Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Novel biosensor using split-luciferase for detecting vitamin D receptor ligands based on the interaction between vitamin D receptor and coactivator

Hiroki Mano [a], Masashi Takano [b], Shinichi Ikushiro [c], Atsushi Kittaka [b], Toshiyuki Sakaki [a, *]

[a] Department of Pharmaceutical Engineering, Faculty of Engineering, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama, 939-0398, Japan
[b] Faculty of Pharmaceutical Sciences, Teikyo University, Itabashi, Tokyo, 173-8605, Japan
[c] Department of Biotechnology, Faculty of Engineering, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama, 939-0398, Japan

ARTICLE INFO

Article history:
Received 12 September 2018
Accepted 19 September 2018
Available online xxx

Keywords:
Vitamin D
Vitamin D receptor
Bioluminescent sensor
Split-luciferase
Rickets

ABSTRACT

Vitamin D receptor (VDR) ligands, such as 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$] and its analogs, have been investigated for their potential clinical use in the treatment of various diseases such as type I rickets, osteoporosis, psoriasis, leukemia, and cancer. Previously, we reported a split-luciferase-based biosensor that can detect VDR ligands and assess their affinity for the ligand binding domain (LBD) of the VDR in a short time. However, a further increase in its sensitivity was required to detect plasma levels of 1α,25$(OH)_2D_3$ and its analogs. In this study, a novel type of biosensor called LXXLL + LBD was successfully developed. Here, the split luciferase forms a functional complex based on the intermolecular interaction between the LXXLL motif and the ligand-bound form of the LBD. This biosensor has an approximately 10-fold increase in the light intensity compared to the previous versions. Additionally, the binding affinity of the vitamin D analogs for the wild-type and the rickets-associated mutant R274L of VDR was evaluated.

1. Introduction

The active form of vitamin D_3, 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$], and its analogs are well known as ligands of the vitamin D receptor (VDR) [1]. After 1α,25$(OH)_2D_3$ binds to the ligand binding domain (LBD) of VDR, the subsequent complex interacts with the heterodimeric partner retinoid X receptor (RXR) and the coactivators (e.g., steroid receptor coactivating factor 1 (SRC-1), vitamin D receptor interacting protein 205 (DRIP205), and transcriptional intermediary factor 2 (TIF2)) via a specific small amino acid sequence known as the LXXLL motif [2–4]. This complex regulates the expression of many vitamin D-dependent genes associated with calcium (Ca)- and phosphate-homeostasis, cell differentiation, and immunology [5–7]. Therefore, several analogs of 1α,25$(OH)_2D_3$ have been synthesized and investigated clinically for the treatment of osteoporotic and rickets diseases as well as for psoriatic skin diseases [8,9]. Recently, vitamin D and its analogs have been suggested to be promising agents for the treatment of several cancers, cognitive disorders, and Alzheimer's disease [9–12]. Accordingly, the development of a screening system for VDR analogs that can conveniently assess their affinity for VDR is essential.

We previously reported a split-luciferase based biosensor that can detect 25$(OH)D_3$ and 1α,25$(OH)_2D_3$ [13]. This biosensor consists of the chimeric fusion protein of *firefly* luciferase with the ligand-binding domain (LBD) of VDR and the LXXLL motif of the coactivator. The biosensor has the following detection mechanism. When 25$(OH)D_3$ or 1α,25$(OH)_2D_3$ binds to the LBD, the split luciferase forms an active complex with the insertion of the LXXLL motif and the LBD. The complex subsequently emits light with a detection limit of about 0.1 nM for 1α,25$(OH)_2D_3$. However, our aim was to detect very low concentrations (<0.1 nM) of 1α,25$(OH)_2D_3$ in the blood. Thus, a further increase in the sensitivity was required.

Abbreviations: CYP, Cytochrome P450; VDR, vitamin D receptor; LBD, ligand binding domain; LucN, N-terminal domain of luciferase; LucC, C-terminal domain of luciferase; SRC-1, steroid receptor coactivating factor 1; RXRα, retinoid X receptor α; VDRE, vitamin D responsive element; TIF2, transcriptional factor 2; DRIP205, vitamin D receptor interacting protein 205.

* Corresponding author.
E-mail address: tsakaki@pu-toyama.ac.jp (T. Sakaki).

https://doi.org/10.1016/j.bbrc.2018.09.122
0006-291X/

In this study, a novel type of split-luciferase-based biosensor was developed, that can detect very low concentrations of 1α,25(OH)$_2$D$_3$ depending on the intermolecular interaction between the LXXLL motif and the LBD. This novel type of biosensor was denoted as the LXXLL + LBD biosensor, and its sensitivity and dynamic range were examined. The Arg274Leu (R274L) mutation in the VDR identified in patients with HVDRR reduces their affinity for 1α,25(OH)$_2$D$_3$ by 1000-fold [14–16]. Vitamin D analogs that have a high affinity for this variant form of the VDR may be used to treat these patients. Therefore, the LXXLL + LBD(R274L) biosensor that can evaluate the affinity of various analogs for the VDR(R274L) was developed.

2. Materials and methods

2.1. Materials

Ethanol (EtOH) was purchased from Wako Pure Chemicals (Osaka, Japan) and 1α,25(OH)$_2$D$_3$ was purchased from Cayman Chemical. Co (Ann Arbor, MI, USA). The vitamin D analogs AH-1, (24*R*−OH)AH-1 and (24*S*−OH)AH-1 were synthesized by Kittaka's group (Teikyo University, Tokyo, Japan) [17–19]. D-luciferin was purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA).

