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The unusual mechanism of inhibition of the p90 ribosomal S6 kinase (RSK) by flavonol rhamnosides

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Abstract

All known protein kinases share a bilobal kinase domain with well conserved structural elements. Because of significant structural similarities of nucleotide binding pocket, the development of highly selective kinase inhibitors is a very challenging task. Flavonols, naturally occurring plant metabolites, have long been known to inhibit kinases by mimicking the adenine moiety. Interestingly, recent data show that some flavonol glycosides are more selective, although underlying mechanisms were unknown. Crystallographic data from our laboratory revealed that the N-terminal kinase domain of p90 ribosomal S6 kinase, isoform 2, binds three different flavonol rhamnosides in a highly unusual manner, distinct from other kinase inhibitor interactions. The kinase domain undergoes a reorganization of several structural elements in response to the binding of the inhibitors. Specifically, the main β-sheet of the N-lobe undergoes a twisting rotation by ~56° around an axis passing through the N- and C-lobes, leading to the restructuring of the canonical ATP-binding pocket into pockets sterically adapted to the inhibitor shape. The flavonol rhamnosides appear to adopt compact, but strained conformations with the rhamnose moiety swept under the B-ring of flavonol, unlike the structure of the free counterparts in solution. These data suggest that the flavonol glycoside scaffold could be used as a template for new inhibitors selective for the RSK family.

Keywords

RSK kinase; SL0101; quercitrin; flavonol; inhibitor; induced fit; conformational selection

1. Introduction

Protein kinases constitute one of the largest gene families, accounting for about 2% of the human genome [1]. They phosphorylate Ser, Thr and Tyr residues on specific target proteins, regulating a plethora of biological processes, such as differentiation, mitosis, muscle contraction, etc. A number of pathological phenomena, notably various forms of cancer, have been linked to mutations and disregulation of kinases [2] opening the way to therapeutic strategies using selective inhibition of medically relevant kinases. While, in general terms, protein kinases constitute a very diverse family of large, multidomain proteins, the catalytic function is confined to a ~300 amino acid domain, which provides all the machinery required for phosphorylation [3]. Other structural elements in kinases

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typically serve either as regulatory or targeting modules. The overall structural organization of kinase domain and specifically that of the active site which includes the ATP pocket, is highly conserved [3]. All protein kinase domains consist of a smaller, mostly β-structured N-lobe, a short hinge fragment and an α-helical, stable C-lobe [4]. The gorge between the two lobes harbors the ATP-binding site. Therein lies a major problem for drug design, because the ATP binding site is on the one hand highly suitable for inhibitor design, but on the other offers no easy way to achieve selectivity for the desired enzyme [5, 6].

One of the solutions to the problem is to exploit the unusual malleability of the kinase domain tertiary structure, which lacks the single canonical hydrophobic core, characteristic for most globular proteins, but instead contains distinct and separate `spines' that can easily reorganize to yield a spectrum of conformations, many of which are catalytically inactive [4, 7]. By choosing small molecules with chemical scaffolds that bind to such conformers, and at the same time block the ATP-binding site either by conformational selection or through an induced fit mechanism, it is possible to design drugs that show the required selectivity and potency [8, 9]. One of the examples of such a selective inhibitor which met with great success in the clinic is Gleevec [8].

