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Preparation of highly pure daidzin on oligo-β-cyclodextrin-Sepharose HP and investigation of chromatographic behavior of isoflavones by molecular docking

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ABSTRACT

A novel method using column chromatography on oligo-β-cyclodextrin-Sepharose HP for the preparation of high purity daidzin from crude soybean samples was proposed in this work. The isoflavone of daidzin in sample A and B was purified under the optimum mobile phase composed of methanol/acetic acid/water = 20.0/8.0/72.0 (v/v/v) at a flow-rate of 1.0 mL/min in one-step operation with a purity of 97.2% and 98.1%, a recovery of 95.3% and 96.3% respectively. The target products in isolated fraction were detected and characterized by HPLC analysis and ESI-MS spectrum. Preparative separation with sample-load of up to 2.42 mg/mL medium gave satisfactory results for daidzin with the purities over 97% and recoveries approximately 90%. Molecular docking simulations were utilized to help demonstrate the inclusion complexation between β-cyclodextrin and the isoflavones in samples through inclusion geometries and calculations of the binding energies. The prediction of the elution orders with AUTODOCK and SURFLEX-DOCK were validated by the chromatographic results.

1. Introduction

Isoflavones are secondary metabolites of plants that are produced in abundance in soybeans [1]. Numerous epidemiological studies and several clinical trials have indicated the benefits of soy-isoflavones for human health [2–5]. According to these reports, soy-isoflavones have been associated with a variety of biological activities involved in estrogenic [6,7], anti-oxidative [8], anti-osteoporosis [9] and anticarcinogenic [10]. Dietary intake of isoflavones can reduce the risks of hormone-dependent and independent cancers and cardiovascular diseases, alleviate menopausal symptoms such as hot flashes and osteoporosis, and prevent hereditary chronic nose bleeds and autoimmune diseases [11]. In soybeans, there are twelve main isoflavones including daidzin (D), genistin (G), glycitin (Gly) and their respective aglycone, acetyl and malonyl forms [12]. These isoflavones mostly exist as glycosides that are compounds with sugar in a proportion of approximately 3:6:1 for daidzin, genistin and glycitin, respectively [13]. Recent research has suggested that isoflavone aglycones might be more biologically active than their respective glycosides in view of their effective absorption [14–16].

Due to the multiple beneficial effects of soy isoflavones on human health, interest in soy ingredients has increased all over the world. The potential of isoflavones as health-enhancing dietary compounds is to be evaluated. Commercial preparations of isoflavone extracts from soybean are sold as nutritional supplements. Thus, it is necessary to obtain large quantity of highly purified mono-component of isoflavones for clinical experiments and as standards for the quantitative analyses of isoflavones available in soybeans and in typical soyfoods. Several separation and purification methods for these compounds have been proposed, from classical solvent extraction [17] to methods using new technologies, like ultrasound-assisted extraction [18], supercritical fluid extraction [19,20] and high-speed counter-current chromatography [21,22]. However, some drawbacks of these methods in practice are that the one-step separation purity and yield are comparatively low, special facilities are often required and a large amount of organic solvents should be used. Additionally, time-consuming and multiple steps during the performance restrict the employment of those methods. Therefore, a more efficient and simple method is needed for the purification of soy-isoflavones.

Recently, agarose-based media especially highly cross-linked agarose gel, which have certain advantages such as high chemical stability, adequate porosity and rigidity, and ease of ligand coupling, were reported to be used in the separation and purification of active components from natural products based on both hydrogen bonding and hydrophobic interaction [23–27]. The

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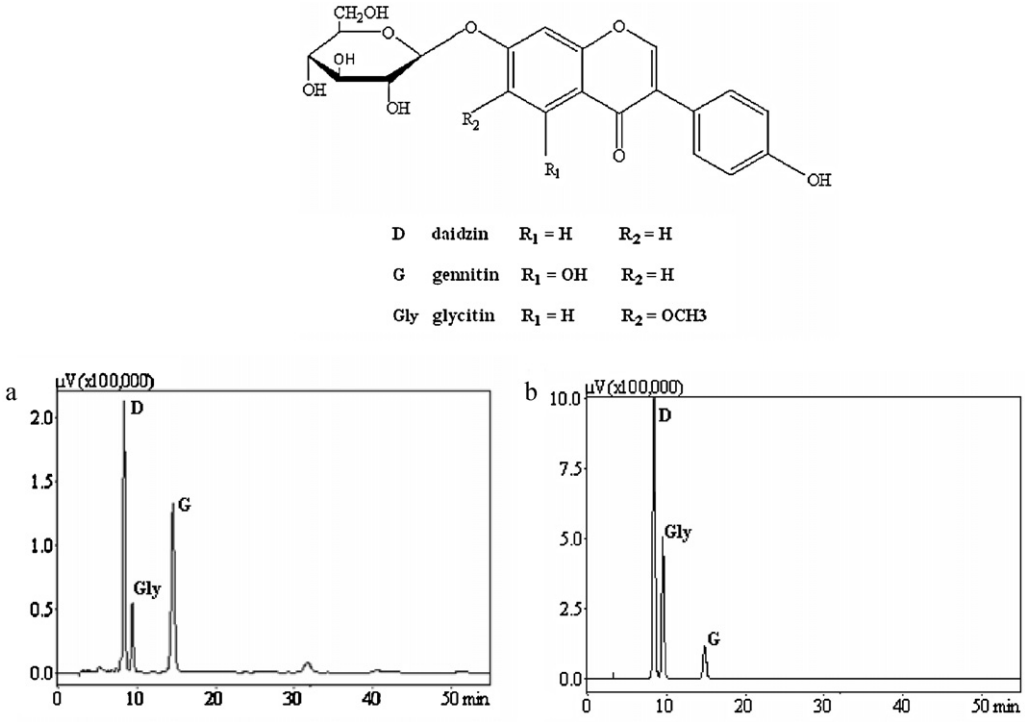

