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Amazing Stability of the Arginine–Phosphate Electrostatic Interaction

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Abstract

Electrostatic interactions between a basic epitope containing adjacent arginine residues and an acidic epitope containing a phosphorylated serine are involved in receptor heteromerization. In the present study, we demonstrate that this arginine–phosphate electrostatic interaction possesses a “covalent-like” stability. Hence, these bonds can withstand fragmentation by mass spectrometric collision-induced dissociation at energies similar to those that fragment covalent bonds and they demonstrate an extremely low dissociation constant by plasmon resonance. The present work also highlights the importance of phosphorylation–dephosphorylation events in the modulation of this electrostatic attraction. Phosphorylation of the acidic epitope, a casein kinase one consensus site, makes it available to interact with the basic epitope. On the other hand, phosphorylation of serine and/or threonine residues adjacent to the basic epitope, a protein kinase A consensus site, slows down the attraction between the epitopes. Although analyzed here in the frame of receptor heteromerization, the arginine–phosphate electrostatic interaction most likely represents a general mechanism in protein–protein interactions.

Keywords

electrostatic interaction; receptor heteromers; phosphorylation; CK1; PKA

1. Introduction

Different mechanisms were suggested for the underlying protein–protein interaction in receptor heteromerization.^{1,2} We have recently demonstrated that heteromerization of adenosine A_{2A} with dopamine D₂ receptors^{3–5} and glutamate NMDA receptors (NR1–1 subunit) with dopamine D₁ receptors^{6,7} depend on epitope–epitope electrostatic interactions and suggested that this is a general mechanism for receptor heteromerization.^{8–10} Striking similarities were found between the epitopes involved in the A_{2A}–D₂ and NMDA–D₁ receptor heteromerization:^{8–10} (1) an arginine (Arg)-rich epitope localized in the N-terminal portion of the third intracellular loop of the D₂ receptor and the C-terminus of the NR1–1 subunit of the NMDA receptor; (2) on the opposing epitopes of the C-termini of both A_{2A} and D₁ receptors, a serine (Ser) residue susceptible of phosphorylation by casein kinase one (CK1) (pSer₃₇₄ and pSer₃₉₇, respectively); (3) a protein kinase A (PKA) consensus site adjacent to the Arg-rich epitope of the D₂ receptor and the NR1–1 subunit of the NMDA receptor. In the present study, by using mass spectrometric collision-induced dissociation and surface plasmon resonance techniques, we demonstrate that the Arg–phosphate electrostatic interaction

possesses a “covalent-like” stability and that its formation can either be induced or impaired as a result of phosphorylation.

2. Materials and Methods

Mass Spectrometry

A Quadrupole-Time-of-Flight (Q-TOF) Global Ultima mass spectrometer (Waters, Milford, MA) was used for ElectroSpray Ionization (ESI) analysis. A flow rate of 5 $\mu\text{L}/\text{min}$ was used to introduce the sample into the mass spectrometer. The mass spectrometer was operated in positive mode with a capillary voltage of 2.9 kV, a sampling cone voltage of 50 V, a source temperature of 100 $^{\circ}\text{C}$, a desolvation temperature of 200 $^{\circ}\text{C}$, a desolvation gas flow rate of 650 L/Hr, and a cone gas flow of 100 L/Hr. For MS/MS analysis, a selection mass window of 6 Da with a collision gas pressure of 7 mbar was employed. Collision energies between 5 and 40 V were used for ion fragmentation. Mass spectra presented are the sum of 50 consecutive 1-s scans. Average intensity values reported in this study are the result of three replicate sample runs. Both a DE-pro Matrix-Assisted Laser Desorption/Ionization (MALDI) and a MALDI–Time-of-Flight–Time-of-Flight (MALDI-TOF-TOF) 4700 (both MALDI are from Applied Biosystems Framingham, MA) were used. All spectra were acquired in positive ion-mode. Both instruments were used to acquire spectra in linear mode and the MALDI-TOF-TOF was used for the collision-induced dissociation work (selection mass window of 5 Da). The MALDI matrix, 6-aza-2-thiothymine (ATT) was purchased from Aldrich (Milwaukee, WI) and prepared fresh daily as a saturated solution in 50% ethanol. A 0.1 or 0.3 μL peptide mixture + a 0.1 or 0.3 μL matrix (ATT) were applied to the MALDI target and allowed to air-dry prior to introduction into the mass spectrometer.

