Short Communication

Strong complexation of water-soluble betulin derivatives with (2-hydroxypropyl)-γ-cyclodextrin studied by affinity capillary electrophoresis

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Abbreviations:

ASB, betulin 3-acetate-28-sulfate; **DPP**, data point position; **DPR**, data point range; **DSB**, betulin 3,28-disulfate; **HVL**, Haarhoff-Van der Linde; **HP-β-CD**, (2-hydroxypropyl)-β-cyclodextrin; **HP-γ-CD**, (2-hydroxypropyl)-γ-cyclodextrin; **ms ACE**, mobility shift affinity capillary electrophoresis; **MUM**, measurement uncertainty multiplier; **nMRR**, normalized maximum response range

Keywords:

Betulin derivatives; Binding constants; Inclusion complexes; Haarhoff-Van der Linde function; High-affinity interaction

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Abstract

The complexation between (2-hydroxypropyl)-γ-cyclodextrin (HP-γ-CD) and water-soluble betulin derivatives, betulin 3,28-disulfate (DSB) and betulin 3-acetate-28-sulfate (ASB), belonging to the class of pentacyclic lupane triterpenoids, was studied using mobility shift affinity capillary electrophoresis (ms ACE). It was found that the complexation is a high-affinity interaction. In this case, a very low amount of HP-γ-CD should be added to the background electrolyte (BGE), and triangular peaks are observed as a result of ligand deficiency in the sample zone. Le Saux et al. showed in 2005 that using the parameter a_1 of the Haarhoff-Van der Linde (HVL) function instead of the migration time measured at the peak apex eliminates the effect of ligand deficiency on effective electrophoretic mobility. Therefore, the electrophoretic mobilities of asymmetrical peaks of DSB and ASB were calculated in this way. The obtained experimental data correspond to 1:1 complexes. The calculated values of binding constants logarithms at 25 °C are 6.70 ± 0.05 and 7.03 ± 0.10 for the HP-γ-CD complexes of DSB and ASB, respectively.

Pentacyclic lupane triterpenoids, in particular betulin and its derivatives (Fig. 1) exhibit antitumor, antimicrobial and other types of biological activities [1]. The bioavailability of such compounds can be increased by obtaining inclusion complexes with cyclodextrins (CD). Complexation of betulin derivatives with CDs has not been studied enough. Recently, the interaction of water-soluble betulin derivatives with β-CD [2] and HP-β-CD [3], and the interaction of betulinic and betulonic acids (water-insoluble) with β-CD [2] and HP-β-CD and HP-γ-CD [4-6] have been studied.

One of the techniques to study complexation is ms ACE [7-12]. The approach is based on recording several electropherograms of analyte using BGEs with different ligand content and on calculating binding constants from the dependencies of electrophoretic mobility on ligand concentration. Kinetically labile complexes give one peak in electropherograms, the effective electrophoretic mobility of which is the average weighted over the mole fractions of species. For the case when a studied compound D forms a 1:1 complex with CD, the effective electrophoretic mobility, μ_{eff}, multiplied by a factor allowing the correction for viscosity change, υ, depends on the CD concentration [CD] as follows:

$$v_i \cdot \mu_{eff,\ i} = \frac{\mu_D + \mu_{11} K_{11} [CD]_i}{1 + K_{11} [CD]_i} \quad (1)$$

where μ_D and μ_{11} are the ionic mobilities of D and the complex D/CD, respectively. Dubský et al suggested the CEval software for data processing and statistical evaluations in ACE [11]. The accuracy and precision of ms ACE binding studies can be greatly improved by optimizing the measurement conditions. Parameters such as data point range (DPR), data point position (DPP), and normalized maximum response range (nMRR) are of great importance [12]:

$$DPR = R_{C_n} - R_{C_1} \quad (2)$$

$$DPP = (R_{C_n} + R_{C_1})/2 \quad (3)$$

$$nMRR = \frac{\mu_{11} - \mu_D}{SD(\mu_{K_D})} \quad (4)$$

where R_{C_n} and R_{C_1} are the mole fractions of complexed analyte at the highest and lowest ligand concentration, respectively, $SD(\mu_{K_D})$ is the standard deviation (SD) of the effective mobility at a ligand concentration equal to the dissociation constant value, K_D (K_D = 1/K_{11}). DPP and DPR should be chosen as high as possible; values < 0.1 should be avoided. The measurement uncertainty can be minimized by using at least five data points which cover more than 40% of the binding hyperbola in its upper part. The values of nMRR should not be less than 250. For the most optimal measurement conditions, the measurement uncertainty multiplier (MUM), a ratio of the uncertainty of K_D values to the uncertainty of measuring the input data, approximately equals to 1.5.