2.2. Construction of plasmids

The cDNA encoding LucN (1–415 aa)–NHPMLMNLLKDN-(GGGGS) × 3-LBD (amino acid 121–427 aa)-LucC (416–550 aa) was inserted between *Xho*I and *Not*I site of pIRES2-AcGFP vector (Takara, Otsu, Japan). Resultant plasmid was named pIRES2-LucN-NHPMLMNLLKDN-(GGGGS) × 3-LBD-LucC as described in our previous reports [13]. To generate LucN-NHPMLMNLLKDN, PCR was performed using pIRES2-LucN-NHPMLMNLLKDN-(GGGGS) × 3-LBD-LucC vector as a template with the set of primers 5′-AATTCTCGAGATGGAAGATGCCAAAAACATTAAG-3′ and 5′-ATATGCGGCCGCTAATTATCTTTAAGAAGGTTCATGAGC-3'. To obtain LBD-LucC, PCR was performed using pIRES2-LucN-NHPMLMNLLKDN-(GGGGS) × 3-LBD-LucC vector as a template with the set of primers 5′-ATCTCGAGATGCGGCCCAAGCTGTCTGAGGAGCAGCAG-3′ and 5′-ATATGCGGCCGCTTACACGGCGATCTTGCCGCCCTTCTTG-3'. cDNA encoding LucN-NHPMLMNLLKDN and LBD-LucC were each inserted between *Xho*I and *Not*I site of pIRES2-AcGFP vector. Resultant plasmids were named pIRES2-LucN-NHPMLMNLLKDN and pIRES2-LBD-LucC, respectively. To generate LBD-GS-LucN, LBD and LucN fragments were amplified using pIRES2-LucN-NHPMLMNLLKDN-(GGGGS) × 3-LBD-LucC as a template with the set of primers 5′-ATCTCGAGATGCGGCCCAAGCTGTCTGAGGAGCAGCAG-3′ and 5′-AATTGGATCCGGAGATCTCATTGCCAAACACTTCG-3′, and 5′-TATAGGATTCGAAGATGCCAAAAACATTAAGAAGG-3′ and 5′-TAAGCGGCCGCTTAGTCCTTGTCGATGAGAGCGTTTGTAG-3'. These fragments were inserted between *Xho*I and *Not*I site of pIRES2-AcGFP vector using 3 piece ligation technique. Resultant plasmid was named pIRES2-LBD-GS-LucN. To generate NHPMLMNLLKDN-GS-LucN, an annealed oligonucleotide (5′- TCGAGATGAACCACCCGATGCTCATGAACCTTCTTAAAGATAATG-3′ and 5′- GATCCATTATCTTTAAGAAGGTTCATGAGCATCGGGTGGTTCATC-3′) was inserted into *Xho*I/*Bam*HI site of pIRES2-LBD-GS-LucN vector. Next, to generate pIRES2-LucN-*Sal*I/*Bam*HI-LucC vector using inverse PCR method, inverse PCR was performed with KOD-plus DNA polymerase (Toyobo, Osaka, Japan) using pIRES2-LucN-NHPMLMNLLKDN-(GGGGS) × 3-LBD-LucC vector as a template with the set of primers 5′-ATATGTCGACGTCCTTGTCGATGAGAGCGTTTGTAG-3′ and 5′-ATATGGATCCGGCTGGCTGCACAGCGGCGACATCG-3'. The GS-LucC fragment was inserted into the *Bam*HI/*Not*I site of pIRES2-LBD-GS-LucN vector and resultant plasimd was named pIRES2-LBD-GS-LucC. An annealed oligonucleotide NHPMLMNLLKDN fragment was inserted into *Xho*I/*Bam*HI site of pIRES2-LBD-GS-LucC, and resultant plasmid was named pIRES2-NHPMLMNLLKDN-GS-LucC vector. To amplify the VD-LBD fragment, PCR was performed using pIRES2-LucN-NHPMLMNLLKDN-(GGGGS) × 3-LBD-LucC vector as a template with