We chose the acidic peptide for normalizing the complexes because the basic ones always ionize better and have a better relative abundance mainly 100%. Thus, to partially nullify the effect of suppression and for lack of a better way to normalize we chose to divide the RA of the complex by the RA of its acidic peptide.

Peptides

VLRRRRKRVN, **HELKGVCPPEPGLDDPLAQDGAVGS**, **SAQEpSQGNT**, **RRRRKRVNpTKRpSSR**, **SFKRRRSSK**, **SFKRRRSpSK**, **GpSSEDLKKEEA**, and biotinylated **VLRRRRKRVN** were synthesized by the Sequencing and Synthesis Laboratory at the Johns Hopkins School of Medicine.

Sample Preparation

The amount deposited in the experiment shown in Figure 1 are **VLRRRRKRVN** (25 pmoles), **HELKGVCPPEPGLDDPLAQDGAVGS** (50 pmoles) are **SAQEpSQGNT** (50 pmoles). The spectrum was acquired in positive reflectron mode on an ABI TOF-TOF and is the average of 400 Laser shots. The amount deposited in the experiment shown in Figure 2b are **VLRRRRKRVN** (25 pmoles), **SAQEpSQGNT** (50 pmoles). The spectrum was acquired on an ABI TOF-TOF, the CID was done using air and is the average of 1000 laser shots. Figure 2C and D data was obtained on the Q-TOF using a flow rate of 5 $\mu\text{L}/\text{min}$, each microliter contained **VLRRRRKRVN** (1 pmole) and **SAQEpSQGNT** (15 pmoles). The amount deposited in the experiment shown in Figure 4a **SFKRRRSSK** (10 pmoles), **SFKRRRSpSK** (10 pmoles), **GpSSEDLKKEEA** (10 pmoles). The spectrum was acquired in linear positive mode on an ABI DE-pro and is the average of 50 Laser shots. The amount deposited in the experiment shown in Figure 4b are **SAQEpSQGNT** (100 pmoles), **VLRRRRKRVN** (25 pmoles), **RRRRKRVNpTKRpSSR** (25 pmoles). The spectrum was acquired in positive reflectron mode on an ABI TOF-TOF and is the average of 400 Laser shots.