The aim of this paper was to study the complexation of water-soluble betulin derivatives (Fig. 1) with (2-hydroxypropyl)-γ-cyclodextrin using ms ACE.

The study was carried out using capillary electrophoresis systems with a diode-array detector Agilent ^{3D}CE G1600A and Agilent G7100 (Agilent Technologies, Waldbronn, Germany) of the Krasnoyarsk Regional Center of Research Equipment, Federal Research Center "Krasnoyarsk Science Center SB RAS". Separation was carried out using the thermostated capillary segment as described in [3] and Supporting Information S1. HP-γ-CD with an extent of labeling equal to 0.6 molar substitution (average relative molecular mass 1580) was purchased in Sigma-Aldrich (Moscow, Russia). HP-γ-CD was dissolved in BGEs. The solution of 0.002 % dimethyl sulfoxide (DMSO) dissolved in samples or BGEs was used as an electroosmotic flow (EOF) marker. The water-soluble betulin derivatives were synthesized as described in [2].

Fig. 2A shows examples of the electropherograms obtained. As can be seen from Fig. 2A, for BGEs containing 0.15 and 0.5 μM HP-γ-CD, a baseline rise at the left peak side and a strong distortion of the sample zone are observed, respectively. A significant shift of the peak position and distortion of the sample zone are obtained when the ligand content in BGE is 1 μM. However, when the ligand concentration in BGE is high as compared to the analyte concentration, no change in effective mobility is observed. In mobility shift ACE, the ligand

concentration should be 10-100 times higher than the analyte concentration in order to minimize systematic errors and the influence of the sample matrix [10,13].

In this study, it was found that a decrease in the concentration of the betulin derivatives under study below 20 μM is unreasonable due to the low detection sensitivity. Thus, the distortion of the sample zone (electromigration dispersion) is observed as a result of the ligand deficiency in this zone due to the strong complexation of the compounds with HP-γ-CD. This distortion could be caused by the slow rate kinetics. However, the complexes studied are kinetically labile because the complexes were not appeared in electropherograms recorded using BGE without the HP-γ-CD addition (to obtain the complexes, an excess amount of HP-γ-CD was added to samples as described in [5]), that is, the complexes quickly decompose in the ligand absence in BGE.

There are several approaches in order to correctly calculate binding constants for the case when the analyte concentration is lower than the ligand concentration. In 2002, Galbusera et al. proposed a very complicated and tangled approach based on solving differential equations [14]. Other approaches for calculating the ligand concentration reduction in the sample zone have also been suggested [8]. In 2005, Le Saux et al. showed that using the parameter a_1 of the HVL function instead of the migration time measured at the peak apex allows eliminating the effect of ligand deficiency on effective electrophoretic mobility [15]. The HVL function was previously used to calculate the correct values of electrophoretic mobility for the triangular peaks due to the electromigration dispersion that are observed at high analyte concentration. For peak fitting with the HVL function, the CEval software can be used [11]. The correct electrophoretic mobility can also be calculated from the simulation of effects of complex-formation or from a theoretically evaluated parameter, the nonlinear electromigration mobility slope, and the mobility obtained from the peak apex [16-18].

