in streptozotocin induced diabetic rats (16) and diabetic AT1-receptor knock-out mice (17). This receptor is also involved in the activation of brain renin-angiotensin system (26) and mutation in its gene (*(P)RR/ATP6ap2* gene) is associated with X-linked mental retardation in humans with no reports of renal or cardiovascular dysfunctions (27).

Therefore, considering the pathological consequences mediated by receptor-ligand interaction, a (P)RR blocker would definitely help to reduce the chance of either its such detrimental direct effects and/or the long-term complications of diabetes/hypertension. In this regard, decoy peptide region of prorenin prosegment would have been an ideal choice for its practical use as (P)RR blocker (1, 12,13, 15-17, 28-30). However, contrasting results from different laboratories have weaken the acceptance of decoy as the inhibitor of (P)RR (19,20,31). As a result, first and foremost, we need to understand the binding mechanism of these ligands to (P)RR. So far, studies have reported the binding mechanism of renin and prorenin to the receptor by giving emphasis on the structures of the ligands (28,29,32). On the other hand, due to lack of the three dimensional structure of the (P)RR, the primary structure of the receptor did not get attention despite that several anti- (P)RR antibodies designed from the middle part (^{107}DSVANSIHSLFSEET^{121}) and C-terminus (^{221}EIGKRYGEDSEQFRD^{235} and ^{237}SKILVDALQKFADD^{250}; close to the N-terminus of (P)RR transmembrane part) regions of (pro)renin receptor have been used for various studies (28,29) as shown in Figure 1. Thus, in this review the interaction between renin/prorenin and the (P)RR would be discussed not only from the structural point of view of the ligands but also of the receptor.

3. (PRO)RENIN BINDING PROTEINS

Several groups of researchers have reported about the prorenin binding proteins and calculated the binding affinity (reciprocal of dissociation constant, K_D) of (pro)renin to those proteins. Takahashi *et al* reported a renin binding protein, RnBP, that specifically binds renin (with a K_D value of 0.2 nM) not other aspartyl proteases like cathepsin D or pepsin through a leucine zipper (a peptide motif involved in protein-protein interaction) (33). Recombinant RnBP injected into the circulation inactivates renin that results in a substantial drop in blood pressure because RnBP binding to renin strongly inhibits cleavage of AOGEN to ANG I (33). Sealey *et al* reported a high affinity binding protein for both the renin and prorenin known as prorenin binding protein (ProBP) (34). This protein has more than four times higher affinity for its ligand prorenin (K_D = 200 pM) than renin (K_D = 900 pM) (34). Mannose-6-phosphate receptors can bind and internalize renin/prorenin in cardiac myocytes (35) and fibroblasts (36) that requires glycosylation of renin. The internalized prorenin by the mannose-6-phosphate receptors in human umbilical vein endothelial cells can be activated proteolytically but not the surface bound prorenin (37). Van den Eijnden *et al* revealed that prorenin bound to mannose-6-phosphate on HUVECs with high affinity (K_D = 0.9 nM), internalized and proteolytically degraded into renin (38). In addition, mannose-6-phosphate independent receptor can take up unglycosylated renin in cardiac tissues of TGR (mREN2)27 transgenic rats that express the *ren*-2d renin gene (39).

Most of these binding proteins and/or receptors with greater affinity can only act as the binding proteins of renin/prorenin (32,33). Their interaction neither can generate angiotensin-II nor can initiate second messenger pathways. Rather, these proteins act as the clearing agent of renin/prorenin by internalizing the ligands into the cells and thus, degrading them e.g., mannose-6-phosphate receptors (34-38). The (P)RR, on the other hand, perform dual effects after binding to its ligand (renin/prorenin) by generating Ang-I and inducing signal transduction (2,17-20,31).

