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Research Article

Non-Sulfated and Sulfated Sulfakinins Utilize Ligand-Specific Interactions with DSK-R2 and a Conserved Mechanism for Selectivity to Influence Gut Motility and Locomotion

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Abstract

Peptides impact biology; establishing how these ligands interact with their receptors may provide an approach to influence health. In identifying these interactions it is important to consider peptides often exist as a family of structurally-related, biologically-distinct ligands that may signal through multiple G protein-coupled receptors (GPCRs). This complexity must be addressed to avoid ambiguity when deciphering signaling. Sulfakinins comprise a peptide family that uniquely influence biology, yet, little is known about their ligand-receptor interactions, a critical first step in signaling. *Drosophila melanogaster* sulfakinin generates four bioactive products; drosulfakinin I (DSK I; FDDYGHMRF-NH_2), DSK II (GGDDQFDDYGHMRF-NH_2), and the sulfated tyrosyl forms of these two peptides. All four ligands bind GPCRs designated DSK-R1 and DSK-R2; however, how these biologically-unique ligands and their receptors interact remains relatively unexplored. We established non-sulfated (ns) DSK I and nsDSK II are more effective than sulfated (s) DSK I and sDSK II on adult and larval gut motility, and sDSK I and nsDSK I influence larval locomotion; however, sDSK II and nsDSK II do not. We hypothesized the ligands form unique DSK-R interactions to mediate peptide-specific effects on gut motility and on locomotion. We compared ligand-receptor interactions to structure-activity data to test our hypothesis. We identified a polar contact to the 3-7 lock and an aromatic contact between TMs 2 and 3 on DSK-R2 critical for gut motility; a salt bridge to D347 of DSK-R2 crucial for locomotion. Sulfakinins and their receptors are similar in structure and activity to cholecystokinin (CCK); M317 in DSK-R2 was accordant with the selectivity of nsDSK I and nsDSK II in larval gut motility, reminiscent of the role M195 on ECL2 of CCK-R1 plays in signaling. These findings indicate drosulfakinin signaling involves ligand-specific interactions to DSK-R2 utilizing a conserved mechanism behind selectivity to generate peptide-specific influence on gut motility and locomotion.

ABBREVIATIONS

CCK: Cholecystokinin; DSK: Drosulfakinin; ECL: Extracellular Loop; GPCR: G Protein-Coupled Receptor

INTRODUCTION

Peptides that impact biology are often encoded in precursors that undergo processing to yield multiple, structurally-related but biologically unique products. The complexity of these products may be increased by post-translational processing of a single amino acid residue; sulfation of tyrosine is an example of a modification that generates additional structurally-related peptides that are functionally distinct [1]. In addition, the peptides often bind to one or more G protein-coupled receptors (GPCRs) to transduce a signal uniquely based on ligand-protein interactions that reflect a novel structure-activity relationship. It is essential to address this complexity when establishing peptidergic signaling to avoid ambiguity in interpreting data.

Identifying peptide-receptor protein interactions is an approach to delineate mechanisms that underlie transduction in order to provide data for the design of functional agonists and antagonists to influence biology. Molecular docking identifies interactions to predict whether a ligand or analog may bind and activate a transduction process. Structure-activity data identify residues critical for the effect of a peptide; results which are independent, yet complementary to docking, thus providing a comparative evaluation of the predictions that were based on ligand-protein interactions.

Cholecystokinin (CCK), neuropeptide Y (NPY) and somatostatin (SST) are examples of peptides that affect multiple

biological processes through ligand-specific interactions with a GPCR [1-3]. Here, we investigate molecular docking of invertebrate sulfakinin (SK) peptides to help explain their structure-activity relationship (SAR) in gut motility and locomotion. Invertebrate sulfakinins are similar to vertebrate cholecystokinin in precursor organization and processing, peptide distribution, structure, and activities, and receptor sequences [4-12].

Sulfakinins influence numerous, critical biological processes including gut motility, locomotion, feeding, and heart rate [4-12]. Sulfakinins are processed from a polyprotein to yield multiple, structurally-related peptides with a C-terminal structure represented by (D/E) DYGHMRF-NH_2. *Drosophila melanogaster* sulfakinin encodes two drosulfakinin (DSK) peptides, DSK I (FDDYGHMRF-NH_2) and DSK II (GGDDQFDDYGHMRF-NH_2). Post-translational processing generates bioactive sulfated (s) and non-sulfated (ns) DSK peptides [8-12]. The drosulfakinins bind to GPCRs, DSK-R1 and DSK-R2 [13-15].