Among the possible chemical scaffolds used as a starting point for kinase inhibitor design, are the flavonols, natural compounds of plant origin that are known for their ability to mimic adenine, and to inhibit ATP-binding enzymes, including a wide range of kinases. However, naturally occurring flavonols tend to inhibit a broad spectrum of kinases with low selectivity, because while they replace the ATP in a competitive manner, they lack elements that would confer selectivity. Interestingly, flavonols exist in plants as glycosides, and some of these more complex compounds have also been shown to inhibit kinases, remarkably with higher specificity than their aglycone counterparts. For example, a kaempferol rhamnoside (also known as SL0101) had been recently identified as a selective inhibitor of the p90 ribosomal S6 kinases (RSK) [10–12]. This is interesting for two reasons. First, this observation raises the possibility that flavonol glycosides, or more generally flavonols conjugated on the 3-hydroxyl, may be exploited as a potentially useful novel scaffold for kinase inhibitor design, at least for some kinase families. Second, this observation hints at a possibility of designing a selective brand of inhibitors for RSK kinases, which have been widely implicated in tumor biology [13, 14]. Such inhibitors might offer a potent new tool in the fight against several forms of cancer. However, until recently, there was no structural information regarding how SL0101, or any other flavonol glycosides, might interact with kinases to achieve specificity, and how their binding mode differs from that of pure flavonols. The work in our laboratory resulted in the elucidation of the crystal structures of the N-terminal (biologically active) catalytic domain of the RSK2 kinase isoform inhibited by SL0101, as well as by two closely related compounds, i.e. afzelin and quercitrin [15]. Unexpectedly, our data show that the binding of flavonol rhamnosides to the $RSK2^{NTKD}$ is accompanied by an unprecedented structural reorganization of the entire N-lobe of the kinase domain, and a dramatic change in the mutual disposition of the N- and C-terminal lobes. Although it is not clear if the observed structural reorganization is a result of an extreme induced fit event, or if conformational selection is at work, these new structures expand in a significant way our understanding of the malleability of the kinase tertiary scaffold, and open new avenues in the search for selective, biomedically relevant inhibitors. In this short review, we discuss the structural features of known flavonol-kinase complexes; we present a brief overview of the RSK kinase family, and finally review the new information regarding the structures of the RSK2 N-terminal catalytic domain in complex with the flavonol rhamnoside inhibitors.

2. Flavonols and their glycosides as kinase inhibitors

2.1. Inhibition of kinases by flavonols

Flavonols, derivatives of 3-hydroxy-2-phenylchromen-4-one (Fig. 1 A), are among the most ubiquitous secondary metabolites in plants [16]. The functions of flavonols in plants include filtration of ultraviolet light, regulation of nodulation and pollen fertility and coloration of leaves, flowers and fruits. Flavonols have been shown to possess strong antioxidant properties in vitro and many of the benefits associated with plant-rich diets are thought to be mediated at least in part by flavonols [17]. Flavonols have also been recognized as inhibitors of protein kinases and ATP-ases [18]. This biochemical property stems from the molecular structure of the flavonols, whose planar AC heterocyclic ring system is similar in size and shape to the adenine moiety of ATP. Kinases recognize adenine primarily through hydrogen bonds between the aminopyrimidine part of adenine and the main chain group of the hinge region between the N- and C-lobes of the kinase domain (Fig 1B), although the binding is also mediated by several non-polar interactions with conserved hydrophobic residues from N- and C-lobe [19]. A number of crystal structures of complexes of protein kinase domains with bound flavonols have been deposited in the PDB (Table 1), allowing for the visualization of how the flavonols are able to compete with ATP. Although the H-bonding potential of flavonols is different from that of adenine, its two hydroxyl groups are capable of forming hydrogen bonds with the main chain atoms of the hinge region of the kinase mimicking aminopyrimidine part of adenine. For the flavonols bound to kinases, the heterocyclic AC-system typically lies in the same plane as the adenine moiety of ATP, but the orientation of the molecule around the axis perpendicular to that plane varies between complexes, so as to engage A, C or B ring in hydrogen bonding with amide and/or carbonyl atoms of the hinge region (Fig. 1, C–E). In rare cases, select side chains of the kinase form additional hydrogen bonds with flavonol's hydroxyls. Details of the interactions of flavonols with kinases, as visualized by crystal structures, are summarized in Table 1.

2.2. Inhibition of kinases by flavonol derivatives

Despite variations in their mode of binding to kinases, all unconjugated flavonols belong to Type I class of kinase inhibitors, i.e. those that are ATP-competitive and bind to the active conformation of kinases [6]. Thus, these compounds are generally weak and non-selective inhibitors probably because the relative simplicity of the flavonol backbone translates into a generic recognition mechanism, while a limited number of possible conformers does not allow for the precise fit of the molecule to the binding site. Interestingly, certain more complex, conjugated flavonols are known to be highly potent and selective kinase inhibitors. In fact, natural flavonols are mono- or polyglycosylated in plants at positions 3 and 7, usually by glucose or rhamnose, or by oligosaccharides [16]. The carbohydrate moieties confer solubility on the hydrophobic flavonols, but create potential steric hindrance for interactions at the ATP-binding sites. In particular, the 7-glycosides are no longer capable of mimicking adenine due to the steric hindrance resulting from the addition of the carbohydrate substituent to the A-ring. Nevertheless, several flavonol glycosides have the ability to specifically inhibit select protein kinases. For example, the natural flavonol glycoside hyperin (quercetin 3-galactoside) was found to inhibit CDK2 and CDK5 kinases with IC_{50} in the low μM range [20]. Other synthetic derivatives of flavonoids also show potential as drug leads. For example, flavopiridol (alvocidib) inhibits cyclin dependent kinases with IC_{50} in low nM range [21]. Flavopiridol was derived from the alkaloid rohitukine and is the first inhibitor of cyclin dependent kinases to be studied in phase II clinical trials to treat several types of cancer [21].