3.1. Binding of prorenin to the (pro)renin receptor, (P)RR

Specific receptor for human renin on human mesangial cells was first proposed by Nguyen *et al* in 1996 that demonstrated binding of ^{125}I-recombinant human renin to primary and immortalized human mesangial cells in a time-dependent and saturable manner (40). Renin binds to the receptor with a very high affinity (K_D = 0.4 and 1.0 nM for primary and immortalized mesangial cells, respectively) that causes mesangial activation by increasing plasminogen activator inhibitor-1 antigen (40). Later, same researcher cloned human (pro)renin receptor, (P)RR in 2002 for the first time that binds both renin and prorenin (2). Over expressed human and rat (P)RR on the COS-7 cells bind to their respective recombinant prorenin with K_D values of 1.8 and 0.89 nM, respectively (1). Using recombinant rat (P)RR expressed in the baculovirus expression system, the binding affinity of rat prorenin was almost 3 times higher (K_D = 8.0 nM) than that of mature rat renin (K_D =20.0 nM) (4).

The human (P)RR when expressed in rat vascular smooth muscle cells binds prorenin with ~6 nM

Table 1. Binding kinetics of recombinant prorenin molecule to the (pro)renin receptor associated with different anti- (P)RR antibodies in surface plasmon resonance.

Antibody recognition site in (P)RR	Antibodies	Association rate constant (k_a)	Dissociation rate constant (k_d)	Dissociation constants (K_D)
^{107}DSVANSIHSLFSEET^{121}*	Anti-107/121	$1.5x10^{6}$	$4.3x10^{-3}$	$2.9x10^{-9}$
^{221}EIGKRYGEDSEQFRD^{235}*	Anti-221/235	$1.7x10^{7}$	0.0202	$1.2x10^{-9}$
^{237}SKILVDALQKFADD^{250}	Anti-237-250	$1.29x10^{7}$	0.0224	$1.74x10^{-9}$
His-His-His-His-His-His* (tagged at the C-terminal end)	Anti-His tag	$3.6x10^{6}$	0.028	$7.8x10^{-9}$

* (28,29)

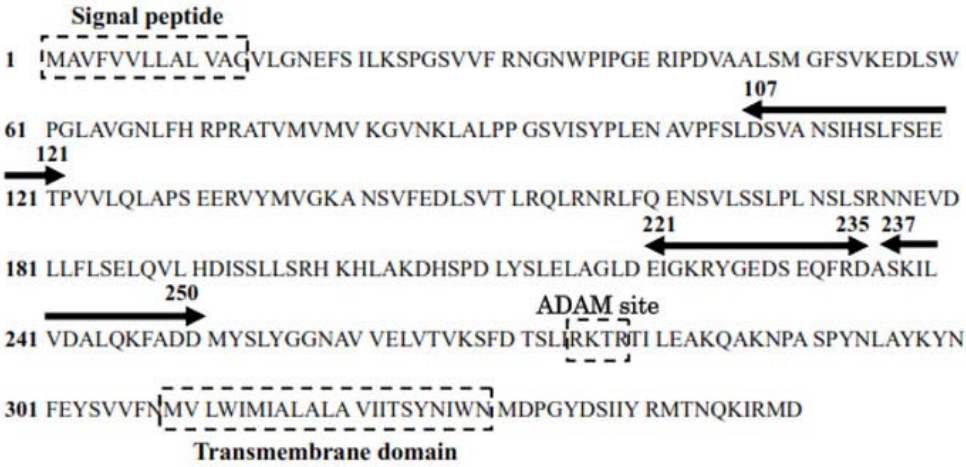

Figure 1. Primary structure of human (pro)renin receptor showing signal peptide, ADAM cutting site, transmembrane domain and the epitope sequences (indicated by the arrows). The epitope sequences from the middle part (^{107}DSVANSIHSLFSEET^{121}) and C-terminus (^{221}EIGKRYGEDSEQFRD^{235} and ^{237}SKILVDALQKFADD^{250}; close to the N-terminus of (P)RR transmembrane part) regions of (pro)renin receptor were used to develop anti- (P)RR antibodies. These antibodies were designated as anti-107/121, anti-221/235 and anti-237/250 antibodies, respectively and purified by affinity chromatography.

and such binding results enhanced Ang-I generation (3). Recently, using plasmon surface resonance (SPR) and recombinant (P)RR (32.5 kDa with six histidine residues tagged at the C-terminus lacking the transmembrane sequence, synthesized by cell free *in vitro* system) the binding affinities of renin and prorenin for the (P)RR were estimated to be 4.0 and 1.2 nM, respectively (29). These values are also in agreement (nanomolar order) with the values reported by other workers.

