Journal of Molecular Graphics and Modelling 28 (2010) 604–611

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

journal homepage: www.elsevier.com/locate/JMGM

Interaction of brassinolide with essential amino acid residues: A theoretical approach

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ARTICLE INFO

Article history:
Received 11 November 2009
Received in revised form 16 December 2009
Accepted 17 December 2009
Available online 4 January 2010

Keywords:
Brassinosteroids
Brassinolide
Amino acids
Biological activity
Density functional theory

ABSTRACT

The interaction of the most active natural brassinosteroid, brassinolide, with the twenty natural amino acids is studied applying the multiple minima hypersurface method to model the molecular interactions explicitly. The resulting thermodynamic data gives useful information about the amino acids with the greatest association for brassinolide and the stabilities of such complexes. Density functional theory (DFT) optimizations were further carried out to test the performance of semiempirical calculations. Additional calculations with a more accurate DFT method were performed to explore the formation of this type of molecular complexes. The semiempirical geometries and stability order of these complexes are in good agreement with the DFT calculations. Each group of amino acids possesses a preferential zone of interaction with brassinolide, forming the polar-charged amino acids the most stable complexes. This study could contribute to future investigations of the interaction of brassinosteroids with the receptor protein in plants.

1. Introduction

Brassinosteroids (BR) are a group of plant-originated steroidal lactones that promote growth [1]. These molecules are important plant regulators in multiple developmental processes at nanomolar and micromolar concentrations, including cell division, cell elongation, vascular differentiation, reproductive development and modulation of gene expression [2]. They are found at low concentrations throughout the plant kingdom and are widely distributed in both reproductive and vegetative tissues [3,4].

To date, 61 naturally occurring BR have been discovered [3]. Natural brassinosteroids identified so far have a common 5α-cholestan skeleton and their structural variations come from the kind and orientation of the oxygenated functions in rings A and B. These modifications are produced by oxidation and reduction reactions during biosynthesis. All hitherto known native BR possess a 22R,23R diol structural feature in the side chain moiety which is essential for high biological activity [5].

Brassinolide (Br) *[(22R,23R,24S)-2α,3α,22,23-tetrahydroxy-24-methyl-homo-7-oxa-5α-cholestan-6-one]* (see Fig. 1) is the most active brassinosteroid [6]. It has 2α,3α-vicinal hydroxyl groups at the A-ring (Region 1). Moreover, the lateral chain exhibits another diol group with R configuration at C_{22}/C_{23} (Region 2) and 24S methyl substitution. A lactone group in C_6/C_7 (Region 3) is also important for the biological activity of Br.

BR biosynthesis is produced in the endoplasmic reticulum but its recognition occurs outside the cell. Therefore, these phytohormones must move from the inside to the outside of the cell where they are recognized by either the same cell or neighbouring ones [4]. The investigation of BR signalling, which has become a pressing research priority in recent years [7], is related to the study of BR biosynthesis. In plants, BRI1 (brasinosteroid receptor insensitive 1), which is a leucine rich repeat (LRR) receptor kinase localized in the plasma membrane, is a critical component of a receptor complex for BR. The BRI1 gene encodes a receptor serine/threonine kinase with an extracellular domain containing 25 LRR. This domain is interrupted by a 70-amino-acid island domain (ID) located between the 21 and 22 LRR [8]. The analysis of Br binding to BRI1 has provided further pieces of evidence indicating that the extracellular domain of BRI1 recognizes Br. It has also been shown

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doi:10.1016/j.jmgm.2009.12.006

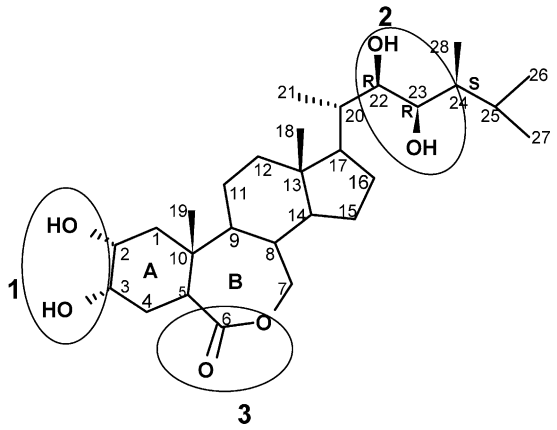

Fig. 1. Structure of brassinolide indicating specific zones of hydrophilic interaction.

that Br-binding activity ceases when the 70-amino acid ID is mutated. In contrast, mutations on the kinase domain of BRI1 have no effects on Br binding. These observations strongly indicate that the 70-amino-acid ID in the extracellular domain of BRI1 is able to detect BR [9]. Despite significant progress in understanding the molecular and cellular effects of BR, key issues about their biological activity and mode of action remain unknown [7]. Since the 3D structure of the brassinolide receptor is not accurately known, the Br docking into the binding site is speculative and indirect methods must be used [10,11]. Knowledge of the interaction of BR with amino acids is undoubtedly essential to understand how the binding of these phytohormones with the BRI1 receptor in plants occurs.

