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Effect of two simultaneous aza-β^3-amino acid substitutions on recognition of peptide substrates by cAMP dependent protein kinase catalytic subunit

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ABSTRACT

Peptidomimetic analogs of the hexapeptide RRASVA, containing simultaneously two aza-β^3-amino acid residues in different positions of this sequence, except for the phosphorylatable serine residue, were synthesized and tested as substrates for the cAMP-dependent protein kinase catalytic subunit. All these peptidomimetics were phosphorylated by the enzyme and this reaction was characterized by the K_m and k_{cat} values as well as by the second-order rate constants k_{II}. Affinity and reactivity of all peptidomimetics was lower than that for the parent peptide RRASVA. The effect of backbone modification was dependent upon the positions where these two aza-β^3 residues were located, although the sequence of amino acid side groups remained the same in all compounds. It was found that the influence of two backbone modifications in the substrate structure was not described additively, i.e. the effect of each structural alteration was dependent upon the position of the second modification. The results were in agreement with the concept of specificity-determining clusters in the sequence of peptide and peptidomimetic ligands, which predominantly determine the molecular recognition of these ligands by their target sites and therefore serve as major modification points for the design of activity of peptidomimetic ligands.

1. Introduction

A significant part of the substrate specificity of protein kinases is determined by the amino acid sequence around the substrate phosphorylation site and essential recognition motifs have been suggested for many kinases [1]. However, in most of these studies recognition of natural L-amino acid sequences by protein kinases has been considered, whereas – according to our present knowledge – the influence of peptide backbone modification has been analyzed only for the protein kinase A and protein kinase C catalyzed reactions with a series of D-amino acid-containing peptides [2,3], and more recently for the protein kinase A catalyzed phosphorylation of peptidomimetic substrates, each containing one aza-β^3-amino acid residue [4].

The last-named study revealed that the substitution of natural amino acids with their aza-β^3-analogs in the parent peptide RRASVA might have a strong influence on the effectiveness of substrate phosphorylation, although the sequence of peptide side chains remained the same [4]. Secondly, the effect of introduction of aza-β^3-amino acid residues was significantly influenced by the position of this modification [4]. Therefore, it was suggested that specificity-determining clusters which play major part for recognition of substrates, could be defined also on the backbone level. This conclusion may have impact on the design of peptidomimetic ligands in general. For example, if the recognition pattern of the peptidomimetic ligand should be close to the prototype peptide, the specificity cluster must be kept intact. On the contrary, if the objective of peptidomimetics design is to diminish interaction of this ligand with its target site, the specificity cluster is the best place for backbone modification. These rules are useful for design of ligands with orthogonal properties against two different binding sites: for example, lowering sensitivity against proteolysis and retaining binding effectiveness against the drug target site. For further analysis of principles of molecular recognition of backbone modifications, we synthesized a new series of peptide RRASVA analogs containing simultaneously two aza-β^3-amino acids in different positions and studied the protein kinase A catalyzed phosphorylation of these compounds.

2. Experimental

2.1. Chemicals

γ-[^{32}P]ATP was purchased from Amersham (UK). The catalytic subunit C_{alpha} of mouse cAMP-dependent protein kinase (protein

Abbreviations: Protein kinase A, cAMP-dependent protein kinase catalytic subunit, EC 2.7.11.11; BSA, Bovine serum albumin; HATU, (2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate).

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kinase A), recombinantly expressed in *Escherichia coli*, 30 U/mg, 0.1 mg/mL, lot 040916, was purchased from Biaffin GmbH & Co. KG (Germany). ATP, TRIS/HCl, BSA and H_3PO_4 were purchased from Sigma–Aldrich (USA). Phosphocellulose paper P81 was acquired from Whatman (UK). $MgCl_2$ was purchased from Acros (Germany). The peptide substrate RRASVA of purity above 95% was purchased from GL Biochem Ltd. (Shanghai, China) and was characterized by HRMS spectrum and HPLC. Peptidomimetic substrates were synthesized and characterized in this work as described below. Chemicals for peptide synthesis were obtained from commercial suppliers (Senn Chemicals, Iris Biotech GmbH, Fluka, and Novabiochem) and were used without further purification. Analytical thin-layer chromatography was carried out on pre-coated silica gel plates (60 F_{254}, Merck). Flash column chromatography was carried out using silica gel 60 (230–400 mesh ASTM) from Merck & Co., Germany.

