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Computer modeling of ligand-receptor interactions – enkephalin analogues and delta-opioid receptor

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Since, Hughes determined endogenous opioid pentapeptides – enkephalins, large number of synthetic analogues were prepared. Many analogues of enkephalins were synthesized by our group in addition. In our previous study we established a relationship between the replacement in position 2 in endogenous enkephalins and their δ-opioid receptor selectivity.

Computer modeling was used in this study to analyze binding affinity of a series of δ-opioid selective enkephalin analogues to the model of δ-opioid receptor, published in PDB (id: 1OZC). MolDoc SE algorithm implicated in the software program Molegro Virtual Docker was used.

Basing on docking results was established that: 1) all encephalin analogues have good binding affinity to δ-opioid receptor by forming H-bonds with specific amino acid residue in the receptor pocket; and 2) the rank of the derivatives obtained with this approach is rather different compared with the rank of their biological *in vitro* assay activity. These results reveal further steps for the computer modeling of selective encephalin analogues such as: 1) development of a novel optimization procedure; and 2) application of a different algorithm and software.

Key words: enkephalins, delta-opioid receptor, computer modeling, docking

INTRODUCTION

It is well established that there are at least three major opioid receptor types in the brain and periphery. These receptors are referred to as μ–, δ-, and κ-opioid receptor and have distinct pharmacological profiles, anatomical distribution, and functions [1–4]. [Met^5]- and [Leu^5] – enkephalins have high affinities for δ-opioid receptors. Many analogues of enkephalins were synthesized and their biological activity was evaluated, in order to establish selective ligand to δ-opioid receptor (DOR) [5–8]. This process is time consuming, very expensive and it involves many specialists: chemists, biologists, and medics. Computational approach is innovative and rational method, in which chemical synthesis and biological screening is replaced by virtual screening. It makes possible screening a huge number of compounds in a short period of time in a low cost. Therefore, the target of our work is the DOR.

Here we present a computer assisted modeling of ligand – receptor interactions, in our case δ-opioid selective ligands with DOR. Our aim is to check the reliability of three dimensional (3D) models of the DOR using the experimental data obtained with *in vitro* assay and the parameters calculated from docking approach.

METHODOLOGICAL APPROACHES

1. Objects/Ligands:

DPDPE ([D-Pen^{2,5}]-enkephalin, selective δ-opioid receptor agonist) [5];

endogenous opioid pentapeptides ([Leu^5]- and [Met^5]-enkephalin) and their analogues are presented in Table 1 [5–8].

Table 1. Ligands used in our study.

Ligand	Primary structure
DPDPE	Tyr-D-Pen-Gly-Phe-D-Pen
[Leu^5]-enk	Tyr-Gly-Gly-Phe-Leu
[Met^5]-enk	Tyr-Gly-Gly-Phe-Met
[Cys(Bzl)^2, Leu^5]-enk	Tyr-Cys(Bzl)-Gly-Phe-Leu
[Cys(Bzl)^2, Met^5]-enk	Tyr-Cys(Bzl)-Gly-Phe-Met
[Cys(O$_2$NH$_2$)^2, Leu^5]-enk	Tyr-Cys(O$_2$NH$_2$)-Gly-Phe-Leu
[Cys(O$_2$NH$_2$)^2, Met^5]-enk	Tyr-Cys(O$_2$NH$_2$)-Gly-Phe-Met
[DCys(O$_2$NH$_2$)^2, Leu^5]-enk	Tyr-D-Cys(O$_2$NH$_2$)-Gly-Phe-Leu
[DCys(O$_2$NH$_2$)^2, Met^5]-enk	Tyr-D-Cys(O$_2$NH$_2$)-Gly-Phe-Met
[HCys(O$_2$NH$_2$)^2, Leu^5]-enk	Tyr-HCys(O$_2$NH$_2$)-Gly-Phe-Leu
[HCys(O$_2$NH$_2$)^2, Met^5]-enk	Tyr-HCys(O$_2$NH$_2$)-Gly-Phe-Met

Target: human δ-opioid receptor (DOR), published in PDB (id: 1OZC), [9].