This work deals with the study of the interaction of Br with the essential amino acids as an important step in understanding how the interaction of these phytohormones with the BRI1 receptor protein occurs. This work approaches the Br–amino acid interaction independent of the different cavities where they could be located in the interaction with BRI1.

2. Methodology

In order to explore the conformational space in the interaction of Br with the twenty naturally selected amino acids, the *multiple minima hypersurfaces* (MMH) procedure is applied [12–17]. MMH has been successfully employed in the study of several systems [14,18–24]. This methodology is used to explore the energy hypersurface and subsequently find stationary points of minimum energy which significantly contribute to the thermodynamic properties of the system. This procedure combines quantum mechanical methods for energy calculations with statistical mechanics to obtain thermodynamic quantities related to the molecular association process.

To simulate the electronic density of amino acids in proteins a model system shown in Fig. 2 is used, with R being the lateral chain that determines a given amino acid (aa). In this model, peptide bonds are simulated with methylamine ($NHCH_3$) and acetyl groups ($COCH_3$). To analyze the results, amino acids are classified into five groups according to polarity and the character of the R moiety: non-polar aliphatic, non-polar aromatic, sulphur, polar-uncharged and polar-charged amino acids. Three areas of possible interactions have been indicated in Fig. 2.

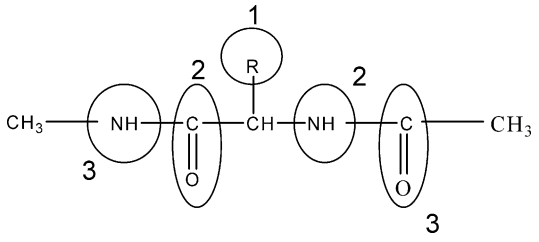

Fig. 2. Example of a blocked amino acid indicating specific zones of interaction.

It has been previously shown by our group that the AM1 semiempirical method [25] effectively reproduces the geometry of natural brassinosteroids and some analogues [24,26]. After having optimized the structure of Br and the 20 amino acids with the AM1 method, a set of 50 random complexes of Br and each aa were generated using the GRANADA program [15], and later optimized with the AM1 method as implemented in the MOPAC v. 6.0 program [27]. The eigenvector following routine for searching minima was used and all convergence thresholds were increased 100 times with respect to the default values. The MMOK option for optimizing the peptidic bonds by means of a local molecular mechanic term was also used because it is better suited to reproduce the planar geometry of the peptidic bond and the experimental values of the rotational barriers [28]. The partition function for each Br–aa system is calculated from the electronic energies of the 50 optimized complexes taking as the reference state the isolated molecules (Br and a given aa). Any thermodynamic property of the association process (Br + aa → Br–aa), *e.g.*, the association energy (ΔE^{assoc}) and Gibbs free energy (ΔG^{assoc}), can be calculated from the partition function of the system [12–14].

The MMH procedure was used to determine the amino acids with the highest absolute association energy for Br. Additional complexes between Br and each of these amino acids were created. AM1 optimizations and frequency calculations were carried out to ensure that all the relevant regions of interaction between Br and the amino acids were explored.

B3LYP is one of the most widely used density functionals in biological systems and still remains a valid and particularly efficient alternative for the "average" quantum chemistry problems [29]. Therefore B3LYP/6-31G geometry optimizations and frequency calculations were performed. There are, however, still some problems with most common density functionals, and is the description of non-bonded interactions in which dispersion plays an important role [29]. The M05-2X density functional [30] has been proved to successfully describe non-covalent interactions for biological systems and is used in this work to test the performance of B3LYP and semiempirical calculations. Then single-point energy calculations using B3LYP and M05-2X density functionals at the 6-311 + G(d) level of theory were further carried out on the B3LYP geometries. These calculations were performed with the Gaussian 03 package [31].

The nomenclature used to identify the complexes is Br($u;v$)–aa($x;y$), where u and v are the Br regions interacting with regions x and y of the given aa. For example, Br(1)–GLU(1) identifies the complex in which region 1 of Br (see Fig. 1) interacts with region 1 (the lateral chain) of glutamic acid (GLU) (see Fig. 2). When a chemical group is specified, the interaction occurs with such a group: *e.g.*, Br(1)OH_2–GLU(1) identifies the complex in which the OH group in position 2 (region 1) of Br interacts with region 1 of GLU.

3. Results and discussion

The hypersurface of the Br–aa complexes was initially explored following the MMH procedure. Examples of AM1 structures of these complexes along with their relative populations are shown in Figs. 3 and S1–S5 of the Electronic Supplementary Material. Table 1 shows the calculated association energy (ΔE^{assoc}) and Gibbs free energy (ΔG^{assoc}) of Br with each aa. The MMH procedure leads to the following Br–aa affinity order: GLU > ASP > ARG > LYS > HIS

