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Interactions of helquats with chiral acidic aromatic analytes investigated by partial-filling affinity capillary electrophoresis

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ABSTRACT

Noncovalent molecular interactions between helquats, a new class of dicationic helical extended diquats, and several chiral acidic aromatic drugs and catalysts have been investigated using partial-filling affinity capillary electrophoresis (PF-ACE). Helquats dissolved at 1 mM concentration in the aqueous background electrolyte (40 mM Tris, 20 mM acetic acid, pH 8.1) were introduced as ligand zones of variable length (0–130 mm) into the hydroxypropylcellulose coated fused silica capillary whereas 0.1 mM solutions of negatively charged chiral drugs or catalysts (warfarin, ibuprofen, mandelic acid, etodolac, binaphthyl phosphate and 11 other acidic aromatic compounds) were applied as a short analyte zone at the injection capillary end. After application of electric field, analyte and ligand migrated against each other and in case of their interactions, migration time of the analyte was increasing with increasing length of the ligand zone. From the tested compounds, only isomers of those exhibiting helical chirality and/or possessing conjugated aromatic systems were enantioselectively separated through their differential interactions with helquats. Some compounds with conjugated aromatic groups interacted with helquats moderately strongly but non-enantiospecifically. Small compounds with single benzene ring exhibited no or very weak non-enantiospecific interactions. PF-ACE method allowed to determine binding constants of the analyte-helquat complexes from the changes of migration times of the analytes. Binding constants of the weakest complexes of the analytes with helquats were less than 50 L/mol, whereas binding constants of the strongest complexes were in the range 1 000–1 400 L/mol.

1. Introduction

Study of non-covalent molecular interactions is important due to their high significance in various (bio)chemical and biological processes, separation procedures and analytical methods, including chiral separations. Strength of the non-covalent interactions between two interacting compounds – analyte A and ligand L – is characterized by the apparent binding (association, formation, stability) constant, further called only the binding constant, K_b, defined by the Eq. (2) below. The binding constants of complexes can be determined by a variety of physicochemical methods, such as UV–vis and fluorescence spectrophotometry [1], mass spectrometry (MS) [2], nuclear magnetic resonance (NMR) [3], equilibrium dialysis [4], calorimetry [5], electrochemistry [6], chromatography [7] and electrophoresis [8], see reviews [2,9]. Some of them are based on utilization of separation techniques under equilibrium conditions. From these methods, affinity capillary electrophoresis (ACE) features several important benefits, such as high separation efficiency, mass sensitivity, speed of analysis and presence of interacting compounds in a free solution with easy control of their concentration. Perfect purity of analytes is not necessary if the impurities do not interfere with the zones of analytes. Moreover, interactions of more than one analyte with ligand present in the background electrolyte (BGE) can be studied simultaneously if the analytes do not influence each other [10–12]. Analysis can be performed in aqueous [5,13], hydro-organic [14,15] or non-aqueous media [16,17]. In the classical mobility shift ACE, the BGE solutions with different ligand concentrations are used, and the binding constant of the analyte-ligand complex is calculated from changes in

Abbreviations: ACE, affinity capillary electrophoresis; BGE, background electrolyte; BNP, 1,1′-binaphthyl-2,2′-diyl hydrogenphosphate; EOF, electroosmotic flow; HPC, hydroxypropylcellulose; PF-ACE, partial filling affinity capillary electrophoresis; SDS, sodium dodecyl sulfate.

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effective mobility of the analyte related to the concentration of the ligand in the BGE [18–21].