Fig. 1. HPLC chromatograms of the crude soybean extracts sample A (a) and B (b) on C18 column. Flow rate: 1.0 mL/min; mobile phase: methanol/acetic acid/water = 38.0/3.1/58.9 (v/v/v).

reasonable interpretation was presented that differences in cross-linking reagents and synthesis procedures would lead to a very large number of different structural elements, and introduce a variety of polar groups allowing cumulative cooperative interaction between the polysaccharide gel media and the ingredient of the separated samples, thus to give rise to chromatographic retardation [25]. On account of the ability to form inclusion complexes with the gust molecules whose sizes are suitable to its cavity, β-cyclodextrin (β-CD) and its derivatives are extensively used as ligand of stationary phases in chromatography. The introduced β-CD moieties may enhance the isolation selectivity of some molecules and result in chromatographic retardation on the coupled media. In our previous reports we have synthesized several β-CD coupled agarose gel media, and their applications for the one-step separation of active ingredient in natural products have also been exhibited [28].

In this work, the present aim was to prepare high purity daidzin from the two given crude soybean extracts samples which contained abundance of this isoflavone checked by HPLC. It was difficult to separate daidzin from other isoflavones especially glycitin because of the similarity of the properties in physics and chemistry and the molecular structure as shown in Fig. 1. The purified individual component of daidzin from the samples and its aglycone, i.e., daidzein obtained through the hydrolysis with hydrochloric acid will be used in the next experiment. Tentatively, the medium coupled by epichlorohydrin-polymerized β-CD ligand and its base matrix Sepharose HP were utilized for isolating daidzin by adsorption chromatography. The data revealed that several individual components of isoflavones in the samples achieved baseline separation on the medium of oligo-β-CD substituted Sepharose HP (oligo-β-CD-Sepharose HP). This paper described the successful purification of daidzin by oligo-β-CD-Sepharose HP chromatography. Moreover, a computational molecular docking simulations approach was used to illuminate the chromatographic behavior of isoflavones in samples on this β-CD coupled medium.

2. Materials and methods

2.1. Materials and solvents

The isoflavones standards were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Two samples A and B of the crude soybean extracts were kindly provided by College of Life Science and Technology, Beijing University of Chemical Technology. Allyl-group substituted Sepharose HP (34 μm average particle diameter) was a gift from GE Healthcare Bio-Sciences (Uppsala, Sweden). Methanol (Merck, Darmstadt, Germany) used was HPLC grade. Ethanol, acetic acid (HAc), sodium hydroxide (NaOH), and others were of analytical grade and obtained from Beijing Chemicals Factory (Beijing, China). Water supplied by a Milli-Q water purifier system from Milipore (Bedford, MA, USA) was used in all chromatographic separations and analyses.

2.2. Sample preparation

The crude soybean samples A and B were obtained using acetone circumfluence extraction and the hybrid extraction of acetone and ethyl acetate from soybean. An amount of 200 mg crude soybean extracts sample A or B was dissolved in 100 mL of mobile phase in order to optimize the separation work. All samples were prefiltrated using a 0.45 μm syringe filter supplied by Xinya Equipment Company (Shanghai, China) to remove dust. The sample solutions were stored in refrigeratory at 4 °C before injection into the columns. For the study of column loading capacity, sample concentrations varied from 20 mg/mL to 100 mg/mL.

2.3. Chromatography purification

The process of coupling oligomerized β-CD to brominated allyl-group substituted Sepharose HP was described in detail in reference [28]. The synthesized medium oligo-β-CD-Sepharose HP (β-cyclodextrin content about 31 mg/mL) and its base matrix