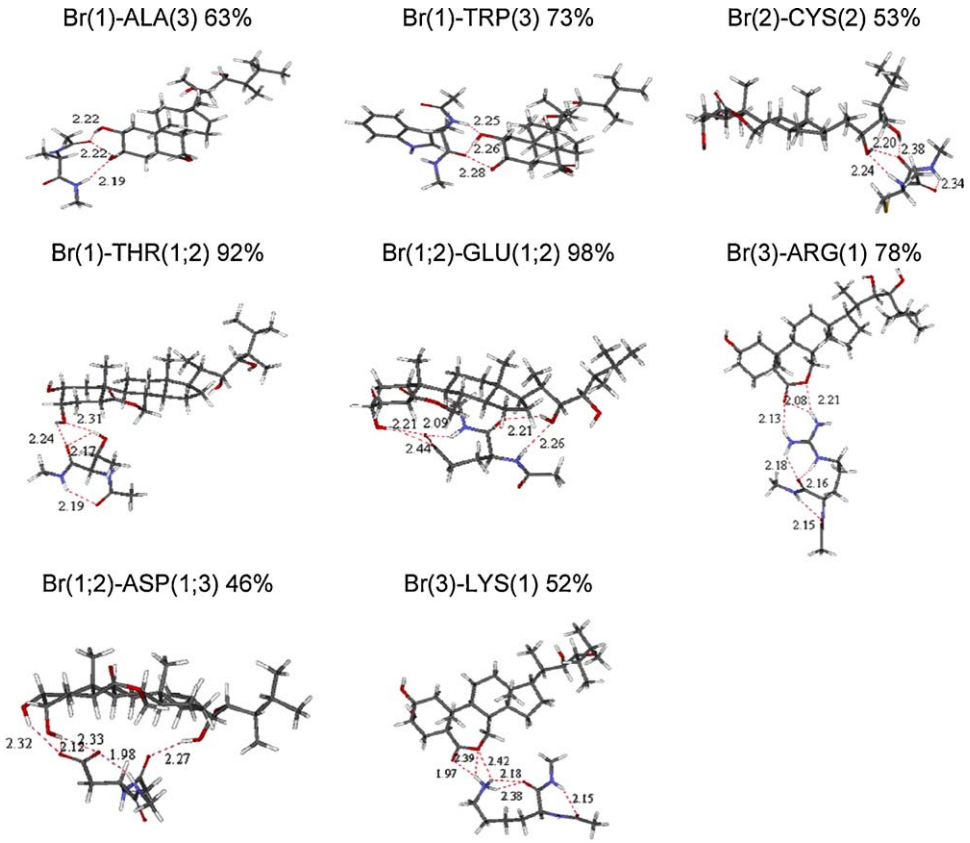

Fig. 3. AM1 structures and relative populations of the most stable complexes of brassinolide (Br) with the different types of amino acids obtained by applying the MMH methodology.

> THR > ALA > VAL > SER > ILE > GLN > LEU > GLY > TRP > PHE > CYS > ASN > MET > PRO > TYR. Recently, the receptor of ecdysteroids, an animal steroid hormone that shows structural similarities with BR has been isolated, and some of the amino acids implicated in the binding pocket agree with the highest associated amino acid residues obtained (*e.g.*, GLU, ARG, THR, and ALA) [32].

Table 1
Calculated ΔE^{assoc} and ΔG^{assoc} (kcal/mol at 298.15 K) of the brassinolide complexes with the twenty natural amino acid residues obtained at the AM1 level applying the MMH methodology.

Amino acids	ΔE^{assoc}	ΔG^{assoc}
Aliphatic		
GLY	−9.27	−10.96
ALA	−11.34	−12.42
ILE	−10.95	−11.85
LEU	−10.08	−11.06
VAL	−11.31	−12.23
PRO	−6.99	−8.45
Aromatic		
PHE	−8.89	−10.02
TRP	−8.91	−10.12
TYR	−6.74	−8.79
Sulphur		
CYS	−8.77	−9.98
MET	−7.77	−9.01
Polar uncharged		
SER	−11.26	−12.63
ASN	−8.73	−10.60
GLN	−10.29	−11.33
THR	−12.91	−13.71
Polar charged		
GLU	−37.22	−37.87
ASP	−32.09	−33.32
ARG	−29.51	−30.82
LYS	−22.07	−23.62
HIS	−18.91	−20.43

In the group of aliphatic non-polar amino acids, Br–ALA and Br–VAL are the complexes with the greatest association. With the exception of the Br–PRO complex, all the complexes with this group of amino acids possess similar ΔE^{assoc} and ΔG^{assoc} values. The lateral chain of these amino acids is non-polar, therefore, as expected, the strongest interactions with the hydrophilic regions of Br occur mainly through the backbone (zones 2 or 3, see Fig. 2) of these amino acids; see, for example, Br(1)–ALA(3) (63%), Br(2)–VAL(2) (88%), Br(1)–ILE(2) (89%), Br(1)–LEU(3) (85%), and Br(1)–PRO(2) (54%) (Figs. 3 and 1S). In the complex with the highest relative population between Br and ALA, Br(1)–ALA(3) (see Fig. 3), the diol group of ring A of Br interacts with the simulated backbone of ALA. The complex Br(2)–VAL(2) (see Fig. S1) is also very stable, which indicates that the interaction with the diol group of the lateral chain of Br is also favoured. Complexes with an important contribution to the partition function in which the interaction is produced between the oxalactone group of Br and the backbone of the amino acid were only obtained in the cases of GLY and ALA (*e.g.*, Br(3)–GLY(2) and Br(3)–ALA(2), see Fig. S1). This type of interaction cannot take place when the length of the aliphatic lateral chain increases due to steric hindrance with the methyl group in C_{18}.

