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BIOCHEMISTRY

Computational modeling of cAMP-dependent protein kinase allostery

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Abstract. Allosteric regulation by ATP of peptide binding with a cAMP-dependent protein kinase catalytic subunit was computationally modeled by combining conventional docking analysis and molecular dynamics calculations. It was found that the peptide docking energy was dependent on peptide structure, and, moreover, this energy was also different for the free enzyme and the enzyme–ATP complex. This difference was used to model the allosteric effect of ATP on peptide binding. The same computational analysis revealed that ligand binding reduced the root-mean-square fluctuation (RMSF) values of the enzyme backbone αC atoms, pointing to a ligand-induced reduction in intrinsic conformational dynamics of the protein. As this stiffening of the conformation was induced by the binding of ATP as well as peptides, and its magnitude was in correlation with the ligand binding energy, it was suggested that the modulation of protein conformational dynamics may be responsible for the allosteric regulation of binding effectiveness through the alteration of ligand off-rate from the binding site. This means that the atomic network of interactions, which determines the molecular recognition of the peptide substrate in its binding site, is not changed by allostery, but the intensity of these interactions is affected. This change modulates the overall ligand binding effectiveness and is recognized as an allosteric effect.

Keywords: protein kinase A, modeling of substrate binding, allosteric regulation of substrate binding, protein conformational dynamics, allostery mechanism.

Abbreviations:
ATP – adenosine triphosphate
cAMP – cyclic adenosine monophosphate
PKAc – catalytic subunit of cAMP-dependent protein kinase
RMSF – root-mean-square fluctuation values of protein backbone Cα atoms
RMSD – root-mean-square deviation of atomic positions of protein backbone
AMP-PNP – β,γ-imidoadenosine 5′-triphosphate
PKI(5-24) – peptide inhibitor TTYADFIASGRTGRRNAIHD

INTRODUCTION

The classical models of allostery were formulated for oligomeric proteins, assuming that effector binding affects substrate binding on another subunit (Monod et al. 1965; Koshland et al. 1966). Later, the concept of allostery was extended to monomeric proteins, which have distinct binding sites for two ligands. This definition also includes bi-substrate enzymes (Cui and Karplus 2008; Laskowski et al. 2009; Cornish-Bowden 2014; Changeux 2011). Protein kinases belong to this group of enzymes as the in-line transfer of a phosphoryl group from ATP to a phosphorylatable peptide/protein assumes that both these substrates are simultaneously bound with the enzyme (Wang and Cole 2014). Direct evidence that allosteric interaction between ATP and the peptide substrate binding sites manifests in the catalytic subunit of cAMP-dependent protein kinase (PKAc) was presented by Masterson et al. (2008). Thereafter, the idea of allosteric regulation in PKAc was expanded by Kuznetsov and Järv (2009),

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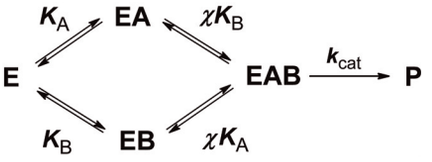
who presented a comprehensive survey of the available experimental data about allosteric interactions in this enzyme. This analysis revealed that the interaction of peptide substrates or inhibitors with PKAc is influenced by the simultaneous binding of ATP or its analogs and, conversely, the binding effectiveness of ATP is governed by the presence of peptides (Kuznetsov and Järv 2009).

The kinetic mechanism of this phenomenon can be summarized by the following reactions scheme, formulated by I. W. Segel in 1975 (Segel 1975).



(1)

In this scheme, E stands for enzyme, A is ATP and B is a peptide substrate; the parameters K_A and K_B describe the interactions of substrates A and B with the free enzyme E; χ characterizes the allosteric effect in the formation of the ternary complex EAB; k_{cat} stands for the observed rate constant of the catalytic steps; and P describes sum of the reaction products.