Thereby, for asymmetrical peaks, the electrophoretic mobilities of DSB and ASB were calculated using the parameter a_1 of the HVL function. The fitting was carried out using MS

Excel and CEval. In the rectangle in Fig. 2A, an example of the DSB peak fitted with the HVL function is presented; the vertical lines indicate the migration time corresponding to the parameter a_1 of the HVL function. The peaks obtained with BGEs containing 0.15 and 0.5 μM HP-γ-CD did not fit to the HVL function and were not used in the calculations. This leads to the unavoidable narrow DPR, especially for ASB, and proximity of DPP to 1 (Table 1 and Supporting Information S2). In addition, SD of the effective mobility is not the most optimal due to the relatively low signal-to-noise ratio (S/N ~ 10), which results in nMRR < 250 (Table 1). However, an increase of the compound concentration for increasing the S/N ratio leads to the fact that the peaks do not fit to the HVL function even at 1 μM HP-γ-CD concentration in BGE.

The DSB and ASB peaks were symmetrical when the HP-γ-CD concentration in BGEs was higher than 7.5 μM and in BGE without the HP-γ-CD addition. In Fig. 2B, dependencies of the viscosity corrected electrophoretic mobility of DSB and ASB on the HP-γ-CD concentration in BGEs (points), calculated as described in Supporting Information S1, are shown. The obtained experimental data on electrophoretic mobilities correspond to 1:1 complexes (Supporting Information S3). In addition, indirect evidence for the 1:1 complexes is the fact that the mobilities of the complexes are almost 2 times lower than the compound mobilities. Such ratio (1.8-2) is observed for the HP-β-CD and HP-γ-CD complexes of compounds having broad hydrophobic regions in molecules, such as betulin derivatives [3] and glyco- and tauro-conjugated bile salts [19].

The binding constants, ionic mobilities, and 95 % confidence intervals were determined from the nonlinear regression fitting by (i) the CEval software (v.5.6.3, Prague, Czech Republic, http://echmet.natur.cuni.cz/ [12]), (ii) by Statistica 13 (StatSoft, Tulsa, OK, USA), and (iii) by OriginPro 8.1 (OriginLab Corporation, Northampton, USA). The results of all the programs were identical within error (Supporting Information S4). The calculated values of binding constants logarithms are 6.70 ± 0.05 for the HP-γ-CD complexes of DSB and 7.03 ± 0.10 for the HP-γ-CD complexes of ASB, respectively. The electrophoretic mobilities μ_D and μ_{11} are 27.9 ± 0.2 and

15.0 ± 0.1 10^{-9} m^2 V^{-1} s^{-1} for DSB and 14.2 ± 0.1 and 7.72 ± 0.04 10^{-9} m^2 V^{-1} s^{-1} for ASB, respectively. As can be seen from Fig. 2B, theoretical curves and experimental points well agree. Unfortunately, the inability to eliminate the effect of ligand deficiency on the effective electrophoretic mobility at a concentration of HP-γ-CD less than 1 μM results in non-optimal measuring parameters and relatively high MUM values (Table 1 and Supporting Information S2). It should be noted that the values obtained relate to the complexation of anionic forms of betulin derivatives with HP-γ-CD. The studied betulin derivatives are salts of sulfonic acids (Fig. 1) that are the most strongly acidic class of uncharged organic compounds with pK_a values < 0 [20]. That is, in aqueous solutions, sulfonate groups are fully dissociated.

Thus, the high-affinity interaction between HP-γ-CD and water-soluble betulin derivatives, betulin 3,28-disulfate and betulin 3-acetate-28-sulfate, was studied using ms ACE. For asymmetrical peaks distorted due to the electromigration dispersion, the electrophoretic mobilities were calculated using the parameter a_1 of the HVL function. The binding constants logarithms are 6.70 ± 0.05 and 7.03 ± 0.10 for the complexes of DSB and ASB, respectively.

The authors have declared no conflict of interest.

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Figure captions

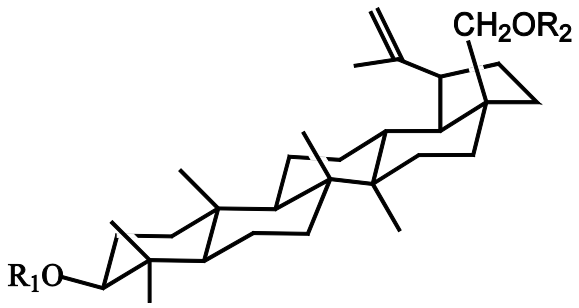

Figure 1. Structural formula of sodium salts of DSB and ASB. R_1, R_2 = SO_3Na for DSB and R_1 = $COCH_3$, R_2 = SO_3Na for ASB.

