Theoretical Assessment of Chiral Recognition of Whelk-O1 for Chiral Compounds

[1]YU CAO, [2,3]XIANLONG WANG, [1]SHUN YAO, [1]QIANG CHENG AND [1]HANG SONG*
[1]*Department of Pharmaceutical and Biological Engineering*, *Sichuan University*, *610065 Chengdu*, *China.*
[2]*School of Life Science and Technology*, *University of Electronic Science and Technology of China*, *Chengdu*, *China.*
[3]*Department of Chemistry*, *Bryn Mawr College*, *101 N Merion Ave*, *Bryn Mawr*, *PA 19010*, *United States.*
hangsong@scu.edu.cn*

(Received on 23rd February 2011, accepted in revised form 27th June 2011)

Summary: In order to perform a qualitative analysis of chiral separation and explore the separation mechanism, the docking method was used to study the intermolecular interactions between the Whelk-O1 HPLC chiral stationary phase and the given enantiomers. Thirteen typical chiral analytes were selected and docked with Whelk-O1 using AutoDock 4.0 and the simulation results were in accordance with the three-point interaction theory. By comparison with the experimental results, the method was showed to be a feasible and efficient way to predict the elution order of the enantiomers of chiral compounds.

Introduction

Whelk-O1, a brush-type chiral stationary phase (CSP), has been widely used in separation of enantiomers [1-4] and in the evaluation of new CSP as a reference [5, 6]. In recent years, Whelk-O1 has become an important subject in the study of chiral separation mechanism [7, 8]. With the rapid development of computer science and technology, computational chemistry has been effectively used to study the separation mechanism. Density functional theory (DFT) and molecular dynamics (MD) methods were employed to optimize the compound structures and simulate the chiral separation process by Zhao *et al.* [7]. Molecular dynamic (MD) simulation was applied to reproduce the chiral separation factor and the elution order [8]. However, it is usually time-consuming [9]. Alberto *et al.* [10] explored a simple chemoinformatic method to predict whether the enantiomers could be separated by Whelk-O1 CSP. The method was based on a nonlinear model built upon a large amount of experimental data and structural features of the analytes. But it did not calculate the intermolecular forces between the enantiomers and CSP. The applications of chemoinformatic techniques in the enantioselective molecular recognition were reviewed by Alberto *et al.* [11]. To overcome the disadvantages of methods above, researchers desire to develop a simpler and more efficient method, which should not only be able to predict whether the enantiomers can be separated, but also be able to analyze the separation mechanism and the elution order.

Recently, AutoDock, as a common docking software mainly used to dock ligands with protein receptor, was found to have good performance in chiral separation simulation on the cyclodextrin CSP [12, 13]. However, the AutoDock has not been applied in brush-type CSPs in present reports. In order to accelerate the simulation speed, AutoDock adapts a grid-based approach to approximate the energy calculations with the energy function. The time to perform the calculation using this approach is only proportional to the number of atoms in the ligand. Therefore, it makes the overall energy calculation and thus the entire docking simulation can be very fast [14].

Although the 'chirality debate' and the mechanism of enantioselection have been investigated for many years [15-17], there was seldom study on the mechanism through calculating the pair-wise interactions between atoms of enantiomers and a CSP for clearly explaining the chiral separation in theory without guess and assumption. The three-point model is one of most famous chiral separation theories and is frequently applied by chiral researcher [8]. In this study, a method was developed on the basis of three-point model and the interaction model in this method was set up by calculating interactions between each atom of enantiomers and CSP. The method would perform well in the qualitative analysis with simpler operation and could be conveniently used by common researcher with little computational chemistry knowledge to predict separation results and analyze mechanism of chiral separation.