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2. Docking Procedure

To apply the docking procedure we postulated the following assumptions:

Opioid receptors (ORs) belong to "G-Protein Coupled Receptors (GPCRs)" which have structural similarity with the bacteriorhodopsin. Because the receptor is located in the membrane, 3D structures of GPCRs are unknown. In the absence of crystallographic data, indirect methods, which include site-directed mutagenesis, chimeric studies, the substituted cysteine accessibility method, and affinity labeling studies, have been instrumental in locating key contacts for molecular recognition [10]. As a target of our docking procedure we used model of human DOR, published in PDB (id: 1OZC), [9]. It was found that there are several key amino acid residues which are responsible for ligand binding. First very important residue is aspartate in *trans* membrane helix III. It is conserved among all biogenic amine receptor families. The role of this residue is to bind a free amino group of the ligand. Since the structurally similar phenolic group is often essential for opiate and opioid activity [11], it was believed that the formation of a hydrogen bond might be important for the recognition processing of the opioid receptor family as well. Histidine (His) residue in helix V is very important for hydrogen-bond formation with opioid phenol of Tyr residue. In key positions DOR has Trp in helix VI and Leu in Helix VII.

Basing on these assumptions, ligands were evaluated by external electrostatic interactions and external hydrogen-bond formation, during docking procedure. MolDoc SE algorithm [12] was used with 10 runs for each ligand with energy minimization and hydrogen-bond optimization after docking. Five poses for each ligand were generated. Because receptor did not contain any cavity, procedure of docking was made four times with different constrains, in fact they were four different amino acid residues in binding site of receptor – Asp128, Trp274, His278 and Leu300.

3. Computational tools

In this study we used a model of DOR, published in PDB (id: 1OZC, [9]). Docking studies were performed using Molegro Virtual Docker, run on Windows operating system. Visualizations of enkephalins, enkephalin analogues and of docking poses were made and analyzed on Molegro Molecular Viewer, and evaluation function for efficacy of docking of the ligand and receptor is the following:

$$E_{score} = E_{inter} + E_{intra},$$

where E_{score} is a docking scoring function, E_{inter} – ligand-protein interaction energy, and E_{intra} – internal energy of the ligand [12]. Values of the scoring function and its components were presented in Table 2.

4. Correlations

In order to find relationship between sets of data derived from in vitro assay and docking results, we tried to predict it with a help of the Spearman correlation, using GraphPad Prism 3.0. Spearman's rank correlation coefficient is a non-parametric measure of statistical dependence between two variables. It assesses how well the relationship between two variables can be described using a monotonic function. If there are no repeated data values, a perfect Spearman correlation of +1 or −1 occurs when each of the variables is a perfect monotone function of the other. To interpret Spearman, for values of r_s of 0.9 to 1, the correlation is very strong; between 0.7 and 0.89, correlation is strong; between 0.5 and 0.69, correlation is moderate; between 0.3 and 0.49, correlation is moderate to low; between 0.16 and 0.29, correlation is weak to low; and below 0.16, correlation is too low to be meaningful [13]. It can be calculated by the equation:

$$\rho = 1 - \frac{6\sum d_i^2}{n(n^2-1)},$$

where differences $d_i = x_i - y_i$ between the ranks of each observation on the two variables are calculated, and n is the number of the variables in each set.

RESULTS

1. Docking results

Docking program generates five pose for each analogue. Total energy of the ligand-receptor complex was calculated and hydrogen-bond interactions were evaluated.

Analyzing these docking results, we choose the best pose for each ligand with the lowest value of the scoring function. The data are presented in Table 2. The range of the values obtained was between -137.509 to 41.3876 kcal/mol. The lowest potential energy is characteristic for the complex of DOR with [Leu^5]-enk and the highest for [$Cys(O_2NH_2)^2$, Met^5]-enk.

Table 2. Ligands in ascending order of the scoring function (E_{score}) obtained with docking.