Br complexes with aromatic amino acids possess lower absolute ΔE^{assoc} and ΔG^{assoc} values than those obtained for the complexes with aliphatic amino acids. TRP and PHE show the highest association for Br. The interaction occurs preferentially between the diol groups of ring A and the lateral chain of Br and the backbone of these amino acids. An example of this is the Br–TRP complex with the highest relative population (Br(1)–TRP(3), 73%, see Fig. 3), and the complexes Br(1)–PHE(3) (76%) and Br(2)–PHE(2) (12%) (see Fig. S2). The complex Br(3)–TRP(1) has a low contribution to the partition function (0.7%), which indicates that

the interaction with the lateral chain of TRP is not favoured. The complexes with TYR – the least stable of this group – with the highest relative populations, Br(1)–TYR(1) and Br(2)–TYR(1), show this particular aa interacting through its lateral chain (see Fig. S2). These results seem to indicate that the interaction of Br with the lateral chain of aromatic amino acids is not as favoured as with their backbone.

The complexes between Br and sulphur amino acids (MET and CYS) where the interaction occurs through the sulphur atom (*e.g.*, Br(1)–CYS(1), 2e-4%, see Fig. S3) possess very low stability and do not contribute to the partition function. The strongest interaction is produced between the backbone of these amino acids and the hydrophilic zones of Br, *e.g.*, Br(2)–CYS(2), Br(3)–CYS(2), Br(2)–MET(2), Br(3)–MET(2) and Br(1)–MET(2) (see Figs. 3 and S3). Sulphur amino acids tend to show lower associations to Br than the aromatic amino acids.

The polar-uncharged amino acids interact with Br preferentially through their polar-uncharged lateral chains, as expected. These complexes show similar or slightly higher association stabilities than those with aliphatic amino acids. The complexes with THR and SER exhibit the greatest stabilities within this group of amino acids. Br(1)–THR(1;2) (92%) and Br(1)–SER(1;3) (65%), which show two zones of interaction between Br and the aa, are the complexes in these two groups with highest relative population. In Br(1)–THR(1;2) (see Fig. 3) the interaction occurs between the –OH in C_3 of Br and the hydroxyl group of the lateral chain and the carbonyl group near C_α of THR. In the case of Br(1)–SER(1;3) (see Fig. S4), the diol functionality of ring A of Br interacts with the –OH group of the lateral chain and the backbone of SER. Br(3)–ASN(2) (57%) and Br(1)–GLN(1) (84%) are the complexes of ASN and GLN with the highest relative populations (see Fig. S4).

The complexes between Br and the polar-charged amino acids show the greatest stabilities as reflected by the highest absolute values of ΔE^{assoc} and ΔG^{assoc} in Table 1. The interaction with Br is always produced through the polar-charged lateral chain of these amino acids. The acid amino acids, GLU and ASP, show the highest association for Br. In these complexes, *e.g.*, Br(1;2)–GLU(1;2) (98%) and Br(1;2)–ASP(1;3) (46%) (see Fig. 3), Br and the amino acids – which appear extended below the Br structure – interact through two distinct areas in each molecule. In Br(1;2)–GLU(1;2), the hydroxyl groups in C_3 and C_{22} of Br interact with the polar lateral chain (region 1) and the backbone (region 2) of GLU, respectively. In the Br(1;2)–ASP(1;3) complex, the diol group of Br and the –OH in C_{22} interact with the polar lateral chain of ASP and the extended backbone (region 3), respectively. The complex Br(1)–ASP(1) (46%, see Fig. S5) also shows a significant contribution to the partition function of the Br–ASP system.

The minima found by the MMH procedure show the interaction between the basic amino acids (ARG, LYS and HIS) and the oxalactone functionality of Br, *e.g.*, Br(3)–ARG(1) (78%), Br(3)–LYS(1) (52%) and Br(3)–HIS(1) (47%) (see Figs. 3 and S5). The complexes Br–ARG show the highest association in this group. The complexes where the interaction is produced between the diol groups of the lateral chain and ring A of Br and these amino acids possess very low stabilities and, therefore, quite low contributions to the partition function.

To ensure all significant Br–aa complexes were taken into account, additional complexes between Br and the amino acids with the highest affinity, *i.e.*, GLU, ASP, ARG and LYS, were further explored, first with the AM1 method and later on with the B3LYP and M05-2X functionals, as described in Section 2. Eight Br–GLU complexes were considered together with five complexes between Br and the each of the other amino acids.

Tables 2–5 display the energy (ΔE) and Gibbs free energy (ΔG) of the association process for the different complexes found between Br and the previously mentioned amino acids at 298.15 K. The values reported correspond to the four levels of theory considered: AM1, B3LYP/6-31G, B3LYP/6-311 + G(d)//B3LYP/6-31G and M05-2X/6-311 + G(d)//B3LYP/6-31G. The B3LYP/6-31G structures of these complexes are shown in Figs. 4–7. The complexes marked with an asterisk are the lowest-energy complexes previously found following the MMH procedure.

