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Volodymyr Tanin[1]
Vsevolod Tanchuk[2]

DOCKING STUDIES OF TYPICAL CONFORMATIONS OF PROTEIN TYROSINE PHOSPHATASE 1B

Institute of Bioorganic Chemistry and Petrochemistry, National Academy of Sciences of Ukraine
Murmanska street 1, 02660, Kyiv-94, Ukraine
E-mails: [1]vtaninva@gmail.com; [2]v_tanchuk@yahoo.com

Abstract. *Typical conformations of protein tyrosine phosphatase 1B have been tested by docking known inhibitors. It was found that though there is no preferable conformation, there is a suitable conformation for each inhibitor.*

Keywords: binding sites; clustering; conformations; docking; phosphatase 1B; structure similarity.

1. Introduction

Human protein tyrosine phosphatases regulate a number of biochemical processes which depend on dephosphorylation of phosphotyrosine residues in proteins [19, 22, 24].

Intracellular Protein Tyrosine Phosphatase 1B (PTP1B) is known to be involved in insulin receptor dephosphorylation and considered a negative regulator of insulin signal transduction [9].

It means that PTP1B is one of the most promising therapeutic targets for potential treatment of type 2 diabetes and obesity [10].

There is constantly growing interest in developing inhibitors of this enzyme [24, 25].

Derivatives of carboxylic, phosphonic, sulfonic acids [3, 13, 21, 26], heterocyclic and other compounds [11, 12] have been tested as PTP1B inhibitors.

As it is known computer simulations play an important role in drug design.

In the case of PTP1B such methods has been already applied.

Several active compounds have been studied using computer-based approaches, including molecular docking [7, 8].

Molecular docking is also an important tool used to understand detailed mechanisms of inhibitor binding to the enzyme [1, 15, 16, 17, 18].

PTP1B is a one or rare cases when the enzyme is represented by a large amount of data in the PDB data bank [2].

This is another proof of the significance of the enzyme, but it also a challenge for the investigator.

Computer simulations usually rely on multi-dimentional optimization.

This make them heavily dependent on starting conditions since the energy surfaces are very complicated with a great number of local minima.

2. Analysis of publications

The investigation of the conformations of PTP1B in complexes with different inhibitors has already been published [20]. new approach that allows investigation of selected parts of macromolecules (especially enzymes) has been proposed.

The approach allows to study the mobility of selected parts of enzymes (amino acid residues in the active site) on the basis of X-ray images.

Crystal structures of enzymes and mobility of amino acid residues of enzymes are known to serve as a framework for understanding the mechanism of enzyme catalysis and can provide a structural basis for the search for potent and selective inhibitors and obtained during, such investigation results may help in further understanding of enzyme functioning and provide starting points for the computer simulations of inhibitor binding.

As it is known, molecular simulations (docking, molecular dynamics, even quantum chemistry) play an important role in drug design.

On the other hand modeling of molecules and especially macromolecules is a great challenge due to their high complexity and flexibility.

The results of computer simulations are highly dependent on starting conditions (they tend to find the nearest local minimum).

This means that finding of relevant starting conditions is really important.

It was also found that using one conformation of an enzyme is not enough to cover all possible modes of its interaction.

It was suggested to use an ensemble of starting conformations.

The conformations were usually generated by computer and clustered [23].

It has been already suggested to use multiple conformations from crystallographic sources [4].

It was also suggested to use conformations of an enzyme, obtained by experimental methods, cluster them and use cluster centroids as an ensemble of starting conformations for further computer simulations.

Experimental data are different from data obtained by computer simulations.

In many cases they are not complete or require some special treatment.

At the same time it was found in the case of PTP1B that conformation of a free enzyme is substantially different from the conformations of the enzyme in the enzyme-inhibitor complexes (the so called induced fit) [20].

It means that experimental data (PDB) give more solid grounds for further investigations.

Unfortunately there are usually only few structures and investigators are happy to have at least something to start, but in some cases (PTP1B, thrombin) there are a lot of structures and an investigator has to choose.

PTP1B is represented by more than 100 structures in the RSCB protein data bank.

The PDB database is constantly growing and there will be more and more cases like PTP1B.

So, the authors think that the proposed approach will be more demanded.

It is not possible to use all existing crystal structures of enzyme for efficient screening.

This led us to the conclusion that existing data on binding sites from the PDB files should be divided into classes based on the similarity of the binding profile.

