ISSN 1993-6842 (on-line); ISSN 0233-7657 (print)
Biopolymers and Cell. 2023. Vol. 39. N 1. P 24–32
https://doi.org/10.7124/bc.000A87

UDC 577.152.6

Computational Modeling of the Nanocomposite Complex of EMAP II Cytokine with TiO_2 Nanoparticles

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Aim. Computational modeling of the complex of endothelial monocyte activating polypeptide II (EMAP II) with TiO_2 nanoparticle. Since the environment of malignant cells may be characterized by a low pH, TiO_2 nanoparticles will be able to release the bound compound under the pH changes. This allows considering the TiO_2 nanoparticles as means for the targeted EMAP II delivery. **Methods.** Computational modeling of the complexes of EMAP II with TiO_2 using molecular docking approach, characterization of complexes. **Results.** Spatial structures of the complexes of EMAP II protein with TiO_2 nanoparticles have been modeled and analyzed. The results obtained by EMAP II cytokine docking against a 5 nm TiO_2 nanoparticle indicated that TiO_2 nanoparticles are likely to prevent the formation of aggregates by blocking the unstructured ^{34}DVGEIAPR^{41} loop and the hydrophobic tryptophan "pocket" in EMAP II structure. Also, TiO_2 nanoparticles are likely to reduce the conformational flexibility of EMAP II molecule by involving a significant part of amino acid residues in the formation of the nanocomposite complex. **Conclusion.** In the complex of EMAP II cytokine with 5 nm TiO_2 nanoparticles, the binding of TiO_2 to areas on the protein surface responsible for the formation of protein aggregates can prevent the aggregation and stabilize the structure of EMAP II in solution.

Keywords: cytokine, EMAP II, nanoparticle, titanium dioxide, TiO_2, computational modeling, molecular docking.

Introduction

Protein drugs represent a unique and versatile class of biotherapeutic agents with high biological activities and specificities. This rapidly developing biotechnology makes it possible to produce various proteins on a large scale and in a reproducible manner. Some proteins have shown high antitumor activity and have become the alternatives to cytotoxic chemotherapeutic agents for cancer treatment. At the present stage, the development of new stable

non-toxic drugs of targeted action based on recombinant proteins is required [1]. One of the most promising areas of targeted therapy is the use of antiangiogenic and proapoptotic proteins in the form of nanocomposite complexes, which can specifically target tumor cells and inhibit pathological processes [2].

Among a fairly wide range of biological applications of nanoparticles, in particular TiO_2 nanoparticles, one of the most promising is their use for targeted delivery of biologically active substances [3,4]. This is due to a number of properties inherent in nanoparticles, in particular, the ability to accumulate several or many molecules of bioactive substance on their surfaces depending on the ratio of sizes of nanoparticle and bound molecule. A wide range of substances are capable of interacting with the nanoparticle surface — from low molecular weight organic compounds to large protein molecules. The presence of a certain selectivity for target cells and the ability to penetrate these cells, as well as the ability to release bound substance as a result of changes in environmental cell conditions, in particular in response to changes of extracellular pH, makes it promising to use TiO_2 nanoparticles for targeted delivery of antitumor drugs giving the fact that the environment inside the malignantly transformed cells is usually characterized by low pH values. Several "smart" stimuli-sensitive drug delivery systems responding to certain stimuli like pH, temperature, and magnetic force were proposed [4]. It is well known that the acidic extracellular pH is a major feature of tumor tissue and extracellular acidification is primarily considered to occur due to lactate secretion from anaerobic glycolysis. Tumors have successfully adapted to acidic pH and use it for cellular activation, this increases drug resistance and leads to more aggressive behavior [5]. Therefore, an anticancer protein delivery system should retain the drug at pH 7.5 but release it at acidic pH 5.0–6.0. The nanosized protein delivery system can be internalized by tumor cells through the endocytosis pathway, thus improving effective release of the anticancer drug in the tumor tissue [6].