Recently, another natural flavonol glycoside, SL0101, obtained from the Amazon plant *Forsteronia refracta* was shown to selectively inhibit a specific family of kinases, the p90

ribosomal (RSK) kinases [10]. SL0101 is one of only two commercially available selective inhibitors for the N-terminal domain of RSK (the second is the unrelated compound BI-D1870 [22, 23]), and constitutes a useful reagent to dissect the involvement of RSK kinases in various biological processes. For example, it was shown that proliferation of cell lines modeling prostate and breast cancer was inhibited by SL0101 while no similar inhibitory effect was observed with non-cancer cells [10, 24]. These studies suggest that anti-cancer drugs may be developed on the basis of SL0101 and perhaps some other related flavonol glycosides. However, development of inhibitors based on SL0101 scaffold has been so far hampered by the absence of structural information that would rationalize the specificity and affinity of interactions of flavonol glycosides with RSK kinases.

3. The RSK kinase family

3.1 Structure and regulation of RSK kinases

Protein kinases are typically multidomain proteins, with the catalytic kinase domain flanked by diverse regulatory modules, such as, for example, C1 and C2 domains in protein kinase C [25]. Six unusual human protein kinases contain two catalytic domains in a tandem, and no other modules; these are the p90 ribosomal S6 kinases (RSK), of which there are four homologous isoforms (RSK1-4) encoded by distinct genes, and two homologous mitogen- and stress-activated kinases, MSK1 and MSK2 [25, 26]. The catalytic tandem consists of an N-terminal domain which shows homology to the AGC family of kinase domains [25] and a C-terminal domain which in turn is homologous to the Ca^{2+}/calmodulin dependent kinase family [27, 28]. Space constraints do not allow us to discuss the MSK kinases further in this paper.

The C-terminal domains of RSK kinases serve as switches that activate the N-terminal kinase domains (NTKD), which are the physiologically active modules that phosphorylate the cognate targets [25, 26, 29]. The four RSK isoforms share pair-wise 73–80% amino acid similarity and exhibit a common pathway of activation. Briefly, RSK kinases are downstream effectors of the extracellular signal activated kinase 1/2 (ERK1/2) [29]. The ERK1/2 activate the C-terminal kinase domain by phosphorylation of Thr577 (RSK2 numbering) which triggers autophosphorylation of Ser386 in the hydrophobic motif, creating a docking site for the PDK1 kinase (Fig. 2A). The latter binds to this site and phosphorylates Ser227 within the activation loop with concomitant catalytic activation of NTKD to within 10% of its potential [26]. To achieve the maximum catalytic competence, an additional phosphorylation of Ser369 in the so-called turn motif by ERK1/2, or in some cases by another heterologous kinase, is required [30]. RSK4 does not seem to require activation by PDK1 [31] leaving it constitutively active in most cells.

Structural information about RSK kinases is limited to their isolated catalytic domains. Crystal structures have been determined for the N-terminal domain of RSK1 in complexes with three inhibitory compounds [32]; and for the N-terminal domain of RSK2 in complex with AMPPNP [33]. There are also crystal structures available for the C-terminal kinase domain of RSK2 [34], and RSK1 [35] but as this domain is of tangential relevance to our review, we will not describe those studies further.