In this paper, the allosteric effect of ATP on the docking energy of a series of peptide substrates with PKAc was modeled computationally, and the results of these calculations were compared with the experimental data presented in our previous papers (Kuznetsov and Järv 2008; Kivi et al. 2014). To overcome the limitations of conventional docking algorithms, which do not consider alteration of the protein structure by ligand binding (Chen 2015), a time-effective approach was proposed in which docking analysis was alternately combined with optimization of the protein structure by molecular dynamics calculations. It was found that the calculated peptide docking energy was dependent on peptide structure and was also different in the free enzyme and the enzyme–ATP complex. Therefore, the allosteric effect of ATP on peptide binding was modeled by these calculations. On the basis of this computational analysis, a putative molecular mechanism of the allosteric regulation of ligand binding affinity is proposed in this study, and the implications of this mechanism on ligand molecular recognition are discussed.

METHODS

Initial files for computational analysis

The input files used for modeling PKAc were built by starting from the X-ray structure of the enzyme ternary complex with the ATP-analog inhibitor AMP-PNP and peptide inhibitor PKI(5-24) (Zheng et al. 1993), listed as "1ATP" in the PDB database (www.pdb.org). This complex was selected because most amino acids are well localized in this structure. For modeling of the apoenzyme, both ligands were deleted from this structure, whereas the peptide was deleted and AMP-PNP was replaced by ATP to obtain the input file for the PKAc–ATP complex. Two physiologically meaningful Mg ions, which were replaced by Mn ions for the X-ray analysis, were also restored in the protein structures. All these procedures were described in detail in our previous paper (Izvolski et al. 2013). The peptide and ATP structures were prepared by ChemSketch version 12 software (Advanced Chemistry Development, Inc., Canada) and PyMOL Molecular Graphics System version 1.6 (Schrödinger, LLC, USA) and optimized by molecular dynamics calculations.

Ligand docking study

Conventional AutoDock Vina version 1.1.2 software for ligand docking (Trott and Olson 2010) and molecular dynamics simulation GROMACS version 4.5.4 package (Hess et al. 2008) were used. After the first docking step, the structure of the complex was optimized and the new input file for the protein prepared for the second docking procedure. The steps of docking and molecular dynamics optimization were repeated three times. After these calculation cycles, the protein structure remained unchanged in the enzyme–ligand complex.

The rules for scoring results of peptide positioning were derived proceeding from the mechanism of the phosphoryl group transfer reaction. This reaction requires a close positioning of the peptide serine OH group and the γ-phosphorus atom of ATP at a distance not greater than 5Å (Wang and Cole 2014); this distance was used for filtering the peptide poses. From the docking poses that met the distance criteria, the peptide with the best docking energy was selected for the next step of the analysis. All improperly oriented substrates were excluded from the analysis.

The RMSF values for Cα atoms of the protein backbone were calculated by using the RMSF tool of the GROMACS package (Hess et al. 2008).

Correlation analysis

For correlation analysis, GraphPad Prism software package version 5.0 for Mac OS was used (GraphPad Software Inc., USA).

RESULTS AND DISCUSSION

Computational analysis of peptide binding with PKAc

In the first stage of the *in silico* binding study, the initial structures of the free PKAc and the ATP complex with this enzyme were optimized by molecular dynamics

Table 1. Calculated energies of peptide docking with a cAMP-dependent protein kinase catalytic subunit (E_{E+B}), with its complex with ATP (E_{EA+B}), and experimental pK_B and pχ values from the experimental study by Kuznetsov and Järv (2008), characterizing the dissociation of the enzyme–peptide complex (EB) and the allosteric effect of ATP on this equilibrium, as shown in (1)

Peptide		E_{EA+B} kcal/mol	E_{E+B} kcal/mol	* pK_B	* pχ
I	RRYSV	–8.3	–7.8	5.68	1.02
II	RRASVA	–8.0	–7.6	4.60	0.72
III	LRRASLG	–7.9	–7.6	4.39	0.44
IV	RKRSRKE	–7.3	–7.0	3.92	0.31
V	LRKASLG	–7.2	–7.1	3.64	0.17
VI	LARASLG	–6.6	–6.7	2.72	–0.14
VII	LRAASLG	–5.8	–6.1	2.19	–0.48

* Results compiled from the work by Kuznetsov and Järv (2008).