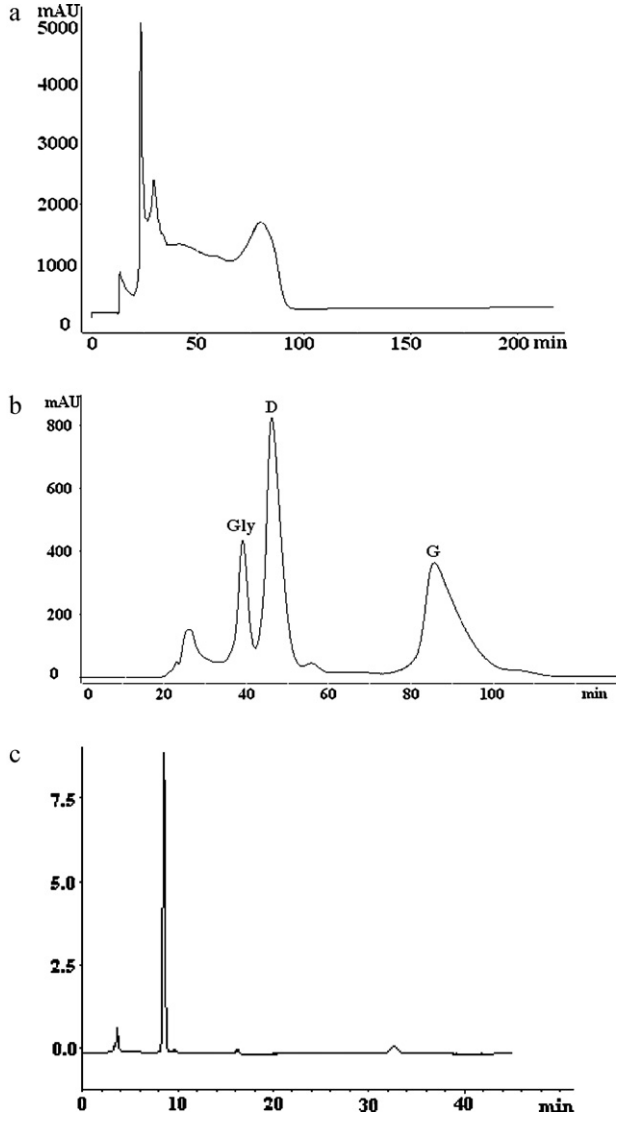

Fig. 2. The chromatograms of separation of sample A on (a) Sepharose HP, (b) oligo-β-CD-Sepharose HP. Flow rate: 1.0 mL/min, mobile phase: methanol/acetic acid/water = 20.0/8.0/72.0 (v/v/v). (c) HPLC analysis of peak D in (b). Column: RPC18; flow rate: 1.0 mL/min; mobile phase: methanol/acetic acid/water = 38.0/3.1/58.9 (v/v/v).

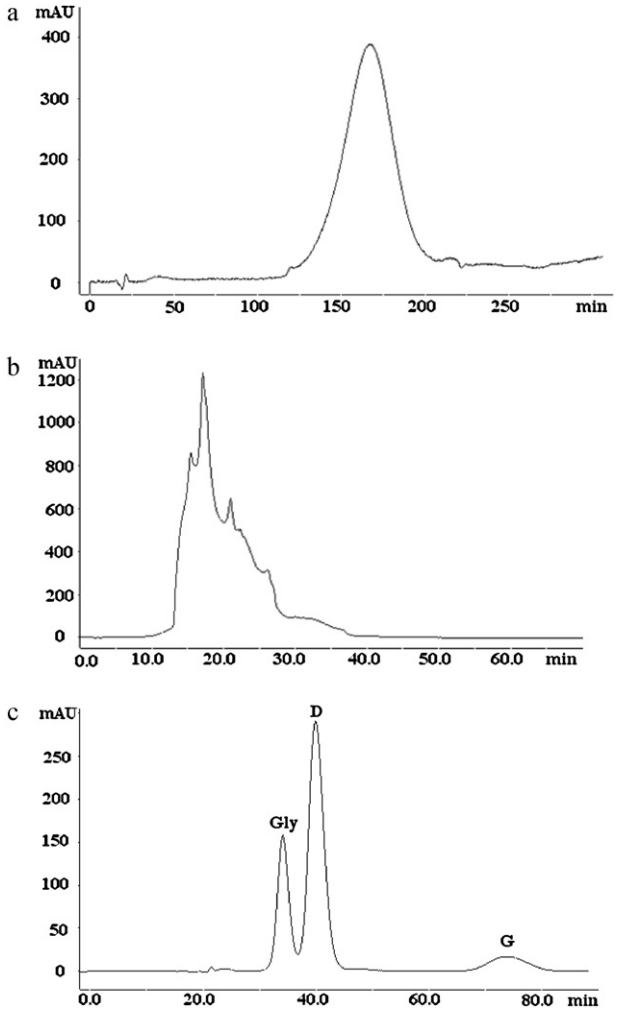

Fig. 3. Effect of mobile phases on the separation of sample B on oligo-β-CD-Sepharose HP medium (a) acetic acid/water = 1.0/99.0 (v/v), (b) methanol/acetic acid/water = 27.0/0.4/72.6 (v/v/v), (c) methanol/acetic acid/water = 20.0/8.0/72.0 (v/v/v).

Sepharose HP were packed in a 420 × 10 mm I.D. column with the total volumes of 33 mL. Chromatographic operations using the two columns were performed on ÄKTA Purifier BASIC 100 pH/C chromatography system composed of a model P-900 pump, a model UV-900 UV-monitor and fraction collector Frac-900. One milliliter sample was loaded to the column and equilibrated in isocratic chromatographic mode at a mobile phase flow velocity of 1.0 mL/min unless special mentioned. The effluent was monitored with the UV detector at 254 nm and the peak fractions were collected according to the elution profile. Fractions of the target product were collected for HPLC assay and MS identification. For the sake of restoration of the original function, the used columns were regenerated with a sufficient amount of 0.5 M NaOH and 30% (v/v) HAc after ten cycles. The columns were stored in 20% (v/v) ethanol.

2.4. HPLC analysis

HPLC was employed to analyze the crude samples, standard samples and eluted peak fractions of the experiments eluted from the purification steps. The HPLC system was an Alltech binary gradient HPLC system equipped with a reversed phase C18 column (250 × 4.6 mm I.D., 5 μm) from Beijing Analytical Instrument Apparatus Factory, Beijing, China. A 20 μL sample was injected each time and the UV detection wavelength was set at 254 nm applied to monitor the eluate. The column was at ambient temperature. The mobile phase consisted of methanol/acetic acid/water = 38.0/3.1/58.9 (v/v/v). In order to quantify eluted component concentrations, a series of daidzin standard solutions were analyzed using the HPLC assay. The obtained working calibration curve at 254 nm shows a good linear fit over the range of 0.2–300 μg/mL. The regression line was $Y = 11587.5X + 4.577$,

Table 1
Effect of sample-load on the daidzin purification from sample A and B using oligo-β-CD-Sepharose HP column.

Sample-load (mg/mL medium)	Purity (%)		Recovery (%)		Resolution (R)	
	A[a]	B	A	B	A	B
0.61	97.8	98.1	93.5	96.1	1.22	1.26
1.21	97.6	98.0	92.5	95.2	1.16	1.20
1.81	97.3	97.8	91.2	93.4	1.08	1.14
2.42	97.1	97.4	89.8	91.5	0.95	1.02
2.72	97.1	97.2	78.3	81.2	0.83	0.91

[a] A and B meant sample A and sample B.