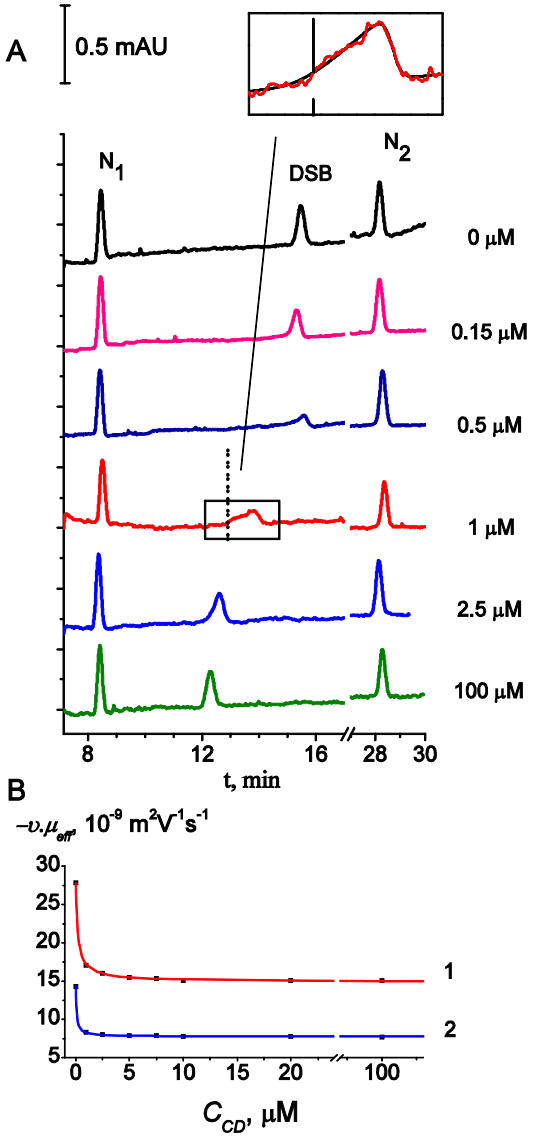

Figure 2. (A) Effect of the HP-γ-CD concentration in BGE on the shape and position of the DSB peak and (B) dependences of the viscosity corrected electrophoretic mobility of DSB (1) and ASB (2) (calculated using the parameter a1 of the HVL function for asymmetrical peaks) on the HP-γ-CD concentration in BGE. The DSB and ASB concentration is 20 µM. In rectangle, the DSB peak fitted with the HVL function are shown. The vertical lines indicate the migration time corresponding to the parameter a_1 of the HVL function. N_1 and N_2 are the DMSO peaks from 1st and 2nd injections. Capillary, 80.5/72 cm of total/effective lengths and 50 µm id. Capillary temperature, 25 ± 0.04 ºC. Voltage, + 30 kV. Detection, 200 nm. Sample injection, 10 sec*50 mbar. BGEs, 10 mM disodium tetraborate decahydrate with pH 9.18 with the addition of 0 - 100 µM HP-γ-CD.

Table

Table 1. Parameters for measuring the effective electrophoretic mobilities to determine binding constants and the estimated values of MUM

Compound	DPR	DPP	nMRR[a)]	MUM[b)]
DSB	0.17	0.92	205	7-11
ASB	0.09	0.96	154	22-33

[a)] nMRR, was calculated using $SD(\mu_{C_L=1\mu M})$ instead of $SD(\mu_{K_D})$ because 1 µM is the lowest ligand content in BGE at which the effective mobility can be calculated by HVL fitting; while $K_D(DSB) = 10^{-6.70} = 2.0 \cdot 10^{-7}$, $K_D\,(ASB) = 10^{-7.03} = 9.3 \cdot 10^{-8}$ (Supporting Information S2).

[b)] MUM was calculated as described in Supporting Information S2.