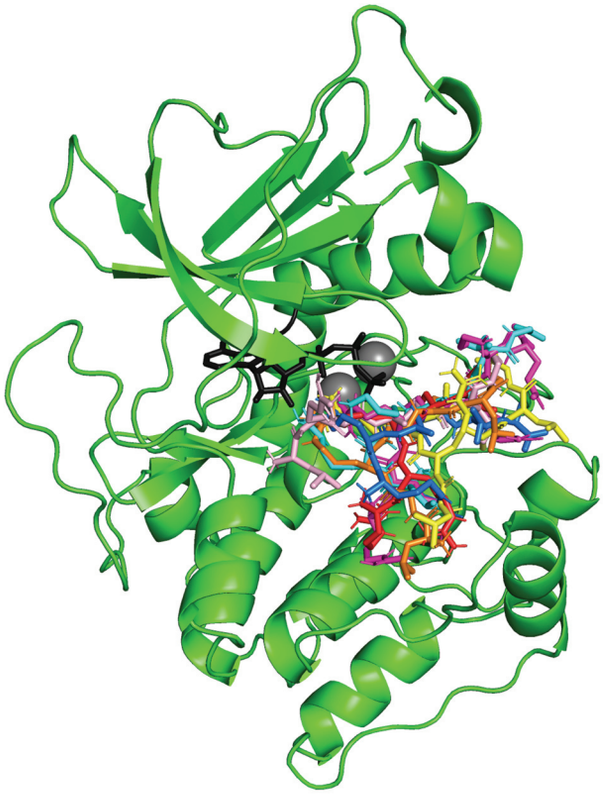


Fig. 1. Docking of short peptide substrates with the complex of PKAc (green ribbon structure), ATP (black stick structure) and magnesium ions (grey spheres). The stick structures of peptide substrates are coloured as follows: RRYSV – red, RRASVA – marine, LRRASLG – yellow, RKRSRKE – magenta, LRKASLG – cyan, LARASLG – orange, LRAASLG – pink. The peptides are docked in the binding area located between two lobes of the enzyme molecule.

calculations until the RMSD value leveled off after 60–100 ns. Then the most populated structural clusters of the free enzyme and the enzyme–ATP complex were selected (Izvolski et al 2013) and were used for peptide docking. The relevant peptide positions were selected from the docking results, proceeding from the stereochemistry of the phosphoryl group transfer reaction, which requires close positioning of the serine OH group and the γ-phosphorus atom of ATP at a distance no greater than 5Å (Wang and Cole 2014). As ATP was absent in the apo-enzyme, the Mg ions located in the active site were used for the scoring of peptide position in this case. From all the docking poses that met the distance criteria, the peptide with the best docking energy was selected for the next step of the analysis.

In the next step, the structures of the protein–peptide and protein–ATP–peptide complexes were again optimized by molecular dynamics calculations, and the most populated clusters of these structures were selected (Izvolski et al. 2013). Thereafter, the bound peptide was deleted from these optimized structures to obtain a new input file for the next docking step. The results of the second docking analysis were again filtered by using the proximity criterion of the reacting groups, and the docking energy values for the relevant peptide positions were calculated.

These alternate steps of structure optimization and ligand docking were repeated three times until the structure of the most populated protein clusters remained practically unchanged, suggesting that structural perturbations induced by ligand binding had been taken into consideration by this algorithm. The docking study algorithm was used for a series of peptide substrates of PKAc. The structure of these peptides and the results of calculations are summarized in Table 1. The docking positions of the listed peptides are illustrated in Fig. 1. It can be seen that all peptides are docked between the two lobes of the enzyme molecule, where localization of the specific peptide inhibitor PKI(5-24) has been observed in an X-ray study (Zheng et al. 1993).