When compared to the well-studied PKA (protein kinase A, Fig. 1B), the N-terminal kinase domain of RSK2 ($RSK2^{NTKD}$), shows intriguing differences with respect to the organization of the N-lobe (Fig. 1C). Notably, in the active conformation of PKA, the αC helix which plays an important role in the catalysis of phosphoryl transfer, is packed against the main 5-stranded β-sheet. In contrast, in $RSK2^{NTKD}$ a unique 3-stranded β-sheet is assembled from parts of the activation segment, from the RSK-specific N-terminal extension of the kinase domain, and the region homologous to the αB helix of PKA. The remaining residues

expected to form the αC-helix are disordered [33]. Similar structure was reported for MSK1 crystallized without the nucleotide [36], but was thought to represent an autoinhibited species. In contrast, mutagenesis studies suggest that 3-stranded β-sheet is important for catalytic function of RSK2 [33] raising the possibility that the β-sheet may carry out some functions of displaced αC helix. Specifically, it has been suggested that the canonical salt-bridge Glu118-Lys100 cannot form in RSK2 and is replaced by a novel interaction, Lys216-Asp211. It is also puzzling that the complex with AMPPNP contains no Mg^{2+} [33]. Nevertheless, for the purposes of this review, the overall architecture of the NTKD and its mode of ATP binding, are similar to the overall paradigm established for AGC kinases [25].

2.2 Biomedical significance of RSK kinases

Among RSK kinases, RSK2 has been studied by far most extensively and a number of functions such as transcriptional regulation and cell proliferation, growth and survival have been ascribed to this isoform [29, 37], The loss of RSK2 function due to inactivating mutations and truncations results in X-linked mental retardation known as Coffin-Lowry syndrome [38]. In line with human genetic data, RSK2 knockout mice show deficiencies in learning and co-ordination but are otherwise normal [39]. In recent years RSK kinases, especially RSK2, have been widely implicated in tumor biology [13, 14]. In general, most data indicate that misregulation of RSK kinases, while unlikely to be a leading cause of cancer on its own, may severely worsen many aspects of the disease. RSK1 was identified by kinome-wide siRNA screen to be a key modulator of lung cancer metastasis [40]. In a different experimental setup, up-regulated RSK2 activity has been linked to invasiveness of epithelial cells [41]. Activation of RSK2 has been shown to increase cell survival through direct phosphorylation and inactivation of pro-apoptotic protein Bad in non-transformed neurons [42] and in lung cancer cells [13]. Ample indirect evidence also link RSK kinases to cancer: increased enzymatic activity and/or expression and phosphorylation levels of RSK kinases were reported in skin [43], breast [10, 44], prostate [24], head and neck [45] cancers and leukemia [46]. In short, available data suggest that with the progression of cancer, the ERK1/2 kinase pathway becomes often up-regulated in cancer cells due to increased levels of stress, which leads to sustained activation of RSK1 and RSK2. Sustained activation of RSK kinases leads to increased proliferation rate, survival, motility and metastatic spread of cancer cells, all of which adversely affect patient prognosis. While RSK kinases have been increasingly recognized as targets for anti-cancer drug discovery [47], efforts to design specific inhibitors with clinical potential have not been very successful. To date, only two distinct, RSK selective inhibitors targeting the NTKD have been identified. The dihydropteridinone derivative BI-D1870 [23] was found to inhibit all RSK kinases with IC_{50} in low nM range, while not significantly inhibiting any other of about 40 tested kinases even at 100-fold higher concentrations. Another inhibitor, the naturally occurring flavonol rhamnoside of plant origin, named SL0101, kaempferol-3-O-(3″,4″-di-O-acetyl)-α-L-rhamnopyranoside [10] also shows significant selectivity and potency towards RSK kinases [22]. Until recently, the structural underpinnings of this unusual selectivity of both inhibitors have been completely unknown.

4. Crystal structures of the NTKD of RSK2 inhibited by flavonol rhamnosides

To gain insights into the mechanism by which SL0101 inhibits RSK kinases we crystallized a complex of the N-terminal kinase domain of RSK2 ($RSK2^{NTKD}$) with the inhibitor [15]. High resolution of diffraction data (1.53 Å) allowed for a detailed analysis of the stereochemistry of SL0101 and its mode of interaction with RSK2. As expected, the SL0101 molecule is bound, between N- and C-terminal lobes in the ATP-binding gorge. When the SL0101- and AMP-PNP-bound structures are compared (Fig. 3A and B respectively), the α-