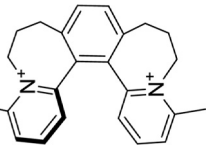
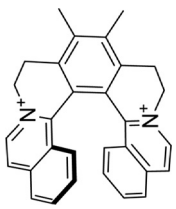
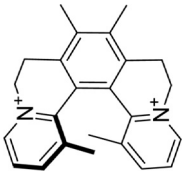
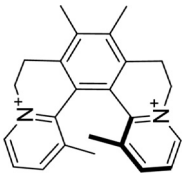
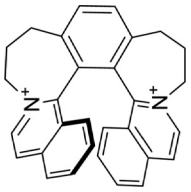
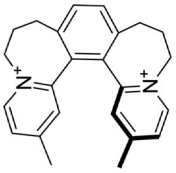
Partial-filling affinity capillary electrophoresis (PF-ACE) is a modification of ACE, where only part of the capillary is filled with a solution of the ligand in the BGE and the analyte is the compound introduced as a short zone at the injection capillary end [22–25]. There are several modes of PF-ACE, for their review see ref. [24]. The most common of them, the so called flow-through PF-ACE [23,24] was used in this study. In this mode, the analyte migrates through the zone of the ligand and from the changes of its migration time due to its interaction with the ligand, the binding constant of the analyte-ligand complex can be evaluated. In PF-ACE, concentration of the ligand in the BGE is kept constant and variable parameter is the substance amount of the ligand expressed as the length of the ligand zone. This technique has several advantages over classical ACE. The method was developed to avoid interference of UV absorbing ligand with detection of the analytes [26]. Due to the use of short ligand zones rather than adding different ligand concentration to the BGE, the consumption of often valuable ligands is much less than in the classical ACE [22]. The method utilizes only one low concentration of the ligand and analyte solutions, hence no corrections for viscosity and ionic strength of the BGE are usually required. The binding constants can be calculated from a slope of linear dependence of analyte migration time changes on substance amount of ligand in the BGE, particularly on the length of the ligand zone [27–29].

Most compounds present in living systems are chiral and often only one of their enantiomers possesses biological activity. While enzyme mediated biosynthesis is highly enantiospecific and results in single enantiomer product, in vitro chemical syntheses usually produce mixture of enantiomers. Most of the physicochemical properties of both enantiomers are identical but their biological activity may differ significantly. While one stereoisomer may be an effective drug, the other isomer may act as a toxic or carcinogenic compound [30,31]. Such difference rises need for analysis and quantification of enantiomeric composition of mixtures of chiral compounds especially in pharmaceutical industry, but control of enantiomeric purity is important in most chemical syntheses.

One of the methods used for chiral analysis is ACE. Separation of enantiomers is based upon their differential interactions with chiral selector dissolved in the BGE. Formation of diastereomeric complexes with different affinities between enantiomeric analytes and chiral selector and/or with different electrophoretic mobilities results in separation of enantiomers in two zones in the applied electric field. There are many compounds used as chiral selectors in CE and other separation methods, such as cyclodextrins and their neutral or charged derivatives [32,33], cyclofructans [34,35], chiral surfactants [36], peptide macrocyclic antibiotics, oligo- and polysaccharides and proteins, see reviews [37–39]. Search for new chiral selectors is necessary in order to achieve a chiral separation of a variety of compounds, which are not effectively separated by currently available selectors.

Helquats (helical extended diquats) were recently introduced as a new class of functional organic molecules with potential application in molecular electronics, material science, catalysis and separation methods. They represent structural link between helicenes [40–43] and viologens [44], which implies helical chirality of helicenes (helicity *P* and *M*) and dicationic character of viologens [45–50], see Table 1. Two quaternary ammonium moieties are responsible for permanent double positive charge of helquat molecules within a wide pH range and they also contribute to relatively good solubility in aqueous media despite rather large hydrophobic multi-ring aromatic system. These compounds do not contain any chiral atom and their chiral properties are based only on their helical asymmetry. Their enantiopurity can be effectively checked by a fast high-efficient capillary electrophoresis method

Table 1
List of enantiopure helquats used in this study.

Compound code[a]	(*P*)-HQ1	(*M*)-HQ1	(*P*)-HQ2	(*M*)-HQ2	(*P*)-HQ3	(*M*)-HQ4	(*P*)-HQ5
Structure							
M_r[b]	414.54	414.54	342.48	342.48	414.54	342.48	342.48

[a] In compound code, *P* stands for *plus* and indicates the right-handed helicity; *M* stands for *minus* and indicates the left-handed helicity.
[b] *Mr* is related to the presented helquat dicationic forms; for simplicity, the trifluoromethanesulfonate (triflate) counterions (M_r = 149.08) are omitted.