As phosphorylation of these peptides has also been studied experimentally (Kuznetsov and Järv 2008), the same table lists the pK_B and pχ values, which characterize the dissociation of the peptides from their complex with the enzyme and the allosteric effect of ATP on peptide binding effectiveness, respectively (1).

Docking energy and peptide structure

It can be seen in Table 1 that peptide docking energies, calculated for the free enzyme (E_{E+B}) and the enzyme–ATP

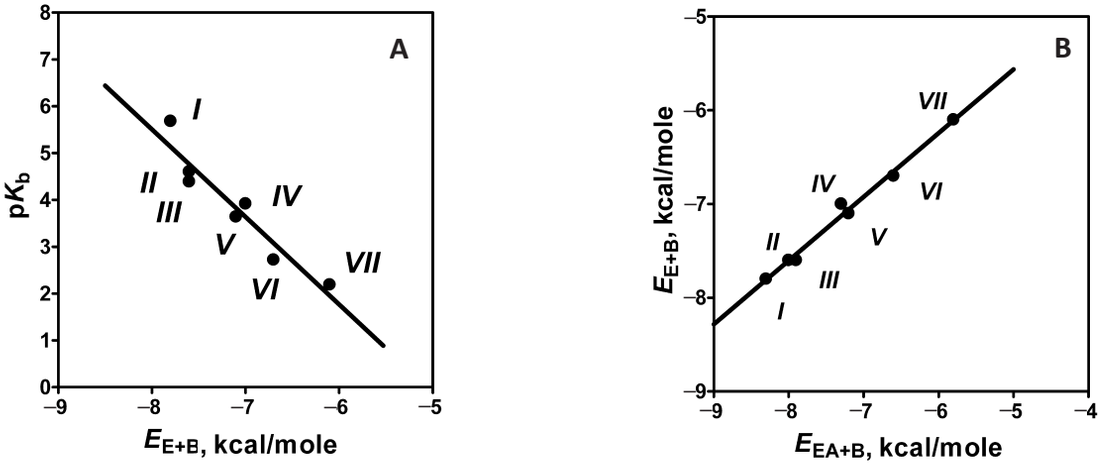


Fig. 2. A –the plot of peptide docking energies vs experimental dissociation constants pK_B , which characterize the dissociation of the enzyme–substrate complex and were determined by Kuznetsov and Järv (2008). B – the comparison of peptide docking energies for the free PKAc and its complex with ATP, listed in Table 1.

complex (E_{EA+B}), depend on the peptide structure. Moreover, a linear similarity plot can be observed between the calculated docking energies E_{E+B} and the experimental pK_B values, characterizing the dissociation-free energy of the peptide complex with PKAc (Fig. 2A). The linear plot includes peptides with two arginine residues preceding the susceptible-to-phosphorylation serine residue – a motif that is recognized as an important structural element of the PKAc minimum substrate (Zetterqvist et al. 1976; Zetterqvist and Ragnarsson 1982; Järv and Ragnarsson 1991). Consequently, the specificity pattern of the peptide substrate binding step can be adequately modeled *in silico*. This conclusion was previously confirmed by the results of computational analysis of PKAc specificity against short peptide substrates (Mena-Ulecia et al. 2014), listing the inter-atomic interactions that are responsible for molecular recognition of the peptide structure by the enzyme active center. Although the physical meaning of this conclusion is not very obvious because the entropy factor may play some role in PKAc interaction with peptides (Kivi et al. 2014), it would not be surprising if the observed differences in short peptide binding effectiveness were predominantly enthalpy driven. Certainly, this conclusion cannot be automatically extended to peptide binding with PKAc in general, and it would be interesting to perform a similar analysis in the case of the binding of long peptides.