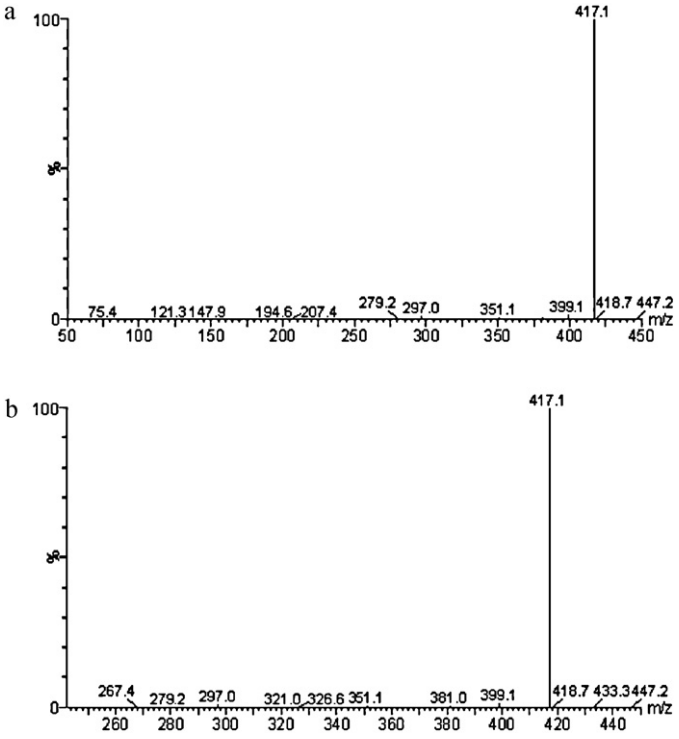

Fig. 4. Mass spectra of daidzin peaks isolated from oligo-β-CD-Sepharose HP column as in (a) Fig. 2 (b) peak D (b) Fig. 3 (c) peak D. The assay was performed on Waters Quattro Premier XE tandem quadrupole mass spectrometer. The capillary voltage and sample cone voltage were 3.5 kV and 30 V respectively. The source temperature was 100 °C, and desolvation gas temperature was set to 350 °C at a flow rate of 7.5 × 100 mL/min (N_2). The mass spectrometer was scanned from m/z = 100–600 in positive ion mode.

R^2 = 0.9994, where Y and X are the peak area and the concentration of daidzin (μg/mL), respectively.

2.5. MS structure identification

The isoflavone components in the supplied samples have different molecular weight so that structure identification by their $[M+H]^+$ ions suffices to determine them. ESI-MS was performed on a Waters Quattro Premier XE tandem quadrupole mass spectrometer (Waters, Milford, MA, USA). The instrument was set to collect data in multiple reactions monitoring (MRM) mode using electrospray ionization (ESI), switching between positive- and negative-ion mode during the run. The ionization source parameters were as follows: capillary voltage, 3.5 kV; sample cone voltage, 30 V; source temperature, 100 °C; desolvation gas temperature, 350 °C at a flow rate of 7.5 × 100 mL/min (N_2). Product ion spectra were obtained by selecting the protonated or deprotonated ions for collision. Data acquisition and processing were performed using MassLynx V4.1.

2.6. Molecular docking simulations

The docking program AUTODOCK 4.0 was used to perform the automated molecular docking [29]. A Lamarckian Genetic Algorithm (LGA) in combination with a grid-based energy evaluation method were used for pre-calculating grid maps according to the interatomic potentials of all atom types present in the host and guest molecules, including the Lennard–Jones potentials for van der Waals interactions and Coulomb potentials for electrostatic interactions. A grid map of dimensions 60 Å × 60 Å × 60 Å, with a grid spacing of 0.375 Å, was placed to cover the β-CD molecule. With the help of AutoDockTools [30], the atomic partial charges were calculated by the Gasteiger–Marsili method [31] and other docking parameters were set as default. For analysis, the configuration with lowest binding energy was selected as the representative. Since the Autodock score which was designed for protein–ligand interactions is optimized to reproduce docked configurations in protein–ligand crystal structures, our docking models were evaluated using another scoring function of the SURFLEX-DOCK module of SYBYL-X 1.1 program package [32]. The small molecules of isoflavones were subjected to subsequent minimization using Tripos force field of SYBYL-X 1.1 for ligands and β-CD molecule for a receptor.

3. Results and discussion

3.1. Comparison of the separation of daidzin from crude soybean sample on Sepharose HP and oligo-β-CD-Sepharose HP

Two resulting HPLC chromatograms of the crude soybean extracts sample A and B were shown in Fig. 1(a) and (b). Three known isoflavones of daidzin, genistin and glycitin in the supplied samples were resolved where peak D contained the target product of daidzin confirmed through compared with the sample solution of standard using HPLC analysis. The peak marked G and Gly contained genistin and glycitin respectively, determined ditto. Obviously, daidzin represented the maximal quantity in the given samples among the three main constituents of isoflavones by extraction process. The purity of daidzin in sample A and B was 51.8% and 65.3%, respectively, based on HPLC peak area percentage. In this part, sample A was used as separated material.