Endothelial monocyte-activating polypeptide II (EMAP II) is a cytokine that shows antitumor and anti-inflammatory activity, it participates in angiogenesis, embryogenesis, and some pathological processes, and induces apoptosis of endothelial cells [7–10]. Previously the tendency of this polypeptide to form aggregates of different sizes in solution was shown, even at room temperature [11]. This tendency increases with increasing temperature, which leads to a constant increase in the size of the aggregates [11, 12]. In addition to the targeted delivery, TiO_2 nanoparticles can potentially be used as a stabilizer and to counteract the aggregation of EMAP II.

The data on the spatial structure of complexes obtained by computer simulation are quite important for understanding the mechanisms of interaction of EMAP II with various nanoparticles. In this article, we present the study of the interaction of TiO_2 nanoparticles with antitumor cytokine EMAP II using the molecular docking method and analysis of the complexes.

Materials and Methods

Modeling of the spatial structure of TiO_2 nanoparticles

The spatial structure of TiO_2 nanoparticles with a diameter of 5 nm has been reconstructed. The nanoparticle modeling procedure was

as follows: an elementary lattice of anatase crystal (one of the natural polymorphic modifications of titanium dioxide) was multiplied to fill a cubic space of 5.1x5.1x5.1 nm to create a 5 nm titanium dioxide nanoparticle. The procedure of construction and multiplication of the crystal was performed using VESTA software (Visualization for Electronic and Structural Analysis) [13]. In the center of the prepared cubic nanoparticle, a region with a diameter of 5 nm was limited, after which the atoms outside the selected sphere were removed. Therefore we obtained a spherical TiO_2 nanoparticle with a diameter of 5 nm.

Modeling of the complex of EMAP II cytokine with TiO_2 nanoparticle

The EMAP II spatial structure determined by X-ray diffraction analysis and deposited in the Protein Data Bank spatial structure database (PDB, https://www.rcsb.org/) was used for the docking procedure. Since the EMAP II structure in the PDB base is deposited in the absence of hydrogen atoms, the corresponding protonation and optimization of the protein structure were performed using the UCSF Chimera software package [14].

The spatial structure of the complex was simulated using the PATCHDOCK web server [15]. The server's working algorithm consists of three stages:

1. Molecular Shape Representation — the computation of the molecular surface with the following detection of geometric "patches" (concave, convex and flat surface areas) by a segmentation algorithm. After filtration, only the patches with "hot spot" are chosen.

2. Surface Patch Matching is carried out using a combination of Geometric Hashing and Pose-Clustering matching techniques. The patches detected on the surface of the receptor in the previous step are matched with correspondent matches on the surface of the ligand. Concave patches are matched with convex and flat patches with any type of patches.

3. Filtering and Scoring — the candidate complexes from the previous step are examined. All complexes with unacceptable penetrations of the atoms of the receptor to the atoms of the ligand are discarded and the remaining candidates are ranked according to a geometric shape complementarity score [16].

A complete scan and analysis of the regions of the interacting components were performed. Electrostatic potentials on the surface of the receptor and ligand were calculated, resulting in about 200 complexes with the calculated energy of interaction between the nanoparticle and the protein. Estimation of interaction energy was performed using PATCHDOCK ACE score (Atomic contact energy). Atomic Contact Energy is an atomic desolvation energy measure that is defined over the energy of replacing a protein-atom/water contact, with a protein-atom/ligand-atom contact. The pre-determined score of effective contact energy between atom type i and type j is defined as

$$T[i,j] = -\ln \ln \frac{\frac{N_{i,j}}{C_{i,j}}}{\left(\frac{N_{i,0}}{C_{i,0}}\right) \times \left(\frac{N_{i,0}}{C_{j,0}}\right)}$$

where type 0 corresponds to the solvent. The number of i-j contact ($N_{i,j}$) and the number of i-0 contact ($N_{j,0}$) are estimates of the actual contact numbers of known complexes. In addition, $C_{i,j}$ and $C_{i,0}$ are defined as the expected numbers of ij contact and i-0 contact.