Non-sulfated DSK I and nsDSK II are more effective than sDSK I and sDSK II at decreasing adult and larval *D. melanogaster* gut motility, whereas sDSK I and nsDSK I influence larval locomotion, but nsDSK II and sDSK II do not. We hypothesized the ligands form unique DSK-R interactions to mediate peptide-specific effects on gut motility and on locomotion. To test our prediction, sDSK I and sDSK II were docked to DSK-R1 and DSK-R2 to compare to the contacts made by nsDSK I and nsDSK II to DSK-R1 and DSK-R2 [16]. The results identified novel peptide-specific and receptor-dependent interactions, consistent with our SAR data. Additionally, a methionine on DSK-R2 resembled M195 of cholecystokinin A receptor (CCK-A or CCK-R1), which plays a critical role in sulfated and non-sulfated CCK selectivity [17], consistent with a conserved mechanism underlying drosulfakinin signaling.

MATERIALS AND METHODS

Receptor modeling

The protocol for modeling GPCRs was previously described [18]. Primary sequences for DSK-Rs were obtained [13] and submitted to I-TASSER [19,20]. Models were refined in Mod Refiner [21]. To prepare for docking, extracellular loops (ECLs) and the extracellular tail were removed and a binding pocket of less than 27000 cubic angstroms was defined using AutoDock Tools-1.5.6.

Molecular docking

The docking protocol was previously described [18]. Ligands were built in PyMOL 1.7.0.3 and prepared for docking using AutoDock Tools-1.5.6 [22] which generated 10 runs of 20 modes each for a total of 200 poses. Examined independently by two researchers, a best pose was based on quality, quantity of physicochemical ligand-receptor interactions, and frequency of conformation.

RESULTS AND DISCUSSION

We docked sDSK I and sDSK II to DSK-R1 and DSK-R2 and compared these interactions to nsDSK I and nsDSK II docked to DSK-R1 and DSK-R2 [16]. Molecular docking and SAR data provide insight into ligand-receptor interactions that underlie peptide-specific effects. Our strategy would not, however, establish whether ligand affinity, ligand efficacy, or different DSK-R isoforms accounted for the observed diversity in peptidergic signaling; to our knowledge there is no published report that describes sulfakinin receptor isoforms. Docking and SAR yield information relevant to a critical first step in signaling, not the entirety of the transduction pathway.

Sulfated DSK I docked to DSK-R1

The sulfate sterically hindered sulfo-Y6 of sDSK I from docking in the bottom of the DSK-R1 binding pocket where, in the absence of the sulfate moiety, Y6 of nsDSK I docked. Despite this re-orientation, the sulfated peptide retained most of the non-sulfated peptide-receptor interactions (Figure 1). Sulfo-Y6 contacted the hydrophobic residues between TMs 2 and 3, with the sulfate pointed upward and available for ECL contact. F9 T-stacked with the transmission switch, W443, but shifted relative to nsDSK I, and pi-stacked with F1. Hydrogen bond propagation originated at the top of the pocket near TMs 2 and 7 through D2 and D3, but minimally contacted the 3-7 lock, Q191 and Y477. M7 was pointed toward the ECLs. H6 formed a salt bridge with E181 at the top of TM3, restricting C184 from contacting the ECLs. R8 shifted to contact the polar residues on TMs 5 and 6, and the C-terminal amide hydrogen bonded with D281 on TM5.

Sulfated DSK II docked to DSK-R1

Sulfotyrosine re-oriented sDSK II on DSK-R1, (Figure 2) allowing the sulfate to form favorable intramolecular salt bridges with R13. F6 was re-positioned from the TM2-TM3 pocket and weakly contacted F187. F14 T-stacked to W443, and sulfo-Y9 interacted with hydrophobic residues at the top of TM6. The aspartic acids were positioned at the top of the binding pocket and were available for interaction with the ECLs.

Sulfated DSK II did not interact directly with the 3-7 lock, although a polar network began with the aspartates and extended through the C-terminal amide, positioned near the 3-7 lock. M12 of sDSK II contacted hydrophobic residues lower in the binding pocket but was blocked from contacting the ECLs. H11 formed a salt bridge to E181 and, along with the GG N terminus, blocked C184 from ECL contact. Sulfo-Y9 and R13 retained polar contacts on TM5 and TM6, including a salt bridge to D281, a unique contact among the DSKs on DSK-R1.

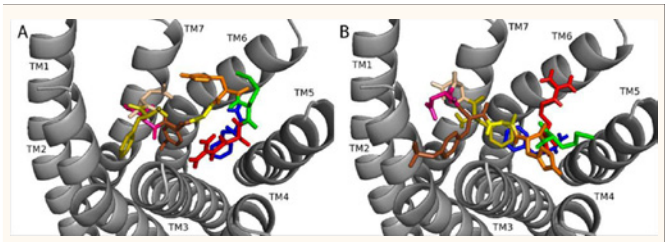

Figure 1 (A) Non-sulfated DSK I and (B) sDSK I docked to DSK-R1: the receptor is a gray ribbon and the residues in the ligands are: F1, olive; D2, wheat; D3, hot pink; (sulfo-)Y4, brown; G5, yellow; H6, orange; M7, green; R8, red; F9-NH2, blue. The sulfate moiety changed peptide-protein interactions; yet, a polar network around TMs 3 and 7, a salt bridge to E181, and aromatic stacking with the transmission switch were retained.