Based on the data obtained from our previous studies about β-CD coupled agarose gel media, the β-CD coupled medium oligo-β-CD-Sepharose HP was experimentally selected to carry out the chromatographic purification of daidzin from samples in this study. Meanwhile, as comparison, in this work cross-linked agarose gel Sepharose HP i.e., the base matrix, which was used in the synthesis of oligo-β-CD coupled Sepharose HP, was also selected to perform the chromatographic experiments. The retention behavior of separating the main isoflavones of sample A on the coupled and non-coupled matrices was investigated by using various different mobile phase. Fig. 2(a) and (b) exhibited the optimal profiles on the two media, respectively. It indicated that the oligo-β-CD-Sepharose HP column possessed the ability to separate daidzin from the other isoflavones in sample A, whereas the main constituents of sample A were only partially resolved on Sepharose HP as shown in Fig. 2(a). The base matrix could not do as efficiently as its coupled medium. This chromatographic result might be ascribed to the ligand of oligomerized β-CD which enhances the local concentration of the β-CD moieties thus increasing the interaction strength between the target product and the medium [28]. The peak marked D in Fig. 2(b) contains daidzin ascertained by C18 column analysis compared with the retention time of standard sample. The result demonstrated that daidzin was thoroughly separated from the other isoflavones in sample A on oligo-β-CD-Sepharose HP column with a purity of 97.2% and a recovery of 95.3%, as determined by HPLC analysis in Fig. 2(c). In this work, the medium oligo-β-CD-Sepharose HP is more suitable than its base matrix Sepharose HP in purifying daidzin.

3.2. Optimization of the mobile phase composition on oligo-β-CD-Sepharose HP column

In this part, sample B was supplied for performing chromatographic separation on the oligo-β-CD-Sepharose HP column for purifying daidzin. In order to facilitate the purification process and reduce the cost of production, isocratic elution mode and

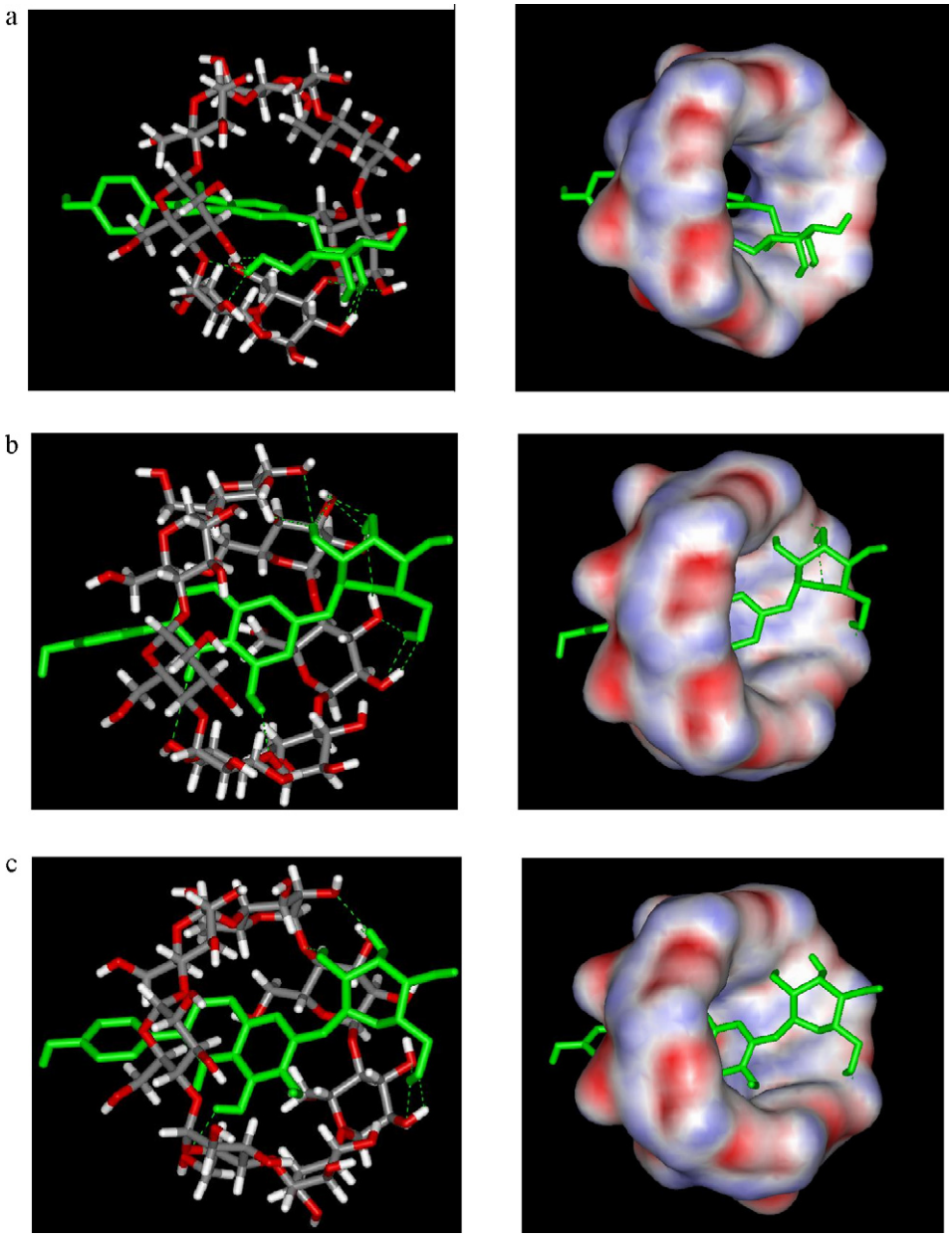

Fig. 5. The configurations of the most stable complexes between β-CD and the isoflavones (green stick) of (a) glycitin, (b) daidzin and (c) genistin. The right view is the left view adding surface colored by electrostatic charge. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

relatively simple solvent system were designed. According to references [25,28], the delocalized electrons of the carboxylic group of acetic acid impose a quenching effect on the adsorption of aromatic group containing solutes on tightly cross-linked polysaccharide gel media. Thus this effect can be used to advantage for the separation of low molecular weight substances. For the sake of validating an optimum mobile phase composition, acetic acid was at first chosen as eluent for the purification of daidzin on oligo-β-CD-Sepharose HP column. Increasing the amount of acetic acid in mobile phase from acetic acid/water = 0.5/99.5 (v/v) to acetic acid/water = 20.0/80.0 (v/v), however, the quenching effect on the adsorption was unobvious. Fig. 3(a) illustrated the chromatographic separation of the crude soybean extract sample B using acetic acid aqueous solvent system composed of acetic acid/water = 1.0/99.0 (v/v). Although a longer elution time of 200 min went through, no useful result of separation of daidzin was obtained.

