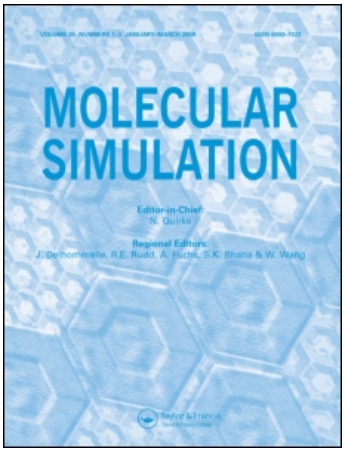

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3D-QSAR WITH CoMFA MODEL OF PROLYLENDOPEPTIDASE SUBSTRATES

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The prolylendopeptidase (PEP) is the proteolytic enzyme, which plays an essential role in the regulation of some processes in central nervous system, such as memory, learning and behavior. It was shown that PEP activity changes at different diseases, like Parkinsons or Alzheimer's diseases, and some PEP inhibitors are used in therapy. At present time the discovery of new types of PEP inhibitors are the actual task.

In this study the structure of PEP active site was analyzed by 3D-QSAR with CoMFA methods using of 12 PEP substrates. The designed pharmacophore model assumes that substrates interact with PEP active site by pyrrolidol ring of proline residue and by hydrogen bonding.

The 3-D-QSAR + CoMFA model of PEP substrates propose that the hydrophobic bonds play the essential role in substrate interaction with enzyme. This model reveals the important steric and electrostatic areas around the molecules and the presence of substituents controls the PEP activity for substrates. Analysis of obtained data allows to assume, that substrate binding in PEP active site causes essential perturbations of substrate structure. This effect mainly depends on chemical nature of the amino acid side chain, located near to proline.

Keywords: Prolylendopeptidase; 3D-QSAR; CoMFA; pharmacophore; active site; substrate

INTRODUCTION

The prolylendopeptidase (PEP) is the proteolytic enzyme, which participates in the regulation of the several processes of the central nervous systems (CNS). This enzyme plays an essential role in the regulation of the memory, learning and behaviour [1]. The function of PEP is a control of the endogenous peptide concentration in organism [2]. Changes of PEP activity are

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shown at different diseases, such as Parkinson's and Alzheimer's disease, some types of toxic substance abuse, *etc.* [3]. The reliable increasing of PEP activity at some diseases, such as various types of amnesia and in declining years, was determined [4].

Some PEP inhibitors have antiamnestic activity and they are successfully used for Alzheimer's disease therapy, for elimination galoperidol-induced catalepsy, improving the cognitive function at normal and pathological ageing, and can be applied for treatment of some types of toxic substance abuse [5, 6]. So the search of new PEP inhibitors is the actual task.

At present the methods of computer simulations are used for acceleration of new compounds finding [7]. The quantitative structure-activity relationship (QSAR) method is used when the three-dimension (3D) structure of target macromolecule is absents (as for PEP). The last modern QSAR modifications, such as three-dimensional QSAR (3D-QSAR), which takes into account the 3-D structure of molecules, is more preferable for this task.

The objective of the study was to design the pharmacophore and 3D-QSAR with CoMFA models for determination of structural features of PEP substrates, important for their binding with enzyme active site.

MATERIALS AND METHODS

The calculations were carried out on Silicon Graphics Workstation Indigo-2 (R4400, XZ) using Sybyl 6.3 software (Tripos Inc., USA).

Substrate specificity and structures of 20 molecules, used in the analysis, are shown in Table I. Hydrolysable by enzyme molecules, which contain the protective group – BOC on C-end, were used in model design.

The molecular models were constructed and their geometries were optimised using the standard Tripos force field. The atomic charges calculation was made by Gasteiger-Huckel method and used in the subsequent analysis. Values of molecular hydrophobicity were calculated by method of Moriguchi [8].

The pharmacophore model was design using "DISCO" program. The values of tolerance were designated in analysis from 0.2 to 2 angstroms.