In general, the chiral enantiomers can be separated by CSP because that the combination ability with CSP for *R*-enantiomer and *S*-enantiomer is

*To whom all correspondence should be addressed.

different. The combination energies of the enantiomers with CSP were defined by the interaction energies between the enantiomers and CSP as follows.

$$\Delta G = \Delta G_{vdw} + \Delta G_{hbond} + \Delta G_{elec} + \Delta G_{tor} + \Delta G_{sol} \quad (1)\ [18]$$

The equation was detailed explained by Morris and Thomsen [18, 19]. Here the internal or intramolecular interaction energy of the ligand is not included in ΔG. Changes in ligand conformation will affect the outcome of the docking, so the changes should be taken into consideration. In AutoDock, the binding free energy includes ΔG and the energy difference arising from the conformation changes of ligands. In AutoDock output files, the binding free energy ($\Delta G_{binding}$) between enantiomers and CSP was divided into seven parts: van der waals energy (ΔG_{vdw}), electrostatic energy (ΔG_{elec}), hydrogen bonding energy (ΔG_{hbond}), desolvation energy (ΔG_{sol}), torsional free energy (ΔG_{tor}), final total internal energy (ΔG_{final}) and unbond system's energy (ΔG_{unbond}). For convenience, the total of ΔG_{vdw}, ΔG_{hbond} and ΔG_{sol} in this text was expressed by $\Delta G_{vdw+hbond+sol}$. Final total internal energy was the internal energy of the ligand in the complex. Unbond system's energy was actually intramolecular interaction energy of the ligand when it existed alone and this energy was invariant in the whole docking procedure. Conformation of the chiral molecules in these two states exist difference, so the energy in these two states must be separately considered in calculating docking energy. The binding free energy can be expressed as follows.

$$\Delta G_{binding} = \Delta G_{vdw+hbond+sol} + \Delta G_{elec} + \Delta G_{tor} + \Delta G_{final} - \Delta G_{unbond} \quad (2)$$

The difference of $\Delta G_{binding}$ for enantiomers ($\Delta\Delta G_{binding}$) decides whether enantiomers can be separated by CSP. The bigger the absolute value of $\Delta\Delta G_{binding}$ is, the easier enantiomers can be separated by CSP. The $\Delta G_{binding}$ of enantiomers can decide the elution order. The smaller value of $\Delta G_{binding}$ is, the larger combination ability of compound with CSP is and thus the later enantiomer is eluted.

Results and Discussion

Calculation Results

The thirteen enantiomers of distinct structural features in this study are listed in Table-1 [20-26]. Table-2 shows the calculated $\Delta G_{binding}$ and retention time from references of chiral enantiomers. For enantiomers **1~9**, the elution orders decided by calculated $\Delta G_{binding}$ were in accordance with the reported elution orders. The $\Delta G_{binding}$ for enantiomers of **11~12** only had slight differences, which agreed with the fact that they were difficult to be separated in experiment. The calculated $\Delta\Delta G_{binding}$ of **10** showed that enantiomers **10** should be separated by Whelk-O1 CSP, but they can not be separated in experiment. The elution order decided by calucation for **13** was opposite to the experimental result.

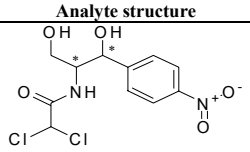
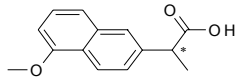
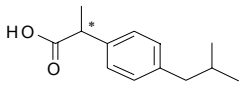
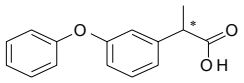
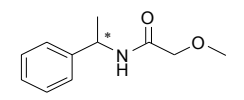
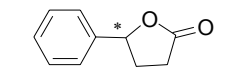
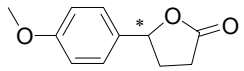
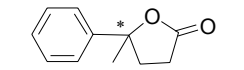
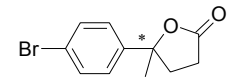
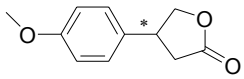
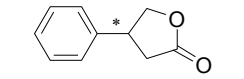
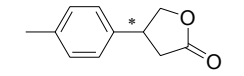
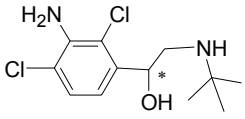
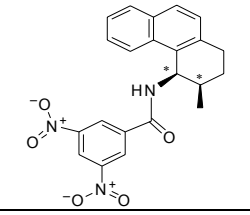
The absolute value of $\Delta\Delta G_{binding}$ of the enantiomers **1~9** were varying from 0.10 to 0.68 kcal/mol referring to Table-2. According to the references [27, 28], the difference range of 0.10~0.68 kcal/mol between the enantiomers were in the range of chiral separation energy. The absolute value of $\Delta\Delta G_{binding}$ of **11~12** were smaller than 0.1 kcal/mol. These small values exceeded the valid range, so enantiomers **11~12** could be hardly separated by Whelk-O1. The difference of binding free energy with CSP between *R*-**10** and *S*-**10** was larger than 0.1 kcal/mol, which did not agree with the experimental result that they can hardly be separated by Whelk-O1. The structure of **10** is very similar to **11** and their structures are equivalent in all regards except that the hydrogen in the latter is replaced by methoxy group in the former. But the calculation result of **11** was in agreement with the experimental result while that of **10** was not. The dynamic factor in experiment might be the cause of the problematic result. The binding free energy of *S*-**13** was lower than that of *R*-**13** while *R*-**13** was more retained by Whelk-O1 in the experiment. The result of **13** with two chlorine atoms attached to benzene ring did not agree with the experimental value.