Plasmon Resonance

Surface plasmon resonance measurements were made using Biacore 3000 with sensor chip SA or custom sensor chip Neutravidin CM4. The running buffer was HBS-P (Biacore, BR-1001–87) containing 10 mM HEPES, pH 7.4, 150 mM NaCl, 0.005% Surfactant P20. The regeneration buffers were 0.05% SDS, and HBS-P adjusted to 500 mM NaCl. Neutravidin (Pierce, Rockford, IL) was immobilized to sensor chip CM4 using amine coupling via an immobilization wizard. Sensor chip CM4 (BR-1000–39) has a carboxymethylated dextran hydrogel containing approximately 30% less carboxymethylation than sensor chip CM5. Reactive *N*-hydroxysuccinimide (NHS) esters were introduced on the surface through modification of the carboxymethyl groups by an 8 min injection of a mixed solution of 0.4 M EDC (1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide) and 0.1 M NHS (BR-1000–50). The Neutravidin was diluted in 10 mM sodium acetate pH 5.0 (BR-1003–51) to a final concentration of 50 µg/mL and injected for 10 min. After the coupling step, any remaining reactive esters on the surface were blocked with a 7-min injection of 1 M ethanolamine, pH 8.5. The flow rate was 10 µL/min and the detection temperature was 25 °C. Using this procedure, immobilization densities for Neutravidin ranged from 9000 to 10 000 RU. The biotinylated-**VLRRRRKRVN** peptide was diluted to 100 µg/mL in HBS-P and injected for 5 min over one flow cell. The amount of peptide captured using this condition was 1500 RU (resonance Units). Biotin binding sites on the reference surface and any remaining free sites on the active surface were blocked with free biotin. A kinetics application wizard was employed for measuring the peptide–peptide interactions. Before running the method, the newly prepared sensor chip surface was preconditioned by three 30-s injections of the regeneration buffers. The preconditioning step ensures that any change in the ligand density or activity due to regeneration occurs before the assay is initiated. **SAQEpSQGNT** was diluted into HBS-P to concentrations of 0.3, 0.612, 1.25, 2.5, 5, and 10 µM. Each peptide concentration was injected at a flow rate of 30 µL/min over the reference and active surfaces for 3 min, and the complex decay was measured for 3 min. Regeneration of the surface was achieved by a 30-s injection of 0.05% SDS followed by a 30 s injection of 500 mM NaCl HBS-P at a flow rate of 30 µL/min. The detection temperature was 25 °C. All data processing and analysis were performed using Biacore's BIA-evaluation software version 4.1.

3. Results

Basics of the Arg-Phosphate Electrostatic Interaction

We previously used soft ionization techniques (MALDI and ESI–MS) to demonstrate the ionization of noncovalent complexes (NCXs) made by salt bridge formation between peptides containing two or more adjacent basic residues (e.g., Arg-Arg) and peptides containing two or more adjacent acidic residues, such as glutamate-glutamate (Glu-Glu) or aspartate-aspartate (Asp-Asp) or a phosphorylated Ser or threonine (Thr) residues.^{11–13} We also showed that a phosphate group forms a more stable interaction with the Arg guanidinium group than two adjacent carboxyl groups from either Asp or Glu.¹¹ Although we previously suggested that adjacent Asp (DD) in the C-terminus of the A_{2A} receptor could possibly be the site of interaction with the D₂ receptor, the site was not conserved and as shown in the mass spectrum in Figure 1, the relative abundance (RA) of the NCXs that the Arg-rich D₂ receptor epitope (**215VLRRRRKRVN₂₂₄**) did form with the A_{2A} receptor epitope containing the pSer₃₇₄ (**370SAQEpSQGNT₃₇₈**) was far higher than that formed with the A_{2A} receptor epitope containing the adjacent Asp residues (**388HELGVCPEPPGLDDPLAQDGA₄₁₂**), even though the epitope containing the adjacent Asp is more electronegative than the phosphorylated epitope (total charge at physiological pH, 7.4: –4 and –3, respectively; GPMW 6.1 software, Denmark).

The guanidinium group at the terminus of the Arg side chain defines a plane in which the central carbon, three nitrogen and five hydrogens all reside (Figure 2a). It has a delocalized positive charge which is distributed over the entire group, resulting in triple resonance stabilization. Its pK_a is 12.5 and hence it is ionized over the entire pH range in which proteins exist in nature. The two highest occupied nonbonding molecular orbitals of the guanidinium group are degenerate in energy, thus distributing two pairs of electrons evenly over the three nitrogen atoms as shown by its resonance. The net positive charge can be neutralized by removing a proton. On the other hand, the phosphate in phospho-Ser $\delta\pi$ molecular orbital systems spreads the negative charge over two or more oxygens because their acid dissociation constants are quite acidic ($pK_{a1} = 0.9$ and $pK_{a2} = 6.1$) due to inductive electron withdrawal. Guanidine-anion binding is often hydrogen bond driven, an enthalpically favorable event, and offers a balance of favorable attraction between the epitopes (Figure 2a). Another possible arrangement could take into account the fact that the guanidinium cation is flat and trigonal, and the phosphate ester is tetrahedral about the phosphorus, with three equivalent oxygen atoms sharing one or two negative charges. Looking at the P atom exactly opposite (180°) from the P-O ester bond to serine, the three negatively charged oxygens appear to be 120° apart, exactly matching the 120° disposition of the partially positive N atoms of the guanidinium ion. So possibly the best positioning of these groups might be an arrangement in which one oxygen sits exactly above one nitrogen.