Polar interactions are always accompanied by dispersive interactions. AM1 and B3LYP have shown problems when describing medium-range dispersion-like interactions [33,34]. Therefore, we have used the M05-2X density functional to validate the performance of AM1 and B3LYP calculations on these systems. The M05-2X density functional has been proved to successfully describe non-covalent interactions for biological systems, especially weak interactions, hydrogen bonding, π–π stacking and interaction energies of nucleobases. The good performance of M05-2X comes from its improved correlation functional, which gives a better description of the medium-range part of non-covalent interactions [35].

Table 2
Change in internal thermal energy and Gibbs free energy (in kcal/mol) of the association process for the brassinolide (Br) complexes with glutamic acid (GLU) at 298.15 K.

Complexes		AM1	B3LYP/6-31G	B3LYP/6-311 + G(d)// B3LYP/6-31G	M05-2X/6-311 + G(d)// B3LYP/6-31G
Br(1)–GLU(1)	ΔE	−24.48	−57.53	−39.08	−47.11
	ΔG	−12.75	−42.26	−23.81	−31.84
Br(1)OH_2–GLU(1)	ΔE	−27.95	−48.48	−33.32	−38.90
	ΔG	−14.97	−34.88	−19.72	−25.30
Br(1)OH_3–GLU(1)	ΔE	−31.73	−55.02	−37.76	−45.54
	ΔG	−17.63	−41.72	−24.45	−32.24
Br(2)–GLU(1)	ΔE	−19.28	−38.77	−27.08	−32.32
	ΔG	−9.91	−27.24	−15.55	−20.79
Br(1;2)–GLU(1;2)[a]	ΔE	−34.73	−56.40	−41.50	−58.72
	ΔG	−16.80	−39.74	−24.83	−42.06
Br(1;2)–GLU(3;1)	ΔE	−29.66	−57.37	−37.00	−46.84
	ΔG	−14.57	−40.60	−20.23	−30.07
Br(1;2)–GLU(1;3)	ΔE	−24.74	−50.21	−32.74	−41.51
	ΔG	−10.56	−34.26	−16.78	−25.56
Br(3)–GLU(2)	ΔE	−10.58	−23.17	−11.60	−20.46
	ΔG	2.23	−9.24	2.33	−6.53

[a]Structure obtained by applying the MMH methodology.

Table 3
Change in internal thermal energy and Gibbs free energy (in kcal/mol) of the association process for the brassinolide (Br) complexes with aspartic acid (ASP) at 298.15 K.

Complexes		AM1	B3LYP/6-31G	B3LYP/6-311 + G(d)// B3LYP/6-31G	M05-2X/6-311 + G(d)// B3LYP/6-31G
Br(1)–ASP(1)	ΔE	−19.49	−30.02	−20.45	−26.08
	ΔG	−7.34	−17.64	−8.07	−13.70
Br(2)–ASP(1)	ΔE	−17.90	−32.58	−23.30	−28.33
	ΔG	−7.53	−22.38	−13.10	−18.13
Br(3)–ASP(2)	ΔE	−9.69	−14.23	−5.71	−14.94
	ΔG	3.31	−0.72	7.79	−1.44
Br(1;2)–ASP(1;3)[a]	ΔE	−29.72	−46.54	−30.97	−39.95
	ΔG	−15.86	−31.85	−16.27	−25.25
Br(1;2)–ASP(2;1)	ΔE	−25.09	−44.21	−24.23	−37.32
	ΔG	−8.28	−26.91	−6.93	−20.02

[a] Structure obtained by applying the MMH methodology.

Table 4
Change in internal thermal energy and Gibbs free energy (in kcal/mol) of the association process for the brassinolide (Br) complexes with arginine (ARG) at 298.15 K.

Complexes		AM1	B3LYP/6-31G	B3LYP/6-311 + G(d)// B3LYP/6-31G	M05-2X/6-311 + G(d)// B3LYP/6-31G
Br(1)–ARG(1)	ΔE	−18.64	−22.15	−17.27	−18.73
	ΔG	−9.17	−12.03	−7.16	−8.62
Br(2)–ARG(1)	ΔE	−15.16	−14.09	−10.37	−11.79
	ΔG	−3.41	−5.42	−1.69	−3.12
Br(3)–ARG(1)[a]	ΔE	−24.39	−26.63	−22.85	−24.57
	ΔG	−13.90	−17.19	−13.41	−15.13
Br(1;2)–ARG(1;2)	ΔE	−8.41	−14.41	−7.05	−11.57
	ΔG	5.84	−0.83	6.53	2.01
Br(1;2)–ARG(3;1)	ΔE	−10.74	−18.48	−8.24	−14.35
	ΔG	2.84	−5.24	5.00	−1.11

[a] Structure obtained by applying the MMH methodology.

Table 5
Change in internal thermal energy and Gibbs free energy (in kcal/mol) of the association process for the brassinolide (Br) complexes with lysine (LYS) at 298.15 K.

