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Sille Ehala
*Academy of Sciences of the Czech Republic*

Rajendra Rathore
*Marquette University*, rajendra.rathore@marquette.edu

Emanuel Makrlik
*University of West Bohemia*

Petr Toman
*Academy of Sciences of the Czech Republic*

Václav Kašička
*Academy of Sciences of the Czech Republic*

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# Affinity capillary electrophoretic study of $K^+/Na^+$ selectivity of hexaarylbenzene-based polyaromatic receptor

Sille Ehala[a], Rajendra Rathore[b], Emanuel Makrlík[c], Petr Toman[d], Václav Kašička[a] *

[a]*Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., Flemingovo 2, 166 10 Prague 6, Czech Republic*

[b]*Department of Chemistry, Marquette University, P.O. Box. 1881, Milwaukee, Wisconsin 53201-1881, USA*

[c]*Faculty of Applied Sciences, University of West Bohemia, Husova 11, 306 14 Pilsen, Czech Republic*

[d]*Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, v.v.i., Heyrovsky sq. 2, 162 06 Prague 6 , Czech Republic*

[b]*Second affiliation, Address, City and Postcode, Country*

**Abstract**

Affinity capillary electrophoretic (ACE) study has proved the selectivity of hexaarylbenzene-based polyaromatic receptor (R) for $K^+$ ion over $Na^+$ ion. The apparent binding constants of the R complexes with $K^+$ and $Na^+$ ions were determined from the dependence of effective electrophoretic mobility of R on the concentration of the above alkali metal ions in the background electrolyte using a non-linear regression analysis. The apparent binding constants ($K_b$) of the K–$R^+$ and Na–$R^+$ complexes in methanolic medium were evaluated as log $K_b = 3.20 \pm 0.22$ for the K–$R^+$ complex, and log $K_b \cong -0.7$ for the Na–$R^+$ complex.

*Keywords:* Affinity capillary electrophoresis, hexaarylbenzene-based receptor; binding constant, $K^+$ complex, $Na^+$ complex

## 1. Introduction

Hexaarylbenzene (HAB) derivatives attract recently a great attention because of their unique propeller-shaped structure and potential application in molecular electronics and nanotechnology. It has been previously described by employing NMR spectroscopy and X-ray crystallography that HAB-based receptor (R) (see Fig. 1) binds a single potassium cation because it synergistically interacts with the polar ethereal fence and with the central benzene ring via cation–$\pi$ interaction [1]. Cation-$\pi$ interaction is well-established phenomenon in gas phase, and in solid state [2] and is known to play an important role in the stabilization of tertiary structures of various proteins [3]. However, according to the above study [1] an accurate binding constant for the formation of K–$R^+$ complex could not be determined by NMR method as it showed complete capture of the $K^+$ ion and suggested that the binding constant is too large to be measured by NMR spectroscopy. Recently, capillary electrophoresis (CE), especially in the mode of

* Corresponding author: V. Kasicka. Tel.: +420-220-183-239; fax: +420-220-183-592.
*E-mail address*: kasicka@uochb.cas.cz.

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affinity capillary electrophoresis (ACE), has become a powerful analytical tool for the studying of non-covalent interactions and for the determination of binding constants of various bimolecular complexes in aqueous, non-aqueous or mixed medias [4-9]. ACE possesses some advantages over other analytical techniques, such as requirement of only minute quantities of material, relatively short analysis times, and ability to employ nonpure samples provided that ACE can separate the analyte of interest from the impurities.

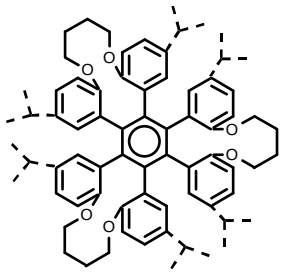

Fig. 1. Structure of the HAB-based receptor

Herein, ACE was employed to test the ability of HAB-based receptor (R) to selectively bind $K^+$ over $Na^+$ ion. Additionally, the binding (stability) constants of the $K–R^+$ and $Na–R^+$ complexes in methanolic medium were determined. The apparent binding constants were obtained from the dependences of effective electrophoretic mobility of R on the concentration of potassium/sodium ion in the background electrolyte (BGE) using non-linear regression analysis. Prior to regression analysis, the effective mobilities (measured by ACE at ambient temperature 23-25°C) were corrected to reference temperature, 25°C, following the procedure described elsewhere [10].

