Characteristic Properties of Genipin as an Activator in Neuronal Nitric Oxide Synthase

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The present investigation was undertaken to compare the structural and electronic properties of genipin with those of tetrahydrobiopterin (H_4B), an essential cofactor in neuronal nitric oxide synthase (nNOS), and 4-aminotetrahydrobiopterin (4-amino-H_4B), an inhibitor of nNOS, using computer-assisted molecular modeling techniques. Molecular modeling, superimposing, and docking simulation, in addition to LUMO-energy calculation, revealed that genipin has structural and electronic properties that markedly resemble those of H_4B.

Key words —— genipin, tetrahydrobiopterin, 4-aminotetrahydrobiopterin, molecular modeling, neuronal nitric oxide synthase

INTRODUCTION

Iridoid compounds are known to possess various biological activities, such as antimicrobial,[1] anticancer,[2] hemodynamic,[3] choleretic,[4] and hepatoprotective effects.[5] In previous studies, we have shown that iridoid compounds, such as geniposide, gardenoside, catalpol, aucubin, and their aglycones, have neuritogenic activity in PC12 h cells,[6] which are a subclone of rat pheochromocytoma cells.[7] Genipin, the aglycon of geniposide, is a particularly potent inducer of neurite outgrowth. Our subsequent investigations of this series have shown that genipin also increased survival in Neuro2a cells, a neuronal cell line, exposed to serum deprivation-induced cytotoxicity,[8] and markedly induced neurite outgrowth through a nitric oxide synthase (NOS)-guanosine 3′,5′-cyclic monophosphate dependent protein kinase pathway followed by extracellular signal-regulated kinase (ERK) activation in rat pheochromocytoma PC12 h cells.[9,10] It is possible that neuronal NOS (nNOS) is the target molecule for the neuritogenic action of genipin. Therefore, we hypothesized that genipin may directly bind to and activate nNOS, as well as possess neurotrophic factor-like activity, similar to that of nerve growth factor or other neurotrophins.[11]

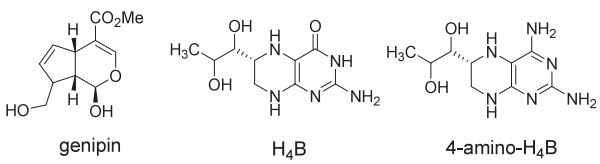

Fig. 1. Structures of Genipin, H_4B and 4-amino-H_4B

As we were interested in studying the characteristic properties of genipin as an activator of nNOS, the present investigation was undertaken to compare the structural and electronic properties of genipin with those of tetrahydrobiopterin (H_4B) and 4-aminotetrahydrobiopterin (4-amino-H_4B) using computer-assisted molecular modeling techniques (Fig. 1).

MATERIALS AND METHODS

Calculations of molecular orbital and molecular electrostatic potentials were performed using the PM5 method of the MOPAC program. Construction of the molecules, superimposition, and docking simulation were performed using a Scigress Explorer ver. 7.5 parameter set developed by Fujitsu Co. (Chiba, Japan). X-ray crystallographic data for H_4B and 4-amino-H_4B in complex nNOS were obtained from Protein Data Bank ID: 1QW6.[12]

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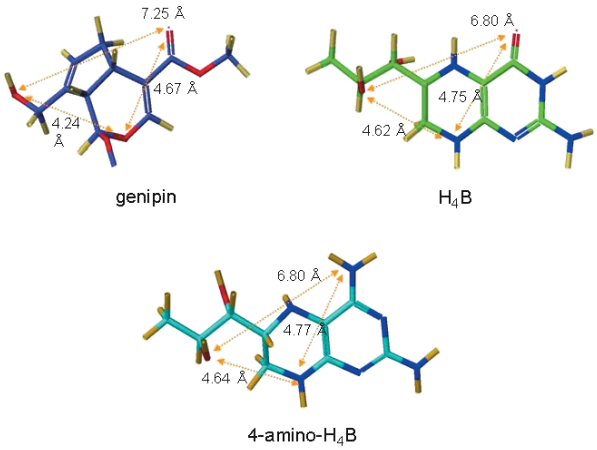

Fig. 2. 3D Structures of the Most Stable Conformers of Genipin, H_4B and 4-amino-H_4B

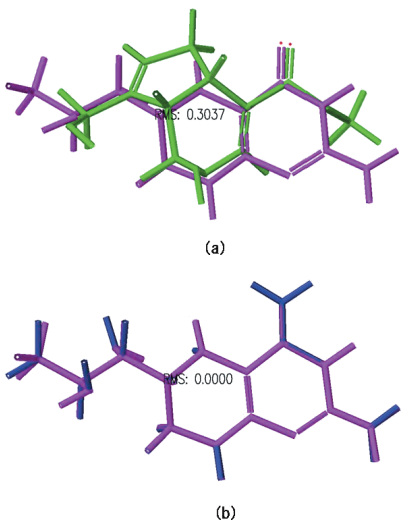

Fig. 3. Superimposition Profiles

(a) Superimposition of genipin on H_4B pharmacophore; (b) Superimposition of H_4B on 4-amino-H_4B pharmacophore.