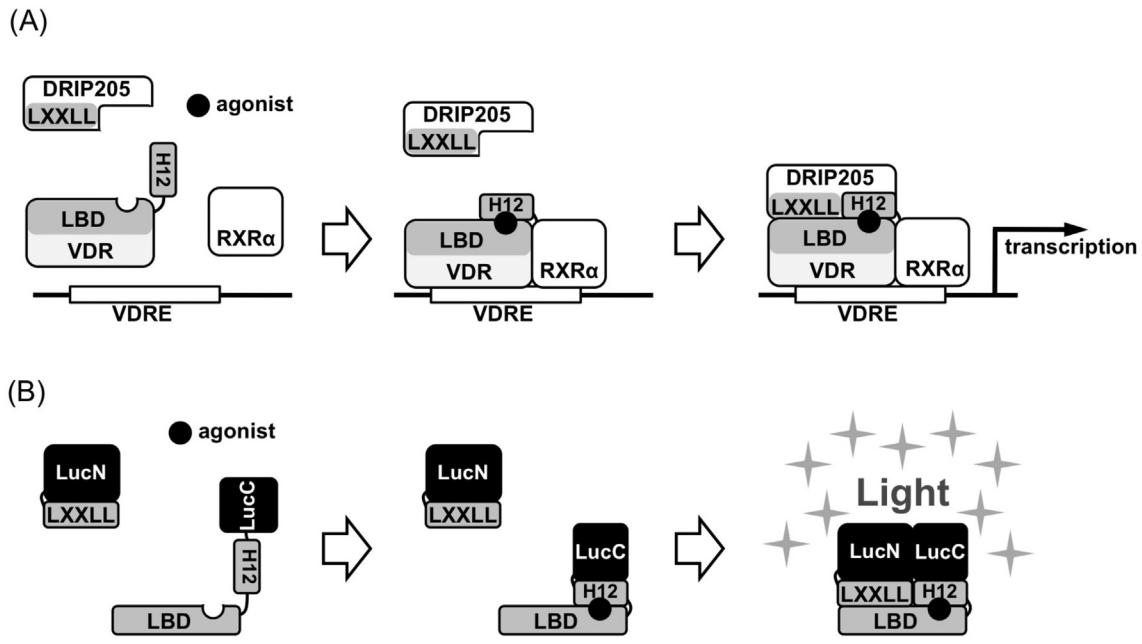

Fig. 1. Schematic diagram of the biosensor to detect VDR ligands based on interaction between LXXLL and LBD. (A) Interaction between LXXLL and LBD caused by agonist binding. When the agonist binds to the LBD of VDR, the position of helix 12 (H12) in LBD is dramatically changed to cause the complex formation between DRIP205 and VDR via an LXXLL peptide motif. (B) Putative detection mechanism of VDR agonists using split luciferase technique. The biosensor proteins consist of the N- and C-terminal domains of luciferase (LucN and LucC), LXXLL peptide, and LBD of VDR. Binding of the agonist to the LBD-LucC may cause a positional change of helix12 in LBD. Then, the LucN-LXXLL and LBD-LucC forms a functional complex to exhibit the luciferase activity.

Please cite this article in press as: H. Mano, et al., Novel biosensor using split-luciferase for detecting vitamin D receptor ligands based on the interaction between vitamin D receptor and coactivator, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.09.122

the set of primers 5′-ATATGTCGACCGGCCCAAGCTGTCTGAGGAGCAGC-3′ and 5′-ATATGCGGCCGCTCAGGAGATCTCATTGCCAAACAC-3′, the PCR product was inserted into *Sal*I/*Not*I site of pIRES2-LucN-*Sal*I/*Bam*HI-LucC, and resultant plasmid was named pIRES2-LucN-VD-LBD vector. To amplify the LucC fragment, PCR was performed using pIRES2-LucN-NHPMLMNLLKDN-(GGGGS) × 3-LBD-LucC vector as a template with the set of primers 5′-AATCTCGAGATGGGCTGGCTGCACAGCGGC-3′ and 5′-TATAGTCGACCACGGCGATCTTGCCGCCCTTCTTG-3′, the PCR product was inserted into *Xho*I/*Sal*I site of pIRES2-LucN-VD-LBD, and resultant plasmid was named pIRES2-LucC-VD-LBD vector. An annealed oligonucleotide (5′-TCGACAACCACCCGATGCTCATGAACCTTCTTAAAGATAATTAAGC-3′ and 5′-GGCCGCTTAATTATCTTTAAGAAGGTTCATGAGCATCGGGTGGTTG-3′) encoding VDNHPMLMNLLKDN was inserted into *Sal*I/*Not*I site of pIRES2-LBD-VD-LBD, and resultant plasmid was named pIRES2-LucC-VD-VDNHPMLMNLLKDN vector. To obtain LBD(R274L)-LucC, PCR was performed with KOD plus-neo (Toyobo, Osaka, Japan) using pIRES2-LBD-LucC vector as the template with the set of primers 5′-CATCATGTTGCTCTCCAATGAGTCCTTCAC-3′ and 5′-ACTCATTGGAGAGCAACATGATGACCTCAATG-3’. Resultant plasmid was named pIRES2-LBD(R274L)-LucC vector. In this study, the PCR was performed with KOD plus-neo except for inverse PCR method. All the constructs were confirmed by DNA sequencing using ABI3130 (Applied Biosystems, Tokyo, Japan).

2.3. Cell culture and DNA transfection

African green monkey kidney (COS-7) cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Nacalai Tesque, Inc., Japan) supplemented with 10% heat-inactivated fetal bovine serum (FBS) containing phenol red (Nacalai Tesque, Inc., Japan) at 37 °C in an incubator with 5% CO_2. The cells were harvested and transiently transfected with each of the plasmids encoding biosensor using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, U.S.A.) in accordance with the manufacture's instructions. Then, transfected cells were seeded onto 96-well plates (1.5×10^4 cells/well). At 24 h after transfection, the culture medium was replaced with DMEM without phenol red (Nacalai Tesque, Inc., Japan) supplemented with 5% charcoal-stripped FBS (Gibco, Rockville, MD, U.S.A.). At 48 h after transfection, the cells were used for luciferase complementary assay.