Stability of the Arg-Phosphate Electrostatic Interaction

In previous work, we have shown that the residues involved in the electrostatic interactions are resistant to enzymatic digests as they are not accessible for cleavage.¹² A mixture of the D₂ receptor epitope **215VLRRRKRVN224** (1351.9 Da) and the A_{2A} receptor epitope **370SAQEPsQGNT378** (1000.3 Da) was analyzed using both Matrix Assisted Laser Desorption/Ionization (MALDI) and ElectroSpray Ionization (ESI) Mass Spectrometry (MS). The molecular ion (MH^+) of the NCX was fragmented by collision-induced dissociation (CID) in the MALDI Time-of-Flight-Time-of-Flight (TOF-TOF) and the ESI Quadrupole-Time-of-Flight (Q-TOF) instruments. The MALDI CID spectrum showed no fragments of the parent peptides, just their MH^+ at m/z 1001.34 and 1352.87 as well as the NCX at m/z 2353.23. The base peak (RA 100%) in that spectrum was that of the Arg-rich epitope. The spectrum was very similar to the one previously obtained in linear mode without CID,⁸ although the NCX peak had a lower RA, and a new peak with a RA of 63% appeared at m/z 1433.25 (Figure 2b). The MH^+ of the new peak is 80 units larger than the MH^+ of the basic peptide, which corresponds to the D₂ receptor epitope + HPO_3 from the pSer of the A_{2A} receptor epitope (Figure 2a). The ESI CID spectrum of the NCX showed the same MH^+ as the ones seen in the MALDI spectrum (data not shown). An increased dissociation of the NCX was observed in the MALDI by progressively increasing the fluency of the laser (data not shown). While the RA of both D₂ receptor-epitope and D₂ receptor-epitope + HPO_3 increased for a while, the former kept increasing while the later eventually started to decrease. With ESI, it was observed that there was very little dissociation of the NCX below 25 V, from 25 to 30 V the dissociation increased and the RA of the D₂ receptor-epitope and D₂ receptor-epitope + HPO_3 kept increasing. At 35 V, the RA of the D₂ receptor epitope remained the same while that of D₂ receptor-epitope + HPO_3 started to decrease, and at 40 V the RA of both the D₂ receptor epitope and the D₂ receptor epitope + HPO_3 was significantly decreased (Figure 2c and 2d). Thus, these MALDI and ESI CID data show that the epitopes forming the NCX do not fragment into amino acid residues, but instead they dissociate into the parent peptides. When subjected to CID, the parent peptides totally fragmented at a voltage of 25 V. Thus, we show that the complexes stability was such that at voltages higher than the ones that completely fragment the parent peptides, the complex only dissociates. Thermodynamics principles dictate that a covalent bond is far more stable than a noncovalent electrostatic bond. However, our experimental data showed that the phosphate-guanidinium interaction was so stable that the covalent bond between the

oxygen from the phosphorylated serine and HPO_3 fragmented before the noncovalent complex between the guanidinium and the phosphate was disrupted (Figure 2a,b), as shown by the MH^+ at m/z 1433.25.