helical cores of the C-lobes are highly similar with an r.m.s. difference of only 0.56 Å for main chain atoms. In contrast, the N-terminal lobe in SL0101 complex undergoes a structural reorganization compared to the canonical tertiary fold, so that the ATP-binding site acquires novel characteristics as a hydrophobic pocket with a surface highly complementary to that of SL0101. The details of the structural reorganization of N-lobe are described elsewhere [15] and are only briefly summarized below. The main 5 stranded β-sheet of the N-lobe rotates by ~56 ° compared to the typical ATP-bound structure, and the two antiparallel β-strands that form the P-loop separate from the rest of the β-sheet to accommodate the intruding inhibitor. In particular, Phe79, at the tip of the P-loop (a conserved, hydrophobic residue that in all protein kinases has the function of shielding the phosphoryl transfer site from solvent) engages in an intimate π-π stacking interaction with the C ring of SL0101. Such close interaction of the P-loop with adenine mimicking fragment of the inhibitor is rare [15]. The unusual, 3-stranded β-sheet seen in the AMPPNP bound structure undergoes a complete topological reorganization with the βB strand (normally a portion of the αB-helix) reversing the orientation and moving from the center of the sheet to the solvent exposed side. Other structural features of $RSK2^{NTKD}$ also undergo modifications: the DFG motif assumes an *out* conformation, large portion of the activation segment and αC helix become ordered, compared to the AMPPNP-bound structure; finally, the hinge loop and αD helix are pulled closer to active site.

Given the highly unusual mode of binding of SL0101 to RSK2 observed in the crystal structure, we deemed it necessary to probe the mechanism of binding in solution. Although this work is still in progress, we have completed experiments which strongly support the notion that the crystal structure is representative of the complex in solution. We mutated Phe79, which plays a crucial role in the recognition of the flavonol moiety by engaging the C ring in a π-π stacking interaction, to alanine, and confirmed that the F79A mutant of RSK2 is resistant to inhibition by SL0101, but retains catalytic activity [15].

Interestingly, the structural reorganization of the kinase domain appears to force the SL0101 inhibitor to adopt a compact, strained conformation to fit into the newly reorganized pocket. Although there is no known structure of free SL0101, there is a crystal structure available for a close relative, quercitrin [48], and several other flavonol glucosides. Typically, these compounds (with three principal degrees of freedom, Fig 4A) adopt an open, trefoil structure that minimizes contact between the B ring and the pyranoside moiety (Fig 4B). In the complex with the RSK kinase, however, the rhamnose ring of SL0101 is packed snugly against the B ring with close contacts (Fig 4C). This could suggest, that the interaction between $RSK2^{NTKD}$ and SL0101 follows the induced fit mechanism, with SL0101 binding to a canonical kinase structure, and undergoing a conformational transition to a strained variant as the protein adapts its tertiary fold.

In order to investigate if related flavonol rhamnosides inhibit RSK2 and bind to the N-terminal kinase in the same manner as SL0101, we crystallized the complexes of $RSK2^{NTKD}$ with afzelin and quercitrin. Afzelin is closely related to SL0101, and differs only by the absence of the two acetyl groups on the rhamnose moiety. Quercitrin is another analogue, similar to afzelin, in which the flavonol moiety is not kaempferol, but quercetin, characterized by an additional hydroxyl group in the 3'-position of the B-ring. Both crystal structures were solved and refined at high resolution [15] (Derewenda et al, Acta Cryst D 2013, in press). For all practical purposes they are virtually identical to the structure of the complex harboring SL0101, with the inhibitors exhibiting the same, strained conformation (Fig 4 D, E). This shows that the acetyl groups have a negligible effect on binding mode, even though they seem to affect the kinetics of the interaction [49]. Moreover, the additional hydroxyl present in quercitrin can be accommodated, albeit the structure of the $RSK2^{NTKD}$/quercitrin complex clearly shows minor structural perturbations due to steric effects

(Derewenda, et al, Acta Cryst D 2013, in press). Specifically, the presence of an additional hydroxyl in the B ring of quercitrin introduces a steric clash with Leu155 of RSK2 making the B ring rotate away from Leu155 by ~15°. As a consequence, the rhamnose moiety swings away to accommodate the rotation of the B ring, so that φ and ψ dihedral angles change by ~−10° and ~5° respectively.