For a given configuration, the ACE score is a summation of each of the atom pairs (one from each subunit) within threshold distance *d*. If the sets of atoms from the two subunits are denoted as S_1 and S_2, respectively, then the ACE is computed as

$$E_{ACE} = \sum_{s \epsilon S_1,\ t \epsilon S_2\ ||s-t||,\ \leq d} T\,[s,t]$$

where $|s-t|$ is the Euclidean distance between *s* and *t*, and $T[s,t]$ is the pre-determined score of the atom pair *s* and *t*. The ACE score can be considered an estimate of the change in desolvation energy of the two molecules in going from the unbound state to the complex. A lower ACE value implies a lower (and hence more favorable) desolvation free energy [16]. Highly separate improvement and optimization of the structure of the complexes were carried out using Mod Refiner program [16]. The final verification of the models of macromolecule structures was performed using MolProbity program [17]. Visualization and analysis of the spatial structure of complexes were performed using UCSF Chimera software [14].

Results and Discussion

Modeling of the spatial structure of TiO_2 nanoparticles

The spatial structure of titanium dioxide nanoparticle modeling was performed using the software package VESTA (Visualization for Electronic and Structural Analysis). To create an elementary cell of TiO_2 crystal (Fig. 1A), the parameters of crystal length (A, B, C) and angles (α, β, γ) were entered according to the parameters of the anatase lattice (A = B = 3.785, C = 9.514, ∠α = ∠β = ∠γ = = 90°), where A, B, C are the lengths of the crystal lattice, and ∠α, ∠β, ∠γ are the dimensions of the angles of the crystal. The type of crystal lattice I_{41} / AMD was chosen as the spatial group of the crystal.

The next step was to expand the elementary cell TiO_2 in the directions of the XYZ

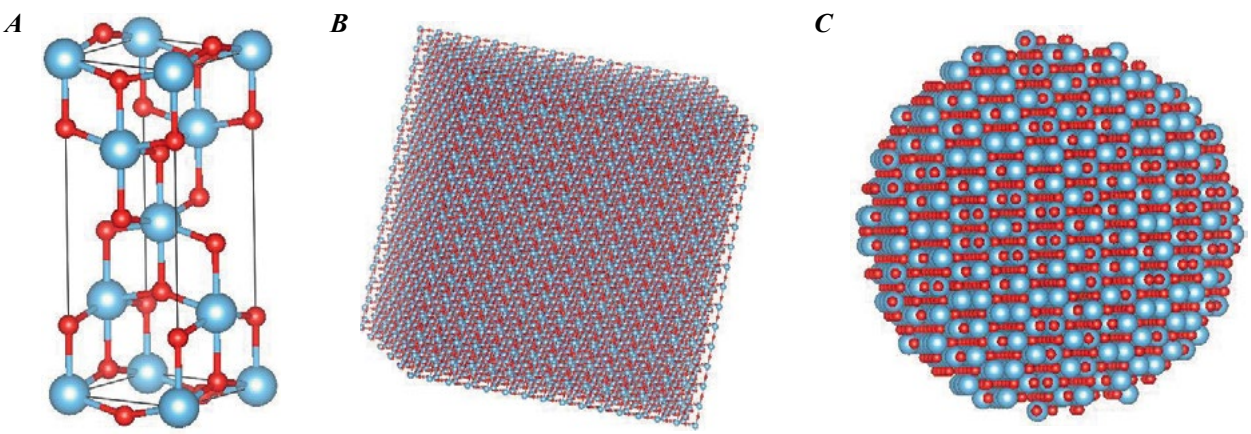

Fig. 1. Crystalline structure of TiO_2 (A) — Elemental cell of anatase crystal (TiO_2), (B) — Multiple elementary TiO_2 cell expanded in the directions of the XYZ plane by 5 nm, (C) — Spherical TiO_2 nanoparticle of 5 nm. Titanium atoms are shown in blue and oxygen atoms in red.

