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Molecular Structure and Docking Analysis of the Integrin Inhibitor Cyclo (Arg-Gly-Asp-D-Phe-Val) Peptide

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Abstract - All possible conformations of Cyclo (Arg-Gly-Asp-D-Phe-Val) ($C_{26}H_{38}N_8O_7$) with antitumor activity were determined and the most stable conformer elucidated by MMFF method using the Spartan06 program. The differences in energy between the conformers were determined, and the low energy conformers, which are required for determination of the biological activity, were characterized. Since as an anticancer candidate, the investigated molecule is expected to be an integrin inhibitor, a molecular docking study was conducted with $\alpha_v\beta_3$ integrin to determine the mechanism of interaction. As a result of docking simulations, the binding affinity (ΔG = -10.5 kcal/mol) and the interactions between the investigated peptide and target integrin were determined.

Keywords: Antitumor activity, Spartan06 program, Integrin inhibitor, Docking simulation

1. Introduction

Integrins are transmembrane receptors that connect the actin cytoskeleton to the extracellular matrix (ECM). Integrins are multifunctional heterodimers that regulate cell-cell and cell-matrix interactions via signaling and are involved in a number of critical functions, including cell cycle and proliferation control, in part through collaboration with growth factor receptors [1]. This connection is dynamically rearranged as a result of mechanical, chemokine, and growth factor signals. The $\alpha_v\beta_3$ integrin functions as a receptor for vitronectin [2], a cell cell-adhesive protein like fibronectin. The domain 10 of module III (III_{10}) of fibronectin comprises the Arginine-Glycine-Asparagine RGD pattern that $\alpha_V\beta_3$ recognizes. For this reason Arg-Gly-Asp (RGD) containing ligands are important for the interactions with integrins. Integrins are heterodimeric transmembrane receptors and mediate cell adhesion. Many cell types' growth and survival have been shown to be dependent on integrin-mediated adherence to extracellular matrix (ECM) protein 1 in recent years [3]. Cell-matrix interactions mediated by integrins control a wide range of biological processes [4]. Cell adhesion, migration, invasion, proliferation, and survival/anoikis are all regulated by integrins' interactions with their substrates, which are also related to tumor growth and metastatic growth [5]. The $\alpha_v\beta_3$ integrin expression in several tumor tissues has strongly suggested that this receptor may have a role in tumor growth, especially in invasive cancers that preferentially metastasize to bone, such as breast and prostate carcinomas [6]. Integrins are being recognized as significant players in tumor biology and may be valuable targets for tumor therapy as more is discovered about the impact of a tumor cell's microenvironment on survival and invasive potential [7]. Recent research has found that $\alpha_v\beta_3$ expression enhances breast cancer spontaneous bone metastasis (Takayama et al., 2005; Sloan et al., 2006), and the functional status of integrin αvβ3 is crucial in many aspects of this process [8].

Following prior research tying its expression to the window of implantation and its absence to infertility problems such as luteal phase insufficiency, endometriosis, hydrosalpinx, and unexplained infertility, the $\alpha_v\beta_3$ integrin has sparked renewed attention [9]. The most common cause of mortality from gynecological illness is ovarian cancer [10]. Women who have ovarian cancer that is solely localized at the time of diagnosis have a good cure rate [11]. However, only around one-fifth of ovarian cancers are identified at this stage, with the majority of cases being discovered after cancer has spread to other parts of the body. Many cancer types, including ovarian cancer cell lines and original tissues from ovarian cancer patients, express $\alpha_v\beta_3$ integrin [12]. There has been a link found between $\alpha_v\beta_3$ integrin expression and ovarian tumor development. The vast majority of tumor vasculature in ovarian carcinomas express this receptor, regardless of v3 expression in the initial tumor [13]. Prostate cancer development has been connected to $\alpha_v\beta_3$ integrin, which has implications for angiogenesis, survival, and invasion. According to the in vitro studies [13,14], integrins helped prostate cancer cells adhere together, migrate through a variety of ECM substrates, and cross the endothelium barrier. The $\alpha_v\beta_3$ integrin receptor, which binds a variety of ligands via an RGD sequence, is prevalent in normal vasculature but is over expressed in tumor vasculature, making it a possible antiangiogenic drug target [15]. The $\alpha_v\beta_3$ integrin expression and activation have also been associated to breast tumors that are more metastatic and aggressive [16]. When $\alpha_v\beta_3$ activity is suppressed by mAb and cyclic RGD peptides, endothelial mortality, angiogenesis suppression, and enhanced endothelial monolayer permeability have all been observed. Inhibiting $\alpha_v\beta_3$ activity has been associated to decreased tumor development in breast cancer xenografts and melanoma xenografts [17].

In this research, the most stable conformation of the Cyclo (Arg-Gly-Asp-D-Phe-Val) peptide was firstly determined. Afterwards, by molecular docking simulations, the binding affinity and the interaction modes of the most stable conformer with $\alpha_v\beta_3$ integrin were revealed.