## 2. Experimental

### *2.1. Chemicals*

All chemicals used were of analytical reagent grade. Methanol was obtained from Penta (Chrudim, Czech Republic); sodium hydroxide, sodium chloride and potassium chloride were supplied by Lachema (Brno, Czech Republic); mesityl oxide (MO) and Tris were supplied by Merck (Germany), chloroacetic acid was obtained from Fluka (Buchs, Switzerland); sodium chloroacetate was purchased from Aldrich (Steinheim, Germany). The HAB-based receptor (R) was synthesized in the group of R. Rathore, for more details, see ref. [1].

### *2.2. Instrumentation*

For the ACE experiments, an adapted home-made CE apparatus [11] equipped with a UV photometric detector monitoring absorbance at 206 nm was used. The ACE separations were performed in the internally uncoated fused silica capillary with total/effective length 306/200 mm, id/od 50/375 μm. Separations were performed at ambient temperature 23-25°C. Chromatography station Clarity (DataApex, Prague, CR) was used for data acquisition and program Origin 6.1 (OriginLab Corp., Northampton, MA, USA) was employed for the non-linear regression analysis. BGE consisted of 50 mM $ClCH_2COOH$ and 25 mM Tris, containing various concentrations of potassium (0-1.0 mM) or sodium chloride (0-30.0 mM) in methanol. The pH value of the BGE according to the conventional pH scale, described by Porras *et al.* [12], was 7.8 (the p$K_a$ value of chloroacetic acid in methanol at 25°C is 7.8 [12]). Analyte, receptor R (20 μM) in $Cl_2CH_2/CH_3OH$, and electroosmotic flow (EOF) marker, mesityl oxide (2.5 mM) in $CH_3OH$, were consecutively introduced into the capillary, by pneumatically induced pressure (10 mbar), for 5 s each. The applied separation voltage was +12 kV (anode at injection end) and the electric current was in the

range 10–12 μA (BGEs containing KCl). Before the first use and between the series of analyses in different BGEs, the capillary was conditioned by subsequent rinsing with water (2 min), 0.1 M aqueous NaOH (10 min), water (2 min), methanol (10 min), and BGE (20 min). Between the runs in the same BGE, the capillary was rinsed with methanol (2 min), water (1 min), 0.1 M NaOH (1 min), water (1 min), methanol (1 min), and BGE (4 min). All rinses were performed by pressure 1 bar.

## 3. Results and discussion

### *3.1 Selection of ACE conditions*

Receptor R absorbs UV light, therefore, in the current study, it was used as an analyte and $K^+$ or $Na^+$ ions were added to the BGE in the form of chlorides. While studying the binding parameters of the particular complex it is preferable to choose BGE, the constituents of which do not interact with any components of the complex. However, in practice it is often difficult if not impossible to fulfill this condition. In this case, BGEs, the constituents of which only very weakly interact with the complex components, should be applied. The theoretical treatment of the interacting equilibria and migration behavior of analyte interacting with more than one of the BGE components in CE was described by Peng *et al*. [13], and is also followed in this work. In the current work, Tris–chloroacetate buffer was employed as BGE for evaluation of the $K^+/Na^+$ selectivity of receptor R by ACE.

### *3.2. Determination of binding constant by ACE*

#### *3.2.1. K-$R^+$ complex*

The ACE method for the estimation of the binding constant involved measuring the change of effective mobility of R as a function of $K^+$ ion concentration in the BGE. Fig. 2 shows series of electropherograms of R (Fig. 2a) or its complex with $K^+$ (Fig. 2b-e) at different concentrations of $K^+$ ions in the BGE. Peak MO corresponds to mesityl oxide (EOF marker).