Detailed Analysis of Binding Free Energy

The detailed analysis of binding free energy was illustrated by taking the fenoprofen as an example. As mentioned in introduction section, the binding free energy was divided into seven parts. The seven energies between the *R*- or *S*-fenoprofen and the CSP are showed in Table-3. The binding free energy between the enantiomers and the CSP is defined as Eq. (2). The difference of the binding free energies between the *R*- and *S*-enantiomer complexes could determine whether the compound can be separated by Whelk-O1. In the seven energies in Eq. (2), the difference of ΔG_{final} of *R*- and *S*-fenoprofen was 0.1 kcal/mol. ΔG_{tor} and ΔG_{unbond} of *R*- and *S*-fenoprofen were equal. The biggest difference of binding free energy was attributed to $\Delta G_{vdw+hbond+sol}$ and ΔG_{elec}. Table-3 shows that the $\Delta G_{vdw+hbond+sol}$ is the key force for fenoprofen' separation by the CSP, which is thought belonging to the main forces in chiral

separation [29].

Table-1: The structures of analytes and CSP.

Number	Analyte structure	Analyte Name	Reference
1		Chloramphenicol	[20]
2		Naproxen	[21,22]
3		Ibuprofen	[23]
4		Fenoprofen	[23]
5		2-Methoxy-N-((R)-1-phenylethyl)aceta-mide	[24]
6		5-Phenyl-dihydro-furan-2-one	[25]
7		5-(4-Methoxy-phenyl)-dihydro-furan-2-one	[25]
8		5-Methyl-5-phenyl-dihydro-furan-2-one	[25]
9		5-(4-Bromo-phenyl)-5-methyl-dihydro-furan-2-one	[25]
10		4-(4-Methoxy-phenyl)-dihydro-furan-2-one	[25]
11		4-Phenyl-dihydro-furan-2-one	[25]
12		4-(4-Methyl-phenyl)-dihydro-furan-2-one	[25]
13		Clenbuterol	[26]
CSP		(*R*,*R*)-Whelk-O1	