2. Methods and Calculations

Conformational analysis was performed by using The Spartan06 software [18] and the MMFF technique [19]. To determine the probable binding sites on the surface of the receptor, The CAVER software [20] was used. Molecular docking investigations were performed using AutoDock-Vina software on the identified active sites [21].

3. Results and Discussions

3.1. Structure

As a result of conformational analysis five lowest energy conformers of Cyclo (Arg-Gly-Asp-D-Phe-Val) were determined. The relative energies of these most stable conformers are given in Table 1 and their molecular structures are shown in Figure 1.

Table 1. The energies of the most stable five conformations of Cyclo (Arg-Gly-Asp-D-Phe-Val) peptide.

Conformers	Total energy (kj/mol)	Relative energy (kj/mol)
(I)	-816.4	0
(II)	-814.2	2.2
(III)	-812.61	3.79
(IV)	-812.53	3.87
(V)	-811.45	4.95

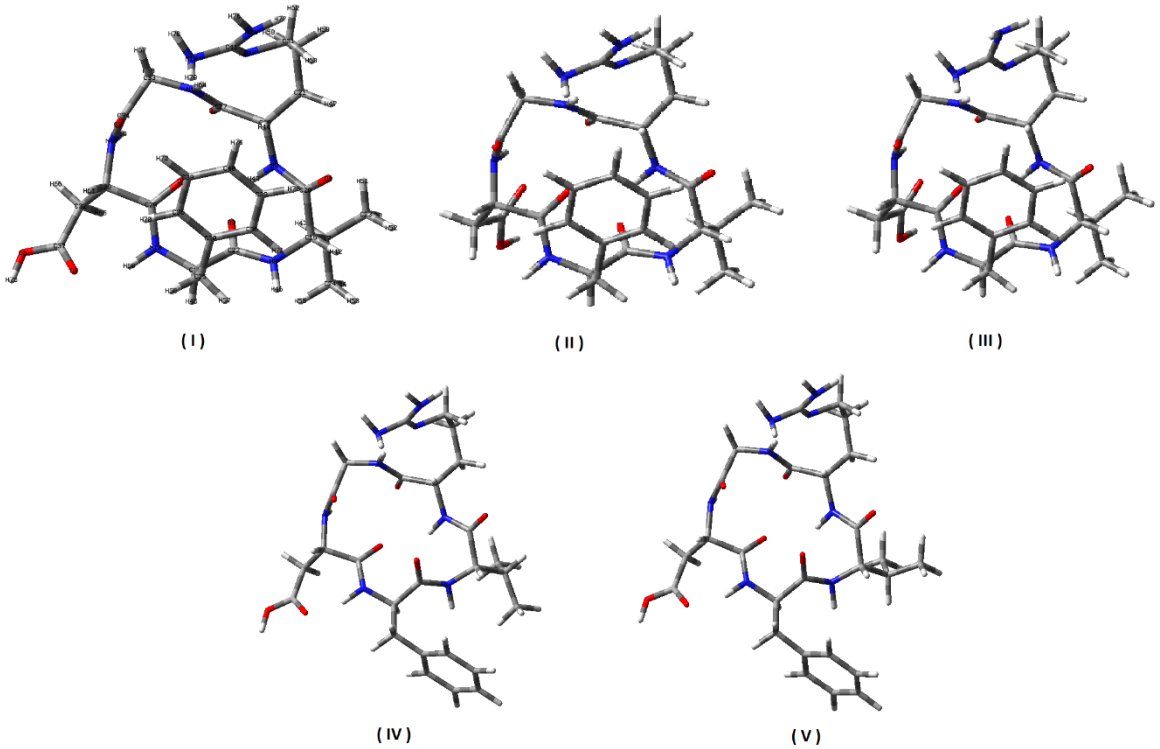

Figure 1. The molecular structures of the obtained five lowest energy conformers of Cyclo (Arg-Gly-Asp-D-Phe-Val) by conformational analysis.

3.2. Molecular Docking

Molecular docking simulations in target protein $\alpha_v\beta_3$ integrin were calculated to evaluate the anti-proliferation effect of the Cyclo (Arg-Gly-Asp-D-Phe-Val) for the anticancer activity. After preparing the $\alpha_v\beta_3$ integrin (PDB ID: 1JV2) for molecular docking [22], docking simulations of Cyclo (Arg-Gly-Asp-D-Phe-Val) to the $\alpha_v\beta_3$ integrin for the most active site were performed with a binding affinity of -10.5 kcal/mol. The 3D docked view of Cyclo (Arg-Gly-Asp-D-Phe-Val) at the most active region of $\alpha_v\beta_3$ integrin is shown in Figure 2. The Cyclo (Arg-Gly-Asp-D-Phe-Val) - $\alpha_v\beta_3$ integrin complex interaction diagrams are also shown. The revealed interactions between Cyclo (Arg-Gly-Asp-D-Phe-Val) ligand and the target protein $\alpha_v\beta_3$ integrin were as follows:

