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## **Molecular recognition by a short partial peptide of adrenergic receptor - Bottom-up approach**

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### **Abstract**

Receptor-neurotransmitter molecular recognition is key of neurotransmission. Although crystal structures are known, the recognition mechanism is not clear, and thus a trial-and-error approach is only a sure method to identify ligands (e.g. drugs) that trigger the receptor. Here, we measured ultraviolet and infrared spectra of complexes between a partial peptide SIVSF, the binding motif of a catechol ring in the adrenergic receptor, and various ligands. The ultraviolet spectra show that two isomers coexist in the complex of SIVSF with properly recognized ligands, such as protonated adrenaline (adrenalineH<sup>+</sup>). From infrared spectra, they are assigned to catechol-bound and ammonium-bound structures. The catechol-bound structure disappears when the ligand is replaced by non-proper molecules, such as noradrenalineH<sup>+</sup>. The results suggest that the molecular recognition is determined by local interactions. This provides a new possibility of screening to find a molecule like medicine that activates the receptor.

Neurotransmitters and hormones propagate signals and regulate physiological activities. This process is driven by molecular recognition between the receptor and ligands (neurotransmitters and hormones); thus, this mechanism attracts many researchers, particularly from the drug design field. So far, structural biological approaches by X-ray crystallography<sup>[1-3]</sup> and NMR<sup>[4-5]</sup> as well as *in silico* approaches by molecular dynamics simulations<sup>[6-8]</sup> have been reported. However, the basic principle of the recognition has not yet been made clear. X-ray diffraction methods provide structural information concerning how the proper ligand is bound in the receptor. The problem is that it is almost impossible to crystalize the receptor at the time when it refuses an unfeasible ligand. Thus, it is difficult to understand the mechanism of how other molecules are not recognized. NMR can detect only stable species. Thus, it is difficult to get the information about how to reject molecules other than the proper ligand. Although computer power is increasing, it is still challenging to reproduce intermolecular interactions between a receptor and molecules with sufficient accuracy. Therefore, a brute-force approach, i.e. trial-and-error, is still the most reliable method used to discover new drugs that trigger some receptors.

Here, we would like to report a bottom-up approach to understand the molecular recognition mechanism<sup>[9]</sup>. It is known that a receptor binds a ligand only by some specific parts (receptor pocket). Thus, it would be possible to study the recognition mechanism by using a partial peptide that corresponds to the receptor pocket. Theoretically, such an approach has been established for protein structure prediction (the fragment assembly method)<sup>[10-11]</sup>, in which the structure of a whole protein is built up by assembling the structures of partial sequences (fragments). But, experimentally, such an approach is difficult in the solution phase because the structure of the partial peptide is disturbed strongly by the surrounding solvent molecules. Particularly, many receptors locate in membrane where no solvent molecules exist, and thus the structure of the partial peptide in solution would be far from the original. In the present work, we introduced the partial peptides into an isolated condition in a vacuum, where no solvent existed. The circumstance is similar to that in membrane<sup>[12]</sup>, and then their structures are essentially determined by their intramolecular interactions. Thus, we expected that it would be possible to study the recognition mechanism based on the molecular complex between the partial peptide and the various ligands including both proper and non-proper ligand molecules.

The combination of electrospray ionization (ESI) and a cryogenic cold ion trap mass spectrometer enables us to get clear structural information of biomolecular ions by combining various laser spectroscopies<sup>[13-16]</sup>. In the cold ion trap (~10K), any conformational fluctuation of biomolecules is significantly suppressed. The mass-spectrometric techniques can be used to select molecules of interest, such as the complex of a partial peptide and a certain ligand. Furthermore, several laser spectroscopies can be applied to isolated molecular complexes to measure their ultraviolet (UV) and infrared (IR) spectra. Under the gas phase isolated condition, normal absorption spectroscopies are hardly applied because of a low concentration. Thus, instead of direct detection of any photo absorption, photo dissociation following photo excitation is used to detect photo absorption. In this work, UV spectra were measured by detecting photo fragments as functions of the frequency of a UV laser, which are called UV photo dissociation (UVPD) spectra. On the other hand, absorption of a single IR photon hardly dissociates the covalent bonds. To measure IR spectra, depopulations of a zero vibrational level by IR excitation are detected by using the UVPD method. Such a method is called IR-UV ion dip spectroscopy, which is one of the most important methods because it enables us to measure isomer-selected IR spectra<sup>[17]</sup>. Details of these spectroscopic techniques and the experimental setup<sup>[18]</sup> are described in Supporting Information together with Fig. S1.