*:chiral center

Interaction Models

The interactions between each atom of the analytes and Whelk-O1 were examined according to the docking output files. Table-4 reveals the interaction energies between each atom of fenoprofen and Whelk-O1. The three-point interaction models consider that the *R*- and *S*-enantiomers can both form strong forces with CSP in two points. But the strong forces in the third point with CSP only exist in one of the enantiomers because of the different configurations. In the calculation result, *R*- and *S*-enantiomer of fenoprofen both formed π–π stack and one H-bond with Whelk-O1 in two points respectively (see Fig. 1). The largest different energy existed between H1 of the enantiomers and Whelk-O1. *S*-enantiomer of fenoprofen had its H1 near the nitro oxygen of Whelk-O1 when the other two-point interactions formed and H1 of *R*-enantiomer was not localized near nitro oxygen of Whelk-O1. So in this point, there were strong interactions only between the *S*-enantiomer and the CSP because of the different configurations. $\Delta G_{vdw+hbond+sol}$ between H1 and CSP was highly variable for enantiomers, e.g. 0.03 kcal/mol for *R*-fenoprofen and -0.41 kcal/mol for *S*-fenoprofen. At the same time, ΔG_{elec} between H1 of the *S*-fenoprofen and the CSP was also lower than that of *R*-fenoprofen. The difference of the electrostatic energy between O29 of fenoprofen and Whelk-O1 could not be neglected. The interaction energy between the two atoms (H1 and O29) of *S*-fenoprofen and CSP was lower than that of *R*-fenoprofen. And in the other two points, the H-bond energy of O9 in *S*-fenoprofen was stronger than that of *R*-fenoprofen and the interaction between the aromatic rings of *S*-fenoprofen and CSP was also stronger than that of *R*-fenoprofen. In conclusion, the difference of three-point interactions between enantiomers finally caused the chiral separation. This quantitative analysis can well explain the mechanism of the chiral separation. Via analyzing the interaction model of other chiral compounds, the separation models of most compounds in this study were found in accordance with the three-point interaction models.

Experimental

General

Naproxen, ibuprofen, fenoprofen, clenbuterol and nine other enantiomers were chosen as the docking objects (see Table-1) on the basis of our previous studies and some published results. The 3D structural coordinates of the CSP and the thirteen enantiomers were obtained from the Cambridge Structural Database (CSD) [30]. The geometries of compounds **1**~**4** were directly taken from CSD and the others were built from their analogues. The enantiomers' structures were generated by the mirror

symmetry operation.

Table-2: The docking results.

Compound	Binding free energy *(kcal/mol)*		Retention time *(min)*		Reference
	R	*S*	*R*	*S*	
Synthomycin	-3.36[a]	-3.57[b]	~16.5	~18.5	[20]
Naproxen[c]	-3.21	-3.09	20.975[d]	42.432	[21,22]
Ibuprofen	-1.87	-1.97	3.07	3.47	[23]
Fenoprofen	-3.83	-4.14	4.08	5.03	[23]
2-Methoxy-N-((R)-1-phenylethyl)acetamide	-3.95	-3.79	11.86	4.97	[24]
5-phenyl-dihydro-furan-2-one	-3.77	-4.36	1.24[α][e]		[25]
5-(4-Methoxy-phenyl)-dihydro-furan-2-one	-3.93	-4.29	1.29[α][e]		[25]
5-Methyl-5-phenyl-dihydro-furan-2-one	-3.93	-4.45	1.16[α][e]		[25]
5-(4-Bromo-phenyl)-5-methyl-dihydro-furan-2-one	-4.11	-4.79	1.09[α][e]		[25]
4-(4-Methoxy-phenyl)-dihydro-furan-2-one	-3.59	-3.83	1.00[α][f]		[25]
4-Phenyl-dihydro-furan-2-one	-3.57	-3.65	1.00[α][f]		[25]
4-(4-Methyl-phenyl)-dihydro-furan-2-one	-3.69	-3.73	1.00[α][f]		[25]
Clenbuterol	-5.2	-5.56	4.425	3.725	[26]

~: the value is gotten from the HPLC pictures in reference.

a: the absolute conformation is *R*,*R*

b: the absolute conformation is *S*,*S*

c: the CSP is (*R*,*R)*-whelk-O1

d: the value is gotten from reference [21]

e: the *R* absolute conformation is first eluted and the value of α is given

f: partial enantiomers resolution was evidenced by rotation via polarimeter although only a single broadened peak was observed [25]

Table-3: The interaction energies between Whelk-O1 and fenoprofen.