The concerted structural reorganization of the N-terminal domain and the inhibitor itself create a unique and highly complementary interface between the two molecules. Eleven amino acids coalesce around the SL0101 molecule, which becomes almost completely buried within the protein moiety, leaving only part of the rhamnoside exposed to solvent (Fig. 5). These residues are Ile50 and Ile52 from the N-terminal β-strand; Phe79 from the P-loop; Leu102, Val131, and Leu147 from the N-lobe; Leu150 from the hinge region; Leu155 from the αD-helix; Leu200; Phe212 from the DFG motif; and Leu 214. Of these amino acids, only Val131, Leu147, Leu150 and Leu200 are in the van der Waals contact with the AMP-PNP molecule in the respective crystal structure [33].

5. Conclusions

In this short review we focused on the three newly determined crystal structures of the RSK2 N-terminal kinase with related flavonol rhamnosides bound in the ATP-site, and compared them to the known complexes of unconjugated, aglycone flavonols with several other protein kinases. Our results are unexpected, because the structural reorganization of the protein moiety surpasses in its magnitude the known conformational adaptation of kinases to type II inhibitors. Importantly, the three structures are virtually identical, strongly suggesting that the complex represents a distinct low-energy species which is highly reproducible, but hitherto unobserved. The mechanism by which the complex forms in solution, i.e. conformational selection vs. induced fit, is still under investigation, and the outcome will have an important impact on our understanding of the malleability of the kinase tertiary fold, and on the potential exploitation of the flavonol rhamnoside scaffold as a lead compound for development of anti-cancer drugs.

Abbreviations

RSK	ribosomal S6 kinase
NTKD	N-terminal kinase domain
CTKD	C-terminal kinase domain
PKA	protein kinase A
AMPPNP	5'-adenylyl-imidodiphosphate

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Highlights

- Recent structural data on mechanism of interaction of flavonol rhamnosides with RSK2 kinase are reviewed
- N-terminal kinase domain of RSK2 kinase undergoes dramatic structural rearrangement as a result of binding to flavonol rhamnosides
- Flavonol rhamnosides are bound in strained conformation

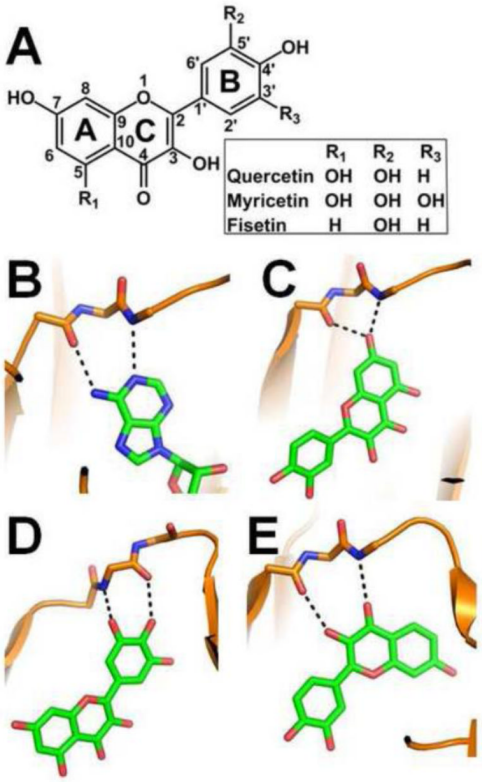

Figure 1.

Interaction of flavonols with hinge regions of kinases. A, General structure of select flavonols found in plants. B, Recognition of adenine ring of ATP by hydrogen bonding with main chain atoms of hinge region as seen in PKA-ATP structure (PDB code: 1ATP). C–E, Unconjugated flavonols mimic adenine ring by forming hydrogen bonds with hinge region of different kinases. Quercetin is recognized through A-ring by kinase STK17B (C, PDB code: 3LM5), myricetin is recognized through B ring by kinase PI3K (D, PDB code: 1E90), while fisetin uses C-ring to from hydrogen bonds with cyclin-dependent kinase 6 (E, PDB code: 1XO2)