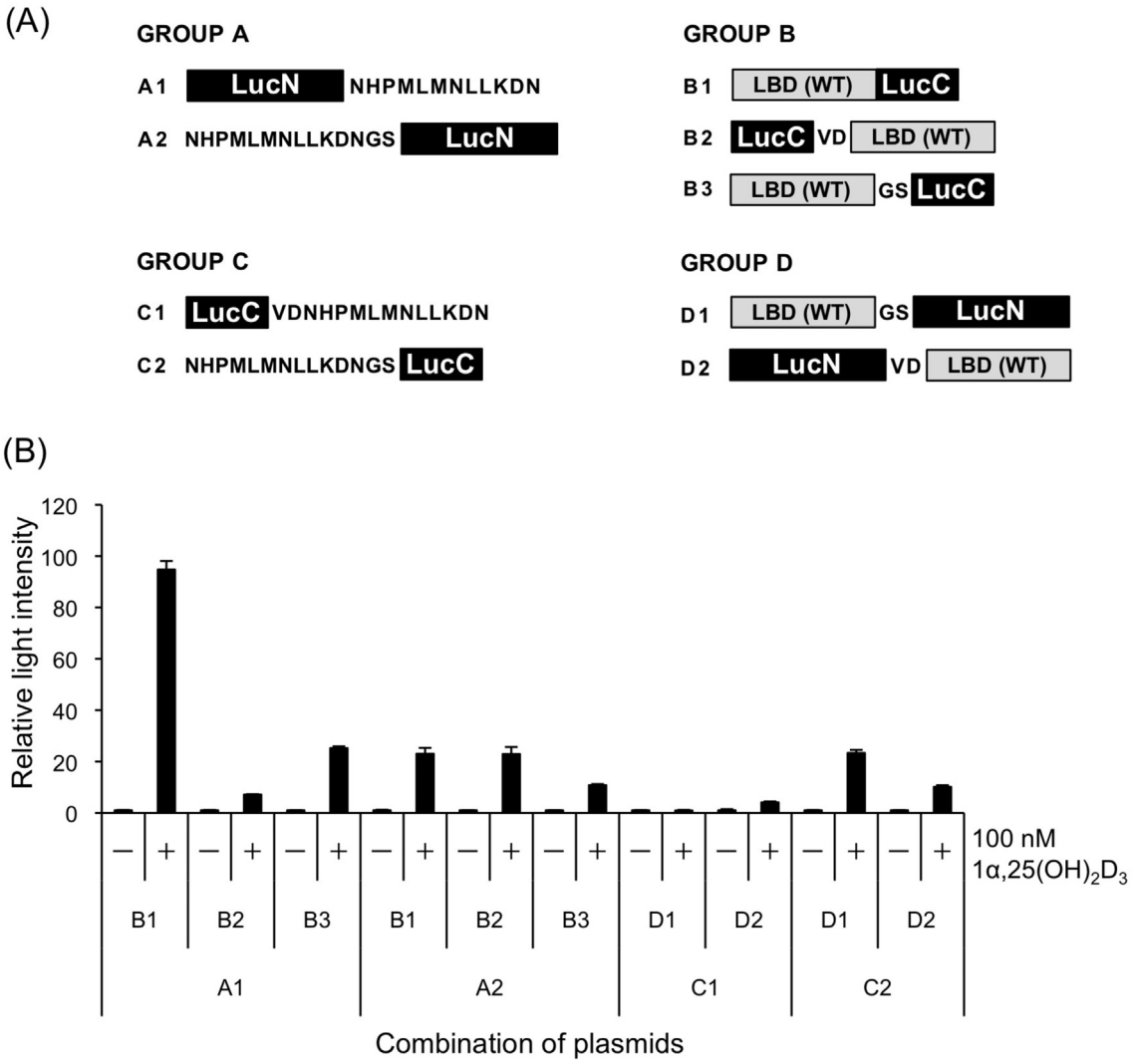

Fig. 2. Development of the split-luciferase-based biosensor to detect VDR agonists. (A) Schematic structures of the biosensor proteins designated as A1-D2. The 6 combinations of Group A with B or the 4 combinations of Group C with D were compared. Each of the biosensor proteins was expressed in COS-7 cells, and the transfected cells were treated with 0.1% EtOH (−) or 100 nM 1α,25$(OH)_2D_3$ (+). (B) The light intensity was measured at 0 min and 90 min after 100 nM 1α,25$(OH)_2D_3$ treatment, and the relative light intensity compared to the control (0.1% EtOH) was shown. Data are represented as mean ± SEM, $n = 4$.

Please cite this article in press as: H. Mano, et al., Novel biosensor using split-luciferase for detecting vitamin D receptor ligands based on the interaction between vitamin D receptor and coactivator, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.09.122

2.4. Luciferase complementary assay

The culture medium was changed to phenol red free Leibovitz's L-15 medium (Gibco, Rockville, MD, U.S.A.) containing 0.5 mM D-luciferin (Thermo scientific, CA, U.S.A.). At 30 min after incubation at room temperature, basal level of the light intensity was measured. Subsequently, EtOH, 1α,25$(OH)_2D_3$, AH-1, (24R–OH)AH-1 and (24S–OH)AH-1 was added to the well at final concentration of 0.1–1000 nM. Because each compound was dissolved in EtOH, the final concentration of EtOH was adjusted to 0.1%, and the 0.1% EtOH added wells were used as negative controls. Measurement of light intensity and calculation of relative light intensity were performed as described in our previous reports [13,20,21].

3. Results and discussion

3.1. Development of a highly sensitive biosensor for the detection of the VDR-coactivator interaction

After 1α,25$(OH)_2D_3$ binding, the LBD of the VDR interacts with a transcription coactivator (SRC-1, TIF-2, and DRIP-205) via the LXXLL motif [2–4]. Then, the resultant coactivator-VDR-RXR complex acts as the transcription factor to activate the vitamin D-dependent gene expression (Fig. 1A). Based on these mechanisms, biosensors to detect the LXXLL-LBD interaction were constructed (Fig. 1B). The biosensors consist of the LBD (121–427 aa) of the VDR, the N– and C-terminal of the *firefly* luciferase fragments [LucN (1–415 aa) and LucC (416–550 aa)] and the LXXLL peptide sequence. This split-luciferase-based biosensor was denoted as the LXXLL + LBD biosensor. When 1α,25$(OH)_2D_3$ binds to the LXXLL + LBD biosensor, the luciferase-light intensity is dramatically increased. The conformational change of helix 12 in the LBD, followed by the interaction of the LXXLL with the LBD, appears to result in a reconstitution of the luciferase activity.