This can be achieved by clustering all available conformations.

Centroids of clusters as representatives of corresponding binding modes can be used as good starting points for molecular simulations.

In our case, we needed a special tool, which is able to compare only selected parts (active sites) of multiple protein structures.

The results of clustering PTP1B conformations are presented in the article [20]. It was found that all existing conformations of the active center can be divided into 5 clusters (Table 1).

It means that there are 5 typical conformation of PTP1B in complex with inhibitor.

Free enzyme adopts a different conformation, which means that it is rather difficult to start simulations from it.

The **goal** of this study was to investigate the difference between the clusters of PTP1B conformations by docking known PTP1B inhibitors.

The authors decided to test this approach and try docking of known PTP1B inhibitors into centroids of all 5 clusters.

The inhibitors were taken from the NIH database [14].

It was assumed that all inhibitors containing phosphonic group should interact with the catalytic site of the PTP1B and their phosphonice groups should be located not far from Cys215.

The database contained 208 unique compounds with phosphonic groups.

In several cases there were several records with the same compound.

It such cases the record with the highest pKi value was chosen.

Some compounds had more than one phoshonic group.

In such cases all possible ways of binding were tested.

As a result 208 compounds gave 258 variants of their binding.

Table 1. Clusters of PDB structures and distribution of the best (most similar to experimental) results between them

Cluster	Centroid (PDB code)	Number of structures	RMSD from ligand-free conformation 2HNP (Å)	WPD-loop	Number of the best docking results for the cluster
1	1NL9	7	1.37	Open	24
2	1PH0	15	1.02	Open	39
3	2CNF	22	2.39	Closed	46
4	1Q6M	11	2.51	Closed	48
5	2CM8	44	2.23	Closed	48

Table 2. Docking results (pKi, $-\log_{10}$ of inhibition constant Ki) for the first 35 of 208 compounds for all clusters

Name	Cluster centroids					Best $R^2 = 0.69$[a] $R^2 = 0.76$[b]	Experimental *pKi*
	1Q6M $R^2 = 0.03$	2CM8 $R^2 = 0.06$	2CNF $R^2 = 0.03$	1NL9 $R^2 = 0.03$	1PH0 $R^2 = 0.02$		
1_1	–	–	3.271	4.818	4.356	–	8.222
1_2	**8.045**	–	3.938	5.787	5.816	**8.045**	8.222
2_1	3.330	4.877	–	5.845	**6.659**	**6.659**	7.921
2_2	9.630	9.248	8.302	5.390	5.721	–	7.921
3_1	–	–	–	3.982	3.462	–	8.222
3_2	**8.273**	7.437	3.183	5.603	5.383	**8.273**	8.222
4_1	6.755	**6.263**	6.703	4.195	3.814	**6.263**	5.770
5_1	–	–	–	4.034	5.024	–	7.959
5_2	**7.517**	6.271	5.684	–	3.432	**7.517**	7.959
6_1	8.265	7.136	6.938	5.581	**5.258**	**5.258**	5.095
7_1	8.463	**8.302**	7.261	5.244	4.716	**8.302**	7.796
7_2	8.925	5.442	6.014	5.713	4.320	–	7.796
8_1	6.703	7.033	**7.591**	5.310	5.478	**7.591**	8.770
8_2	6.835	6.087	6.124	5.801	5.097	–	8.770
9_1	6.571	**7.283**	6.322	5.706	5.677	**7.283**	7.337
9_2	6.366	5.581	6.263	5.515	5.317	–	7.337
10_1	**7.327**	7.173	8.016	3.124	5.170	**7.327**	7.377
10_2	7.987	7.561	5.398	4.100	6.205	–	7.377
11_1	9.945	6.857	**7.686**	5.691	4.628	**7.686**	7.638
12_1	8.786	7.906	**7.877**	6.542	4.980	**7.877**	7.420
13_1	9.065	6.344	6.931	5.897	5.002	–	8.523
13_2	11.529	**8.617**	8.801	6.271	5.288	**8.617**	8.523
14_1	6.483	4.752	5.075	4.356	4.334	–	8.000
14_2	10.979	8.742	**7.752**	5.178	4.474	**7.752**	8.000
15_1	–	–	–	3.403	5.948	–	8.301
15_2	9.989	**9.138**	5.809	5.024	4.048	**9.138**	8.301
16_1	5.845	5.046	6.102	5.508	4.855	–	9.398
16_2	**7.510**	6.505	5.853	6.718	5.603	**7.510**	9.398
17_1	6.630	6.329	6.806	**4.826**	4.305	**4.826**	4.482
18_1	9.432	**7.759**	6.608	3.975	3.755	**7.759**	7.409
19_1	9.542	7.481	**6.601**	5.449	5.471	**6.601**	6.788
20_1	–	–	–	–	–	–	9.000
21_1	9.725	9.028	8.771	6.593	**6.483**	**6.483**	3.456
22_1	7.847	6.439	6.212	5.288	**5.288**	**5.288**	4.824
23_1	7.033	6.733	6.256	5.141	**5.024**	**5.024**	4.456
24_1	8.031	6.395	5.948	**5.002**	5.295	**5.002**	3.777
25_1	7.943	6.681	6.505	4.738	**5.031**	**5.031**	3.347
26_1	7.957	6.454	6.197	4.672	**4.613**	**4.613**	3.892
27_1	6.388	**5.046**	6.168	5.390	5.919	**5.046**	4.237
28_1	7.855	6.168	6.271	**4.708**	4.752	**4.708**	3.345
29_1	7.657	6.329	6.425	4.760	**4.576**	**4.576**	3.109
30_1	7.613	6.931	6.579	**5.552**	5.699	**5.552**	3.867
31_1	7.554	7.349	6.586	5.603	**4.782**	**4.782**	3.755
32_1	6.476	**5.016**	5.765	6.014	5.464	**5.016**	4.052
33_1	7.275	6.718	6.300	4.994	4.965	**4.965**	3.144
34_1	7.055	6.285	6.036	**4.701**	4.818	**4.701**	4.111
35_1	7.583	6.967	6.395	5.383	**5.317**	**5.317**	3.413