3D-QSAR was carried out with CoMFA module of SYBYL. The region was generated automatically by the program. The grid size had a resolution of 2 angstrom. Both steric and electrostatic fields were taken into consideration. The steric and electrostatic potentials were generated using a sp^3 carbon probe and +1 charge. QSAR analysis was carried out in two steps using PLS technique. In the first analysis, using 5 components and a

TABLE I PEP substrates and their K_m, V_{max} values

N	*Structure*	K_m (*mM*)	V_{max} (*nmol/mg* × *min*)
1	BOC–Gly–D–Pro–pNA	no reaction	–
2	BOC–β–Ala–Pro–pNA	no reaction	–
3	BOC–Phe–Pro–pNA	0.24156	0.02199
4	BOC–Arg–Pro–pNA	no reaction	–
5	BOC–D–His–Pro–pNA	no reaction	–
6	BOC–Gly–Pro–pNA	no reaction	–
7	BOC–Lys(Fm)–Pro–pNA	0.20406	0.0172
8	BOC–Lys(TFA)–Pro–pNA	0.13163	0.01783
9	BOC–Cys(Bzl)–Pro–pNA	0.01628	0.01442
10	BOC–Trp–Pro–pNA	0.00202	0.00122
11	Z–Lys(BOC)–Pro–pNA	0.01648	0.00982
12	BOC–Glu(Bzl)–Pro–pNA	0.5719	0.03562
13	BOC–Pro–Pro–pNA	0.04075	0.00186
14	BOC–Ala–Pro–pNA	0.08416	0.01442
15	BOC–Lys(Z)–Pro–pNA	0.01203	0.00809
16	Z–Gly–Pro–pNA	0.27333	0.00917
17	BOC–Ile–Pro–pNA	0.23039	0.3533
18	Bz–Ile–Pro–pNA	0.06681	0.01026
19	Z–Lys–Pro–pNA	0.07291	0.02995
20	BOC–Leu–Pro–pNA	0.54104	0.04685
21	BOC–Gly–Gly–Pro–pNA	0.02681	0.001
22	Brn–Ala–Pro–pNA	0.11847	0.01625

BOC–*t*-butyloxicarbonile, Z–benzyloxicarbonile, pNA–p-nitroanilide, Brn–bornoyl, Bz–benzoyl.

number of cross-validation groups equal to a number of compounds, the optimal number of components was determined. The optimal number of components for the final 3D-QSAR model was chosen as the number of components that corresponds to the minimum cross-validated standard error of estimate (s_{cv}) and $R^2_{cv} > 0.4$. The second run was performed without cross-validation, using the optimal number of components previously determined. The results of the second analysis were used for drawing the coefficients' contour maps.

RESULTS

Active compounds having BOC on C-end of peptides were used for computer modelling. The other compounds were used for model testing. All substrates have p-nitroaniline on N-end of peptide. Before modelling this groups were eliminated from molecule models since they need for photometry determination of substrates hydrolysis and don't take part in substrate binding. Also many natural PEP substrates have NH_3-group at those N-end.

Pharmacophore Model

At first the pharmacophore model of substrates PEP was designed. The twelve compounds (N 03, 07–10, 12–15, 17, 20, and 21 from Tab. I) were used in analysis. This model proposes the ligands groups responsible for active site binding.

Initially 12 pharmacophore models were obtained. All molecules had 8 pharmacophore points: 2 acceptor atoms, 1 donor atom, 1 hydrophobic group on ligand, and also proposed 2 donor and 2 acceptor site on the active site. Acceptor atoms were the atoms of oxygen, and donor atom was the nitrogen. Hydrophobic group belongs to the proline ring.