In Fig. 2, it can be seen that with the increasing concentration of $K^+$ ion in the BGE, the migration time of R is decreasing, i.e. its effective mobility is increasing. This observation confirms that in the BGE containing $K^+$ ion, R interacts with this cation to form positively charged complex moving in the applied electric field. Nevertheless, R is a neutral compound in methanol and should thus migrate together with the EOF marker, if R is present in a free uncomplexed form. However, as can be seen from electropherogram (a) in Fig. 2, even without the presence of $K^+$ ion in the BGE, R migrated a little bit faster than the EOF marker. This observation gives a reason to assume that in the Tris–chloroacetate BGE (25 mM Tris, 50 mM chloroacetic acid) R interacts not only with $K^+$ ion but also with $Tris^+$ cation. Based on the previous study of Rathore *et al.* [1], we assume that 1:1 complex is formed between R and $K^+$ or R and $Tris^+$ cations and that above cations react with R competitively. Since there are no interactions between these two cations, the following equilibria hold:

$$K^+ + R \leftrightarrow K\text{–}R^+ \quad (1)$$

$$Tris^+ + R \leftrightarrow Tris\text{–}R^+ \quad (2)$$

The corresponding equilibrium apparent binding constants are:

$$K_{KR} = \frac{[K\text{-}R^+]}{[K^+][R]} \quad (3)$$

$$K_{TrisR} = \frac{[Tris\text{-}R^+]}{[Tris^+][R]} \quad (4)$$

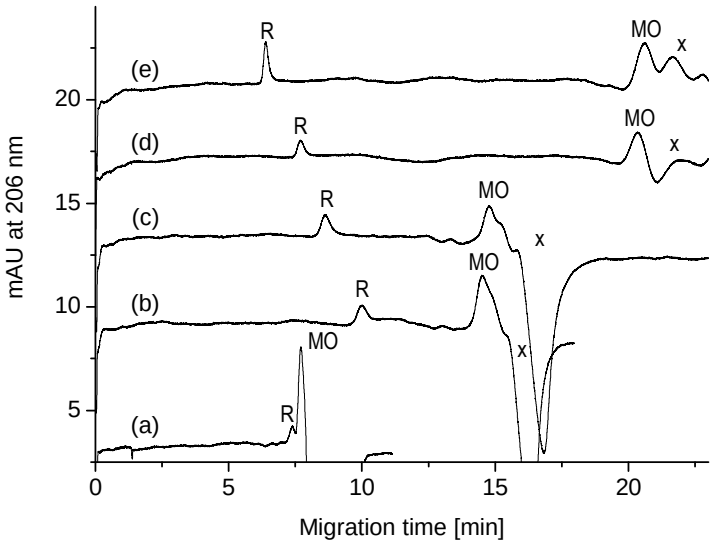


Fig. 2. Typical electropherograms of R in the absence (a) and in the presence (b-e) of potassium ion in the BGE composed of 25 mM Tris, 50 mM $ClCH_2COOH$, and containing various concentrations of KCl: (a) 0, (b) 0.1 mM, (c) 0.2 mM, (d) 0.5 mM, and (e) 1.0 mM. MO, neutral EOF marker; x, system peaks.

where [K–R $^+$], [$K^+$], [Tris–R $^+$], [$Tris^+$] and [R] are the equilibrium concentrations of the K–$R^+$ complex, free $K^+$ ion, Tris–$R^+$ complex, free $Tris^+$ ion and free R, respectively. The migration behavior of R, in the presence of $K^+$ and $Tris^+$ ions can be described by the following equation:

$$m_{\mathrm{R,eff}} = \frac{m_{\mathrm{R}} + K_{\mathrm{KR}}[\mathrm{K}^+]m_{\mathrm{KR}} + K_{\mathrm{TrisR}}[\mathrm{Tris}^+]m_{\mathrm{TrisR}}}{1 + K_{\mathrm{KR}}[\mathrm{K}^+] + K_{\mathrm{TrisR}}[\mathrm{Tris}^+]} \tag{5}$$

where $m_{\mathrm{R}}$, $m_{\mathrm{KR}}$ and $m_{\mathrm{TrisR}}$ are the electrophoretic mobilities of free R, K–$R^+$ and Tris–$R^+$ complexes, respectively. When [$Tris^+$] is constant, Eq. (5) can be simplified to