However, most importantly, it can be concluded from the linear similarity plot between the E_{E+B} and E_{EA+B} values obtained in this study (Fig. 2B) that the pattern of molecular recognition of peptides was the same for the free enzyme and for the enzyme–ATP complex. Therefore, the network of the inter-atomic interactions, which is responsible for molecular recognition of the peptide substrates in the binary and ternary substrate complexes, should be similar, and the presence of ATP has no effect on the network of atomic interactions, and thus on the whole enzyme specificity pattern.

Allosteric effect

The similarity plot for docking energies E_{E+B} and E_{EA+B} in Fig. 2B can be described by the following linear function:

$$E_{E+B} = (0.68 \pm 0.04)\, E_{EA+B} - (2.2 \pm 0.3), \qquad (2)$$

the slope of which is significantly different from (1). It is obvious that the allosteric effect of ATP on peptide binding is not a constant contribution to docking energy but depends on peptide docking energy. In other words, the pχ is larger for ligands that bind more effectively with the protein. The same trend was observed in the experimental data, and the principle "better binding – stronger allostery" was formulated by Kuznetsov and Järv (2008).

The most surprising implication of this analysis is that no new interaction mechanisms between the peptide molecule and the enzyme binding site are needed for the generation of the allosteric effect, and the observed changes in ligand binding energy could be caused solely by changes in the intensity of the existing interactions. This conclusion can be confirmed by the similar specificity pattern of peptide recognition by PKAc in the absence and in the presence of ATP, shown by linear similarity plots in Fig. 2A and B.

An interesting opportunity to explain this phenomenon arose from the results published by Seo et al. (2014). In that paper, evidence was provided that ligand binding affinity could be adjusted through changes in intrinsic

protein conformational dynamics that determine the opening rate of the binding site "cleft" and thus regulate the ligand off-rate. Following this hypothesis, the interrelationship between protein dynamics and ligand binding properties is analyzed in the next part of this work.

Influence of peptide binding on protein conformational dynamics

The RMSF values for protein backbone Cα atoms were calculated and used to characterize the conformational dynamic of PKAc and its complexes with ATP or a peptide, and also with both of these ligands. Initially, these calculations were made by Masterson et al. (2012) and Li et al. (2014) for the protein–peptide (LRRASLG) binary complex and for the ternary complex of this peptide and ATP with the enzyme. These results revealed that for certain amino acids the RMSF values peaked significantly above the background level, and these more flexible protein areas were mainly identified in functionally significant areas of the enzyme. In the case of PKAc, the most dynamic areas were located in the ATP binding site and the glycine-rich loop (amino acids 49–70), around the catalytic loop (amino acids 165–172) and in the peptide binding site (amino acids 187–205). Flexible regions were also found in the enzyme sequence of amino acids 220–230, located on the surface of the large lobe of the enzyme and involved in the interaction with longer peptide substrates.

It was further found that ligand binding with the enzyme reduced the conformational dynamics of the PKAc molecule, and most interestingly, this effect was not localized within the binding sites but was permeated through the whole protein molecule (Li et al. 2014). Similar results were also obtained in our previous work, demonstrating that the enzyme-bound ATP changed the structure of the peptide binding site, and that the peptide binding also affected the structure of the ATP binding site (Izvolski et al. 2013). However, in addition to these results, the present study provides a unique opportunity to analyze the dependence of the conformational dynamics of the PKAc molecule upon ligand structure and binding effectiveness. The results of these calculations are summarized in Fig. 3.