The D_2 receptor-epitope was immobilized on a neutravidin surface and solutions of the $\text{A}_{2\text{A}}$ receptor-epitope at concentrations of 10, 5, 2.5, 1.25, 0.612, and 0.3 μM were injected (plasmon resonance technology). Association and dissociation responses were each measured for 3 min. A specific and stable interaction between the $\text{A}_{2\text{A}}$ receptor epitope and the D_2 receptor epitope was observed. The KD ($k(\text{off})/k(\text{a})$) was 55 μM , the on rate was relatively slow ($k(\text{a})$: 11 ($\text{M}^{-1}\text{s}^{-1}$)) and the off rate was extremely slow ($k(\text{d})$: 6.1×10^{-4} (s^{-1})). The binding responses were fitted to the 1:1 Langmuir model and the data implied that the binding stoichiometries could be greater than 1:1, or bi-phasic association (Figure 3). Considering that the Arg-rich epitope contains 4 adjacent Arg, it is likely that more than one acidic peptide could have interacted with each basic one. Thus using plasmon resonance technology, we have been able to confirm specific and stable interactions between epitopes whose binding appears to be driven by electrostatic attraction.

Phosphorylation-Mediated Regulation of the Arg–Phosphate Electrostatic Interaction

The main signal transduction pathway of D_1 receptors involves PKA activation and it has been shown that D_1 receptor stimulation can modulate NMDA receptor function through PKA-mediated phosphorylation of Ser₈₉₇ of the NR1-1 subunit.¹⁴ Lee et al.⁶ showed that D_1 receptor stimulation decreases the NMDA- D_1 receptor heteromerization involving the NR1-1 subunit. Since Ser₈₉₇ is adjacent to the Arg-rich epitope of the NR1-1 subunit, we tested the possibility that phosphorylation of Ser₈₉₇ could decrease the epitope–epitope electrostatic interaction involved in NMDA- D_1 receptor heteromerization. In fact, as shown in Figure 4a, the addition of a phosphate group to Ser₈₉₇ significantly decreased the ability of the Arg-rich epitope of the NR1-1 subunit (390SFKRRRS_pSK₈₉₈) to form NCXs with the D_1 receptor epitope containing pSer₃₉₇ (396GpSSEDLKKEEA₄₀₈). The analysis of the total charge of these peptides (at physiological pH, 7.4) showed the significant electronegativity of the D_1 receptor epitope (total charge: −4) and the significant electropositivity of the Arg-rich NR1-1 subunit epitope (total charge: +5, without phosphorylation of Ser₃₉₇), which most probably determines the ability of these epitopes to approach each other and form heteromers through epitope–epitope electrostatic interaction. The reduced number of NCXs with the phosphorylated NR1-1 subunit epitope is most probably due to the reduction of the total electropositive charge after phosphorylation (total charge: +3). These results strongly suggest that D_1 receptor-mediated phosphorylation of Ser₈₉₇ decreases NMDA- D_1 receptor heteromerization by decreasing the epitope–epitope electrostatic attraction between the C termini of the D_1 receptor and the NR1-1 subunit of the NMDA receptor (see below).

Close inspection of the residues adjacent to the Arg-rich epitope of the D_2 receptor involved in $\text{A}_{2\text{A}}$ - D_2 receptor heteromerization uncovered a Thr residue (Thr₂₂₅) and a Ser residue (Ser₂₂₈) both susceptible to phosphorylation by PKA (Swiss Prot “Net Phos” Program).¹⁵ As expected, the addition of a phosphate group to Thr₂₂₅ and Ser₂₂₈ significantly decreased the ability of the Arg-rich epitope of the D_2 receptor to form NCXs with the $\text{A}_{2\text{A}}$ receptor epitope containing pSer₃₇₄ (Figure 4b). Again, the decreased number of NCXs might be due to the phosphorylation-induced decrease of the electropositivity of the Arg-rich domain of the D_2 receptor (the total charges for 370SAQEpSQGNT₃₇₈, 215VLRRRRKRNV₂₂₄ and 213RRRRKRNV_pTKRpSSR₂₃₀ were −3, +6 and +5, respectively). As for the D_1 receptor, the main signal transduction pathway of $\text{A}_{2\text{A}}$ receptors involves the activation of PKA.¹⁶ Although it remains to be demonstrated, our results predict that stimulation of $\text{A}_{2\text{A}}$ receptors leads to a decrease in the formation of $\text{A}_{2\text{A}}$ - D_2 receptor heteromers. In conclusion,

its covalent-like stability and its regulation by phosphorylation makes the Arg-phosphate bond a very likely general phenomenon involved in protein–protein interactions.