2.2. Analytical methods

NMR spectra were measured on a 300 MHz instrument (Bruker, Germany) in DMSO-d_6 or $CDCl_3$ as solvent and internal reference. A Waters HPLC system was used for purification and analysis of synthetic products. Purification of aza-β^3-peptides was performed on a C18 XTerra® RP18 (19 × 300 mm, 10 μm) column using water/acetonitrile linear gradient (5–100% B in 40 min, 8 mL/min, rt, 215 nm). The following buffers were used: (eluent A) water containing 0.08% TFA by volume; (eluent B) acetonitrile containing 1% TFA by volume. Characterization of purified aza-β^3-peptide hybrids was performed on a C18 XTerra® (4.6 × 250 mm, 5 μm) column using water/acetonitrile linear gradient (5–100% B in 30 min, 1 mL/min, 30 °C, 215 nm). Mass spectra of the products were measured on a LTQ Orbitrap (Thermo Electron) spectrometer (positive ionization, static nanospray, boron silicate emitters Proxeon, resolution 100,000 at m/z 400, external calibration).

2.3. Synthesis of Fmoc protected aza-β^3-amino acids

Fmoc-aza-β^3-Ala-OH was prepared from methyl hydrazine, and Fmoc-aza-β^3-Val-OH and Fmoc-aza-β^3-Arg(Boc)$_2$-OH were obtained from hydrazine hydrate as described previously [5,6]. All products were purified chromatographically and their structure was verified by NMR spectra. Details of these syntheses as well as the results of product analysis have been given in our previous report and attached supplement [4].

2.4. Synthesis of aza-β^3-amino acid containing peptidomimetics

Peptide analogs were prepared on a 0.1 mmol scale by employing the Fmoc/*t*Bu SPPS strategy on a Liberty Microwave-Enhanced Peptide Synthesizer (CEM GmbH, Germany), using HOBt and TBTU as activators, and DiPEA as base in DMF. The preloaded Fmoc-α-Ala-OH Wang resin (0.40 or 0.81 mmol/g) was used. A coupling time of 10 min was used for the introduction of Fmoc-aza-β^3-amino acids into peptides. For the introduction of Fmoc-Arg(Pbf)-OH, double couplings were performed. Cleavage was made with TFA-TIS-H_2O 95:2.5:2.5 cocktail over 4 h. The yield of aza-β^3-peptides was systematically lower than that of normal peptides, remaining within 50–60% range for isolated di-aza-β^3 products compared to the average yield of above 80% of normal peptides. Crude peptidomimetics were purified chromatographically [7] and the purity of the products pooled was above 95%, as found from the analytical HPLC runs.

2.5. Kinetic measurements

The initial rate of peptide phosphorylation was measured at 30 °C as described previously [8,9]. Briefly, the reaction mixture (final volume 70 μL, 50 mM TRIS/HCl, pH 7.5) contained: 100 μM γ-[^{32}P]ATP, 10–1000 μM peptide or peptidomimetic substrate, 10 mM $MgCl_2$, and 0.3 μg/mL of the enzyme. The stock solution of protein kinase A was diluted up to 500-fold in 50 mM TRIS/HCl buffer (pH 7.5) containing 1 mg/mL BSA. The phosphorylation reaction was initiated by the addition of 10 μL of this solution into the reaction mixture. At known time intervals after starting the reaction, 10 μL aliquots were taken from the reaction mixture and spotted onto pieces of phosphocellulose paper, which were subsequently immersed into cool 75 mM phosphoric acid to stop the reaction. The pieces were then washed three times (15 min each time) with ice-cold 75 mM H_3PO_4 to remove the excess of γ-[^{32}P]ATP and were dried at 120 °C for 15 min. The radioactivity bound onto the paper was measured as Cherenkov radiation using a Beckman LS 7500 scintillation counter. The values of the initial rate of the phosphorylation reaction (v) were calculated from the slopes of the phosphorylation product concentration *vs* time plots. As these experiments were made at constant ATP concentration (100 μM), the kinetic data were analyzed by the classical Michaelis–Menten rate equation,