It can be seen in Fig. 3 that the peptides studied have a rather diverse influence on the RMSF values. However, the general pattern of these plots still revealed characteristic flexibility peaks in functionally important places. Furthermore, it is obvious that ligand binding changed the RMSF value at these sites. For a closer examination of this trend, the ΔRMSF values for the binary and ternary PKAc complexes were calculated for amino acid number 200, which is located in the peptide binding site and is optionally selected for this analysis. Interestingly, a rather good correlation of ΔRMSF values with docking energy E_{E+B} as well as with the allostery parameter pχ can be observed. Moreover, this correlation can be formalized in terms of a linear plot between the ΔRMSF and E_{E+B} values for peptides *I–VI* (Fig. 4). This dependence points to the fact that interrelationship between protein conformational dynamics and modulation of ligand binding affinity, which is described as the allosteric effect pχ, may indeed exist, following the principles formulated by Seo et al. (2014).

Conformational dynamics driven allostery

Results of the computational modeling of PKAc allostery have pointed to the following molecular mechanism for this phenomenon.

Firstly, it should be considered that ligand binding reduces protein conformational dynamics as the formation of additional interactions between the ligand and protein functional groups inevitably reduces the flexibility of the peptide fragments involved. This effect of structure stiffening is distributed over the whole protein molecule, including other protein binding sites, as illustrated by changes in the RMSF plot for the whole protein.

Secondly, it should be considered that the protein conformational dynamics govern the ligand–protein complex dissociation rate (off-rate) through control of the intrinsic opening rate of the binding site "cleft", whereas the ligand association rate (on-rate) is, to a great extent, diffusion controlled (Seo et al. 2014). Therefore, the dissociation constant of the enzyme–ligand complex, which is determined by the ratio of the ligand off-rate and on-rate constants, should be sensitive to an alteration in protein conformational dynamics. This change in the dissociation constant, recognized as an allosteric effect, can be experimentally observed if the binding of at least two ligands with the same protein molecule takes place.

Thirdly, the mechanism of allostery explains the interrelationship between the allosteric effect and ligand binding affinity, presented by linear similarity plots between pχ and pK_A as well as pχ and pK_B, as was shown elsewhere (Kuznetsov and Järv 2009). Following this plot, the principle "better binding – stronger allostery" was formulated by Kuznetsov and Järv (2009).

Fourthly, it is possible that some ligands may disturb the protein structure in such a way that their binding increases its conformational dynamics. Therefore, the existence of a negative allostery can be predicted where ligand binding lowers the effectiveness of binding of another ligand molecule. This situation of the structure-induced inversion of the allosteric effect can be observed in the case of peptide *VII*, which is the worst substrate in the peptide series studied.

Finally, it should be noted that conformational dynamics can govern only part of the ligand binding energy,