Ligand	E_{score}	E_{Inter}	E_{Intra} (vdw)
[Leu^5]-enk	-137.509	-156.052	82.0781
DPDPE	-127.274	-141.104	71.3263
[Cys(O_2NH_2)^2, Leu^5]-enk	-111.597	-157.553	137.362
[DCys(O_2NH_2)^2, Leu^5]-enk	-110.925	-161.443	135.278
[DCys(O_2NH_2)^2, Met^5]-enk	-109.216	-145.814	87.1034
[Met^5]-enk	-107.048	-127.242	85.6935
[Cys(Bzl)^2, Met^5]-enk	-90.9322	-171.597	179.634
[Cys(Bzl)^2, Leu^5]-enk	-71.2288	-130.887	142.195
[HCys(O_2NH_2)^2, Leu^5]-enk	-59.6588	-133.404	303.431
[HCys(O_2NH_2)^2, Met^5]-enk	-44.7916	-80.1617	165.299
[Cys(O_2NH_2)^2, Met^5]-enk	-41.3876	-104.626	133.122

The data presented in Table 3 concerns the number of hydrogen bonds formed during interaction between DOR and the ligands. DPDPE well-known selective DOR agonist binds to the receptor pocket with four hydrogen bonds: Tyr129 forms 3 H-bonds with 2 NH-groups of peptide backbone and with free NH_2-group of Tyr; and Trh213 with CO group from peptide backbone.

Table 3. H-bonds and interactions between ligand and receptor pocket.

Ligand	Number of hydrogen bonds	Other interactions
DPDPE	4	no
[Leu^5]-enk	3	no
[Met^5]-enk	2	Salt bridge
[Cys(Bzl)^2, Leu^5]-enk	3	π-π Trp274 – Phe
[Cys(Bzl)^2, Met^5]-enk	2	π-π Phe218 – Phe
[Cys(O_2NH_2)^2, Leu^5]-enk	3	π-π Trp274 – Tyr, SO_2NH_2 – Asp128, Tyr308
[Cys(O_2NH_2)^2, Met^5]-enk	4	π-π Phe218 – Tyr, SO_2NH_2 – Thr213, Val179
[DCys(O_2NH_2)^2, Leu^5]-enk	6	$2SO_2NH_2$ – Tyr308
[DCys(O_2NH_2)^2, Met^5]-enk	3	SO_2NH_2 – Tyr129
[HCys(O_2NH_2)^2, Leu^5]-enk	5	π-π Phe222 – Phe, SO_2NH_2 – Tyr129
[HCys(O_2NH_2)^2, Met^5]-enk	3	SO_2NH_2 –His278

In the case of [Leu^5]-enk there are 3 H-bonds: Tyr129 with CO-group from peptide backbone and Tyr interact with the receptor by forming H-bonds with Val179 and Thr213 (Figure 1A).

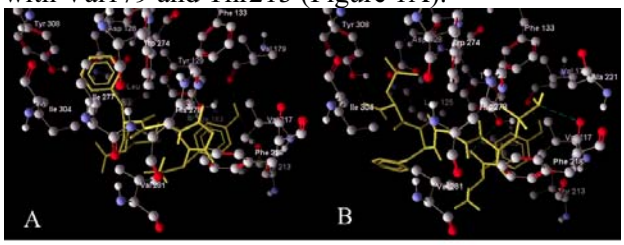

Fig. 1. Interactions in the bidning pocket of DOR with: A) [Leu^5]-enkephalin and B) [DCys(O_2NH_2)^2, Leu^5]-enkephalin

[Met^5]-enk bind to the receptor pocket by forming two H-bonds: Asp128 with NH_3 of Tyr and His278 with Tyr. Additionally it forms the salt bridge with COO^- of Asp128 and NH_3^+ of Tyr.

In the case of [Cys(Bzl)^2] analogues of [Leu^5] and [Met^5]-enkephalins, π-π interactions occur: Phe274 with Phe in [Leu^5], and Phe218 with Phe in [Met^5]-analogue. [Cys(Bzl)^2, Leu^5]-enk forms three H-bonds in DOR pocket – OH group of Tyr forms H-bonds with Val179 and Thr213, and COOH group of Leu with Tyr308. [Cys(Bzl)^2, Met^5]-enk binds receptor with two H-bonds – Tyr129 with NH from peptide backbone and Val217 with OH of Tyr.

Complex of [Cys(O_2NH_2)^2, Leu^5]-enk with DOR has relatively high total potential energy but it binds very strong to the receptor pocket by three H-bonds (SO_2NH_2 with Asp128, Tyr308 and Tyr129 with NH from peptide backbone) and π-π interaction (Trp274 with Tyr).