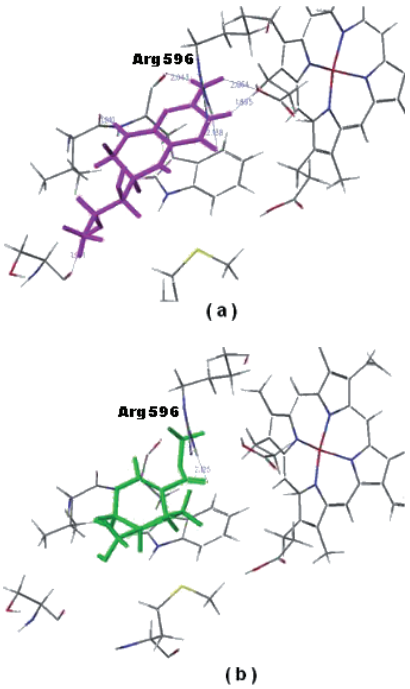

Fig. 4. Docking Profiles

(a) X-ray crystallographic data for H_4B in complex nNOS; (b) Binding conformation of genipin docked inside the binding site in nNOS.

RESULTS AND DISCUSSION

Molecular Modeling and Superimposing Experiments with Genipin, H_4B and 4-amino-H_4B

On the basis of the lowest energy conformation models of genipin, H_4B and 4-amino-H_4B, we found common pharmacophors that consisted of three possible hydrogen bond regions (genipin: oxygen at 2 position, carbonyl oxygen of methoxycarbonyl group at 4 position, oxygen of hydroxymethyl group at 8 position; H_4B: oxygen of carbonyl group at 4 position, nitrogen at 8 position, oxygen of 2-hydroxylpropyl group at 6 position; 4-amino-H_4B: amino nitrogen at 4 position, nitrogen at 8 position, oxygen of 2-hydroxylpropyl group at 6 position), as shown in Fig. 2. Molecular superimpositions upon the three computer-generated lowest energy conformers were generated by fitting each conformer. The fit of genipin to H_4B in its pharmacophoric conformation gave a root mean square fit of 0.3 Å and that of H_4B to 4-amino-H_4B gave a root mean square fit of 0 Å (Fig. 3). Molecular modeling and superimposing experiments showed that genipin was structurally very similar to H_4B and 4-amino-H_4B, indicating a close degree of overlap in their pharmacophoric conformations.

Docking Simulation of Genipin and H_4B with nNOS

Each crystal structure of H_4B and 4-amino-H_4B in complex nNOS revealed the location of both binding sites of nNOS.[13] Figure 4(a) shows x-ray crystallographic data for H_4B in complex nNOS. Adopting this conformation, genipin was computer docked inside the H_4B binding site of nNOS [Fig. 4(b)]. The results indicated that the ester carbonyl oxygen at the 4 position of genipin and the Arg 596 main chain nitrogen in nNOS were located within a hydrogen-bond distance of 2.1 Å.

Electronic Features of Genipin, H_4B, 4-amino-H_4B, and Some nNOS Inhibitors (N^{ω}-propyl-*L*-Alg, W1400, AR-R17477)

It is interesting that genipin and H_4B act as nNOS activators, while 4-amino-H_4B acts as an nNOS inhibitor, even though these compounds were found to have marked structural similarities in the above experimental data. Thus, calculations of the HOMO- and LUMO-energy of genipin, H_4B and 4-amino-H_4B, in addition to some known nNOS inhibitors (N^{ω}-propyl-*L*-Alg, W1400, AR-R17477), were carried out using the PM5 method in the MOPAC program (Table 1). A plot of the HOMO-energy versus the LUMO-energy for these compounds is shown in Fig. 5. These compounds were classified into inhibitors and activators in nNOS, according to whether their LUMO-energy was above or below −0.3 eV, even though the characteristics of their HOMO-energy were not unique. This result supports the possibility that genipin and H_4B may interact with a more electronegative receptor binding site than do 4-amino-H_4B and other known NOS inhibitors.

Table 1. Calculated Parameters for Genipin, H_4B, 4-amino-H_4B and Other nNOS Inhibitors

	HOMO-Energy (eV)	LUMO-Energy (eV)
H_4B	−8.229	−0.497
Genipin	−9.686	−0.573
N^{ω}-propyl-*L*-Alg	−9.518	−0.138
W1400	−9.170	0.347
AR-R17477	−8.965	−0.208
4-amino-H_4B	−8.068	0.089

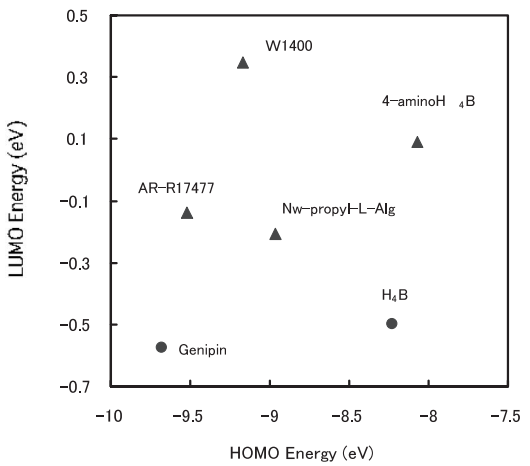

Fig. 5. Parameters Calculated by PM5 for Genipin, H_4B, 4-amino-H_4B and Other nNOS Inhibitors

Molecular modeling, superimposing, and docking experiments, in addition to the calculation of LUMO-energy, were performed to characterize the properties of genipin. It was revealed that genipin has structural and electronic properties that closely resemble those of H_4B. We hope to be able to apply the insights gleaned from these studies to design new nNOS activators possessing strong affinity and potent activity.

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