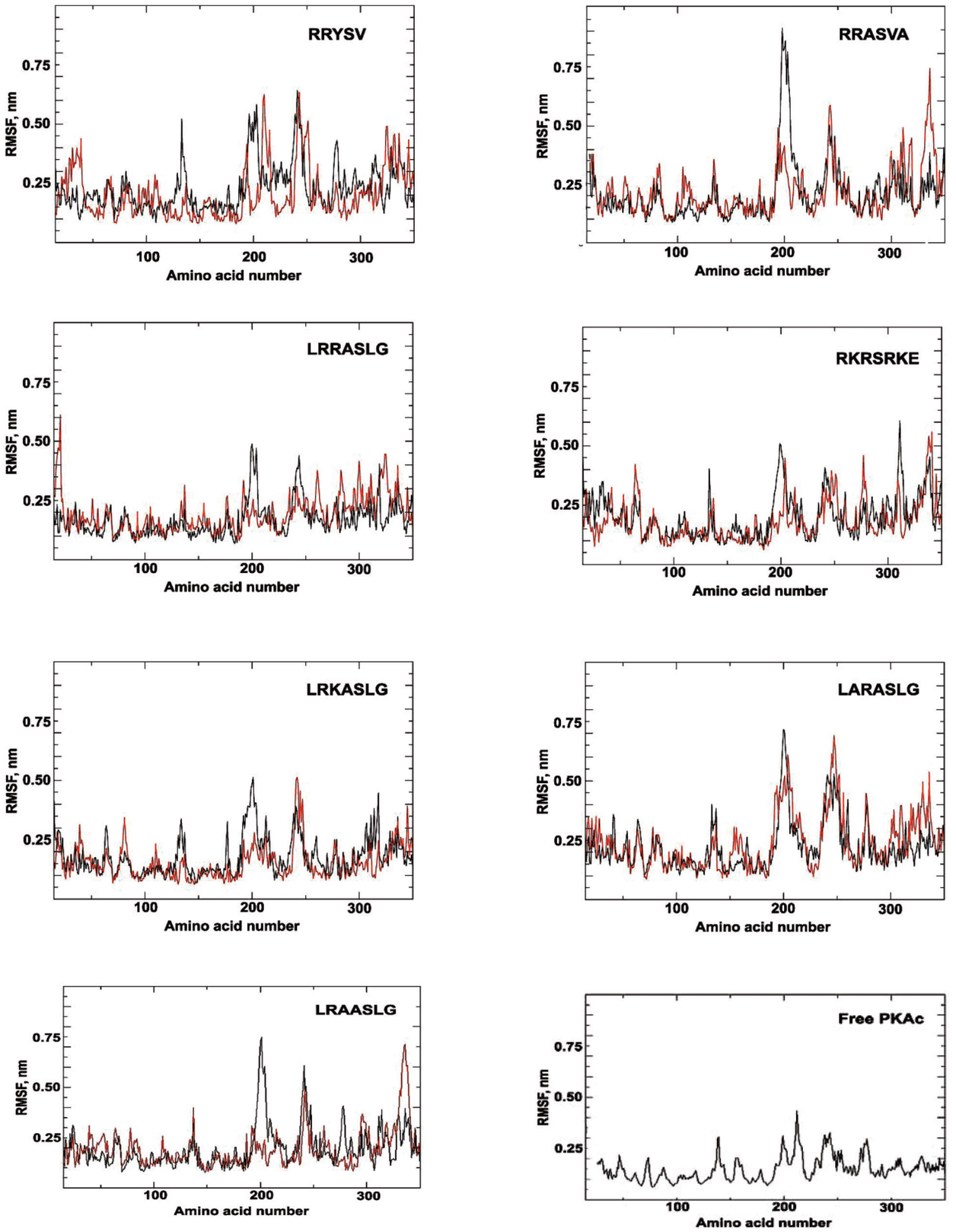


Fig. 3. RMSF plots for PKAc complexes with short substrate peptides (black line); ternary complexes of PKAc with the same peptides and ATP (red line); the same plot for the free enzyme.

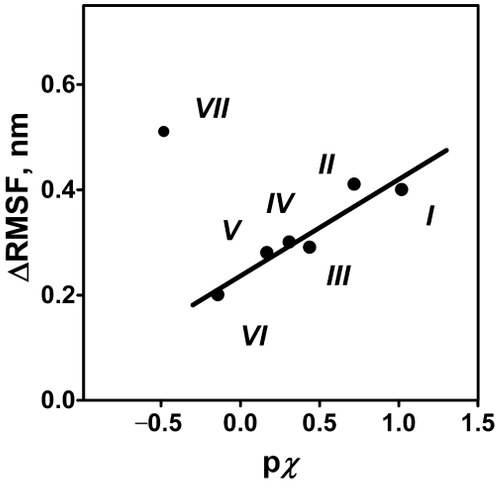


Fig. 4. Correlation between the ΔRMSF values, characterizing the change in backbone mobility of the enzyme–peptide complex due to ATP binding, and the allosteric effect pχ, characterizing the influence of ATP binding on the effectiveness of the enzyme–peptide complex formation. Numbers shown in the Fig. refer to the peptide numbers in Table 1.

while the main contribution should be determined by ligand–protein interactions that determine the molecular recognition of the ligand in its binding site. These contributions, governed by inter-atomic ligand–protein interactions as well as by alterations in protein dynamics, cannot be separated for a single binding site. However, conformational dynamics may play a specific role if this property governs the binding effectiveness of another ligand in a distinct binding site, as happens with PKAc, where peptide binding can be studied in the presence or absence of ATP.