Complexes		AM1	B3LYP/6-31G	B3LYP/6-311 + G(d)// B3LYP/6-31G	M05-2X/6-311 + G(d)// B3LYP/6-31G
Br(1)–LYS(1)	ΔE	−15.00	−20.04	−15.27	−16.83
	ΔG	−6.36	−10.50	−5.73	−7.29
Br(2)–LYS(1)	ΔE	−17.96	−22.18	−16.70	−19.28
	ΔG	−8.57	−12.00	−6.52	−9.10
Br(3)–LYS(1)[a]	ΔE	−20.57	−23.61	−20.11	−21.16
	ΔG	−11.10	−13.97	−10.48	−11.53
Br(1,2)–LYS(1;2)	ΔE	−11.89	−20.07	−7.70	−17.12
	ΔG	1.49	−5.14	7.23	−2.19
Br(1;2)–LYS(3;1)	ΔE	−3.76	−13.47	−3.66	−12.00
	ΔG	8.99	0.07	9.88	1.54

[a] Structure obtained by applying the MMH methodology.

The two most stable Br–GLU complexes at the AM1 and B3LYP/6-311 + G(d) levels of theory, Br(1;2)–GLU(1;2) and Br(1)OH_3–GLU(1), differ by less than 1 kcal/mol, but this energy difference becomes 10 kcal/mol with the M05-2X functional. Br(1;2)-GLU(1;2), the MMH (AM1) complex obtained with the highest relative population, is the most stable complex found at the highest levels of theory (see Table 2 and Fig. 4).

The stability order is almost the same at the four levels of calculation. The three least-stable complexes, Br(1;2)–GLU(1;3), Br(2)–GLU(1) and Br(3)–GLU(2), are equally ranked by AM1, B3LYP/6-31G and B3LYP/6-311 + G(d). M05-2X/6-311 + G(d) also shows the Br(2)–GLU(1) and Br(3)–GLU(2) complexes with the lowest stability, but the complex Br(1;2)–GLU(1;3) is less than 1 kcal/mol more stable than the complex Br(1)OH_2–GLU(1). The interaction of the lateral chain of GLU with the oxalactone functionality of Br is not favoured due to the electrostatic repulsion between the negative partial charges of the oxalactone and the carboxylate groups. Hence, the most stabilizing interaction of the oxalactone group of Br should take place with the –NH group of the backbone of this aa, *e.g.*, Br(3)–GLU(2).

The MMH (AM1) Br–ASP complex with the highest relative population, Br(1;2)–ASP(1;3), is the most stable among the extended set of complexes explored (see Fig. 5) at the four levels of theory (see Table 3). As previously discussed, this complex possesses a double stabilizing Br–ASP interaction.

The structures of the lowest-energy complexes, *e.g.*, Br(1;2)–GLU(1;2) and Br(1;2)–ASP(1;3) at the AM1 (see Fig. 3) and B3LYP/6-31G (see Figs. 4 and 5) levels of calculation, show some differences. In the AM1 complex Br(1;2)–GLU(1;2) the interaction is produced between the entire –COO^- group of the lateral chain of

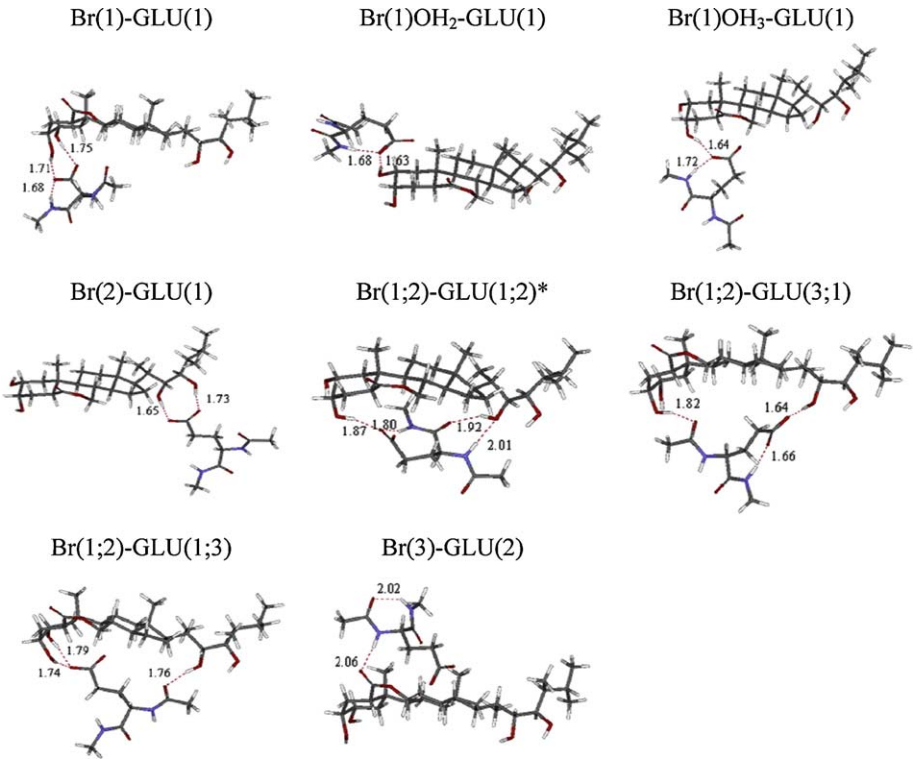

Fig. 4. Preferential positions of interaction (B3LYP/6-31G) between brassinolide (Br) and glutamic acid (GLU). *Structure of the minimum obtained by applying the MMH methodology.