planes by 5 nm (Fig. 1B). We used the VESTA Boundary algorithm to construct additional cells and calculate the corresponding coordinates. The final step was to limit the spherical region with a diameter of 5 nm in the multiplied section of TiO_2, followed by the removal of atoms outside the selected sphere. As a result we obtained a TiO_2 nanoparticle with a diameter of 5 nm (Fig. 1C).

EMAP II – TiO_2 nanoparticle complex modeling

We performed molecular docking of the crystallographic structure of EMAP II protein (PDB code 1EUJ) against the TiO_2 nanoparticle molecule. As a result of modeling the interaction of TiO_2 nanoparticles with EMAP II cytokine, about 200 complexes with different contact interfaces and interaction energy were obtained. The next step was the selection of complexes according to the interaction energy of the protein and the nanoparticle, contact interface area and overall score of the solution. The range of ACE score values for all models varies from — 222.84 to — 396.27 kJ/mol. Analysis of the obtained complexes showed that the formation of a protein complex with a negatively charged TiO_2 nanoparticle occurs mainly with two contact interfaces on the surface of EMAP II molecule. In one case (complex 1) the cytokine motif ^{6}VSRLDLRIGCIIT^{18} was involved in the complex formation together with the unstructured ^{34}DVGEIAPR^{41} loop (Fig. 2), and in the other (complex 2) the hydrophobic environment of the aromatic residue Trp125 (tryptophan "pocket") was involved in complex formation (Fig. 3).

In the complex 1 (Fig. 2) with the surface of 5 nm nanoparticles TiO_2, 15 amino acid residues interact with protein: Arg12, Gly14, Cys15, Ile17, Thr18, Arg20, Asp34, Gly36, Ile38, Ala39, Pro40, Asn58, Val100, Pro101 and Gly102. The ACE of the complex is — 393.85 kJ/mol with an approximate interaction interface area of 36.496Å^2

In the complex 2 (Fig. 3) the surface of 5 nm TiO_2 nanoparticles interact with 13 amino acid residues: Asn49, Ser86, Pro87, Glu88, Trp125, Glu126, Gln129, Pro130, His133, Asn162, Gly164 and Lys166. The ACE of the complex is — 396.27 kJ/mol with an approximate interaction interface area of 42.717Å^2

We previously studied the aggregation properties of the EMAP II cytokine in solution [11]. It was found that the key sites contributing to the formation of protein aggregates in solution are ^{34}DVGEIAPR^{41} site and hydrophobic tryptophan "pocket" [11]. Blocking of these sites leads to a significant reduction in aggregation properties. The obtained results of the docking of cytokine EMAP II against a 5 nm TiO_2 nanoparticle indicate that it is likely that TiO_2 nanoparticles may be able to inhibit the formation of the aggregates by blocking the unstructured ^{34}DVGEIAPR^{41} loop and the hydrophobic tryptophan "pocket" at the EMAP II surface, which are involved in the aggregation process.

As known, EMAP II cytokine is highly homologous to the C-terminal non-catalytic domain of mammalian tyrosyl-tRNA synthetase [18]. The C-terminal module after proteolytic cleavage from the catalytic mini-TyrRS also displays the cytokine functions similarly to EMAP II [19–20]. Both EMAP II and the C-terminal module of TyrRS are potential protein drugs for anticancer therapy in terms of their proapoptotic and antiangiogenic activities.