In case of SPR, the (P)RR was immobilized on the gold plated carboxy methyl-5 (CM-5) sensor chip through amine coupled anti- (P)RR antibodies. These antibodies were designed from different regions of (pro)renin receptor. For example, from the middle part ^{107}DSVANSIHSLFSEET^{121} (named as anti-107/121 antibody) and C-terminus (^{221}EIGKRYGEDSEQFRD^{235} and ^{237}SKILVDALQKFADD^{250} (named as anti-221/235 and 237/250 antibodies, respectively); close to the N-terminus of (P)RR transmembrane part) regions of (pro)renin receptor (28,29) as shown in Figure 1. Using these antibodies, the (P)RR was immobilized and then, allowed to bind to the recombinant prorenin molecule. Depending on the flexibility of the (pro)renin receptor associated with different antibodies, the prorenin would show its binding affinity and this has been reflected through the values of dissociation constants (K_D) presented in Table 1.

The His tag sequence at the C-terminal end of (P)RR was considered like the transmembrane sequence of the native (P)RR and it was hypothesized that immobilization of (P)RR through this antibody would keep the flexibility of the receptor almost similar to that on the membrane expressed with the transmembrane sequence. So, binding of prorenin to the (P)RR (K_D = 7.8 nM) associated with the anti-His tag antibody would indicate the native binding pattern (28). The middle region of the receptor (^{107}DSVANSIHSLFSEET^{121}) when occupied by its antibody (anti-107/121 antibody), the binding of prorenin is not altered (K_D = 2.9 nM) (28). Also, when antibodies binds to their respective antigens near the N-terminal of transmembrane region e.g., sequences of (P)RR from 221-235 and 237-250, the binding affinities of prorenin for the receptor increases 6/7-folds than its hypothesized more flexible state (associated with anti-His tag antibody at the C-terminal end). The reason for increasing the binding affinity after hindering those regions of (P)RR could be due to- i) conformational change of the receptor mediated by the antibody binding to the epitope and/or ii) antibody binding to these regions of (P)RR could reflect the association of other associated proteins (either on the cell membrane or intracellular) that facilitates the low binding affinities *in vivo*. These data therefore indicate that prorenin probably interacts with the N-terminal region (s) of the receptor.