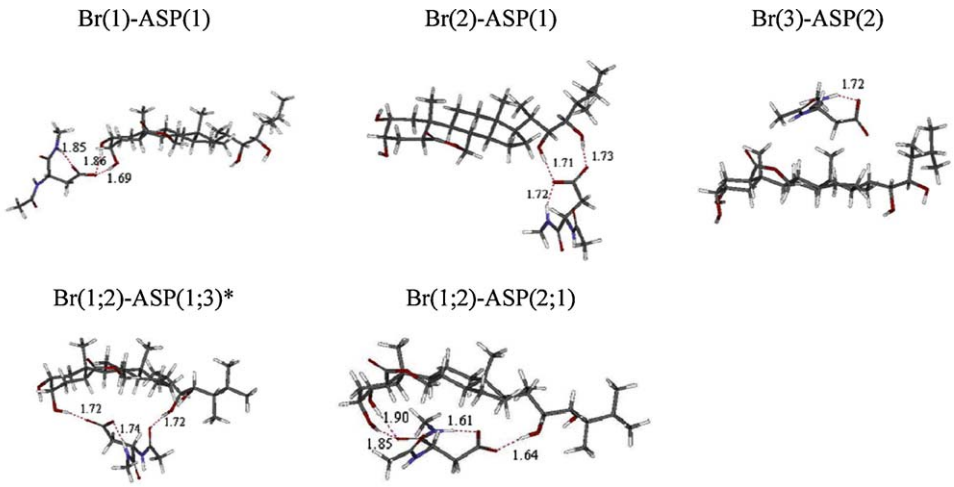

Fig. 5. Preferential positions of interaction (B3LYP/6-31G) between brassinolide (Br) and aspartic acid (ASP). *Structure of the minimum obtained by applying the MMH methodology.

GLU and the –OH in C_3 of Br. When this complex is reoptimized at the B3LYP/6-31G level of theory, the $–COO^-$ group is not entirely participating in the interaction with the –OH in C_3. In the AM1 complex Br(1;2)–ASP(1;3) the entire diol group of ring A of Br interacts with the $–COO^-$ group of the lateral chain of ASP. However, in the B3LYP geometry the –OH in C_3 of Br is the only group interacting with the lateral chain of ASP.

Although the AM1, B3LYP/6-311 + G(d) and M05-2X/6-311 + G(d) stability order for the complexes between Br and the acid amino acids is not exactly the same, there is excellent agreement between the predicted complexes with the greatest and lowest stability. For the Br–ASP complexes, the AM1 stability order agrees with the B3LYP/6-31G and M05-2X/6-311 + G(d) results.

For the Br complexes with basic amino acids, ARG and LYS, the same situation applies. The MMH (AM1) complexes with the highest relative populations, Br(3)–ARG(1) and Br(3)–LYS(1), are also the most stable among the extended set of complexes explored (see Figs. 6 and 7) at the four levels of theory (see Tables 4 and 5). The AM1 stability order of the Br complexes with ARG and LYS coincide with the order predicted by the three DFT calculations.

Br(3)–ARG(1) exhibits the interaction between the carbonyl group of ring B of Br and the $–NH_2$ groups of the lateral chain of ARG. In Br(3)–LYS(1) the carbonyl group of Br interacts with the $–NH_3$ of LYS. Other Br–ARG and Br–LYS complexes showing a double interaction involving two regions of both molecules, *e.g.*, Br(1;2)–

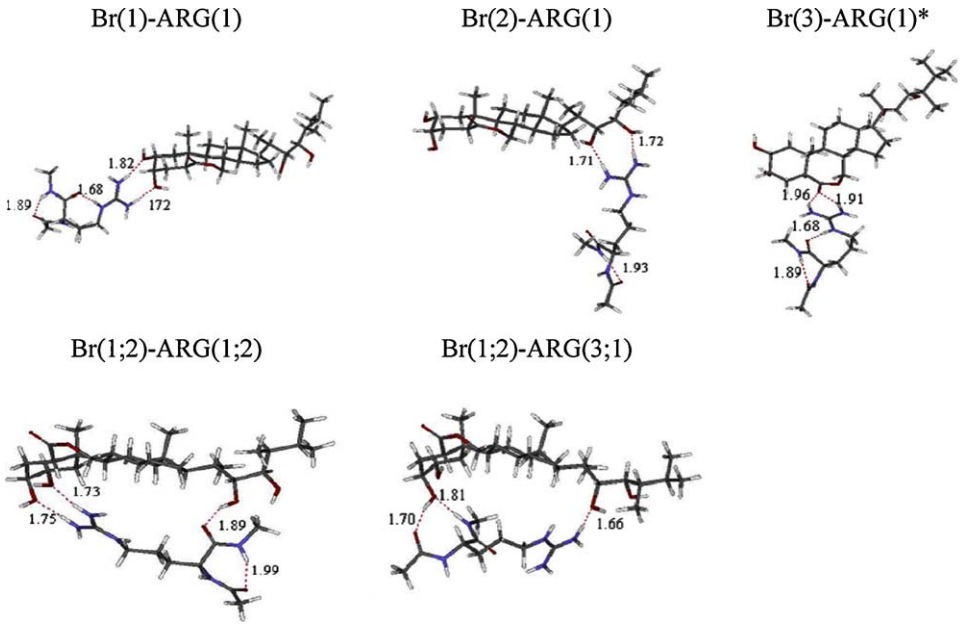

Fig. 6. Preferential positions of interaction (B3LYP/6-31G) between brassinolide (Br) and arginine (ARG). *Structure of the minimum obtained by applying the MMH methodology.