a – Compound name + type of binding (depends on the number of phosphonic groups).
b – 205 compounds without (20, 150, 160).
c – 202 compounds without outliers (152, 155, 202).

3. Results and discussion

Docking was done by a modified version of AutoDock [5].

This version of AutoDock has several specific features, including the ability to impose special constraints on docking poses, apply standard multidimensional optimization techniques and making global optimization by sequential search.

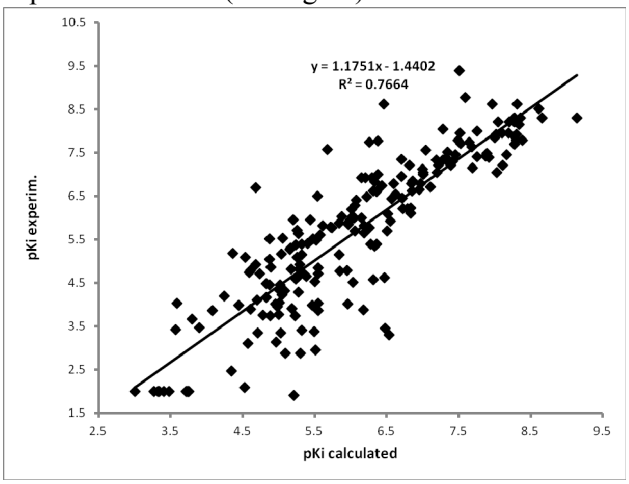
Positions of the atoms of phosphorous were limited to the region around the position of phosphorous (or sometimes sulfur) in the original PDB files of cluster centroids. We allowed maximal deviation of 2 Å.

New approaches to optimization allow to process a large number of molecules in a limited time.

This Table 2 contains the information about all dockings.

Unfortunately we have found that there is no cluster that has good enough correlation with experimental results.

There is also no good correlation between the highest and the lowest calculated values and the experimental ones (see Figure).



Correaltion between calculated and experimental values of pKi

On the other hand each compound has a variant of binding with predicted pKi quiet close to the experimental value.

Furthermore these values correlate with experimental values quiet well (R^2=0.69, R=0.83, RMS = 0.99).

The best (closest to experimental data) results are emphasized by bold characters.

There are 3 cases when AutoDock failed to calculate a reasonable pKi (compounds 20, 150, 160) or gave the results substantially different from experimental (outliers 152, 155, 202) due to some limitation of the method (in most cases it was energy penalty for too many rotating bonds).

Correlation without the outliers is substantially better (R^2=0.76, R=0.88, RMSD = 0.86).

The best results are not usually the highest or the lowest calculated values.

In general docking tends to overestimate.