Proposed Mechanistic Model for Receptor Heteromerization Involving the Arg–Phosphate Electrostatic Interactions

The A_{2A}, D₂ and D₁ receptors belong to the of GPCR family, with seven transmembrane domains.^{2,16} The A_{2A} and D₁ receptors have similar structures, with a long C terminus, and the D₂ receptor is characterized by a long third intracellular loop (Figure 5). The NR1-1 subunit of the NMDA receptor (a ligand-gated ion channel) contains four transmembrane domains (although the second can only be considered partially transmembrane) and long C and N termini¹⁷ (Figure 5). The epitopes involved in A_{2A}-D₂ and NMDA-D₁ receptor heteromerization are situated in their largest intracellular domains (Figure 5). The analysis of the total charge of these domains (at physiological pH, 7.4) showed the D₂ receptor domain to be significantly electropositive with a total charge of +15 for the first 90 amino acids in the N-terminal portion of the third intracellular loop, while the NR1-1 subunit of the NMDA receptor has a total charge of +12 for the last 90 amino acids of the C terminus. On the other hand, a significant electronegativity was found in the corresponding domains of the A_{2A} receptor with a total charge of –9 for the last 90 amino acids of the C-terminus (including pSer₃₇₄) while the D₁ receptor has a total charge of –7 for the last 90 amino acids of the C-terminus (including pSer₃₉₇). This creates optimal conditions for a mutual approach of the domains containing the interacting epitopes. PKA-mediated phosphorylation of the Ser or Thr residues located in the vicinity of the Arg-rich epitopes of the D₂ receptor and NR1–1 unit of the NMDA receptor engenders a decrease in the total electropositivity of the domain (2 less positive charges per phosphorylated residue), which would decrease the attraction to the respective electronegative domains of the C termini of the A_{2A} and D₁ receptors, thus decreasing A_{2A}-D₂ and NMDA-D₁ receptor heteromerization.

4. Discussion

Most physiological events require proteins to interact, as in receptor-G protein cascades and receptor heteromerization. Protein folding is paramount for such functions and, hence, the importance of attractive forces in protein–protein interactions. We have previously shown that the mechanism involved in some receptor heteromerizations is due to the linear alignment of the guanidinium groups of adjacent Arg on one epitope and a phosphorylated amino acid residue on the opposing epitope, resulting in salt bridge formation through electrostatic attraction. The present work highlights the importance of phosphorylation–dephosphorylation events in the modulation of this electrostatic interaction. Thus, phosphorylation of the CK1 consensus site on the acidic epitope makes it available to interact with the Arg-rich basic epitope. On the other hand, phosphorylation of Ser and Thr residues adjacent to the basic epitope, a PKA consensus site, will slow the attraction between the epitopes. In addition, the collision-induced dissociation data show that once the noncovalent bond is established, it leads to a highly thermodynamically favorable conformation, as the noncovalently bound phosphate breaks its covalent bond to the Ser and remains noncovalently bound to the basic epitope. Hence, under the right electrostatic conditions, the Arg-phosphate noncovalent bond possesses a “covalent-like” stability. Although in the present study such bond is analyzed in the frame of receptor heteromerization, epitope–epitope electrostatic interactions most likely represent a general mechanism underlying many biological processes. Emphasizing the importance of Arg and Ser are the following facts: Ninety percent of phosphorylated residues are Ser, and the genetic code shows that Arg and Ser share the highest level of degeneracy with Leucine (six codons).