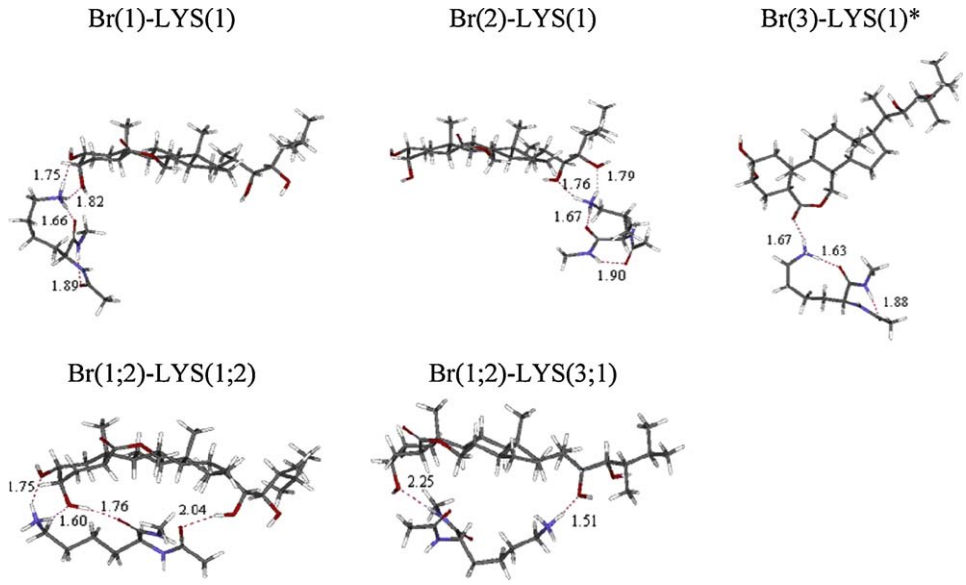

Fig. 7. Preferential positions of interaction (B3LYP/6-31G) between brassinolide (Br) and lysine (LYS). *Structure of the minimum obtained by applying the MMH methodology.

ARG(1;2) and Br(1;2)–ARG(3;1) (see Fig. 6), Br(1;2)–LYS(1;2) and Br(1;2)–LYS(3;1) (see Fig. 7), possess a much lower stability which is probably associated with a significant decrease in entropy during formation.

In spite of the known limitations of AM1 Hamiltonian to correctly describe interactions dominated by dispersion [33], the AM1 results (geometries and energies) have shown to be in excellent agreement with the B3LYP and M05-2X results obtained, especially for the most and least-stable complexes with each amino acid. The values of statistical properties found with AM1 were not significantly different due to error compensation of this method. On the other hand, dispersion-stabilized structures can be obtained after refinement of AM1 geometries with M05-2X density functional.

4. Conclusions

A theoretical model for understanding the interaction between brassinosteroids and amino acids has been established. The MMH methodology, using the semiempirical Hamiltonian AM1, allows the determination of the degree of association between Br and the amino acids based on the thermodynamical properties of the complexes they form. The order of association found is: GLU > ASP > ARG > LYS > HIS > THR > ALA > VAL > SER > ILE > GLN > LEU > GLY > TRP > PHE > CYS > ASN > MET > PRO > TYR. The Br–amino acid association is the strongest with the polar-charged amino acids. The MMH Br–aa association order for the four amino acids with the highest association for Br has been reproduced with the B3LYP and M05-2X density functional methods.

It has also been shown that each group of amino acids possesses a preferential zone of interaction with Br. While polar-charged and uncharged amino acids interact most strongly through their polar lateral chains, the other amino acids prefer their backbones. Br possesses three main zones of hydrophilic interaction with amino acids and the association with one area or another depends on the particular aa. The association of acid amino acids occurs through the diol groups of ring A and the lateral chain of Br, however, the interaction with basic amino acids is produced through the oxalactone group of ring B of this phytohormone.

The M05-2X/6-311 + G(d) results indicate that AM1 and B3LYP are suitable methods for describing not only the geometries of these complexes but also their order of stability. The most stable complexes obtained by means of the MMH-AM1 approach are also the most stable ones when applying the B3LYP and M05-2X functionals with the TZ basis set.

Acknowledgments

The authors are indebted to the financial support from MEC, Spain (Project: CTQ2007-63332), the Universidad de la Habana, the Universidad Autónoma de Madrid, AECID, Spain (Project: D/019558/08) and the Natural Sciences and Engineering Research Council of Canada (NSERC). Thanks are also due to CCC-UAM and Information Technology Services at Thompson Rivers University. NMD acknowledges a sabbatical fellowship from MEC, Spain.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jmgm.2009.12.006.

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