Between Cyclo (Arg-Gly-Asp-D-Phe-Val) and the amino acid residues of Val23, two hydrogen bonds with 2.41, 3.1Å length, and a unfavorable donor-donor interaction with 2.3Å length; Val98, hydrogen bonds with 1.97 and 2.49 Å length; Arg99, 1.63 and 2.49 Å long unfavorable donor-donor interaction and 1.92 Å long hydrogen bond; Ile161, 3.53 Å long carbon hydrogen bond; Val226, 2.26 Å long hydrogen bond; Ala343, 5.23 Å long Pi-alkyl interaction; Ile344, 2.67 Å long hydrogen bond; Pro346, 1.93 Å long hydrogen bond; Met408, 2.48 Å long hydrogen bond; Gly410, 2.76 Å long hydrogen bond are defined.

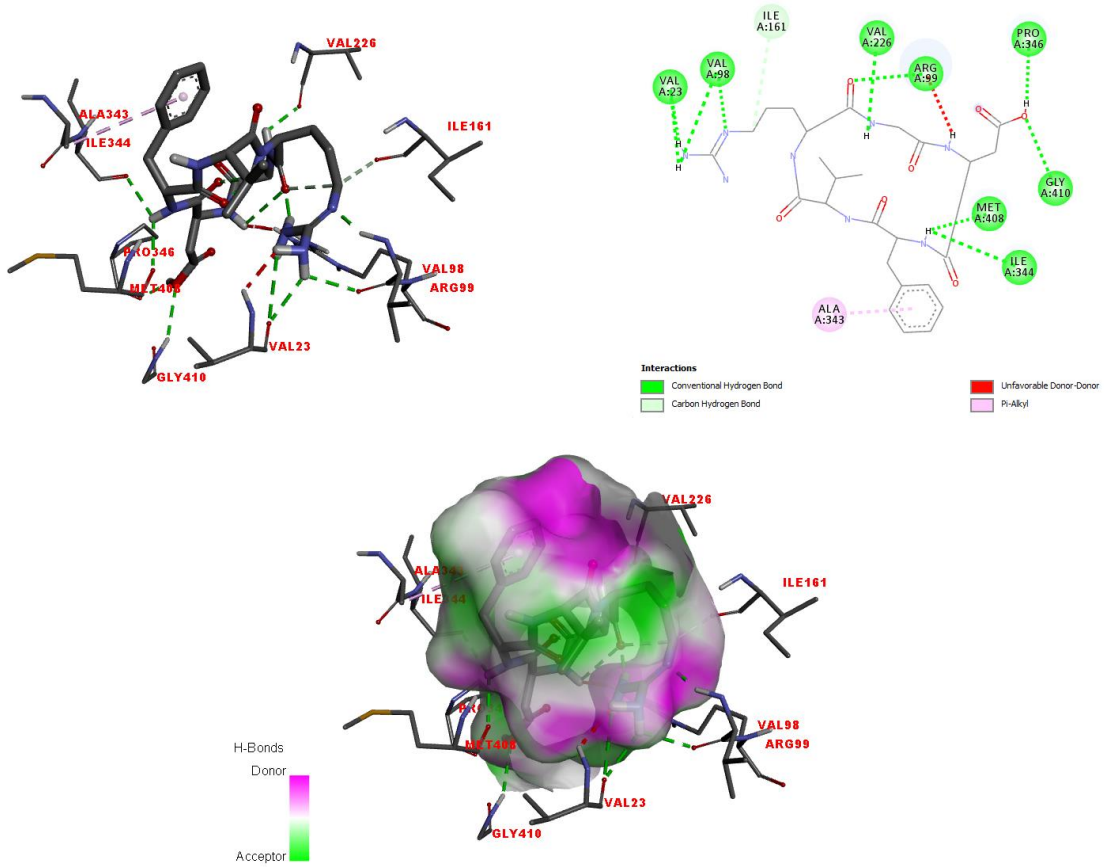

Figure 2. The 3D docked views of the most stable conformer of Cyclo (Arg-Gly-Asp-D-Phe-Val) in active site of $\alpha_v\beta_3$ integrin (-10.5 kcal/mol).

4. Conclusions

The most stable conformer of cyclic pentapeptide, Cyclo(Arg-Gly-Asp-D-Phe-Val), was determined due to the importance of the function-activity relationship for the bioactive molecules. The interaction of the title molecule with $\alpha_v\beta_3$ integrin was investigated by docking simulations, by using the most stable conformer of the Cyclo(Arg-Gly-Asp-D-Phe-Val), to assess its biological activity and to examine the inhibitory effect on integrin. The binding affinity of cyclo (Arg-Gly-Asp-D-Phe-Val) to $\alpha_v\beta_3$ integrin was found to be -10.5 kcal/mol. According to molecular docking predictions, Cyclo (Arg-Gly-Asp-D-Phe-Val) found to have a good inhibitory effect on $\alpha_v\beta_3$ integrin and may exhibit significant anti-tumor properties.

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