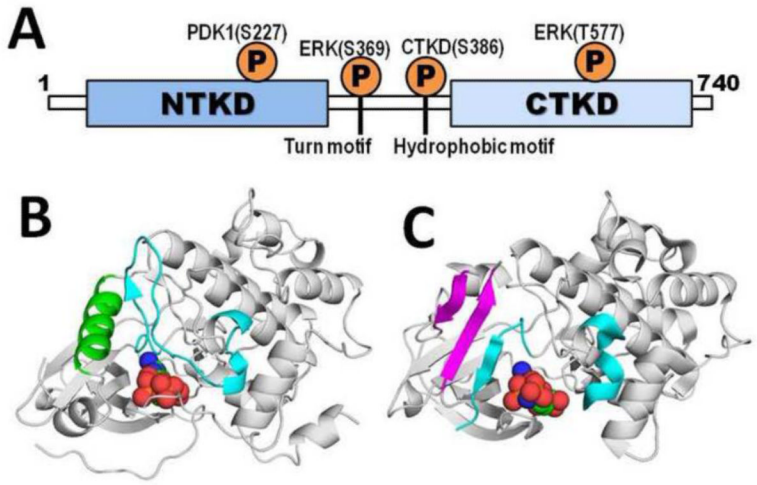

Figure 2.

Structure and regulation of RSK2 kinase. A, Schematic representation of RSK2 with regulatory phosphorylation sites. B, Structure of kinase domain of protein kinase A with bound ATP (PDB code: 1ATP). Activation segment is shown in cyan, αC helix shown in green. C, Structure of N-terminal kinase domain of RSK2 with bound AMPPNP (PDB code: 3G51). Activation segment is shown in cyan and two strands of novel 3-stranded β–sheet are shown in magenta. Note that part of the activation segment folds into β–sheet becoming a component of a novel β–sheet and that αC helix is disordered.

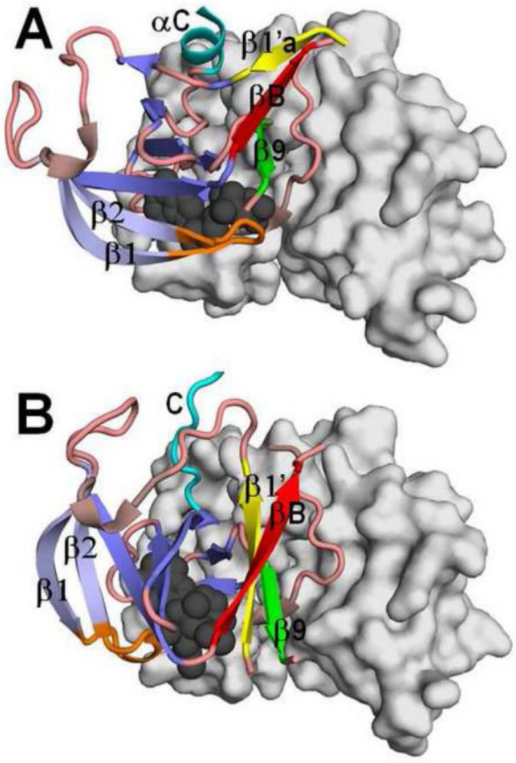

Figure 3.

Structural rearrangements of RSK2^{NTKD} in response to the binding to the SL0101. Cartoon representation of the structure of AMPPNP-RSK2^{NTKD} complex (A, PDB code:3G51) and SL0101-RSK2^{NTKD} complex (B, PDB code: 3UBD) with ligands shown as black spheres. Yellow, red and green strands constitute the unique 3-stranded β–sheet, αC helix is colored cyan, and first two strands of core β–sheet are colored light blue.