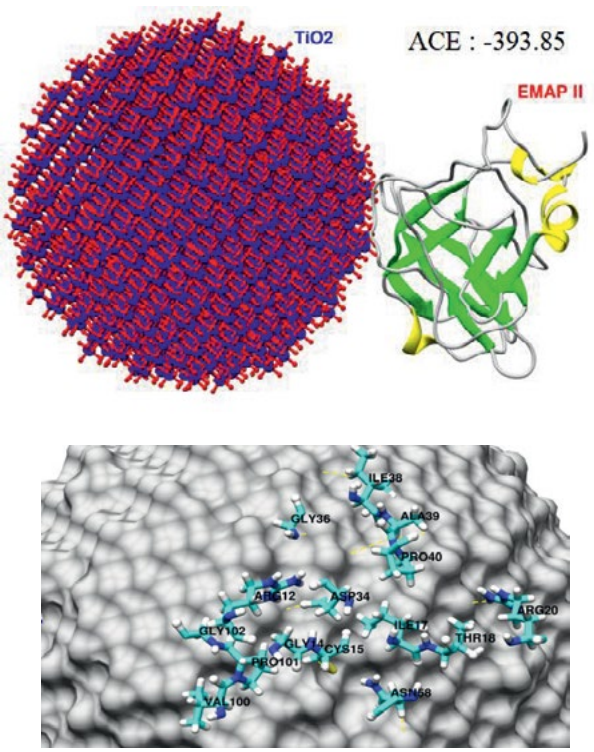

Fig. 2. Structural organization of the complex 1 of TiO_2 nanoparticle with EMAP II and amino acids residues involved in the complex formation.

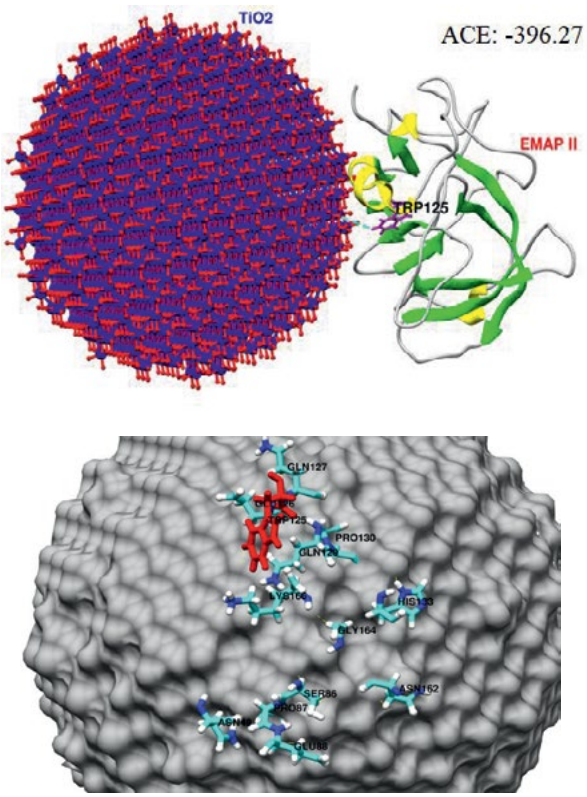

Fig. 3. Structural organization of the complex 2 of TiO_2 nanoparticles with EMAP II and amino acids residues involved in the complex formation.

Earlier, Autodock 4.0.5 program was used in order to evaluate the interaction of titanium dioxide nanoparticles with different proteins [21]. The analysis of docked structures was performed with regard to the most efficient binding with amino acids without any TiO_2 surface modification. The higher negative binding energy revealed strong binding of titanium dioxide with proteins and more specifically TiO_2 nanoparticles showed frequent interaction with positively charged R-group, nonpolar aliphatic R-group, aromatic R-groups, polar uncharged R-groups and negatively charged R-group-containing amino acids [21]. Our data for TiO_2 nanoparticle complex with EMAP II correlate well with these Autodock data.