The interaction energy	*R(kcal/mol)*	*S(kcal/mol)*
$\Delta G_{vdw+hbond+sol}$	-5.40	-5.99
ΔG_{elec}	0.13	0.51
ΔG_{final}	-0.41	-0.51
ΔG_{tor}	1.37	1.37
ΔG_{unbond}	-0.48	-0.48
$\Delta G_{binding}$	-3.83	-4.14

Table-4: The interaction energies between each atom of enantiomers of fenoprofen and Whelk-O1

Atom	$\Delta G_{vdw+hbond+sol}$		The type of force	ΔG_{elec}	
	R	*S*		*R*	*S*
C3	-0.39	-0.42		0.03	0.03
C4	-0.47	-0.46		-0.04	-0.03
C5	-0.46	-0.45		-0.04	-0.04
C6	-0.31	-0.32		-0.01	-0.01
C7	-0.20	-0.26		0.00	0.00
C8	-0.22	-0.30		0.00	0.00
O9	-0.69	-0.78	H-bond of *R* and *S*	0.09	0.11
C10	-0.34	-0.36	π–π stack	-0.03	-0.04
C11	-0.36	-0.29		-0.01	-0.03
C12	-0.32	-0.25		0.00	0.00
C13	-0.24	-0.26		0.00	0.00
C14	-0.25	-0.31		0.00	0.00
C15	-0.29	-0.36		-0.03	-0.02
C31	-0.28	-0.30		-0.07	-0.07
C25	-0.36	-0.35		-0.02	-0.03
C1	-0.18	-0.13		-0.05	-0.10
O2	-0.07	0.02		0.16	0.28
O29	-0.01	0.02		0.22	0.85
H1	0.03	-0.41	Strong interaction of *S*	-0.05	-0.38
Total	-5.41	-5.97		0.15	0.52

Docking

In this study, AutoDock 4.0 was employed to calculate the intermolecular interactions between the enantiomers and the CSPs. AutoDock performs the docking of the analytes to a set of grids which are used to describe the CSP and AutoGrid pre-calculates these grids. The grid box was all the same for the enantiomers and the center of grid box was fixed on macromolecular, so the space was the same for the enantiomers. And the grid box was enlarged until the lowest binding free energy achieved. In the LGA calculation, the parameters in AutoDock began with 'ga' (genetic algorithm) [31]. In this study, the number of ga_run was set in 100 considering a few rotational bonds. The ga_num_evals was set in long ($2.5e^7$) and the ga_pop_size was set in 50. All above docking conditions were the same for all the enantiomers to make the results comparable. The results from the docking were evaluated according to their energy scores, which adopted by most references [32-34].

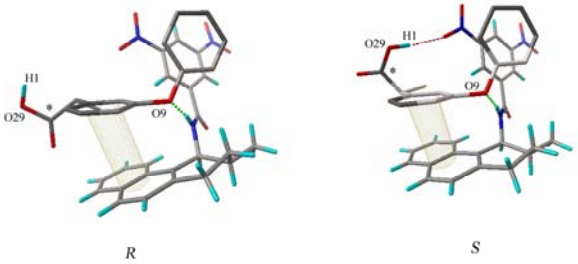


Fig. 1: Interactions between fenoprofen and the CSP. π–π stack interaction (multiple lines), hydrogen bonds (dotted lines), the strong interaction only exists in *S*- configuration (line with dots), the chiral center (*).

Conclusions

In this study, a simple method was developed

to predict chiral separation and elution order of given enantiomers with the whelk-O1 CSP and it was convenient to common researchers for the relative prediction. Thirteen analytes were selected and docked to Whelk-O1 using AutoDock. Most of the docking results agreed with the experimental data. An elaborative analysis of the interactions between the enantiomers and CSP could provide more detailed mechanism of chiral separation. The method can be applied to other brush-type CSPs.

The simulation did well for most enantiomers, but it failed for the analytes which have chlorine atoms attached to the benzene ring. Another limitation of the method was that the researchers need to know the reasonable accurate geometries of CSPs and enantiomers. Further study is supposed to improve the method and extend its application.