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Figure 1.

MALDI spectrum showing the interaction of the basic epitope from the third intracellular loop of the D₂ receptor **VLRRRRKRVN** with the two possible acidic epitopes from the carboxyl terminus of the A_{2A} receptor, **SAEQpSQGN** and **HELGVCPEPPGLDDPLAQDGAVGS**. The normalized relative abundance of the MH⁺ of the noncovalent complexes (NCXs) formed by the D₂ receptor epitope with the phosphorylated and the nonphosphorylated A_{2A} receptor epitopes was 70% and 5%, respectively. Normalization was obtained by dividing the relative abundance of the MH⁺ of the NCX by the relative abundance of the MH⁺ of the corresponding A_{2A} receptor epitope.

Figure 2.

Mass spectrometric collision-induced dissociation (CID) of the NCX formed by the D₂ receptor-epitope **VLRRRKRVN** with the A_{2A} receptor-epitope **SAQEpSQGNT**. **(a)** Cartoon showing the electrostatic interaction between the guanidinium group of Arg (blue) and the phosphate group of the phosphorylated serine (red). The green broken line shows where the covalent bond brakes (green broken line) after collision-induced dissociation to generate **VLRRRKRVN + HPO₃**. **(b)** MALDI-CID of the NCX at m/z 2353.21. **(c)** Data acquired using the ESI mode of a Q-TOF. The Ion-chromatogram shows the NCX (m/z 2353.21) stability at progressively higher collision voltages. **(d)** Data acquired using the ESI mode of a Q-TOF. The blue Ion-chromatogram shows the appearance of the basic epitope **VLRRRKRVN** (m/z = 1352.87). The red Ion-chromatogram shows the appearance of the 80 Daltons larger new species **VLRRRKRVN + HPO₃** (m/z = 1433.25).

Figure 3. Plasmon Resonance sensogram. The D₂ receptor epitope **VLRRRRKRVN** was biotinylated and attached to the chip surface, while increasing concentrations (10, 5, 2.5, 1.25, 0.612, and 0.3 μM) of the phosphorylated A_{2A} receptor epitope **SAQEpSQGNT** were passed over the surface. The data show a slow on rate and an extremely low off rate.

Figure 4.

MALDI spectra showing the formation of noncovalent complexes (NCXs) between the epitopes of the D₁ and A_{2A} receptors with the phosphorylated and nonphosphorylated epitopes of the NR1–1 subunit of the NMDA and D₂ receptors, respectively. (a) The normalized relative abundance of the MH⁺ of the NCXs formed by the D₁ receptor epitope GpSSEDLKKEEA with the epitopes of the NR1-1 subunit SFKRRRSSK and SFKRRRpSSK was 12.9% and 3%, respectively. (b) The normalized relative abundance of the MH⁺ of the NCXs formed by the A_{2A} receptor epitope SAQEpSQGNT with the epitopes of the D₂ receptor VLRRRRKRVN and RRRRKRVNpTKRpSSR was 48.3% and 28.0%, respectively. Normalization was obtained by dividing the relative abundance of the MH⁺ of the NCXs by

the relative abundance of the MH^+ of the corresponding phosphorylated and nonphosphorylated epitopes of the NR1-1 subunit of the NMDA receptor and the D_2 receptor.

Figure 5.

Scheme of the structure of the GPCRs A_{2A}, D₂, and D₁ and the NR1-1 subunit of the ligand-gated ion channel NMDA receptor. The acidic epitopes of both the A_{2A} and D₁ receptors (dark red) are localized in an electronegative domain (light red) of their characteristic long electronegative C termini. The basic epitope of the D₂ receptor (dark blue) is localized in an electropositive domain (light blue) of its characteristic long third intracellular loop. The basic epitope of the NR1-1 subunit (dark blue) is localized in an electropositive domain (light blue) of its long electropositive C terminus. Intramembrane domains are shown in green.