In order to improve this chromatographic operation, new solvent systems were tested. Considering the poor water-solubility of daidzin, the polarity of the mobile phase should be adjusted. Appropriate amount of methanol was added to the mobile phases making the above solvent system slightly more hydrophobic. Solvent systems of acetic acid–water–methanol with different concentration and composition were applied for optimizing the mobile phase. Fig. 3(b) and (c) were the two experimental chromatograms under the different mobile phase conditions of acetic acid–water–methanol solvent systems on oligo-β-CD-Sepharose HP. In Fig. 3(c), peak D contained the target product daidzin confirmed through compared with the sample solution of standard using HPLC analysis. It is evident that solvent system composed of methanol/acetic acid/water = 20.0/8.0/72.0 (v/v/v) is the optimum mobile phase which can make daidzin complete resolution from the other isoflavones, and produce symmetrical peaks and appropriate separation time. After appropriate peak cutting, a daidzin

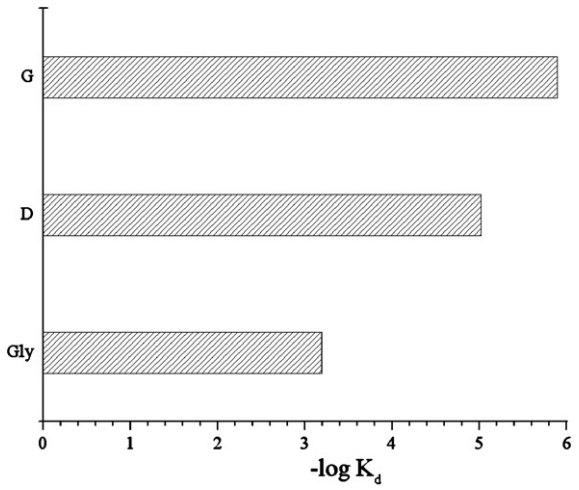

Fig. 6. Scores of the binding affinities at 298 K of the complexes between β-CD and the isoflavones using SURFLEX-DOCK module of SYBYL-X 1.1.

fraction with a purity of 98.1% and recovery of 96.3% was obtained in one step purification from sample B on oligo-β-CD-Sepharose HP medium, as determined by HPLC analysis (data not shown).

3.3. Determination the optimal sample-load of samples

The oligo-β-CD-Sepharose HP medium exhibited higher adsorption selectivity and separation efficiency under the optimum mobile phase compared with data from reports in references [17,21,22] referring to the purification of daizin. In view of improving and scaling up the separation procedure of daidzin, the optimal loading of the column to sample A and sample B was evaluated via increasing the sample-load. The investigation about the effect of the sample-load on the purification of daidzin from the crude soybean extracts was initiated using 20 mg sample A, corresponds to loading 0.61 mg/mL medium, at a flow rate of 1.0 mL/min. Chromatogram illustrated the resolution (R) between daidzin and its closest neighbour peak Gly was almost the same as the sample-load of 0.06 mg/mL medium in Fig. 2(b). Subsequently, the value of the R became more and more smaller along with the increasing of the sample-load. The data resulted from the increase of the sample loading from 0.61 to 2.72 mg/mL medium were shown in Table 1. It was displayed the purities of daidzin which separated from sample A and B exceeded 97% with recoveries approaching 90% when sample-load was less than 2.42 mg/mL column packing, otherwise, poor separation efficiency arise rapidly from further increasing of sample-load. Thus, the column loading could be limited to less than 2.42 mg/mL medium in order to prepare over 97% pure daidzin with recovery of 90% from supplied samples by oligo-β-CD-Sepharose HP column.

Table 2
Binding free energies at 298 K of the complexes between β-CD and the isoflavones obtained from molecular docking with AUTODOCK, and the corresponding retention time of the isoflavones on oligo-β-CD-Sepharose HP medium.

Analyte	$-\Delta G$ (kcal/mol)	Retention time (min)[a]
Glycitin (Gly)	4.27	38.2
Daidzin (D)	4.51	47.6
Genistin (G)	4.85	85.1
Daidzein	5.15	103.7[b]
Genistein	5.26	115.2[b]

[a] Conditions as reported in Fig. 2(b). The data were average values of three measurements carried out.
[b] Retention time of daidzein and genistein was determined using standards of the two isoflavones.

3.4. Identification of the purified fractions

The fractions corresponding to daidzin in Figs. 2(b) and 3(c) peak D were collected and confirmed by comparison with a reference standard of daidzin using HPLC analysis. The results revealed that there was no interference to the daidzin peak by other endogenous components. The further structure identity of the peak fractions in Figs. 2(b) and 3(c) marked as D was characterized by ESI-MS spectrometry. The mass spectra were obtained in positive mode. Daidzin forms a pseudo-molecular ion $[M+H]^+$ with m/z 417 under positive mode, this is in accordance with the molecular weight of daidzin (MW = 416). Fig. 4(a) was the positive ion ESI mass spectrum of the fraction corresponding to daidzin on the chromatogram peak D in Fig. 2(b), and Fig. 4(b) was that of peak D in Fig. 3(c). They all showed an abundant ion peak at m/z 417.1, which were the proton adduct of daidzin $[M+H]^+$ (MW 416 plus 1). Thus, according to the obtained data, it is tentatively safe to conclude that the peak D of Figs. 2(b) and 3(c) is daidzin with high purity.