$$v = \frac{k_{cat}[E][S]}{K_m + [S]}, \tag{1}$$

where [S] stands for peptide or peptidomimetic concentration. In addition, the linear parts of the Michaelis–Menten plots were analyzed to calculate the second-order rate constants k_{II} of the enzyme reaction [10,11], as at [S] < K_m we have:

$$v = \frac{k_{cat}}{K_m}[E][S] = k_{II}[E][S]. \tag{2}$$

2.6. Computational analysis

Peptidomimetic structure modeling was performed using the Spartan 4.0 software suite (Wavefunction, Inc., USA), and the minimum-energy conformations of the studied peptidomimetics were obtained. Conformational searches were made by using molecular mechanics with the additional condition of the aqueous medium for finding optimal geometry. All compounds were represented as zwitterions for these calculations.

The peptidomimetics docking modeling with protein kinase A was carried out by using the AutoDock Vina software (ver. 1.0.3) [12]. The docking compatible structure formats of the protein were prepared by AutoDockTools (ver. 1.5.4) [13], proceeding from the X-ray structure of protein kinase A ternary complex (PDB ID:1CDK), from which the peptide structure was removed. The fitting box with 0.3 Å of grid spacing was defined once and used for all docking calculations. The fitting area covered all protein. The initial structures of peptidomimetics were calculated as described above. However, these structures were significantly modified during the docking process and extensive lists of their docking conformations together with docking energy values were obtained. For all these docking conformations the distance between the γ-phosphorous atom and the oxygen atom of the phosphorylatable serine hydroxyl group of the enzyme-bound substrates were calculated by using Perl script designed by us as an interface to AutoDock Vina software [14]. Further, these distances were used to select "reactive complexes" from the list of all binding complexes, where the phosphoryl group transfer from ATP to substrate could take place. Following the general understanding of the mechanism of the phosphorylation reaction [15], complexes with reasonably short distances between P and O atoms, ranging from 3 to 5 Å, were taken into consideration.

2.7. Data processing

Calculations and statistical analysis of kinetic data were made by using the GraphPad Prism software package (ver. 4.0, GraphPad Software Inc., USA). The kinetic parameters reported are given with standard errors.

3. Results and discussion

The hexapeptide RRASVA is known as the minimum substrate of protein kinase A [1,16]. Proceeding from this parent compound series of peptidomimetics was synthesized, where two amino acids of this sequence were simultaneously replaced with their aza-β^3-analogs. These replacements covered all possible pair-wise combinations in this peptide, except for the substitution of the serine residue which is phosphorylated by the kinase-catalyzed reaction. The synthetic procedures used for preparation of these peptidomimetics were described in the supplementary material of our previous publication [4]. For the introduction of both aza-β^3-amino acids, modified coupling conditions were applied [4]. Briefly, prolonged reaction time was used for addition of aza-β^3-amino acids to peptide, and TBTU was applied as activator for coupling of amino acid next to aza-β^3-derivative. These modifications were important to improve the yield of synthesis, as coupling of aza-β^3-amino acids was not efficient if the conventional solid-phase peptide synthesis protocol was used. The structures of prepared compounds were shown in Table 1 together with the results of HR-MS analysis and the overall yield of syntheses.