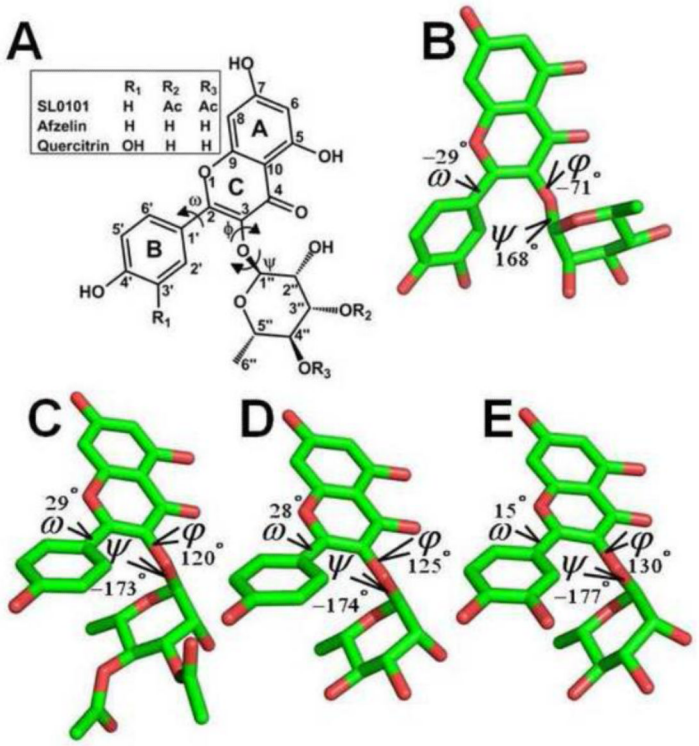

Figure 4.

Conformational changes in flavonol glycosides induced by the binding to RSK2^{NTKD}. A, General structure of flavonol 3-O-glycosides and structures of flavonol glycosides co-crystallized with RSK2^{NTKD}. B, crystal structure of free quercetin (CCDC code: 698519) shows that it adapts open, clover-like structure. The values of three dihedral angles (ω,φ and ψ) describing the three degrees of conformational freedom are shown. C–E, Structures of SL0101 (C, PDB code: 3UBD), afzelin (D, PDB code: 4EL9) and quercitrin (C, PDB code: 4GUE) in complex with RSK2^{NTKD} show that flavonol glycoside adapts compact conformation with carbohydrate moiety swept under the B-ring.

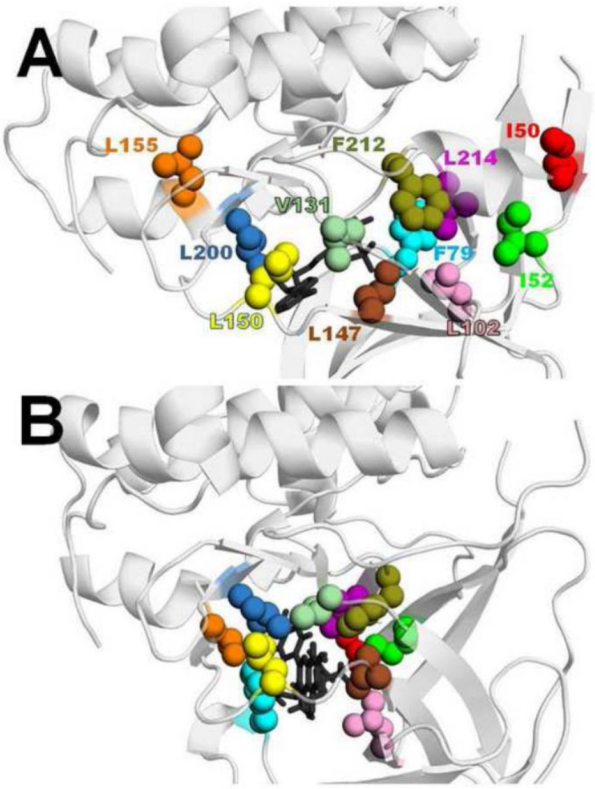

Figure 5.

The reorganization of the 11 hydrophobic residues which form the SL0101 pocket. Main chain atoms of residues forming the hydrophobic pocket for SL0101 structure in RSK2^{NTKD}-AMPPNP complex (A, PDB code: 3G51) and SL0101-RSK2^{NTKD} complex (B, PDB code: 3UBD) are shown in ball representation and the side chains residues are colored individually. Ligands are shown in black stick representation, and the color scheme ind both pictures is identical.

Table 1

Interaction of flavonols with hinge region of kinases

PDB code	Kinase	Flavonol	Ring engaged in H-bonding with hinge region	Residues in the hinge involved
1E90	PI3K	Myricetin	B	V882 (O,N)
1E8W	PI3K	Quercetin	A,C	K883 (O,N)
1XO2	CDK6	Fisetin	C	V101 (N), E99 (O)
2HCK	SRC	Quercetin	A,C	M341 (O,N)
2O3P	Pim1	Quercetin	C	E121 (O)
2O63	Pim1	Myricetin	C	E121 (O)
2O64	Pim1	Quercetagetin	C	E121 (O)
3LM5	STK17B	Quercetin	A	E111 (O), A113 (N)
3V3V	JNK1	Quercetagetin	A, C	D109 (O), M111 (O,N)