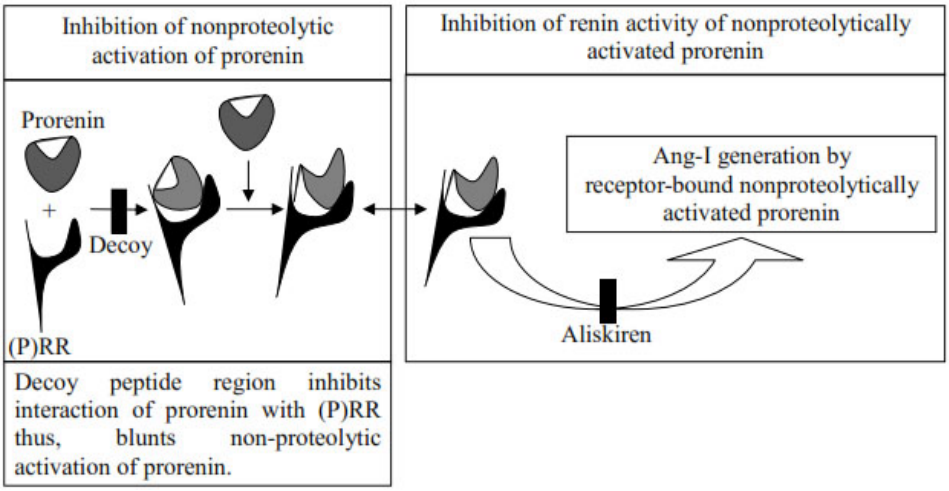

Figure 2. Inhibition of nonproteolytic activation of prorenin by the decoy peptide and renin activity of the nonproteolytically activated prorenin by aliskiren (the first direct renin inhibitor). Lower concentration of decoy peptide was unable to inhibit the activity of the receptor-bound activated prorenin *in vitro* (29) whereas at higher concentration a peptide designed from the N-terminus of prorenin prosegment inhibited renin activity (43).

4. DECOY PEPTIDE: A POSSIBLE (P)RR BLOCKER

The ground work mediated by Suzuki *et al* demonstrated the role of "handle" region peptide ($I^{11P}FLKR^{15P}$, designed from the N-terminal end of prorenin prosegment) in the non-proteolytic activation of prorenin *in vitro* (41). Actually, amino acid residues at the N-terminus of prorenin prosegment have important contribution to maintain the enzymatically active or inactive state of prorenin. Mercure *et al* stated that $R^{10P}IFLKRMPSIR^{20P}$ region played pivotal role to retain the enzymatically inactive state of prorenin (42). Cumin *et al* reported that $L^{13P}KRMP^{17P}$ inhibited renin activity with the K_i and K'_i values of 17.7 and 2.9 μM, respectively (43). Heinrikson *et al* suggested that region $R^{10P}IFLK^{14P}$ was essential for the reversible refolding of the prosegment that leads to prorenin inactivation (44). Suzuki and his group proposed that the region $^{7P}TFKR^{10P}$ (the "gate" region) would probably be dissociated from the renin molecule through the association of region $I^{11P}FLKR^{15P}$ (the "handle" region) with the receptor (41). Using this basic concept, Ichihara *et al* designed a peptide called decoy peptide, $R^{11P}IFLKRMPSI^{19P}$, and administered in the streptozotocin-induced diabetic rats (16). Their work concluded that non-proteolytic activation of prorenin significantly contributes to the activation of kidney renin-angiotensin system (RAS) and the development of diabetic nephropathy. Finally, they proposed beneficial effects of the decoy peptide for preventing diabetic organ damage. On the contrary, other group of researchers using different approaches have failed to demonstrate the same beneficial effects of this peptide on the end-stage organ damage (3,19,20,30). We also demonstrated that decoy peptide at lower concentration could not inhibit renin activity mediated either by mature renin or by the non-proteolytically activated prorenin (Figure 2) rather only inhibited interaction of prorenin with the receptor or in turn inhibited non-proteolytic activation of prorenin (Figure 2) (29).

Our recent *in vitro* studies using surface plasmon resonance (SPR) showed direct binding of the decoy peptide region to the (pro)renin receptor with the dissociation constants (K_D values) ranges from 1.52-6.2 nM (28,29). Further, decoy peptide region also inhibited bindings of rat and human prorenin to their respective receptors expressed on the COS-7 cell membrane with a K_i of 6.6 nM (1). This inhibition was also confirmed using SPR where co-injection of decoy peptide and prorenin through flow cells reduce the binding amount of prorenin to the (P)RR which was reflected by the low resonance signal (28). These data indicated that both prorenin and decoy peptide share a common binding region within the (P)RR molecule. Thus, association of the prorenin molecule to the (pro)renin receptor could plausibly be made through the decoy peptide region not at the C-terminus rather at the N-terminal sequence of the receptor.