We applied this method to complexes of a partial peptide of  $\beta_2$ -adrenergic receptor and its agonists. The  $\beta_2$ -adrenergic receptor is driven mainly by adrenaline (epinephrine), which is one of the catechol amine neurotransmitters. So far, the crystal structure of a complex of adrenaline and  $\beta_2$ -adrenergic receptor has been solved recently<sup>[19]</sup>. The  $\beta_2$ -adrenergic receptor is of the seven-transmembrane type, and the fifth transmembrane  $\alpha$ -helix (TM5) has a binding motif of adrenaline. Its sequence is SIVSF (S: serine, I: isoleucine, V: valine, F: phenylalanine); two serine residues form hydrogen bonds with adjoining phenolic OHs (catechol OHs) of adrenaline<sup>[20]</sup>. Thus, we chemically synthesized a partial peptide, CH<sub>3</sub>CO-SIVSF-NHCH<sub>3</sub> (hereafter simply denoted by SIVSF peptide), whose both termini were protected to avoid any unnecessary hydrogen-bonds by bare termini, which do not originally exist in the receptor<sup>[9, 21]</sup>. By such protection,  $\alpha$ -turn structure (unit structure of an  $\alpha$ -helix) is observed in the gas phase (see Supporting Information).

In this work, we produced a complex of the SIVSF peptide and protonated adrenaline

(adrenalineH<sup>+</sup>), noradrenalineH<sup>+</sup> and dopamineH<sup>+</sup> (see Fig. 1). AdrenalineH<sup>+</sup> is a proper ligand of the  $\beta_2$ -adrenergic receptor, while noradrenalineH<sup>+</sup> and dopamineH<sup>+</sup> are non-proper ones because of their small binding constants (only 10% and 1% of adrenalineH<sup>+</sup>, respectively<sup>[22]</sup>). In addition, we also examined isoprenalineH<sup>+</sup> (isoprenalineH<sup>+</sup>), which has a 10-times higher affinity than adrenalineH<sup>+</sup><sup>[22]</sup>. All of the ligands have a catechol ring, and SIVSF is expected to bind the catechol ring in the receptor, and thus all of them can be bound by SIVSF regardless of the affinity.

As we expected, all of the ligands form the complex with SIVSF, and their UV spectra were measured by the UVPD spectroscopy (Fig. 1). From the absorption frequencies, all of the UV spectra of the complex were assigned to the UV absorption of the catechol ring ( $\pi\pi^*$  transitions)<sup>[23-24]</sup>. Our surprising point is that the UV spectra of the complex of the proper ligands (adrenalineH<sup>+</sup> and isoprenalineH<sup>+</sup>) are similar, while complexes of the non-proper ligands (noradrenalineH<sup>+</sup> and dopamineH<sup>+</sup>) give different spectra, although the UV chromophores are the same. The former gives two absorption bands, indicated by the letters a (red) and b (blue), while the latter shows only a single band corresponding to the a-band. The difference of the UV spectra suggests that the binding structure is different between the two groups.

To obtain geometrical information, isomer-selected IR spectra of the NH and OH stretching region ( $\sim 3\mu\text{m}$ ) of each complex were measured by IR-UV ion dip spectroscopy. Figs. 2a and 2b show IR spectra of the SIVSF-adrenalineH<sup>+</sup> complex measured by fixing the frequency of the UV laser to the a-band (red) and the b-band (blue) in the UV spectrum, respectively. Since they show totally different IR spectra, it can be concluded that the a-band and the b-band arise from different isomers. Here, we call them isomers **a** and **b**. The NH stretching vibrations are mainly observed at less than  $3550\text{ cm}^{-1}$ , which reflect the hydrogen-bonding pattern of the NH bonds in the peptide main chain, i.e. a secondary structure<sup>[25]</sup>. Thus, the secondary structures of SIVSF are significantly different between isomers **a** and **b**.

Similarly, isomer-selected IR spectra of the SIVSF-isoprenalineH<sup>+</sup> complex were measured by probing the a-band and the b-band (Figs. 2c and 2d). As well as SIVSF-adrenalineH<sup>+</sup>, the SIVSF-isoprenalineH<sup>+</sup> complexes also have two isomers, **a** and **b**. Here, the IR spectrum of isomer **b** in each complex (Figs. 2b and 2d, respectively) is very similar. This means that the secondary structure of SIVSF and intermolecular binding pattern are almost the same in both complexes. The common

structure between adrenalineH<sup>+</sup> and isoprenalineH<sup>+</sup> is catechol moiety. Thus, it naturally suggests that both ligands are bound to SIVSF by their catechol ring. This binding pattern (catechol-bound) is also supported by the absence of OH stretching of catechol (3600 and 3660 cm<sup>-1</sup>[24]). This means that both OHs form strong hydrogen bonds with SIVSF. Therefore, we conclude that the isomers **b** are formed by the hydrogen-bonds between catechol OHs of the ligands and SIVSF.