Currently, modern protein-based nanotechnologies offer novel perspective biomedical complexes with unique physicochemical properties for targeted delivery and sustained release of therapeutics at the site of action [3–5, 22, 23]. The pH-induced "smart" drug delivery systems have been greatly developed in recent years, leading to the controllable release of antitumor proteins at the tumor sites since the acidic extracellular pH is a major feature of tumor tissue [22, 23]. pH-responsive nanocomposite composed of self-assembled daunoru-

bicin and TiO_2 nanoparticles was developed for a "smart" stimuli-sensitive drug delivery system [22]. Daunorubicin release from the drug delivery system was significantly accelerated by decreasing pH from 7.4 to 5.0, which is of particular interest to cancer therapy due to the acidic extracellular tumor environment [22]. Application of daunorubicin-TiO_2 nanocomposites resulted in the accumulation of the anticancer drug in tumor cells and induced caspase-dependent apoptosis, enhancing anticancer activity [22]. All these data demonstrate that the use of novel pH-responsive systems based on TiO_2 nanoparticles may be a very promising strategy for the clinical application of anticancer proteins.

Conclusions

Structural modeling of the complexes of TiO_2 nanoparticles with the cytokine EMAP II by molecular docking was performed. Analysis of these complexes showed that the formation of a protein complex with TiO_2 nanoparticle occurs mainly with two contact interfaces on the surface of EMAP II — a part of the cytokine motif ^{6}VSRLDLRIGCIIT^{18} together with an unstructured loop ^{34}DVGEIAPR^{41} in one case and with the microenvironment of Trp125 in another complex.

Our results indicate that TiO_2 is likely to reduce the conformational mobility of the EMAP II molecule, by involving a significant portion of amino acid residues in the formation of the complex, which will stabilize the protein structure, and most importantly, the nanoparticle blocks the sites on the protein surface which are responsible for the formation of protein aggregates. A novel nanocomposite complex of antitumor EMAP II cytokine and TiO_2 nanoparticles is able to expand the possibilities of EMAP II in biomedical applications.

Funding

This work was supported by the Complex program of scientific research of the National Academy of Sciences of Ukraine "Mathematical modeling in interdisciplinary research of processes and systems based on intelligent supercomputer, grid and cloud technologies" (0121U110903).

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Комп'ютерне моделювання комплексу цитокіна EMAP II з наночастинками TiO_2

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Мета. Комп'ютерне моделювання комплексу ендотеліального моноцит — активуючого поліпептиду II (EMAP II) з наночастинками TiO_2. Оскільки середовище злоякісних клітин може характеризуватися низьким pH, наночастинки TiO_2 зможуть вивільняти зв'язані сполуки при зміні pH. Це дозволяє розглядати наночастинки TiO_2 як засоби цільової доставки EMAP II до пухлин. **Методи.** Комп'ютерне моделювання комплексів EMAP II з TiO_2 із використанням молекулярного докінгу та характеристика комплексів. **Результати.** Проведено моделювання та проаналізовано тривимір-

ні структури комплексів білка EMAP II з наночастинками TiO_2. Результати докінгу поліпептиду EMAP II проти наночастинок TiO_2 розміром 5 нм показали, що наночастинки TiO_2 при зв'язуванні з білком, ймовірно, запобігають утворенню білкових агрегатів, блокуючи неструктуровану петлю ^{34}DVGEIAPR^{41} і гідрофобну триптофанову «кишеню» на поверхні EMAP II. Крім того, наночастинки TiO_2, ймовірно, зменшують конформаційну рухливість макромолекули EMAP II, залучаючи значну частину амінокислотних залишків до утворення нанокомпозитного комплексу. **Висновок.** У нанокомпозитному комплексі TiO_2 з цитокіном EMAP II зв'язування TiO_2 з ділянками на поверхні білка, які відповідають за утворення білкових агрегатів, може перешкоджати агрегації та стабілізувати структуру EMAP II у розчині.

Ключові слова: цитокін, EMAP II, наночастинка, діоксид титану, TiO_2, комп'ютерне моделювання, молекулярний докінг.

Received 30.01.2023