Some researchers have reported that the luciferase light intensity of the split luciferase-based biosensor is affected by the domain combinations of the interacting partner, LucN and LucC fragments [22–24]. Therefore, several plasmids encoding the LXXLL + LBD biosensors were constructed that replaced the LucN, LucC, LBD, or LXXLL configuration. Since the amino acid sequence NHPMLMNLLKDN in DRIP205 can bind to the LBD of the VDR, it was selected as the LXXLL motif [4]. All constructs are shown in Fig. 2A. A combination of Group A with Group B or Group C with Group D were cotransfected and coexpressed in the COS-7 cells. The COS-7 cells transfected with each of the LXXLL + LBD biosensors were treated with 0.1% EtOH (control) or 100 nM 1α,25$(OH)_2D_3$. Subsequently, the luciferase light intensity was measured at 90 min after treatment. Of all the combinations of the LXXLL + LBD biosensors, A1+B1 had the highest relative light intensity (Fig. 2B). The relative light intensity of combination A1+B1 increased approximately 90–100-fold in response to 100 nM of 1α,25$(OH)_2D_3$. Thus, the combination A1+B1 was used as the LXXLL + LBD biosensor in the following experiments.

3.2. Dose-dependency of the biosensor in living cells

For the split-luciferase-based biosensors previously created, the detection times ranged from 60 to 120 min in live cells [13,20,21]. Therefore, in the following experiments, a 90 min time point for detection was selected. Next, to investigate the concentration-dependence of 1α,25$(OH)_2D_3$ on the emission intensities, COS-7 cells expressing the LXXLL + LBD biosensor were treated with 0, 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1, 5, 10, or 100 nM 1α,25$(OH)_2D_3$ for 90 min. As shown in Fig. 3, the light intensity significantly increased at concentrations of 0.005 nM of 1α,25$(OH)_2D_3$. Thus, the detection limit was 0.005 nM (5 pM) of 1α,25$(OH)_2D_3$. These results indicate that the sensitivity of the LXXLL + LBD biosensor is higher than that of the previous versions [13].

3.3. Comparison of binding-affinities of the AH-1 and its metabolites

Arg274 (R274), which is located in the LBD of the VDR, serves to tightly fix 1α,25$(OH)_2D_3$ by forming a hydrogen bond with the 1α-hydroxyl group. Thus, the R274L mutation in the VDR identified in HVDRR patients reduces the affinity for 1α,25$(OH)_2D_3$ by 1000-fold [14–16]. The decreased binding affinity of 1α,25$(OH)_2D_3$ for the VDR results in a decreased interaction with RXR and coactivators. Accordingly, transcription is repressed. Thus, in turn, the osteogenesis-related genes (such as the Ca channels, osteopontin, and osteocalcin) are repressed to cause rickets.

In our previous study, we observed that a vitamin D analog, designated AH-1, had a high affinity for the VDR(R274L) [21]. It has been suggested that AH-1 might be useful in the treatment of patients with HVDRR caused by the R274L mutation in the LBD of the VDR. It is well known that the VDR ligands induce the expression of the CYP24A1 genes. The resultant CYP24A1 enzymes convert the VDR ligands into their inactive forms [25,26]. Thus, the metabolism of AH-1 was examined using recombinant human CYP24A1 enzymes expressed in *Escherichia coli* cells. It was observed that AH-1 was mainly converted into two metabolites, (24*R*–OH)AH-1 and (24*S*–OH)AH-1 [19,27]. Although (24*R*–OH)AH-1 showed a higher biological activity than (24*S*–OH)AH-1, their binding affinities for either VDR(WT) or VDR(R274L) remained unknown. Therefore, the affinity of (24*R*–OH)AH-1 and (24*S*–OH)AH-1 for the WT and R274L mutant types of VDRs using the LXXLL + LBD(WT) and LXXLL + LBD(R274L) biosensors, respectively. The COS-7 cells expressing either the LXXLL + LBD(WT) or LXXLL + LBD(R274L) biosensors were treated with various concentrations (0, 0.1, 1, 5, 10, 50, 100, and 1000 nM) of 1α,25$(OH)_2D_3$, AH-1, (24*R*–OH)AH-1, or (24*S*–OH)AH-1 for 90 min. The chemical structures of all the compounds are shown in Fig. 4A–D. All the compounds induced an

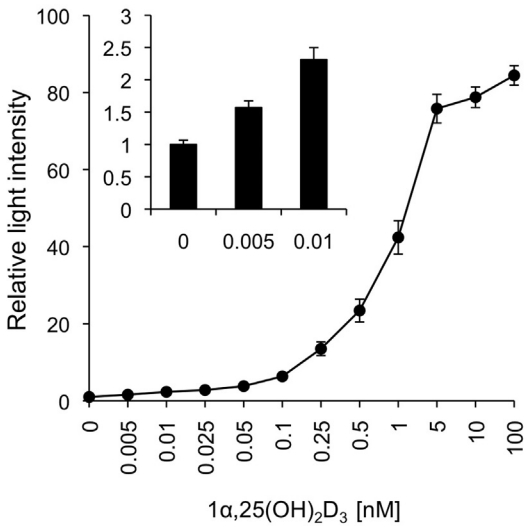

Fig. 3. The relationship between concentration of 1α,25$(OH)_2D_3$ and the relative light intensity of the LXXLL + LBD biosensor in COS-7 cells. The COS-7 cells expressing LXXLL + LBD biosensor proteins were treated with 0.1% EtOH or various concentration of 1α,25$(OH)_2D_3$. The light intensity was measured at 90 min after treatment and the relative light intensity compared to the control (0 nM = 0.1% EtOH) was shown. Data are represented as mean ± SEM, $n = 4$.