On the other hand, isomers **a** in adrenalineH<sup>+</sup> and isoprenalineH<sup>+</sup> complexes give different IR spectra, especially in the NH stretching region, which suggests different secondary structures of SIVSF. Since this structural difference arises from different amino groups of the ligands (adrenaline: methylamine, isoprenaline: isopropylamine), it can be expected that their ammonium groups form hydrogen bonds with the peptide bonds of SIVSF and disturb the secondary structures in the different ways. The number of bands in a region higher than 3550 cm<sup>-1</sup> also confirms this binding type (ammonium-bound). Isomer **a** of the SIVSF-adrenalineH<sup>+</sup> complex has five OH groups in total, and four OH stretching bands appear in this region. This means that almost of all the OH groups do not form strong hydrogen bonds in isomer **a**. From this spectral signature, we conclude that the ligand is bound to SIVSF by hydrogen-bonding of the ammonium group. Detailed assignments of the OH stretching vibrations are fully described in Supporting Information.

The UV spectra of noradrenalineH<sup>+</sup> and dopamineH<sup>+</sup> complexes show broad but intense bands in the region of the a-band (isomer **a**), but no intense peaks in the region of the b-band (isomer **b**). This suggests that they do not have a catechol-bound isomer. To confirm that their structures are ammonium-bound, IR spectra were also measured (Figs. 2e and 2f). The observed IR spectra clearly show the spectral signature of ammonium-bound isomer: four and three OH stretching bands in the region higher than 3550 cm<sup>-1</sup> for noradrenalineH<sup>+</sup> and dopamineH<sup>+</sup> complexes, respectively. Their spectral feature in the NH stretching region are clearly different from that of isomer **b** (catechol-bound, Figs. 2b and 2d). This spectral pattern means that noradrenalineH<sup>+</sup> and dopamineH<sup>+</sup> complexes are ammonium-bound. Here, both complexes show similar vibrational patterns in the NH stretching region. It also confirms their ammonium-bound structures, because both ligands have the same ammonium group.

The results described above are summarized as follows: high-affinity ligands (adrenalineH<sup>+</sup> and isoprenalineH<sup>+</sup>) for the  $\beta_2$ -adrenergic receptor can form catechol- and

ammonium-bound complexes, while non-proper ligands (noradrenalineH<sup>+</sup> and dopamineH<sup>+</sup>) form only ammonium-bound ones. Since the SIVSF peptide is a binding site of catechol OHs in the receptor, it is meaningful that only the proper ligands show the catechol-bound complex. This suggests that the structure of SIVSF in the complex is similar to the native structure ( $\alpha$ -helix, more precisely  $\alpha$ -turn in this case) in the receptor protein.

The IR spectra provide structural information concerning the SIVSF peptide in the complex. If the SIVSF peptide forms an  $\alpha$ -turn structure, the NH group of Phe should form a hydrogen bond with a C=O group of the first Ser<sup>[26]</sup>. Such a hydrogen bond is termed C<sub>13</sub> because it forms a 13-membered cyclic hydrogen bond. The typical frequency of the NH stretch of C<sub>13</sub> is reported to be 3320-3350 cm<sup>-1</sup><sup>[27-28]</sup>. Thus, if the frequency of the NH stretching band of Phe can be determined, we can conclude whether the SIVSF peptide forms the  $\alpha$ -turn structure or not. To assign the NH stretching band of Phe, we used the <sup>15</sup>N labeling method<sup>[16, 29]</sup>. Fig. 3 shows the IR spectra of the catechol-bound complex of adrenalineH<sup>+</sup> with a) SIVSF(<sup>15</sup>N) and that of <sup>14</sup>N isotopomer (same as Fig. 2b). Only the band observed at 3316 cm<sup>-1</sup> in the <sup>14</sup>N isotopomer is shifted by <sup>15</sup>N substitution. Thus, we can assign the band at 3316 cm<sup>-1</sup> to the NH stretch of Phe. This frequency is very close to the typical value of the C<sub>13</sub> NH stretch; we thus conclude that the SIVSF peptide involved in the catechol-bound complex forms  $\alpha$ -turn structure. Thus, the SIVSF peptide preserves the  $\alpha$ -turn structure through the formation of the catechol-bound complex.