It was found that all prepared peptidomimetics were phosphorylated by protein kinase A and this reaction was well described by the Michaelis–Menten rate equation (1), and the K_m and k_{cat} values were obtained for all peptidomimetic substrates at saturating ATP concentration 100 μM (Table 2). Additionally, the linear part of the initial velocity *vs* substrate concentration plots was used for calculation of the second order rate constant k_{II}, as presented by Eq. (2). The physical meaning of k_{II} corresponds to the k_{cat}/K_m ratio, but as this value was calculated from a different set of experimental data, the agreement between these parameters indicated that the kinetics of the phosphorylation reaction was not affected by substrate or product inhibition phenomena. Secondly, as the physical meaning of the second order rate constant k_{II} was not dependent on the rate-limiting step of the enzymatic reaction, these constants have been considered as the most proper kinetic parameters for analysis of substrate reactivity [10].

It can be seen from Table 2 that the introduction of two aza-β^3-amino acids into the structure of the parent peptide significantly decreased the effectiveness of the phosphorylation reaction if compared with phosphorylation of RRASVA itself. And secondly, the effect was clearly dependent on the location of these modifications in the substrate backbone. Notoriously, both kinetic parameters, K_m and k_{cat}, were affected by these modifications, while the changes were more extensive in the former constant, and certainly the effect cumulated in the k_{II} values. For example, if the K_m value increased 140 times and the k_{cat} value decreased 15 times for azaβ^{3}RRazaβ^{3}ASVA if compared with RRASVA phosphorylation, the change in the k_{II} value was almost 2000-fold for this pair of substrates.

Table 1
Peptidomimetic substrates of protein kinase A derived from peptide substrate RRASVA and containing two aza-β^3-amino acids.

Peptidomimetic	Overall yield of synthesis (%)	Experimental* molecular mass (M^+)
azaβ^{3}Razaβ^{3}RASVA	24	689.4165
azaβ^{3}RRazaβ^{3}ASVA	39	689.4165
azaβ^{3}RRASazaβ^{3}VA	19	689.4165
azaβ^{3}RRASVazaβ^{3}A	4	689.4165
Razaβ^{3}Razaβ^{3}ASVA	16	689.4165
Razaβ^{3}RASazaβ^{3}VA	25	689.4165
RRazaβ^{3}ASazaβ^{3}VA	30	689.4165
Razaβ^{3}RASVazaβ^{3}A	22	689.4165
RRazaβ^{3}ASVazaβ^{3}A	25	689.4165
RRASazaβ^{3}Vazaβ^{3}A	28	689.4165

* The calculated molecular mass (M^+) of all compounds is 689.4165.

It was found that the presence of two aza-β^3-amino acids in peptidomimetics had a significantly stronger influence on substrate phosphorylation rate if compared to effects caused by substitution of single amino acid in RRASVA as shown in our previous paper [4]. Moreover, in the latter case the k_{cat} values were only slightly affected by backbone modifications, while the major effect revealed in the K_m values and was probably connected with the substrate binding step. Therefore, molecular recognition of peptidomimetics with one or two modifications in their backbone seemed to possess different mechanism, and for reliable comparison of these data the second-order rate constants could be preferred.

The influence of two modifications in the substrate backbone upon the second-order rate constants was illustrated in Fig. 1, where the results of this study were presented as a 3D chart. For simplicity, these rate constants were normalized with the same parameter for RRASVA. Therefore, the *Z*-axes presented the logarithmic value of the normalized rate constant $\Delta \log k_{II} = \log \frac{k_{II}^{mimetic}}{k_{II}^{RRASVA}}$, while positions of the backbone modifications were shown by *X* and *Y* axes. The diagonal part of this chart, where *X* and *Y* axes denote the same position of the substrate sequence, represented the mono-substituted substrates, studied in [4]. All other positions corresponded to the di-substituted RRASVA derivatives.

It can be seen that the most significant changes in $\Delta \log k_{II}$ values were observed if modifications were made around the second arginine residue located close to the *N*-terminus of substrate molecules. This arginine residue is generally recognized as the most important element of the consensus motif for protein kinase A [17]. And *vice versa*, if C-terminal amino acids were substituted with their aza-β^3-derivatives, the effect was small. It is noteworthy that all derivatives containing modified alanine in the last position of RRASVA seem to belong to this group.