5. IS THERE MORE THAN ONE BINDING SITE IN THE (P)RR MOLECULE?

Recently, we reported high affinity two binding sites within the prorenin molecule through which it can interact with (P)RR- the decoy (RIFLKRMPSI) and the "hinge" (SQGVLKEDVF) regions (29). Fundamental kinetic studies demonstrated that renin, prorenin, decoy region (RIFLKRMPSI, containing the "handle" region sequence) and "hinge" region peptides could bind to the (pro)renin receptor with different nanomolar affinities. However, binding kinetics further revealed that the K_D values for the bindings of prorenin (shown in Table 1) and the decoy peptide to (P)RR (28,29) varied very closely and thus, may be they share the common binding region in (P)RR along with the reason as proposed in the previous section. On the other hand, the value of the "hinge" region peptide for its binding to (P)RR was 17.0 nM (29) that was almost 3-16 times higher than that for decoy binding. Thus, these data suggest the possibility of having separate binding sites for these molecules in the receptor molecule. Also,

considering the positions of decoy and "hinge" region peptides (one is a part of prosegment and the latter one is a core sequence that acts as junction between the C and N domain of mature renin) in the predicted three dimensional structure of prorenin (29), it is quite unlikely for these regions to share a common binding site in (P)RR. Furthermore, the high binding affinity of prorenin to (P)RR compare to renin (3,4,28,29) could be attributed to the presence of two different binding sites.

We also have to consider the fact about the inhibitory effects of decoy peptide (a sequence of prorenin prosegment) on renin binding to (P)RR. The most probable and hypothetical explanation for such inhibitory effect of the peptide was apprehended to the change in local conformation within the (P)RR molecule after decoy binding. Our recent report (45) sheds light on this probable hypothesis from the point of view of the (P)RR ligand, renin. We observed that when aliskiren bound to the active cleft of renin, binding affinity of renin (i.e., renin-aliskiren complex) decreased more than 1000-fold compare to that of native renin (45). So far, aliskiren was known to inhibit renin activity (either mature renin or activated prorenin shown in Figure 2) by hindering the active cleft from angiotensinogen (45,46) rather inhibiting its binding to (P)RR. However, binding of aliskiren to the active cleft of renin by occupying the S3 and S2 subsites (46) could have altered the local conformation within the renin molecule. In addition, the "hinge" region (probable site through which renin interacts with the receptor) is situated just beneath the active cleft (29). As a result of aliskiren binding to the active site, the flexibility of the "hinge" region within the renin molecule most probably has been reduced and this could have been reflected in the lower value of binding affinity that was reported by Biswas *et al* (45). Thus, "hinge" is probably the main region through which renin could interact with (P)RR. These data all together indicated presence of more than one binding site within (P)RR molecule for its ligands.

6. WHERE DO WE STAND NOW? POSSIBLE FUTURE DIRECTION

Though the history of research with renin-angiotensin system is more than 100 years old initiated by Tigerstedt, discovery of (pro)renin receptor in 2002 added an extra flavor and created new avenues for the scientists to rethink about this complex system. This receptor has given a new dimension regarding the enzymatic activities of prorenin (whose plasma concentration is 10 times higher than that of renin) that cleaves the renin substrate angiotensinogen, physiological contribution as well as pathological involvement of prorenin in the pathogenesis of end-stage organ damage disorders, respectively. Nevertheless, some queries still persists regarding the interaction of renin/prorenin with the receptor. Which form of prorenin contribute to the activation of second messenger pathways- receptor-bound inactive or receptor-bound activated prorenin or both? Although, the dissociation constants (K_D) indicated reversible binding of prorenin to the receptor, is it possible for prorenin to refold to its native form after releasing from (P)RR as it was reported in case of acid activated prorenin (47)?

Therefore, determination of the three dimensional structure would certainly answer some of these inquiries. Our results, so far, using different anti- (pro)renin receptor antibodies, have given a slight glimpse about the plausible binding site of prorenin in the receptor molecule. Nonetheless, use of mutated (pro)renin receptor (specially, mutation at the N-terminus of the receptor) would certainly give more concrete idea about the binding sites of prorenin within the receptor molecule till structure evolved from the X-ray crystallography data is available.

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