These 12 models differ only by used conformers for molecule design. We proposed that the most probable is the model in which the molecule 13 with 2 prolines has energy minimal conformation. Proline has the unique structure in comparison with other amino acids because it has minimum conformational mobility. So the presence of 2 prolines in peptide makes molecule 13 more rigid in the set. So it was assumed that molecule 13 interacts with PEP active site in energy minimal conformation. Selected model of pharmacophore is shown in Figure 1. Besides, the molecules

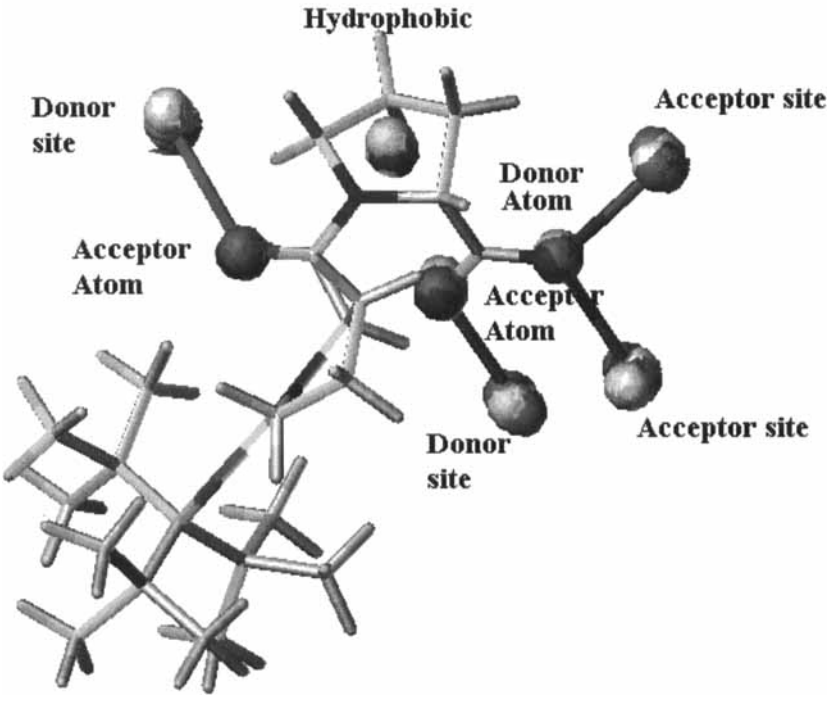

FIGURE 1 Pharmacophore model of prolylendopeptidase substrates. (See Color Plate XIX).

TABLE II 3D-QSAR with CoMFA analysis for PEP substrates

N	*Models*	R^2_{cv}	*n*	R^2	*s*	*F*	s_{cv}	F_{cr}	*Contribution (%)*		
									Steric fields	*Electrostatic fields*	*H-bond*
1	Steric + electrostatic	0,097	3	–	–	–	–	–	–	–	–
2	Steric + electrostatic + log *P*	0,182	3	–	–	–	–	–	–	–	–
3	Steric + electrostatic + H-bond	0,480	3	0,999	0.01	6893	0.634	0.116	15,9	13,4	70,6
4	Steric + electrostatic + H-bond + log *P*	0,335	3	–	–	–	–	–	–	–	–

R^2_{cv} square of the multiple correlation coefficients in analysis with cross-validation.
s_{cv} cross-validated standard error of estimate.
n optimal number of components.
R^2 square of the multiple correlation coefficients without cross-validation.
s standard error.
F significance test.
log logarithm of partition coefficient in octanol/water system.

aligned by this pharmacophore model have the U-liked structure. Such structure was assumed for PEP inhibitors, which have similar structure motif with substrates [9].

3D-QSAR with CoMFA Model

The attempts to design 3D-QSAR with CoMFA model of PEP substrates using only standard force field CoMFA (steric and electrostatic fields) were unsuccessful (Tab. II, model 1).

The important role in binding of substrates and inhibitors to PEP play the hydrophobic interactions [4, 9]. So the values of molecular hydrophobicity ($\log P$) for PEP substrates were calculated. However, obtain model with steric, electrostatic CoMFA fields and with $\log P$ was invalidation (Tab. II, model 2).

The model with good predictive power was designed only after inclusion in the 3D-QSAR with CoMFA analysis the fields for hydrogen bonding (Tab. II, model 3). If the value of hydrophobicity (model 4) was taken into account, statistically significant model was failed. Model 3 predicted, that the contribution of hydrogen bonds in compounds activity are about 70%. For further examination of the predictive property of our model we compared predicted values of K_m with the experimental data for compounds out of the test set (Tab. III). The obtained model quite good predicts the activity of compounds.