Taking together, the idea about specificity clusters seemed to hold out in the presence of two backbone modifications, although in this case these clusters seemed to cover several amino acid positions. On the other hand, however, the effect of the double substitution was significantly bigger compared to the reactivity change of mono-substituted peptidomimetic compounds. This result also indicated that the replacement of more than two amino acids in a short peptide sequence with aza-β^3-amino acids could not be feasible procedure, as may too strongly hamper interaction of peptidomimetics with the binding site.

It is obvious from these experimental data that the contribution of each substitution was dependent upon the position of the second substitution, and the $\Delta \log k_{II}$ values for di-substituted peptidomimetics could not be calculated as the sum of two effects observed in the case of corresponding mono-substituted compounds. This means that the influence of these structural modifications could not be presented by the sum of certain specificity determining interactions, but rather by topological factors, determined by the spatial arrangement of substrate side chains interacting with the protein. For certain, these interactions should be different for different peptidomimetics if compared with the parent peptide.

The influence of the backbone structure on the spatial orientation of side chains of peptidomimetics was demonstrated by computer modeling of di-aza-β^3-substituted RRASVA analogs. Although

Table 2
Phosphorylation of peptidomimetic substrates by protein kinase A catalytic subunit at ATP concentration 100 μM, 30 °C, 50 mM TRIS/HCl, pH 7.5.

	Substrate	K_m (μM)	$10^2 k_{cat}$ (μmol mg^{-1} s^{-1})	$10^2 k_{II}$ (L mg^{-1} s^{-1})
I	azaβ^{3}Razaβ^{3}RASVA	920 ± 127	11.1 ± 1.0	0.0114 ± 0.00079
II	azaβ^{3}RRazaβ^{3}ASVA	1572 ± 333	2.87 ± 0.30	0.00194 ± 0.00014
III	azaβ^{3}RRASazaβ^{3}VA	254 ± 68	1.08 ± 0.11	0.00441 ± 0.00051
IV	azaβ^{3}RRASVazaβ^{3}A	131 ± 38	19.7 ± 2.0	0.121 ± 0.0064
V	Razaβ^{3}Razaβ^{3}ASVA	69 ± 15	2.35 ± 0.15	0.0359 ± 0.0093
VI	Razaβ^{3}RASazaβ^{3}VA	544 ± 122	1.19 ± 0.13	0.00185 ± 0.00049
VII	RRazaβ^{3}ASazaβ^{3}VA	217 ± 46	4.58 ± 0.52	0.0230 ± 0.0012
VIII	Razaβ^{3}RASVazaβ^{3}A	47.4 ± 8.1	4.39 ± 0.27	0.0976 ± 0.0065
IX	RRazaβ^{3}ASVazaβ^{3}A	118 ± 35	14.2 ± 1.7	0.121 ± 0.0094
X	RRASazaβ^{3}Vazaβ^{3}A	73 ± 17	8.80 ± 0.55	0.126 ± 0.013
	RRASVA [4]	11.1 ± 3.5	36 ± 3	3.2 ± 0.1