Please cite this article in press as: H. Mano, et al., Novel biosensor using split-luciferase for detecting vitamin D receptor ligands based on the interaction between vitamin D receptor and coactivator, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.09.122

increase in the light intensity in a concentration-dependent manner using both biosensors, LXXLL + LBD(WT) and LXXLL + LBD(R274L). Using the LXXLL + LBD(WT) biosensor, the concentration-dependence of (24*R*−OH)AH-1 and (24*S*−OH)AH-1 showed a similarity with that of AH-1. Approximately one to less than five nM of 1α,25(OH)$_2$D$_3$ were enough for an 80-fold increase in the relative light intensity. On the other hand, five nM or more of AH-1, (24R−OH)AH-1, and (24*S*−OH)AH-1 were required for an 80-fold increase in the relative light intensity (Fig. 4E). Therefore, the VDR(WT) affinities of AH-1, (24*R*−OH)AH-1, and (24*S*−OH)-AH-1 were lower than 1α,25(OH)$_2$D$_3$. Finally, the VDR(R274L) affinities of 1α,25(OH)$_2$D$_3$, AH-1, (24R−OH)AH-1, and (24*S*−OH)AH-1 were examined and compared using the LXXLL + LBD(R274L) biosensor (Fig. 4F). The affinity of AH-1 for VDR (R274L) was much higher than that of 1α,25(OH)$_2$D$_3$. This result was the same as that obtained with the previous biosensors. However, the affinity of (24*R*−OH) AH-1 and (24*S*−OH)AH-1 was significantly lower than that of AH-1. These results indicate that AH-1 is the ligand with the highest affinity for VDR(R274L) but is inactivated by CYP24A1 whose expression may be induced by AH-1 via the VDR(R274L). It should be noted that this putative mechanism resembles the induction of the CYP24A1 gene expression by 1α,25(OH)$_2$D$_3$ via VDR(WT) followed by inactivation of 1α,25(OH)$_2$D$_3$ by the CYP24A1 enzyme.

In this study, we have successfully developed a highly sensitive biomolecular-type biosensor LXXLL + LBD. This biosensor showed an approximately 10-fold increase in the light intensity compared with our previous fusion-type biosensor [13]. Based on the sensitivity and dynamic range of the LXXLL + LBD biosensor, it could be used to measure 1α,25(OH)$_2$D$_3$ and 25(OH)D$_3$ in human blood. In addition, the affinity of AH-1 and its metabolites for VDR(R274L) could be measured by the LXXLL + LBD(R274L) biosensor. In the future, AH-1 or its analogs might be therapeutic agents for HVDRR patients with VDR(R274L). Additionally, the LXXLL + LBD(R274L) biosensors may be useful for the quantitative analyses of AH-1 and its metabolites.