3.5. Investigation of chromatographic behavior of isoflavones on oligo-β-CD-Sepharose HP using molecular docking

Comparing the chromatograms in Fig. 1(a) with Fig. 2(b), and Fig. 1(b) with Fig. 3(c), distinct phenomena were observed that complete separation of daidzin was realized with different peak orders on column of C18 and oligo-β-CD-Sepharose HP. Three main isoflavones in samples were eluted in a decreasing order of polarity, i.e., D > Gly > G, on octadecyl silica reversed-phase HPLC column that is in good agreement with the textbook knowledge. The situation was changed on oligo-β-CD-Sepharose HP column with the different eluted peak order of Gly > D > G. It was clearly demonstrated that the retention mechanism of purification of daidzin on oligo-β-CD-Sepharose HP was different from that on reversed-phase HPLC column.

As mentioned above, comparative trials were performed on before and after coupled media. Various concentrations and compositions solvent systems were utilized as mobile phases, such as different volume ratios of acetic acid–water, ethanol–water, acetonitrile–water, methanol–water and their mixtures. However, no useful separation of daidzin was obtained on Sepharose HP. In contrast with the partially resolution from other isoflavones on Sepharose HP, the target product daiazin was absolutely purified on the coupled medium oligo-β-CD-Sepharose HP. The obtained separation was tentatively attributed to the introduction of β-CD, especially oligomeric β-CD which caused the ligand concentration of β-CD increased significantly.

β-CD is torus-shaped oligosacchrides made up of seven cyclic-arranged α-1,4-linked D-glycopyranose units. The outside is hydrophilic due to the presence of hydroxyl groups, and the internal cavity is hydrophobic because it is surrounded by glycosidic ethers. This peculiar molecular structure allows β-CD to form inclusion complexes with suitable guest molecules according to their size and shape. The property of molecule recognition has been employed broadly in the area of separation technology including liquid chromatography. On the coupled medium, inclusion complexation between β-CD and the gust might afford the main force with responsibility for the retention. Computational methods provide direct way for theoretically understanding molecular behaviors that are not accessible to experimental approaches in complex systems. There have been many molecular modeling studies on the mechanism of chromatographic separations aimed at both the rationalization and prediction of experimental results.

With respect to the β-CD inclusion complexation, various molecular modeling methods, e.g. molecular mechanics, molecular dynamics and Monte Carlo have been utilized as study methods [33]. Inclusion model of daidzin with β-CD has been investigated