In this work, we measured the UV and IR spectra of the complex of the binding motif in the  $\beta_2$ -adrenergic receptor (SIVSF) with several catecholamine neurotransmitters. It is surprising that even only the binding motif can distinguish proper or non-proper ligands. This suggests a possibility that we can evaluate an ability of molecular recognition, and reveal the mechanism by using only the binding motif, i.e. the bottom-up approach.

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Fig. 1 UVPD spectra of complexes of the SIVSF peptide with a) adrenalineH<sup>+</sup>, b) isoprenalineH<sup>+</sup>, c) noradrenalineH<sup>+</sup> and d) dopamineH<sup>+</sup> measured by detecting a photo fragment m/z = 607.3 which corresponds to the protonated SIVSF peptide.

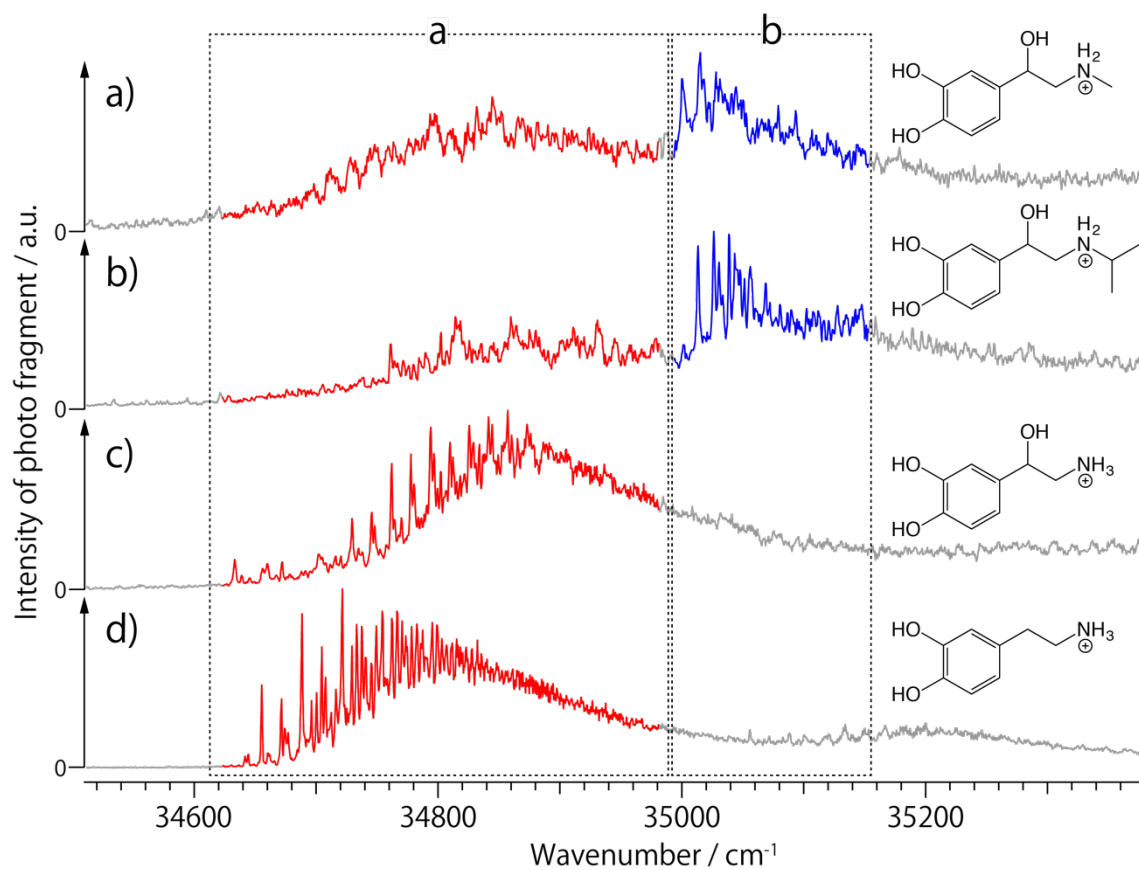