3D Contour maps were analysed from this model. Figure 2 shows the important steric (A), electrostatic (B) and hydrogen bonding (C) regions influencing on substrate specificity. Unfavourable steric fields can be seen in the region, limited the substituent length. Favourable steric fields are situated sideways from substituent, indicating that the long substituents can be placed in active site in curved conformation (Fig. 2A). The favourable region for positive charges are located from the sides of amino

TABLE III Predicted and experimental K_m values for test compounds

		$K_m (mM)$	
I	*Formula*	*Predicted*	*Experimental*
2	BOC–Ala–Pro	0.56	no reaction
6	BOC–Gly–Pro	1.10	no reaction
13	Z–Lys(BOC)–Pro	0.03	0.016
20	Z–Gly–Pro	0.12	0.273
23	Bz–Ile–Pro	0.06	0.067
25	Z–Lys–Pro	0.04	0.073
31	Brn–Ala–Pro	0.09	0.118

acids radicals near to peptide chain, and the other favourable region for negative charges is far from peptide chain in the area of favourable and unfavourable steric fields (Fig. 2B). The contour map of distribution of hydrogen bonds fields shows, that the main site of probable generation

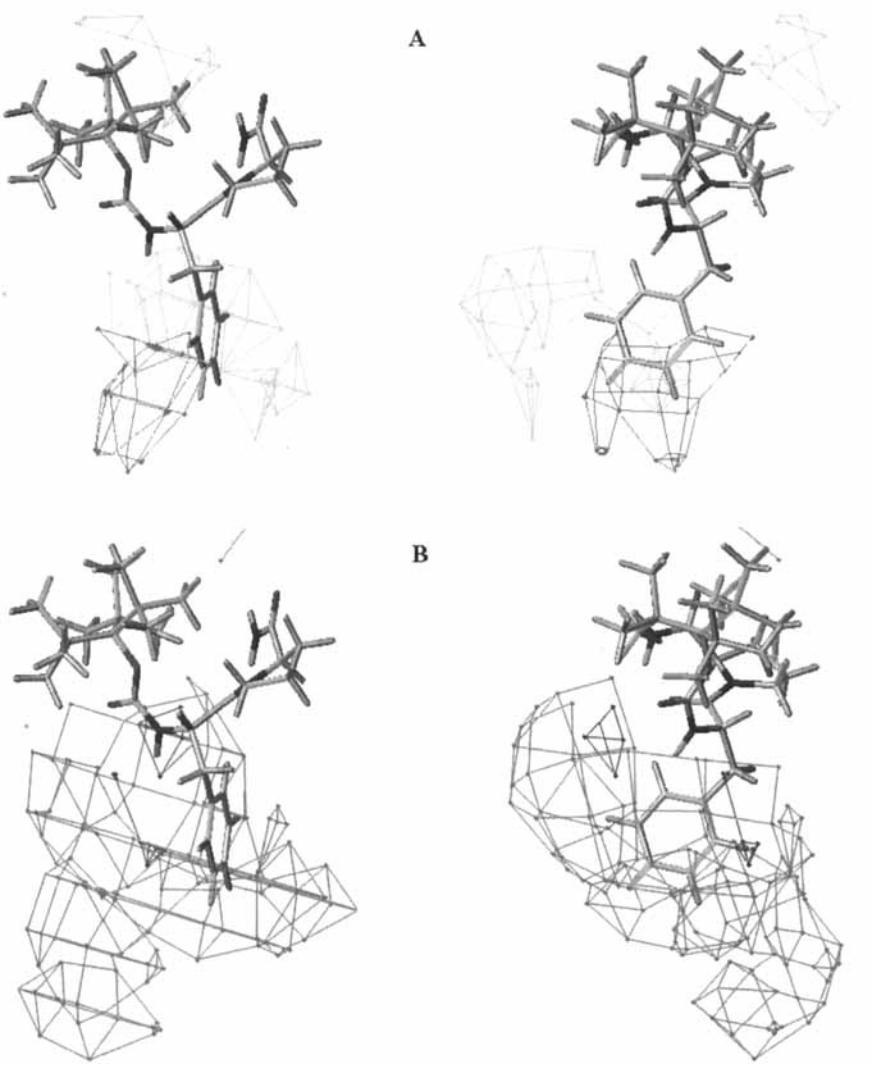

FIGURE 2 Standard dev* coefficients contour plot of CoMFA fields for PEP substrates. A – steric fields: sterically favoured areas for substitute are represented by green lines, sterically disfavoured areas by orange lines; B – electrostatic fields: positive charge favoured areas are represented by blue lines, negative charge favoured areas by red lines; C – areas are represented hydrogen bond fields. (See Color Plate XX).

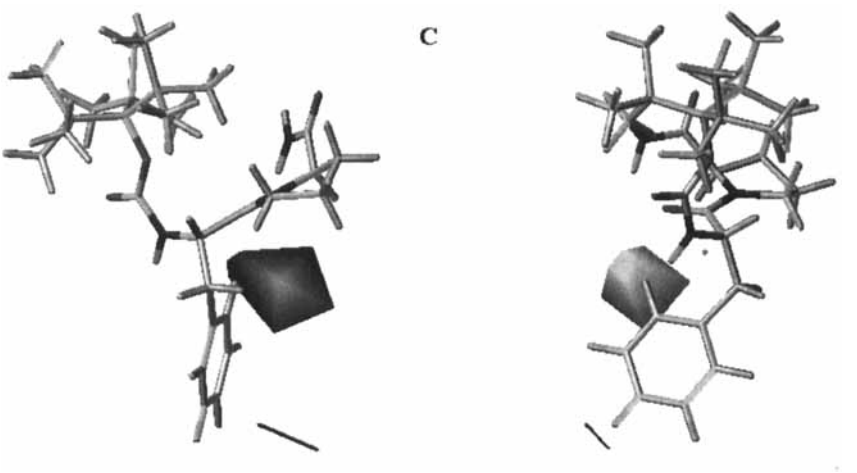

FIGURE 2 (Continued). (See Color Plate XX)

of hydrogen bonds is located in the area of the steric favourable region (Fig. 2C).

DISCUSSION

The designed pharmacophore model assumes that substrates interact with PEP active site by pyrrolidole ring of proline residue and by hydrogen bond of peptide chain of substrates. The side radicals of other amino acids do not participate in molecular recognition. We cannot determine the spatial position of the peptide main chain using pharmacophore model. However, in test set of molecules only the second amino acid residues is changed and the indefinite position of peptide main chain should not influence for designing 3D-QSAR with CoMFA model.