Sulfate by virtue of its size in sDSK I and its charge in sDSK II, mediated re-orientations of the ligands within the DSK-R1 binding pocket. For sDSK I, the sulfate group pulled sulfo-Y6 higher in the binding pocket, allowing F9 to hydrogen bond to D281. This change also shifted the N-terminal polar network higher on TM7. Moreover, the sulfate altered the orientation of sDSK II within the DSK-R1 binding pocket. It substantially decreased the polar contact to TM7, while increasing polar contact around TMs 2 and 3. Therefore, the sulfate affected the conformation and contact sites of sulfakinin peptides docked to DSK-R1.

Sulfated DSK I docked to DSK-R2

Sulfated DSK I docked to DSK-R2 (Figure 3) did not result in an altered ligand conformation. In fact, the N terminus docked in nearly an identical position and orientation to its non-sulfated counterpart, nsDSK I [14]. R8 docked in the polar region around TMs 5 and 6, forming a salt bridge with D347. M7 was rotated down in the binding pocket; it made contact with hydrophobic residues on TMs 3 and 5 and was unavailable to make contact to the ECLs. F9 was positioned between TM4 and TM5; this orientation allowed the C-terminal amide to contact E339. The ligand was unable to form strong contact to the transmission switch, W563, or the 3-7 lock, Q255 and Y597.

Sulfated DSK II docked to DSK-R2

Sulfated DSK II docked to DSK-R2 (Figure 4) resulted in a large scale re-orientation of the ligand within the binding pocket compared to nsDSK II [14]. Sulfo-Y9 made contact with hydrophobic residues at the top of TMs 6 and 7, with the sulfate group likely contacting ECL residues. A polar network originated at the sulfate moiety, propagating downward through D7 and D8 to H11, which contacted the transmission switch and the 3-7 lock. The N-terminal residues were re-oriented from the bottom of the binding pocket to the space Y9 occupied in the non-sulfated ligand, between TMs 2 and 3. R13 contacted polar residues on TMs 5 and 6, and F14 formed hydrophobic contact to TM4. M12 was positioned at the bottom of the binding pocket, unavailable to make ECL contact.

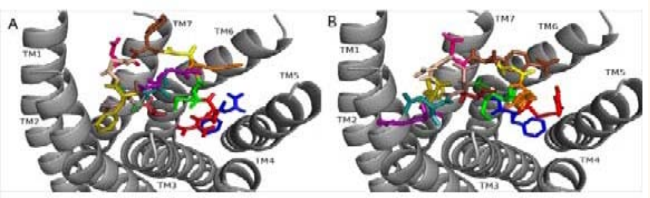

Figure 2 (A) Non-sulfated DSK II and (B) sDSK II docked to DSK-R1: the receptor is a gray ribbon and the residues in the ligand are: G1, firebrick; G2, forest; D3, dirty violet; D4, deep teal; Q5, purple; F6, olive; D7, wheat; D8, hot pink; (sulfo-)Y9, brown; G10, yellow; H11, orange; M12, green; R13, red; F14-NH2, blue. The sulfate driven re-orientation of DSK II led to distinct interactions around the 3-7 lock and increased the hydrophilicity of the residues docked around TMs 2 and 3.

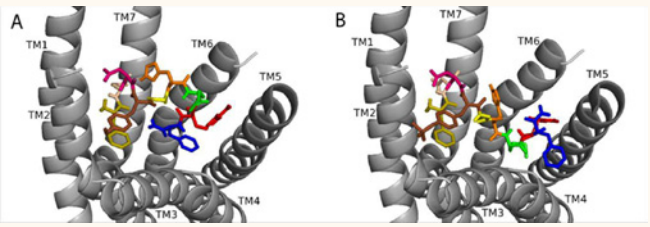

Figure 3 (A) Non-sulfated DSK I and (B) sDSK I docked to DSK-R2: the receptor and ligands are colored as in Fig. 1. The ligands adopted similar poses; the major difference was the sulfated peptide lost aromatic interaction with the transmission switch.