[Cys(O_2NH_2)^2, Met^5]-enk binds to the receptor with four H-bonds. SO_2NH_2 interact with Thr213, Val179, Tyr129 – with NH group from peptide backbone, and His278 – with OH group of Tyr. Additionally π-π interaction occurs between Phe218 and Tyr rings.

The data with [DCys(O_2NH_2)^2, Leu^5]-enk are very different. It forms six H-bonds with the amino acid residues in the receptor pocket. The interactions are as follows: Tyr129 forms two H-bonds with CO groups from peptide backbone, Val179 and Thr213 with OH group of Tyr residue, Val217 with SO_2NH_2, and Tyr308 with COOH group of Leu (Figure 1B).

In the case of [DCys(O_2NH_2)^2, Met^5]-enk just three hydrogen bonds are formed: Tyr129 – CO from backbone, Ile304 – OH (Tyr), and Tyr308 – OH (Tyr).

[HCys(O_2NH_2)^2, Leu^5]-enk forms five H-bonds with receptor pocket. Tyr128 interacts with OH group of Tyr, Ile304 with free NH_3 group, Tyr308 forms two H-bonds with SO_2NH_2 group and one with NH group from peptide backbone. Between Phe222 and Phe rings π-π interaction is established.

The complex [HCys(O_2NH_2)^2, Met^5]-enk – DOR is formed with three H-bonds: OH group of Tyr interact with Val179 and Thr213, and SO_2NH_2 group with His278.

2. Correlations

The Spearman's correlation coefficients for all correlations within data obtained with *in vitro* assay and docking are in the range from –0.1545 to 0.1455 for E_{intra}/K_A correlation and E_{iner}/IC_{50} correlation, respectively. For example, correlation

of IC_{50} and E_{score} is presented on Figure 2. The Spearman's correlation coefficient is 0.1091. This low value shows that the correlation between E_{score} and IC_{50} value is very low.

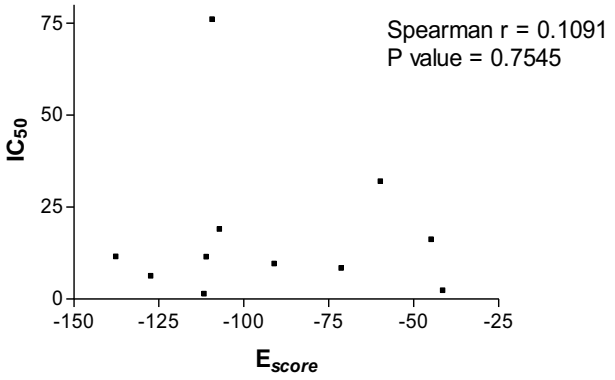

Fig. 2. Figure 2. Spearman correlation for E_{score} and IC_{50} values.

DISCUSSION

The substitution in second position in the encephalin structures with amino acid containing SO_2NH_2 increases additionally the binding of the respective analogue to DOR.

The incorporation in position 2 in the encephalin molecules of Cys(Bzl) does not interfere on their ability to bind to DOR. So, additional interaction due to Bzl group does not appear. Total potential energies of their complexes are similar to the endogenous enkephalin complexes with DOR and number of H-bonds formed is the same.

It appears that Tyr129 is very important amino acid residue in receptor pocket, because ligands interact with this residue. It is able to form H-bonds with its OH group and different functional groups of ligands, such as: OH group of Tyr, NH and CO groups of peptide backbone, and SO_2NH_2 group of amino acid analogue in position 2.

Docking data obtained allow characterizing some structural and chemical properties of the investigated analogues. The results of *in vitro* studies of [Leu^5]- and [Met^5]-enkephalins and their analogues obtained previously were summarized in Table 4. In this table IC_{50} corresponds to the potency of the ligands, the affinity and efficacy are presented by K_A and e_{rel}, respectively. Calculation of the parameters of *in vitro* experiments did not concern directly 3D structure of the receptors. However, in docking procedure 3D structure is the main tool. In this study we applied 3D model of DOR, published in PDB (id: 1OZC).

The ranking of the compounds based on their *in vitro* assay or docking data are rather different because the correlations between them were not established, including between E_{score} and IC_{50}. This fact shows that the increasing of the potency (IC_{50}) of the derivatives does not lead to increasing or decreasing of the value of the scoring function, obtained with docking.