The distribution of the best results over the clusters is shown in Table 1.

These results show that they are more or less evenly distributed between clusters and between closed (the first three) and open WPD loop conformations.

The authors tried to determine the similarity of compounds in each group and similarity of compounds from different groups.

ChemAxon chemical fingerprints were calculated by GenerateMD application from the ChemAxon package [6].

The following parameters were used:

– bit length 1024, maximum number of bits to set for each pattern 7;

– maximal number of bonds 3.

Similarity of compounds was calculated as Tanimoto similarity of the respective bit-strings.

Mean values were calculated for each cluster and for each pair of clusters.

The results are presented in Table 3.

Table 3. Similarity of compounds within each cluster and cross-cluster similarity

Cluster	1Q6M	2CM8	2CNF	1NL9	1PH0
1Q6M	0,660	0,639	0,623	0,570	0,574
2CM8	0,639	0,625	0,613	0,564	0,575
2CNF	0,623	0,613	0,600	0,566	0,572
1NL9	0,570	0,564	0,566	0,554	0,563
1PH0	0,574	0,575	0,572	0,563	0,573

Unfortunately, the similarity is almost the same in all cases. It is not possible to determine from the structure which cluster gives the best docking result.

4. Conlusions

1. It has been proved that the results of docking are heavily dependent on starting conditions.

The results of dockings into different cluster centroids are very different.

2. It was not possible to find a cluster that gave the best docking results for the whole set of compounds.

It all cases the results were rather poor.

3. Nevertheless, each compound had a simulated docking with predicted Ki not very different from the experimental result.

In most cases this was neither the highest nor the lowest Ki value.

Such values gave good correlation between experimental and calculated values for the whole set of compounds.

The bindings with the best predicted Ki values are probably not feasible due to some kinetic or steric reasons.

4. Unfortunately, it was not possible to explain the binding modes of molecules by their structural similarity.

There was no difference between the sets of compounds which have the most similar to experimental results on different clusters.

This probably needs further explanation.

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В.О. Танін[1], В.Ю. Танчук[2]. Дослідження типових конформацій протеїнтирозинфосфатази 1В за допомогою докінгу

Інститут біоорганічної хімії та нафтохімії Національної академії наук України, вул.. Мурманська 1, Київ-94, Україна, 02660

E-mails: [1]vtaninva@gmail.com; [2]v_tanchuk@yahoo.com

За допомогою докінгу відомих інгібіторів досліджено типові конформації протеїнтирозинфосфатази 1В. Найкращої конформації не виявлено, хоча для кожного інгібітора існує найбільш відповідна конформація.

Ключові слова: докінг; кластеризація; конформації; протеїнтирозинфосфатази 1В; сайти зв'язування; структурна подібність.

В.А. Танин[1], В.Ю. Танчук[2]. Исследование типичных конформаций протеинтирозинфосфатазы 1В с помощью докинга

Институт биоорганической химии и нефтехимии Национальной академии наук Украины, ул. Мурманская, 1, Киев-94, Украина, 02660

E-mails: [1]vtaninva@gmail.com; [2]v_tanchuk@yahoo.com

С помошью докинга известных ингибиторов исследованы типичные конформации протеинтирозинфосфатазы 1В. Предпочтительной конформации не найдено, хотя для каждого ингибитора существует наиболее подходящая конформация.

Ключевые слова: докинг; кластеризация; конформации; протеинтирозинфосфатазы 1В; сайты связывания; структурное подобие.

Volodymyr Tanin. Engineer.
Third Department, Institute of Bioorganic Chemistry and Petrochemistry, National Academy of Sciences of Ukraine, Kyiv, Ukraine.
Education: Faculty of Chemistry, specialty engineer of Organic Chemistry and Technology of Organic Substances, National Technical University of Ukraine "Kyiv Polytechnic Institute", Kyiv, Ukraine (2008).
Research area: computer chemistry.
Publications: 15.
E-mail: vtaninva@gmail.com

Vsevolod Tanchuk. Candidate of Chemistry. Senior Researcher.
Third Department, Institute of Bioorganic Chemistry and Petrochemistry, National Academy of Sciences of Ukraine, Kyiv, Ukraine.
Education: Department of Chemistry, specialty - physic chemistry , Kyiv Taras Shevchenko University, Kyiv, Ukraine (1991).
Research area: computer chemistry.
Publications: 100.
E-mail: v_tanchuk@yahoo.com