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DETERMINATION OF DISTRIBUTION CONSTANTS OF ANTIOXIDANTS BETWEEN LIPOSOMES, ALKYLSULFATE MICELLES, OCTANOL AND WATER USING ELECTROKINETIC CHROMATOGRAPHY

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Summary
The *in vivo* effects of the antioxidants are dependent on their lipophilicity, which govern membrane and protein interactions. Two electrokinetic systems, i.e. micellar electrokinetic chromatography (MEKC) and liposome electrokinetic chromatography (LEKC), were used for studying of the lipophilicity of antioxidants. Micelles of sodium decyl sulfate (SDeS) and sodium dodecyl sulfate (SDS) were used as the pseudostationary phase in MEKC and the liposomes were composed of mixtures of 1-palmitoyl-2-oleyl-*sn*-glycerophosphatidylcholine (POPC) and 1-palmitoyl-2-oleoyl-*sn*-phosphatidylserine (POPS) or 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidyl-DL-glycerol (POPG) in LEKC. The retention factors of the studied analytes were determined at pH 7.4. The micelle and liposome/aqueous distribution constants for studied antioxidants were experimentally determined and compared with values of octanol/aqueous distribution constants.

1 Introduction

Antioxidants are secondary metabolites of plants, which can protect organisms from the effect of free radicals. They are able to inhibit oxidative processes and improve the immune function [1]. Polyphenols, including flavonoids, are the most common natural antioxidants in a human diet. Flavonoids can be divided into six groups – flavones, flavonoles, flavanoles, flavanones, isoflavones, and anthocyanines. Phenolic acids can be divided into two groups based on the structure of cinnamic or benzoic acid. *In vivo* effects of these compounds are dependent on their lipophilicity and hydrophilicity. The logarithm of the octanol/water distribution constant, *log* $P_{o/w}$, is the most common way of describing hydrophobicity of compounds. For charged compounds, logarithm of the distribution constant between octanol and aqueous solution with the target pH value is

used [3,4]. For better understanding of the interactions between lipophilic and hydrophilic phases, micelle and liposome/aqueous distribution constant (dependent on van der Waals and hydrogen donor/acceptor interactions) can be determined [5]. MEKC and LEKC were utilized for determination of the distribution constant between micelles, liposomes and aqueous phase at pH 7.4.

The distribution constant, K_D, is characterized by retention factor, k, and the phase ratio, Φ:

$$K_D = \frac{k}{\Phi} \qquad (1)$$

2 Experimental

For LEKC and MEKC studies, the instrument for capillary electrophoresis Agilent 7100 (Agilent, Palo Alto, CA, USA) was used. The uncoated fused-silica capillary with 50 μm I.D. and total length of 36.5 cm (effective length 28 cm) was preconditioned by rinsing for 15 min with 0.1 M NaOH, 15 min with milliQ water, and 15 min (CZE, MEKC) or 5 min (LEKC) with the background electrolyte before the first run. The sample was injected by pressure 50 mbar for 5 s. The applied voltage was 20 kV, the temperature was 25 °C, and the wavelength was set at 214 and 254 nm. All CZE and MEKC experiments were repeated five times, whereas the LEKC experiments were repeated three times.

Phosphate buffer (ionic strength I = 20 mM and pH 7.4) was prepared by mixing the solutions of sodium hydrogen phosphate (purchased from Sigma-Aldrich, St. Louis, MO, USA) and sodium dihydrogen phosphate (from Mallinckrodt Baker, Deventer, The Netherlands). The phosphate buffer was filtered through a 0.45 μm filter. The BGE for MEKC contained SDS or SDeS (Sigma-Aldrich) in phosphate buffer (I = 20 mM) at pH 7.4. The BGE for LEKC was prepared from stock solutions of POPC, POPS, and POPG (all phospholipids were purchased from Avanti Polar Lipids, Alabaster, AL, USA) in chloroform (stored in a freezer), and phosphate buffer (I = 20 mM, pH 7.4). The concentration of the lipid dispersions, i.e., 80:20 mol% POPC/POPS and 80:20 mol% POPC/POPG, were 4 mM, which were diluted to 0.5 mM with phosphate buffer for LEKC analyses.