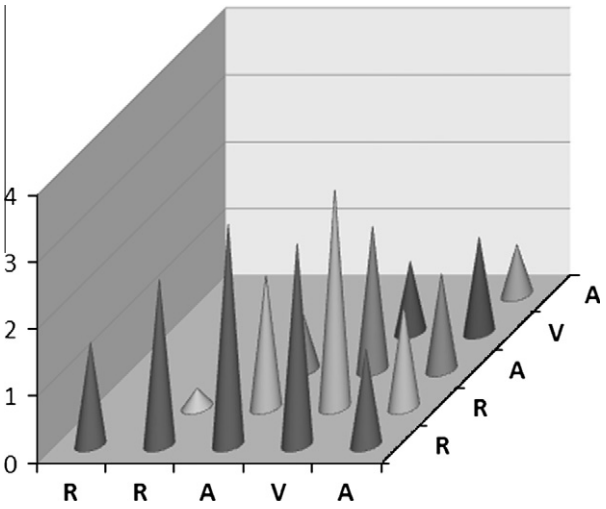

Fig. 1. Chart of position-depending effect of L-amino acids with their aza-β^3 analogs in the peptide substrate RRASVA upon the protein kinase A catalyzed phosphorylation. The effects on the second order rate constants $\Delta \log k_{II} = \log \frac{k_{II}^{mimetic}}{k_{II}^{RRASVA}}$ were plotted against modification positions.

the formation of the loopy structure was characteristic for all these models, it was obvious that amino acid residues possessed different spatial locations. For illustration of this fact, the models of azaβ^{3}RRazaβ^{3}ASVA and RRazaβ^{3}ASVazaβ^{3}A were compared with RRASVA in Fig. 2. It can be seen that arginine residues are significantly differently located in these sequences. Therefore, it was not surprising that the K_m value for azaβ^{3}RRazaβ^{3}ASVA exceeded the same value for RRASVA. On the other hand, however, these differences in the spatial structure did not exclude phosphorylation of these peptidomimetics, pointing to the fact that still close mutual positioning of the ATP γ-phosphate and the serine hydroxyl group was possible in the case of these enzyme-bound peptidomimetics. This close positioning is needed for phosphate transfer reaction from ATP to substrate.

Binding of double-substituted peptidomimetics with the active site of the protein kinase A was modeled by computational docking of these compounds with the enzyme–ATP complex [18]. Extensive lists of various complexes of the enzyme with peptidomimetics were obtained from these calculations, pointing to the possibility that the aza-peptide derivatives could effectively bind not only in the active site of the enzyme, but also in the other areas of the protein molecule. Although the complexes formed outside the enzyme active site might be of interest for further study, in this work we focused on the phosphorylatable complexes formed within the active site. These complexes were selected from the list of all binding poses, assuming that close positioning of the γ-phosphorus atom of the enzyme-bound ATP and the oxygen atom of serine hydroxyl group of peptidomimetics is necessary for the substrate phosphorylation reaction. Therefore, the distance value up to 6 Å between these P and O atoms was settled as the selection rule, proceeding from the mechanism of the nucleophilic substitution reaction at a tetrahedral phosphorus atom [15,19] as well as from the results of the analogous docking modeling for the peptide substrate RRASVA, where the most favorable docking positions yielded the P–O distance around 4 Å.

In the case of all studied peptidomimetics at least few docking complexes were found, where the distance between phosphorus and oxygen atoms remained between 3 and 5 Å. This distance was considered sufficient for the following phosphoryl group transfer reaction. It was noteworthy that these reactive complexes

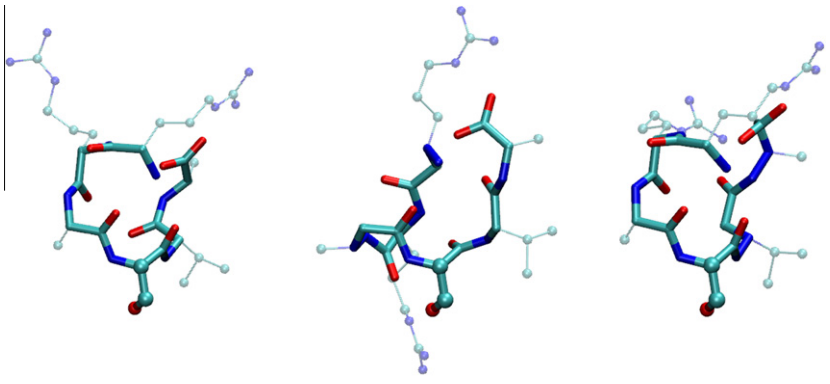

Fig. 2. Comparison of computer models of peptide RRASVA (left) with its peptidomimetic analogs azaβ^{3}RRazaβ^{3}ASVA (center) and RRazaβ^{3}ASVazaβ^{3}A (right). The backbone of these compounds was shown as bold stick model. The serine OH groups of these compounds, shown as bold ball-and-stick model, were similarly oriented in all three molecules to allow comparison of the spatial arrangement of other side groups shown as transparent ball-and-stick models.