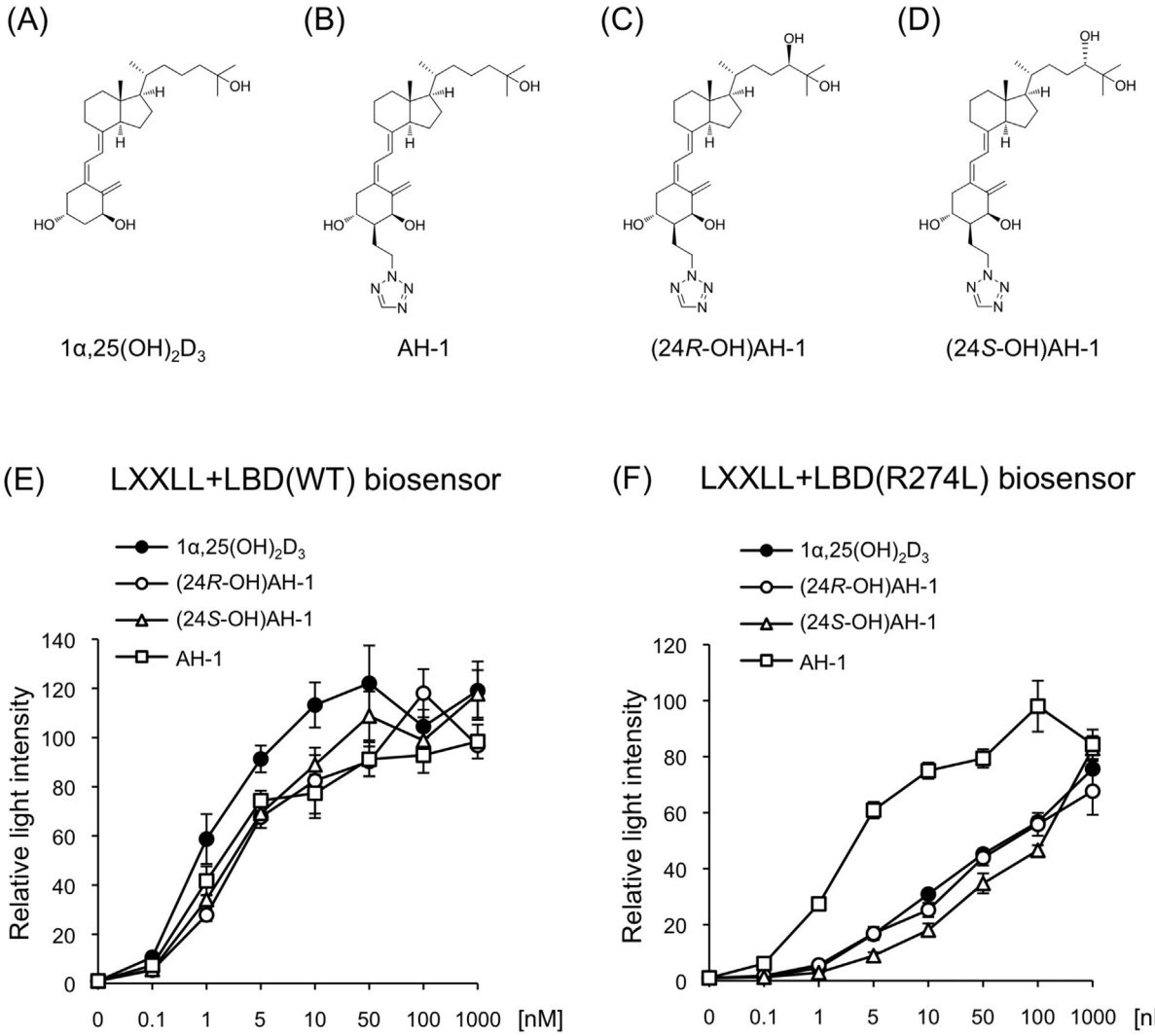

Fig. 4. Comparison of affinity of vitamin D analogs for VDR (WT) or VDR (R274L) using LXXLL + LBD(WT) or LXXLL + LBD(R274L) biosensor in COS-7 cells. Chemical structures of (A) 1α,25(OH)$_2$D$_3$, (B) AH-1, (C) (24*R*−OH)AH-1 and (D) (24*S*−OH)AH-1 were shown. Dose-dependencies of 1α,25(OH)$_2$D$_3$, AH-1, (24*R*−OH)AH-1 or (24*S*−OH)AH-1 on relative light intensity of the LXXLL + LBD(WT) (E) and LXXLL + LBD(R274L) biosensor (F) were shown. The COS-7 cells expressing LXXLL + LBD(WT) or LXXLL + LBD(R274L) biosensor proteins were treated with various concentrations (0−1000 nM) of 1α,25(OH)$_2$D$_3$ AH-1, (24*R*−OH)AH-1 or (24*S*−OH)AH-1 for 90 min. The light intensity was measured at 0 and 90 min after compounds treatment. The relative light intensity compared to the control (0 nM = 0.1% EtOH) was shown. Data are represented as mean ± SEM, (e) 1α,25(OH)$_2$D$_3$:$n = 5$, AH-1:$n = 4$, (24*R*−OH)AH-1:$n = 5$, (24*S*−OH)AH-1 $n = 4$, (f) 1α,25(OH)$_2$D$_3$:$n = 5$, AH-1:$n = 6$, (24*R*−OH)AH-1:$n = 3$, (24*S*−OH)AH-1 $n = 4$.

Please cite this article in press as: H. Mano, et al., Novel biosensor using split-luciferase for detecting vitamin D receptor ligands based on the interaction between vitamin D receptor and coactivator, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.09.122

Funding

This work was supported by Grants-in-Aid from the Japan Society for the Promotion of Science (No. 16H04912 and 16K14904 to T.S.).