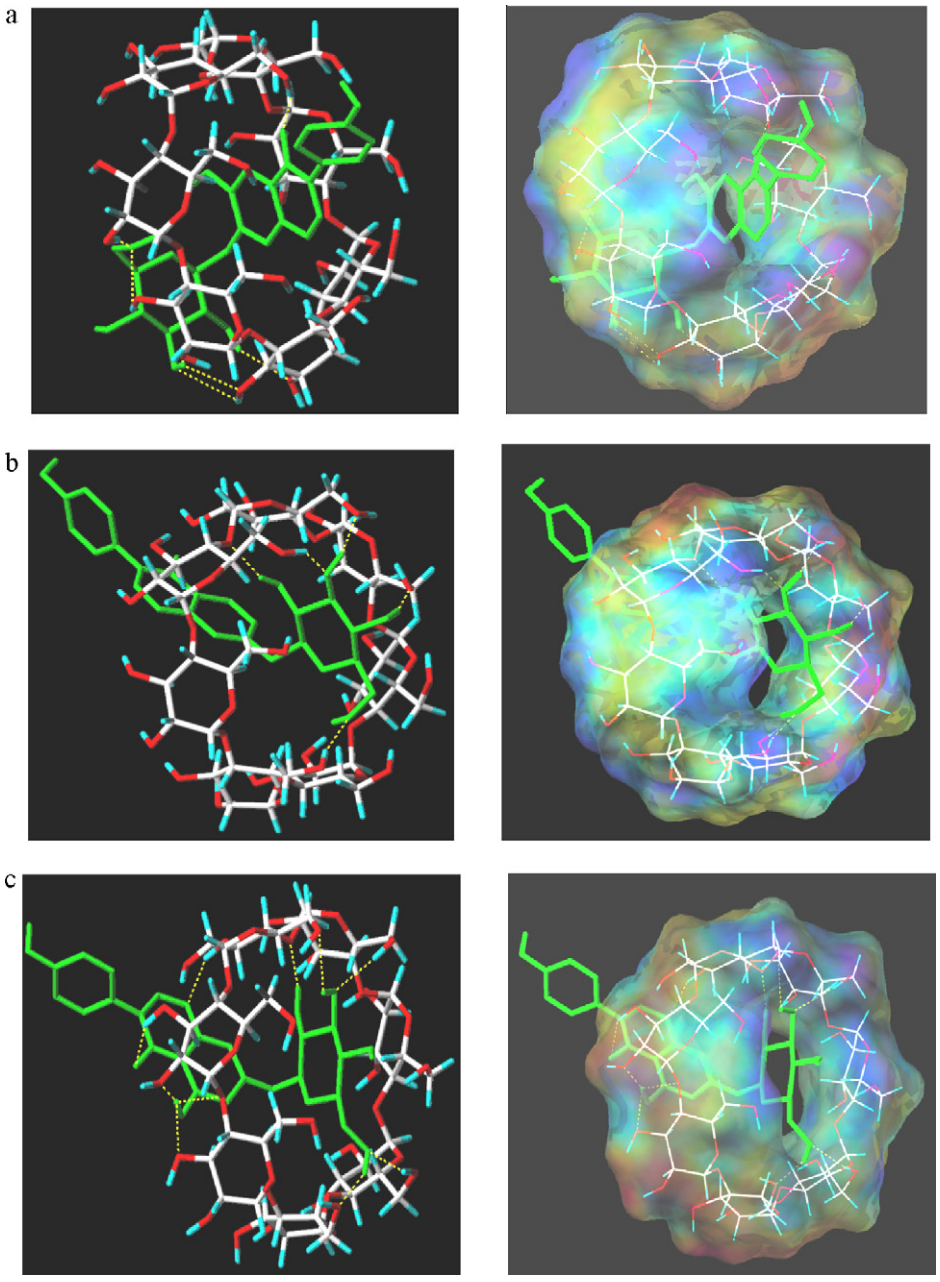

Fig. 7. Superposition of the SURFLEX-DOCK-derived most stable complexes between β-CD and the isoflavones (green stick) of (a) glycitin, (b) daidzin and (c) genistin. The right view is the left view adding surface colored by electrostatic potential. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

by molecular dynamics simulation [34]. Computational results revealed that daidzin can induce a conformational change of β-CD. In this study, we performed molecular docking simulations using a molecular mechanics method with AUTODOCK calculations to investigate inclusion complexes and predict the elution order through the differences in the interaction energies and inclusion geometries. The results of molecular docking with AUTODOCK between β-CD and the three isoflavones in samples were presented for the dominating configuration with minimum binding free energy (ΔG) in Fig. 5. The hydrogen bonding could be determined by AUTODOCK 4.0 as indicated in Fig. 5 (the green dashed lines). The hydrogen bonding mainly arose from the interaction between hydroxyl groups of the β-CD moieties and the phenol groups of the isoflavones. The analytes of glycitin, daidzin and genistin exhibited visible differences in the inclusion geometry in the β-CD cavity, implied the different chromatographic behaviors on the β-CD coupled medium. The binding free energies between β-CD and the three isoflavones obtained from molecular docking with AUTODOCK were shown in Table 2. A higher numerical value of interaction energies meant a stronger interaction between analytes and β-CD, with that a longer retention time predicatively emerged on the β-CD coupled medium. Glycitin had the lowest interaction energy with the host among the three isoflavones. The genistin complex was more stable than the daidzin complex, as shown by the $-\Delta G$ of 4.81 and 4.51 kcal/mol respectively. Thus the actual binding free energy values suggested the elution orders of Gly > D > G. As expected, the aforementioned data of chromatographic experiments about Gly, D, and G as shown in Fig. 2(b), Fig. 3(c), and Table 2 were consistent with the predicted elution orders by simulation. With more compounds, the scope of the

study could be possibly expanded to be a correlation of retention vs. binding, because for three compounds there is a 1 in 6 chance (3!) of this happening randomly, and for five compounds, this chance is 1 in 120. Daidzein and genistein, together with their respective glycoside conjugates of daidzin and genistin, are the main components of dietary soy isoflavones [35], therefore, we added additional related compounds, i.e. daidzein and genistein to the modeling study. The binding free energies (ΔG) between β-CD and the two aglycones were calculated by AUTODOCK given in Table 2. Once again, the longer retention time of daidzein and genistein on the β-CD coupled medium corresponded to their value of $-\Delta G$, namely the elution order was daidzein > genistein.

As CDs are not really exactly like proteins and the kinds of interactions that hold the ligands in place are not really typical for the interactions that hold ligands into protein binding sites, moreover, the scoring method of AUTODOCK has a checkered reputation for predicting free energy of binding, SURFLEX-DOCK scoring function of SYBYL-X 1.1 was selected in this paper for re-scoring molecular docking models between β-CD and the three isoflavones in samples. SURFLEX-DOCK scores are expressed in $-\log K_d$ units to represent binding affinities [36]. The larger value of $-\log K_d$ is in accord with a stronger binding affinity between β-CD and analyte, and might imply a longer retardation time on the β-CD coupled medium. From the numerical values of $-\log K_d$ derived from the scoring function of SURFLEX-DOCK in Fig. 6, it also demonstrated the elution orders of Gly > D > G, which was coincident with the simulation result using AUTODOCK 4.0. The obtained configurations of the most stable complexes between β-CD and the isoflavones from SURFLEX-DOCK were exhibited in Fig. 7. Thus, It can be concluded that the inclusion complexation plays an important role in the purification of daidzin on the oligo-β-CD-Sepharose HP medium.

4. Conclusion

The present paper described the successful separation and purification of daidzin from samples by oligo-β-CD-Sepharose HP chromatography. With the solvent system composed of methanol/acetic acid/water = 20.0/8.0/72.0 (v/v/v) as the optimum mobile phase, the isoflavone of daidzin in sample A and B with a purity of 97.2% and 98.1%, a recovery of 95.3% and 96.3% respectively could be obtained by proper peak cutting. After regenerated by washing with sodium hydroxide and acetic acid aqueous solution in sequence, the reproducibility of the adsorption selectivity and separation efficiency of the coupled media has been remaining in our laboratory without significant change. The results of comparatively chromatographic trials and a molecular docking study elucidated that the inclusion interaction between β-CD and three isoflavones in samples improved the selectivity of the coupled medium oligo-β-CD-Sepharose HP to daidzin. Modeling studies provided a new non-reagents consuming way for mechanism investigation as well as for predicting the chromatographic behavior. To our knowledge, no information about purification of daidzin from crude soybean extracts has so far been reported for β-CD coupled agarose gel media. The method provides a comparative facilitation and selectivity to meet the need of resolving monomeric component of isoflavones from crude soybean extracts.

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