Table 4. IC_{50}, K_A and e_{rel} obtained *in vitro* [12].

Ligand	Mouse vas deferens IC_{50} (nM)	K_A (nM)	e_{rel}
DPDPE	6.18±1.17	180±35	30.2±10.0
[Leu^5]-enk	11.45±2.06	54.9±13.1	5.8±1.0
[Met^5]-enk	18.91±2.15	48.4±7.5	3.6±0.3
[Cys(Bzl)^2, Leu^5]-enk	8.30±1.40	68.5±29.7	9.3±3.2
[Cys(Bzl)^2, Met^5]-enk	9.53±1.20	23.8±3.0	3.5±0.3
[Cys(O_2NH_2)^2, Leu^5]-enk	1.29±0.31	36.4±16.4	29.2±9.5
[Cys(O_2NH_2)^2, Met^5]-enk	2.22±0.45	14.1±5.4	7.3±2.0
[DCys(O_2NH_2)^2, Leu^5]-enk	11.40±2.01	73.4±12.7	7.4±1.9
[DCys(O_2NH_2)^2, Met^5]-enk	75.96±11.67	463±161	7.1±1.8
[HCys(O_2NH_2)^2, Leu^5]-enk	31.92±5.10	76.4±7.1	3.4±0.2
[HCys(O_2NH_2)^2, Met^5]-enk	16.09±1.90	55.7±6.1	4.5±0.3

Obviously for this kind of investigations on ligand – target interactions a novel optimization procedure has to be initiated in further studies. Since we obtained a set of parameters with docking or with *in vitro* bioassay, probably multi-dimensional vectors have to be introduced, such as two-dimensional vector (K_A, e_{rel}) or three-dimensional vector (IC_{50}, K_A, e_{rel}). In the same way docking results could be presented not only with one but with several scoring functions and the vector would be with the following elements - E_{score}, E_{inter}, E_{intra}.

In these two sets of vectors for *in vitro* and docking studies, respectively, it is possible to introduce a partial order, so that these sets become partially ordered sets. Analysis and comparison of maximal elements in the ordered sets could help to understand better the relationship between *in vitro* biological effects and docking studies and to answer whether the models of the biological macromolecules (in our case δ-opioid receptor) correspond to the real 3D structure.

CONCLUSIONS

Basing on docking results obtained with Molegro Virtual Docker it was established that all enkephalin analogues have good binding affinity to δ-opioid receptor. All of the ligands interact by forming many H-bonds with the receptor. Additional interaction between receptor and ligand appears in the case of analogues substituted with amino acid containing SO_2NH_2 group. This study

could not give a definitive answer if the 3D model of DOR corresponds to the real receptor, because there is no correlation between values obtained *in vitro* and docking results.

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КОМПЮТЪРНО МОДЕЛИРАНЕ НА ВЗАИМОДЕЙСТВИЕТО ЛИГАНД-РЕЦЕПТОР – ЕНКЕФАЛИНОВИ АНАЛОЗИ И ДЕЛТА-ОПИОИДЕН РЕЦЕПТОР

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(Резюме)

След определяне на ендогенните опиоидни пептиди – енкефалини от Hughes, са синтезирани голям брой техни синтетични аналози. Нашата група е синтезирала много и различни аналози на енкефалините. В предишно наше изследване е установена връзката между заместванията във втора позиция в ендогенния енкефалин и тяхната селективност по отношение на δ-опиоидния рецептор.

С цел установяване на връзката структура – биологично действие на енкефалинови аналози и δ-опиоидният рецептор е използван докинг. Тъй като липсват кристалографски данни за структурата на δ-опиоидният рецептор, използвахме публикуваният в PBD (id: 1ozc) модел. Използван е MolDoc SE алгоритъм, който лежи в основата на Molegro Virtual Docker. Като лиганди бяха използвани енкефалинови аналози с промяна във втора позиция, за които има данни от *in vitro* изследвания.

В резултат на докинга с получени следните резултати: 1) всички енкефалинови аналози се свързват добре с δ-опиоидния рецептор, като образуват много водородни връзки; 2) подреждането на производните, получено с помощта на докинга, е различно от подреждането им при *in vitro* тестовете. Тези резултати изискват по-нататъшна оптимизация на процедурата за докинг, както и промяна на алгоритъмът и софтуера.