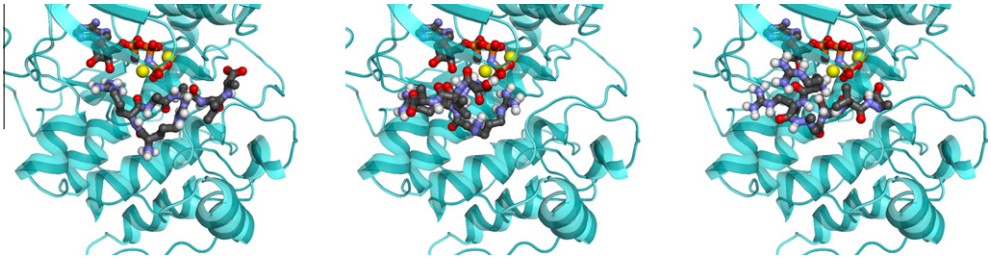

Fig. 3. Docking models for RRASVA (left), azaβ[3]RRazaβ[3]ASVA (center) and RRazaβ[3]ASVazaβ[3]A (right) interaction with the active center of protein kinase A. Protein structure is shown as green ribbon. ATP and substrate molecules are shown by ball-and-stick molecular models with the following color code for atoms: hydrogen – white, carbon – black, nitrogen – blue, oxygen – red, phosphorus – yellow. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

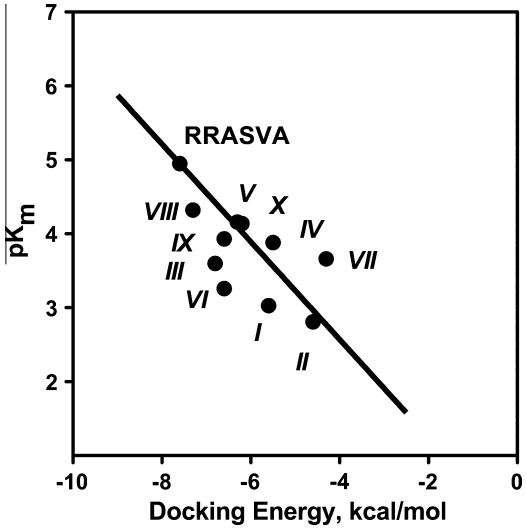

Fig. 4. Comparison of pK_m values for the protein kinase A catalyzed phosphorylation of substrates with docking energy, calculated for the formation of reactive complexes of these compounds with the enzyme. Peptidomimetics are denoted as shown in Table 1.

formed in spite of rather different geometry of RRASVA and peptidomimetic substrates as illustrated in Fig. 2. Therefore, the achievement of close positioning between the P and O atoms should be accompanied by significant structural changes in the peptidomimetic structure, including opening of the loopy structure of ligands. The necessity of this structural change could explain the relatively low binding effectiveness of peptidomimetic substrates with the enzyme. On the other hand, this result was in agreement with the fact that all studied peptidomimetics were still phosphorylated by the enzyme.

The results of docking calculations, where the distance between the γ-phosphorus atom of the enzyme-bound ATP and the oxygen atom of serine hydroxyl group of substrate was used as an additional selection criterion, were illustrated in Fig. 3 for azaβ[3]RRazaβ[3]ASVA and RRazaβ[3]ASVazaβ[3]A. For these substrates, the P–O distances in docking models were shorter than 5 Å and the complexes were characterized by docking energy values −4.6 and −6.6 kcal/mol, respectively. It was important to recapitulate that these complexes were not the most favorable in terms of the docking energy. However, they were the most favorable in terms of the P–O distance, required for the reaction step. Therefore we suggested that these modeling results could be compared with the results of kinetic experiments.