References

1. W. H. Pirkle and K. Z. Gan, *Journal of Chromatography A*, **790**, 65 (1997).
2. M. E. Koscho and W. H. Pirkle, *Tetrahedron: Asymmetry*, **16**, 3345 (2005).
3. A. Magora, S. Abu-Lafi and S. Levin, *Journal of Chromatography A*, **866**, 183 (2000).
4. J. H. Yen, C. C. Tsai and Y. S. Wang, *Ecotoxicology and Environmental Safety*, **55**, 236 (2003).
5. M. H. Hyun, M. H. Kang and S. C. Han, *Journal of Chromatography A*, **868**, 31 (2000).
6. A. Bartolinčić, V. Drušković, A. Šporec and V. Vinković, *Journal of Pharmaceutical and Biomedical Analysis*, **36**, 1003 (2005).
7. C. Zhao and N. M. Cann, *Journal of Chromatography A*, **1149**, 197 (2007).
8. C.F. Zhao, S. Diemert and N. M. Cann, *Journal of Chromatography A*, **1216**, 5968 (2009).
9. R. Thomsen, *Biosystems*, **72**, 57 (2003).
10. A. Del Rio and J. Gasteiger, *Journal of Chromatography A*, **1185**, 49 (2008).
11. A. Del Rio, *Journal of Separation Science*, **32**, 1566 (2009).
12. Z. Q. Zhang, H. He, H. S. Fang, J. H. Yu and P. H. Chuong, *Computers and Applied Chemistry*, **26**, 44 (2009).
13. D. H. Xia and H. Li, *Master's dissertation of Northwest University*, **6**, 16 (2009).
14. P. Szabelski, *Applied Surface Science*, **227**, 94 (2004).
15. A. Del Rio, J. M. Hayes, M. Stein, P. Piras and C. Roussel, *Chirality*, **16**, S1 (2004).
16. M. Lämmerhofer, *Journal of Chromatography A*, **1217**, 814 (2010).
17. I. W. Wainer and J. Caldwell, *Chirality*, **9**, 95 (1997).
18. G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew and A. J. Olson, *Journal of Computational Chemistry*, **19**, 1639 (1998).
19. R. Thomsen, *BioSystems*, **72**, 57 (2003).
20. H. M. He, X. Z. Xu, C. X. Pan, B. C. Shen and X. J. Zhang, *Chinese Journal of Analytical Chemistry*, **33**, 165 (2005).
21. Q. Tang and H. Song, *Master Dissertation of Sichuan University*, **5**, 18 (2004).
22. W. H. Pirkle and C. J. Welch, *Journal of Liquid Chromatography*, **15**, 1947 (1992).
23. Y. M. Liang, H. Song, C. Fu and W. L. Zheng, *Chinese Journal of Analytical Chemistry*, **31**, 1253 (2003).
24. K. Han, J. Park and M. J. Kim, *Journal of Organic Chemistry*, **73**, 4302 (2008).
25. W. H. Pirkle and P. L. Spence, *Journal of Chromatography A*, **775**, 81 (1997).
26. Q. Tang, H. Song, C. Fu, Y. C. Lei and X. Y. Chen, *Chinese Journal of Analytical Chemistry*, **32**, 755 (2004).
27. J. Lesnik, M. Lämmerhofer and W. Lindner, *Analytica Chimica Acta*, **401**, 3 (1999).
28. J. Sun, X. H. Tang, H. Song, Q. Tang and C. Fu, *West China Journal of Pharmaceutical Sciences*, **21**, 30 (2006).
29. Y. Z. Zhou, W. Z. Shan and X. Y. Wang, *Fujian Analysis&Testing*, **16**, 49 (2007).
30. F. H. Allen, *Acta Crystallographica Section B-Structural science*, **58**, 380 (2002).
31. W. Gai, Y. L. Zhang, L. Ai and Y. J. Qiao, *Chinese Journal of Natural Medicines*, **8**, 51 (2010).
32. H. I. Ali, K. Tomita, E. Akaho, H. Kambara, S. Miura, H. Hayakawa, N. Ashida, Y. Kawashima, T. Yamagishi, H. IKeya, F. Yoneda and T. Nagamatsu, *Bioorganic & Medicinal Chemistry*, **15**, 242 (2007).
33. S. M. Reza, M. Azam and M. Sadegh, *Journal of the Chemical Society of Pakistan*, **33**, 464 (2011).
34. S. A. Khan, M. Ahmad and G. Murtaza, *Journal of the Chemical Society of Pakistan*, **33**, 75 (2011).