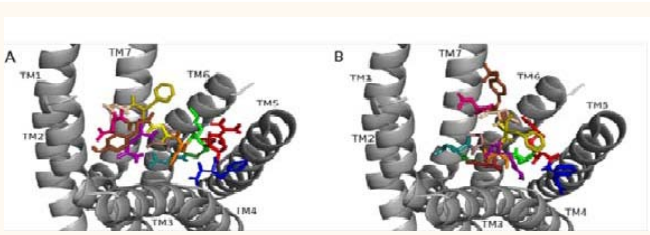

Figure 4 (A) Non-sulfated DSK II and (B) sDSK II docked to DSK-R2: the receptor and ligands are colored as in Figure (2). The sulfate moiety resulted in a re-orientation of the N-terminal extension; however, many contact sites were retained from nsDSK II.

The interactions of sDSK I compared to nsDSK I in the DSK-R2 binding pocket indicated the sulfotyrosine did not affect peptide position; however, the presence of the moiety impacted sDSK II placement compared to nsDSK II. In sDSK II, sulfo-Y9 shifted from a hydrophobic region between TMs 2 and 3 to TMs 6 and 7 which forced F6, D7, and D8 to re-position, resulting in intramolecular interactions rather than contact to the receptor. Sulfated DSK II folded on itself, decreasing the space it occupied compared to nsDSK II. Furthermore, sulfated DSK II formed strong contacts to the transmission switch as well as to the 3-7 lock, and it docked with hydrophobic residues lower in the binding pocket than nsDSK II. The DSK-R2 binding pocket accommodated the bulk of the sulfotyrosine in the shorter ligand; only minor changes in contacts were observed. Longer ligands like nsDSK II and sDSK II filled the binding pocket, and thus, were unable to accommodate the bulk of sulfotyrosine without large-scale changes. Therefore, the sulfate moiety in sDSK II re-orientated this longer ligand which resulted in novel contacts to DSK-R2.

CONCLUSION

Molecular docking showed sDSK I and nsDSK I formed a salt bridge to D347 of TM5 in the DSK-R2 binding pocket, but sDSK II and nsDSK II did not, to indicate drosulfakinin I peptides act through a receptor-specific pathway to influence larval locomotion. Our discovery is novel; it investigated a critical first step in sulfakinin signaling, ligand-receptor binding. This finding is supported by our structure-activity data [8,9] and is in agreement with, and expands upon other work. A previous study implicated DSK-R2 in sulfakinin locomotion signaling [23]; yet the report did not explore all forms of the DSK peptides, sulfated and non-sulfated DSK I and DSK II, nor did it investigate transduction at the molecular level. Also in agreement with our study is the salt bridge sulfo-Y6 of DSK I forms with D221 on TM5 of *Tribolium castaneum* sulfakinin receptor 1 (TcSKR1), but not with TcSKR2 [24], an observation which is consistent with receptor-specific sulfakinin signaling. Additionally, a salt bridge

to D347 is reported to play a critical role in the activation of DSK-R2 [18,23].

The sulfate moiety influences the effects of sulfakinin in larval and adult gut motility. Non-sulfated DSK I formed stronger contact to the 3-7 lock in DSK-R2 than sDSK I, a switch involved in GPCR activation [26,27]; thus, differential docking was consistent with the effects of these peptides in gut motility. Furthermore, the conservation of the nsDSK I, sDSK I, and nsDSK II aromatic contact between TMs 2 and 3 suggests this interaction may be important for transduction. Sulfated DSK II lacked this contact, consistent with its weaker activity in adult gut compared to the activities of the nsDSK I and nsDSK II as well as sDSK I. Thus, direct interactions with the 3-7 lock and aromatic contact between TMs 2 and 3 were consistent with the effects of the sulfated and non-sulfated peptides in adult gut motility.

In larva, the location of the drosulfakinin methionine appeared to be critical for signaling. On DSK-R2, the methionine in nsDSK I and nsDSK II was pointed towards the ECL side of the receptor. However, methionine in sDSK I and sDSK II was located at the bottom of the binding pocket and unable to form interactions with residues outside of the binding pocket. The methionine was positioned near the top of TM6 in non-sulfated ligands, reminiscent of the sulfotyrosine location in CCK-R1, and thus an optimal position to form hydrophobic interactions with residues present on ECL2 [17]. M195 of ECL2 in CCK-R1 is crucial for the selectivity of sulfated versus non-sulfated CCK peptides. Thus, M317 of ECL2 in DSK-R2 is in accordance with a conserved role in the selectivity of sDSK versus nsDSK, potentially interacting with methionine in the non-sulfated peptides to create a rigid ceiling over the binding pocket to stabilize ligand binding. Thus, sulfakinins and cholecystokinins act through peptide-specific signaling and a conserved mechanism in the selectivity of sulfo-ligands reflecting how animals across phylogeny may utilize the complexity of structurally-related, yet biologically-distinct peptides to impact health.

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