The concentration of the stock solution of each standard was 1 g/L in methanol (Mallinckrodt Baker, Deventer, The Netherlands). The concentrations of standards injected for analyses were 25 mg/L in milliQ water, each except for morin, biochanin A, esculin, isoquercitrin, luteolin, and myricetin, which were of 50 mg/L in water/methanol 3:1 (v/v). 0.5 mM thiourea (Sigma) in phosphate buffer was used as a marker of electroosmotic flow.

3 Results and Discussion

The mobilities of the analysed compounds were determined under CZE, MEKC and LEKC conditions. The electrophoretic mobilites of the micelles and liposomes were determined by iterative procedure using a homological series of alkylbenzoates (C1-

C6) [5-7]. The distribution constants of selected flavonoids and phenolic acids between liposomes (80:20 mol% POPC/POPS or 80:20 mol% POPC/POPG), micelles (SDeS and SDS) and phosphate buffer (I = 20 mM, pH 7.4) were determined. Obtained values were compared with literature values of octanol/aqueous distribution constants. The values of log $D_{pH7.4}$ were used for ionized compounds and *log* $P_{o/w}$ values for neutral compounds. log $P_{o/w}$ values were predicted using the ACD/Labs Percepta Platform – PhysCHem Module and the *pKa* values were predicted by Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994-2015 ACD/Labs). Values of *D* were calculated by equation:

$$D = \frac{P_{o/w}}{1+10^{(pH-pK_A)}} \quad (2)$$

Based on the obtained results, the more polar compounds are only weakly retained in POPC/POPG and POPC/POPS liposomes. Significantly higher log K_D values were observed for some phenolic acids (i.e. gallic acid, 4-hydroxybenzoic acid, *p*-coumaric acid, and sinapic acid) when comparing POPC/POPG liposomes with POPC/POPS liposomes. This clearly indicates selective interactions between some of the compounds and the polar head groups of the lipids. Even though both lipids, i.e. POPG and POPS, have an overall negative surface charge, the polar parts of the molecules are dissimilar. POPS has two negatively charged functional groups and one neutralizing positively charged group, whereas POPG only contains one negatively charged phosphate group. The 1,2-diol in POPG might also result in specific interactions with the aforementioned compounds.

For comparison of the LEKC and MEKC separation systems and for characterization of the distribution equilibria, the correlation between the constants determined for SDS micelles and liposomes were evaluated. Both systems provided similar positive slopes of correlation with slightly better correlation of SDS with POPC/POPG, indicated by a higher Pearson's correlation coefficient (0.716 for SDS-POPC/POPG as compared to 0.661 for SDS-POPC/POPS).

4 Conclusions

In the work, CZE, MEKC and LEKC were used for the determination of the distribution constant of the selected flavonoids and phenolic acids. The pseudostationary phases in LEKC were formed by liposomes composed of 80:20 mol% POPC/POPS and 80:20 mol% POPC/POPG. Micelles of SDeS and SDS were used as a pseudostationary phase in MEKC. The obtained distribution constants for the compounds were compared with the literature values of *n*-octanol/aqueous phase. The results show that the distribution constants of phenolic acids and flavonoid glycosides are lower than the distribution constants of the flavonoids. The data showed stronger positive correlation between the two studied liposome systems than between the liposome-micelle system.

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BAL31-NGS IN TELOMERE BIOLOGY

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Summary
We combined a next generation sequencing approach and BAL31 digestion of high molecular weight genomic DNA for purposes of telomere sequence identification *de novo*. Using this method we identified two novel telomere motifs in plants lacking typical plant telomere sequences.

1 Introduction
A wide range of approaches was used for telomere sequence identification in model organisms, including protozoans, yeasts, animals and plants (reviewed in [1]). Each of the models has specific advantages and features. The number of telomere sequential motifs is still increasing and the big picture of telomere motif evolution becomes high resolution image. There are some rare exceptions (e.g. Diptera with telomere retrotransposons), but mostly these telomere motifs are derived from the consensus $T_xA_yG_z$ tandem repeat. Variability, exceptions and unknown telomeres open doors of advanced knowledge, which can be potentially used in biotechnologies, agriculture and medicine. Our effort was to develop method based on a next generation sequencing (NGS) approach for telomere sequence identification in large plant genomes *de novo* (ca. 2 Gb per chromosome).