Fig. 2 IR-UV ion dip spectra measured by probing a) a-band ( $34844\text{ cm}^{-1}$ ) and b) b-band ( $35015\text{ cm}^{-1}$ ) of adrenaline $\text{H}^+$ -SIVSF, c) a-band ( $34860\text{ cm}^{-1}$ ) and d) b-band ( $35013\text{ cm}^{-1}$ ) of isoprenaline $\text{H}^+$ -SIVSF, e) a-band ( $34857\text{ cm}^{-1}$ ) of noradrenaline $\text{H}^+$ -SIVSF and f) a-band ( $34655\text{ cm}^{-1}$ ) of dopamine $\text{H}^+$ -SIVSF complexes. The vertical axis is the normalized absorbance obtained by  $-\log(I_{\text{IRon}}/I_{\text{IRoff}})$ , where  $I_{\text{IRon}}$  and  $I_{\text{IRoff}}$  is photo fragment intensity with and without IR irradiation, respectively.

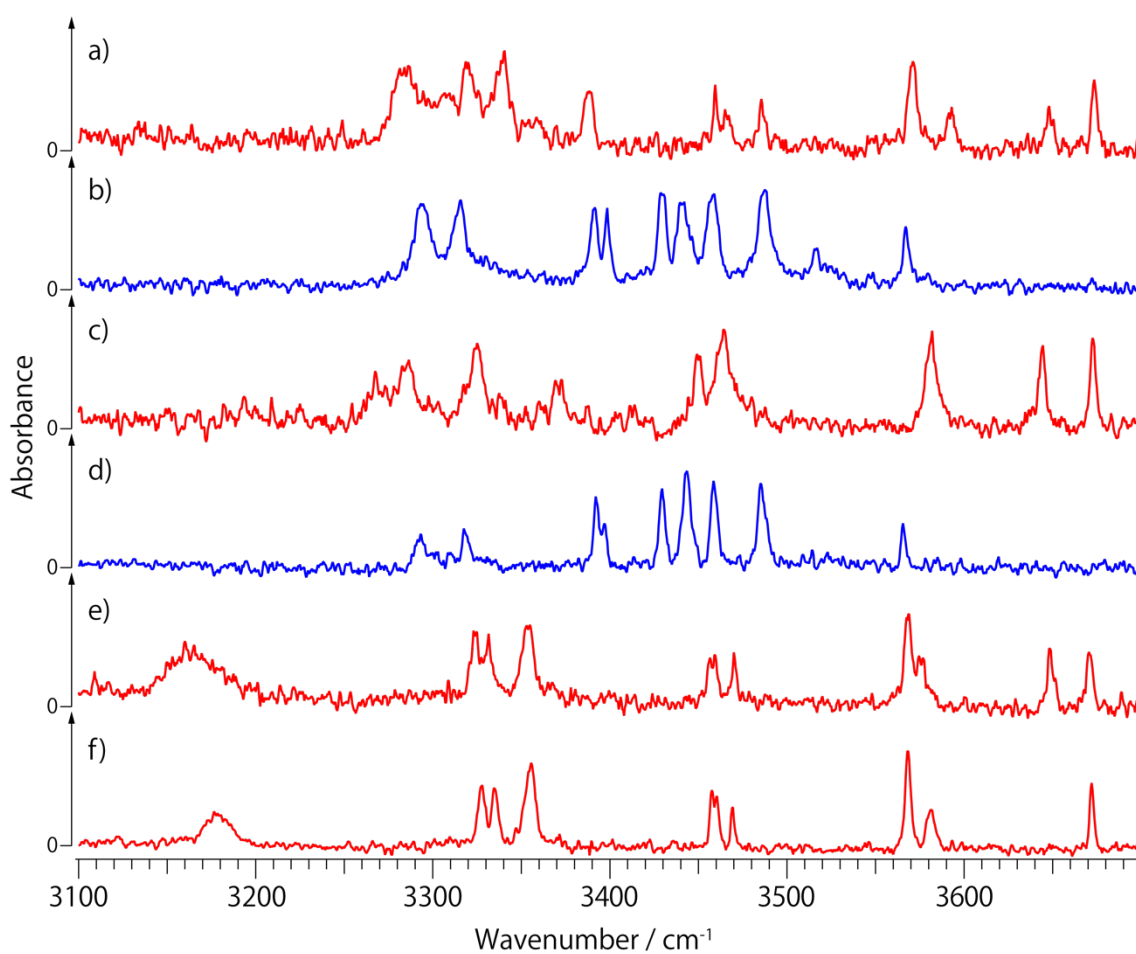


Fig. 3 IR-UV ion dip spectra of a) isomer b of adrenalineH<sup>+</sup>-SIVSF(<sup>15</sup>N) and b) its <sup>14</sup>N isotopomer.