This comparison was made for all studied peptidomimetics in Fig. 4, where docking energies calculated for the formation of reaction complexes were compared with the pK_m values. Besides the data for di-aza-β[3]-substituted RRASVA analogs, this plot included also the data-point for the parent peptide. It can be seen that a rather similar specificity pattern could be observed for the kinetic data and the results of docking energy calculations. Although the presumptive meaning of this plot seems to be clear, universality of this approach will be further validated by extension of similar docking analysis, including data for other peptide and peptidomimetic substrates.

Taking together, the effect of two simultaneous modifications of the peptide backbone could not be calculated as a sum of two effects, each caused by a single modification made in respective positions, and in most cases the effect of modification was amplified in the presence of another aza-β[3]-amino acid. Therefore, recognition of these ligands by their binding site could not be presented in terms of simple additive models, but seems to be governed by topological factors, which determine the fit between a ligand and its target site and could be reasonably well described by computational ligand docking analysis. This conclusion agreed with the hypothesis about specificity-determining clusters of peptidomimetic ligands, as some areas of these chains remained more sensitive against structural modification than other parts of the same molecule.

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References

[1] O. Zetterqvist, U. Ragnarsson, E. Humble, L. Berglund, L. Engström, Biochem. Biophys. Res. Commun. 7 (1976) 696–703.
[2] M. Eller, A. Sepp, R. Toomik, P. Ekman, J. Järv, U. Ragnarsson, L. Engström, Biochem. Int. 25 (1991) 453–460.
[3] M. Eller, J. Järv, R. Toomik, U. Ragnarsson, P. Ekman, L. Engström, J. Biochem. 114 (1993) 177–180.
[4] K. Kisseljova, A. Kuznetsov, M. Baudy-Floc'h, J. Järv, Bioorg. Chem. 38 (5) (2010) 229–233.
[5] O. Busnel, L. Bi, M. Baudy-Floc'h, Tetrahedron Lett. 46 (2005) 7073–7075.
[6] O. Busnel, L. Bi, H. Dali, A. Cheguillaume, S. Chevance, A. Bondon, S. Muller, M. Baudy-Floc'h, J. Org. Chem. 70 (2005) 10701–10708.
[7] A. Dif, F. Boulmedais, M. Pinot, V. Roullier, M. Baudy-Floc'h, F.M. Coquelle, S. Clarke, P. Neveu, F. Vignaux, R.L. Borgne, M. Dahan, Z. Gueroui, V. Marchi-Artzner, J. Am. Chem. Soc. 131 (2009) 14738–14746.
[8] R. Roskoski Jr., Methods Enzymol. 99 (1983) 3–6.
[9] A. Kuznetsov, A. Uri, G. Raidaru, J. Järv, Bioorg. Chem. 32 (2004) 527–535.
[10] D.E. Koshland, Bioorg. Chem. 30 (2002) 211–213.
[11] R. Eisenthal, K.J. Danson, D.W. Hough, Trends Biotechnol. 25 (2007) 247–249.
[12] O. Trott, A.J. Olson, J. Comput. Chem. 31 (2010) 455–461.
[13] M.F. Sanner, J. Mol. Graph. Model. 17 (1999) 57–61.
[14] This script is available at: <ftp://ftp.ut.ee/dock>.
[15] F. Ni, W. Li, Y.M. Li, Y.F. Zhao, Curr. Protein Pept. Sci. 6 (2005) 437–442.
[16] O. Zetterqvist, U. Ragnarsson, FEBS Lett. 139 (1982) 287–290.
[17] A. Kreegipuu, N. Blom, S. Brunak, J. Järv, FEBS Lett. 430 (1998) 45–50.
[18] D. Bossemeyer, R.A. Engh, V. Kinzel, H. Ponstingl, R. Huber, EMBO J. 12 (1993) 849–859.
[19] S.J. Admiraal, D. Herschlag, Chem. Biol. 11 (1995) 729–739.