CONCLUSIONS

A combination of ligand docking with molecular dynamics calculations, used for considering the influence of ligand binding on protein structure, was applied to the modeling of the allosteric effect of ATP on the binding of a series of peptide substrates with PKAc. The docking energy of the peptides was governed by their structure, following the specificity pattern that is characteristic for this enzyme, and was also affected by the presence of ATP that allows modeling of the allosteric effect of this ligand on peptide binding. Interestingly, the allosteric effect is correlated with peptide binding energy that is in turn correlated with changes in protein flexibility, characterized by the root-mean-square fluctuation values. It can be suggested that the allosteric modulation of peptide binding is initiated by changes in protein conformational dynamics that govern the ligand dissociation rate from the enzyme by controlling the intrinsic rate of the binding site conformational opening. The mechanism of allostery does not change the ligand molecular recognition pattern, determined by the atomic network of ligand–protein interactions, but acts through change of the effectiveness (or intensity) of these interactions.

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Photo: private collection

Andrei Izvolski began to show interest in chemistry already at school. During secondary school, he attended an extended chemistry class, gaining advanced subject knowledge. He entered the University of Tartu, Faculty of Physics and Chemistry, which he graduated with a BSc degree in chemistry. His further scientific research was based on biokinetics studies of metals influence on melanocortin receptors interaction with ligands. His PhD work continues with biokinetics on another model protein, namely protein kinase A. Specifically, the goal is to better understand the allosteric effect occurring during ligands binding. The studies combine in situ *experiments and computational modeling based on molecular dynamics simulation.*

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Aleksei Kuznetsov obtained a PhD degree in bioorganic chemistry from the University of Tartu under the supervision of Prof. Jaak Järv. The PhD thesis was dedicated to cAMP-dependent protein kinase kinetics studies by modeling molecular simulations and experimental tools. Subsequently, he continued the research in the field of integrating computational biophysics, modern mathematics and experimental tools to define the protein and ligand interaction behavior for solving the challenges in biological sciences and infectious pathogens. His current work is focused on the development of new bioanalytical methods and adopting them for application in industry.

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cAMP-sõltuva proteiinkinaasi allosteerika modelleerimine arvutil

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Peptiidide sidumise allosteerilist regulatsiooni modelleeriti arvutil, kombineerides ligandi seostumise analüüsi tavalist algoritmi molekulaardünaamiliste arvutustega, mis võimaldas arvesse võtta seostuva ligandi mõju valgu struktuurile. Leiti, et peptiidsete ligandide ensüümiga seostumise energia oleneb peptiidi struktuurist ning on erinev vaba ensüümi ja ensüüm-ATP kompleksi korral. See erinevus iseloomustab valgu sidumisomaduste allosteerilist regulatsiooni.

Sama analüüs näitas, et ligandi sidumine vähendab valgumolekuli peptiidiahelate liikuvust, muutes nende αC aatomite jaoks arvutatud RMSF väärtusi. Kuna peptiidiahelate liikuvuse muutust tingis nii ATP kui peptiidsete ligandide sidumine ja see efekt oli korrelatsioonis ligandi sidumise efektiivsusega, tehti järeldus, et valgumolekuli dünaamika muutumise kaudu saab mõjutada ligandide sidumise efektiivsust ning seega moduleerida allosteerilise efekti suurust. On võimalik, et seejuures muutub eelkõige valk-ligandi kompleksi dissotsiatsiooni kiirus. Samal ajal ei mõjuta selline allosteeriline regulatsioon valk-ligand toimete võrgustikku, mis määrab ligandi molekulaarse äratundmise valgumolekuli aktiivtsentris.