The obtained model was examined with test compounds and it has shown, that the model rather good predicts the most compounds activity. High K_m values were predicted even for compounds for which the activity is not detected (molecules 2 and 6). These compounds distinguish from other molecules by the absent of side radical at second amino acid. This, is probably, the reason for enzyme inability to hydrolyse these peptides. The structure of these compounds does not disagree with designed substrates pharmacophore model, so these peptides can bind to the enzyme active site. It can be explained that interaction of amino acid side radicals with PEP active site leads to the conformation change of enzyme structure,

which produces the possibility for catalytic acting. The possibility of such chanism conforms with data that the most slow stage at PEP substrates hydrolysis is the substrate interaction with PEP active site [10, 11].

It was reported that hydrophobic interaction takes part in binding of PEP substrates and inhibitors [9]. However, the using of substrates $\log P$ values in analysis led to bad results of analysis. It is possible that the hydrophobic region of active site is small enough, and the integral value of hydrophobicity ($\log P$) of the whole molecule can not describe the contribution of hydrophobicity in enzyme ability to hydrolyse its substrates. This data agreed with pharmacophore model designed for PEP inhibitors, where the presence of hydrophobic region on molecules of inhibitor that is placed in local area [9].

The analysis of received data allows to assume, that the substrate binding to the PEP active site causes enzyme essential perturbations, which lead to its activation. The main forces participating in substrates interaction with PEP active site, probably, is hydrogen bonding.

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