References

[1] T. Sakaki, N. Kagawa, K. Yamamoto, K. Inouye, Metabolism of vitamin D3 by cytochromes P450, Front. Biosci. 10 (2005) 119–134.
[2] C. Rachez, M. Gamble, C.P. Chang, G.B. Atkins, M.A. Lazar, L.P. Freedman, The DRIP complex and SRC-1/p160 coactivators share similar nuclear receptor binding determinants but constitute functionally distinct complexes, Mol. Cell Biol. 20 (2000) 2718–2726.
[3] L.A. Zella, C.Y. Chang, D.P. McDonnell, J.W. Pike, The vitamin D receptor interacts preferentially with DRIP205-like LxxLL motifs, Arch. Biochem. Biophys. 460 (2007) 206–212.
[4] B. He, E.M. Wilson, Electrostatic modulation in steroid receptor recruitment of LXXLL and FXXLF motifs, Mol. Cell Biol. 23 (2003) 2135–2150.
[5] O.I. Kolek, E.R. Hines, M.D. Jones, L.K. LeSueur, M.A. Lipko, P.R. Kiela, J.F. Collins, M.R. Haussler, F.K. Ghishan, 1alpha,25-Dihydroxyvitamin D3 upregulates FGF23 gene expression in bone: the final link in a renal-gastrointestinal-skeletal axis that controls phosphate transport, Am. J. Physiol. Gastrointest. Liver Physiol. 289 (2005) G1036–G1042.
[6] S. Samuel, M.D. Sitrin, Vitamin D's role in cell proliferation and differentiation, Nutr. Rev. 66 (2008) S116–S124.
[7] M.T. Cantorna, Y. Zhu, M. Froicu, A. Wittke, Vitamin D status, 1,25-dihydroxyvitamin D3, and the immune system, Am. J. Clin. Nutr. 80 (2004) 1717S–1720S.
[8] T. Matsumoto, Osteoporosis treatment by a new active vitamin D3 compound, eldecalcitol, in Japan, Curr. Osteoporos. Rep. 10 (2012) 248–250.
[9] C. Leyssens, L. Verlinden, A. Verstuyf, The future of vitamin D analogs, Front. Physiol. 5 (2014) 122.
[10] T.L. Briones, H. Darwish, Vitamin D mitigates age-related cognitive decline through the modulation of pro-inflammatory state and decrease in amyloid burden, J. Neuroinflammation 9 (2012) 244.
[11] M.R. Durk, K. Han, E.C. Chow, R. Ahrens, J.T. Henderson, P.E. Fraser, K.S. Pang, 1alpha,25-Dihydroxyvitamin D3 reduces cerebral amyloid-beta accumulation and improves cognition in mouse models of Alzheimer's disease, J. Neurosci. 34 (2014) 7091–7101.
[12] C.S. Latimer, L.D. Brewer, J.L. Searcy, K.C. Chen, J. Popovic, S.D. Kraner, O. Thibault, E.M. Blalock, P.W. Landfield, N.M. Porter, Vitamin D prevents cognitive decline and enhances hippocampal synaptic function in aging rats, Proc. Natl. Acad. Sci. U.S.A. 111 (2014) E4359–E4366.
[13] H. Mano, S. Ikushiro, T. Sakaki, Novel split luciferase-based biosensors for evaluation of vitamin D receptor ligands and their application to estimate CYP27B1 activity in living cells, J. Steroid Biochem. Mol. Biol. 183 (2018) 221–227.
[14] K. Kristjansson, A.R. Rut, M. Hewison, J.L. O'Riordan, M.R. Hughes, Two mutations in the hormone binding domain of the vitamin D receptor cause tissue resistance to 1,25 dihydroxyvitamin D3, J. Clin. Invest. 92 (1993) 12–16.
[15] P.J. Malloy, J.W. Pike, D. Feldman, The vitamin D receptor and the syndrome of hereditary 1,25-dihydroxyvitamin D-resistant rickets, Endocr. Rev. 20 (1999) 156–188.
[16] D. Feldman, P. J.M, Mutations in the vitamin D receptor and hereditary vitamin D-resistant rickets, BoneKEy Rep. 3 (2014) 510.
[17] M. Matsuo, A. Hasegawa, M. Takano, H. Saito, S. Kakuda, T. Chida, K. Takagi, E. Ochiai, K. Horie, Y. Harada, M. Takimoto-Kamimura, K. Takenouchi, D. Sawada, A. Kittaka, Synthesis of 2alpha-heteroarylalkyl active vitamin d3 with therapeutic effect on enhancing bone mineral density in vivo, ACS Med. Chem. Lett. 4 (2013) 671–674.
[18] M. Matsuo, A. Hasegawa, M. Takano, H. Saito, S. Kakuda, K. Takagi, E. Ochiai, K. Horie, M. Takimoto-Kamimura, K. Takenouchi, D. Sawada, A. Kittaka, Design and synthesis of 2alpha-(tetrazolylethyl)-1alpha,25-dihydroxyvitamin D3 as a high affinity ligand for vitamin D receptor, J. Steroid Biochem. Mol. Biol. 144 (Pt A) (2014) 201–203.
[19] M. Takano, K. Yasuda, E. Tohyama, E. Higuchi, T. Sakaki, A. Kittaka, Synthesis of the CYP24A1 major metabolite of 2alpha-[2-(tetrazol-2-yl)ethyl]-1alpha,25-dihydroxyvitamin D3, J. Steroid Biochem. Mol. Biol. 173 (2017) 75–78.
[20] H. Mano, M. Nishikawa, K. Yasuda, S. Ikushiro, N. Saito, M. Takano, A. Kittaka, T. Sakaki, Development of novel bioluminescent sensor to detect and discriminate between vitamin D receptor agonists and antagonists in living cells, Bioconjugate Chem. 26 (2015) 2038–2045.
[21] H. Mano, M. Nishikawa, K. Yasuda, S. Ikushiro, N. Saito, D. Sawada, S. Honzawa, M. Takano, A. Kittaka, T. Sakaki, Novel screening system for high-affinity ligand of heredity vitamin D-resistant rickets-associated vitamin D receptor mutant R274L using bioluminescent sensor, J. Steroid Biochem. Mol. Biol. 167 (2017) 61–66.
[22] T. Ishimoto, T. Ozawa, H. Mori, Real-time monitoring of actin polymerization in living cells using split luciferase, Bioconjugate Chem. 22 (2011) 1136–1144.
[23] M.C. Lake, Q.D. Nguyen, S. Ali, E.O. Aboagye, Development of a novel molecular sensor for imaging estrogen receptor-coactivator protein-protein interactions, PLoS One 7 (2012), e44160.
[24] T. Hashimoto, K.W. Adams, Z. Fan, P.J. McLean, B.T. Hyman, Characterization of oligomer formation of amyloid-beta peptide using a split-luciferase complementation assay, J. Biol. Chem. 286 (2011) 27081–27091.
[25] T. Sakaki, N. Sawada, K. Komai, S. Shiozawa, S. Yamada, K. Yamamoto, Y. Ohyama, K. Inouye, Dual metabolic pathway of 25-hydroxyvitamin D3 catalyzed by human CYP24, Eur. J. Biochem. 267 (2000) 6158–6165.
[26] T. Sakaki, K. Yasuda, A. Kittaka, K. Yamamoto, T.C. Chen, CYP24A1 as a potential target for cancer therapy, Anticancer Agents Med. Chem. 14 (2014) 97–108.
[27] K. Yasuda, E. Tohyama, M. Takano, A. Kittaka, M. Ohta, S. Ikushiro, T. Sakaki, Metabolism of 2alpha-[2-(tetrazol-2-yl)ethyl]-1alpha,25-dihydroxyvitamin D3 by CYP24A1 and biological activity of its 24R-hydroxylated metabolite, J. Steroid Biochem. Mol. Biol. 178 (2018) 333–339.

Please cite this article in press as: H. Mano, et al., Novel biosensor using split-luciferase for detecting vitamin D receptor ligands based on the interaction between vitamin D receptor and coactivator, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.09.122