$$m_{\mathrm{R,eff}} = \frac{m_{\mathrm{R}}^* + K_{\mathrm{KR}}^*[\mathrm{K}^+]m_{\mathrm{KR}}}{1 + K_{\mathrm{KR}}^*[\mathrm{K}^+]} \tag{6}$$

where $m_{\mathrm{R}}^*$ is effective mobility of R in Tris–chloroacetate BGE in the absence of $K^+$ ions, and $K_{\mathrm{KR}}^*$ is the apparent binding constant of the K–$R^+$ complex in the presence of $Tris^+$ cation. Non-zero value of $m_{\mathrm{R}}^*$ results from the interaction between R and $Tris^+$ and its value was determined from the R analysis performed in Tris–chloroacetate BGE, which did not contain any KCl. The effective mobility of R both in the absence of KCl and at different KCl concentrations in the BGE, $m_{\mathrm{R,eff}}$, was calculated from Eq. (7) using the migration time of R, $t_{\mathrm{mig}}$, and that of EOF marker, $t_{\mathrm{eof}}$, respectively, obtained from the ACE experiments:

$$m_{\mathrm{R,eff}} = \frac{L_{\mathrm{tot}} L_{\mathrm{eff}}}{U}\left(\frac{1}{t_{\mathrm{mig}}} - \frac{1}{t_{\mathrm{eof}}}\right) \tag{7}$$

where $L_{\mathrm{tot}}$ and $L_{\mathrm{eff}}$ are the total and effective capillary lengths, respectively, and $U$ is the applied separation voltage. The dependence of mobilities on $K^+$ concentration is presented in Fig. 3. Each individual data point is the mean of

four separate measurements and the error bar represents the standard deviation. The relative standard deviations of the determined electrophoretic mobilities were below 3%.

A non-linear regression analysis using the computer program Origin 6.1 (OriginLab Corp., Northampton, MA, USA) was employed to fit the function given by Eq. (6) to the experimental data. In this fitting procedure, the $c_{K+}$ values are the concentrations of $K^+$ ion in the BGE and the values of $m_{R,eff}$ are the effective mobilities calculated from ACE data according to Eq. (7) and corrected to 25°C following the procedure described in ref. [10]; the values of $K_{KR}$ and $m_{KR}$ are treated as unknown parameters of Eq. (6). The best fit is given in Fig. 3 along with the experimental data. The apparent binding constant ($K_b$) of $K–R^+$ complex was evaluated as $\log K_b = 3.20 \pm 0.22$.

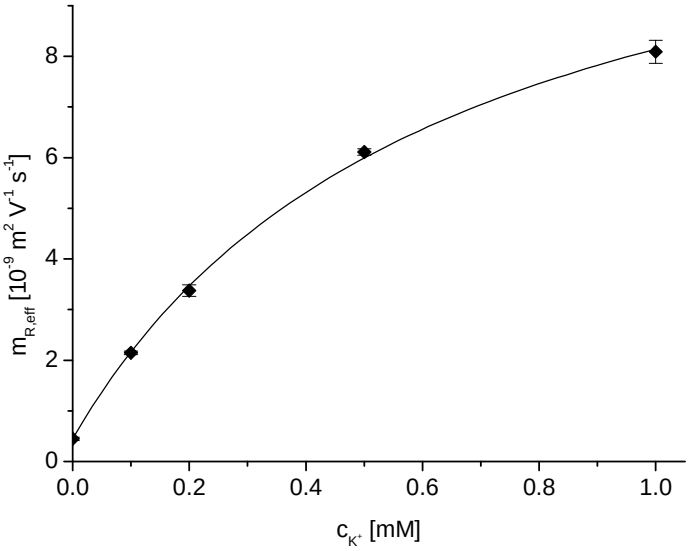


Fig. 3. Dependence of effective mobility of R, $m_{R,eff}$, on potassium ion concentration in the BGE, $c_{K+}$.

*3.2.2. Na-$R^+$ complex*

R interacts with sodium cation so weakly that with the above employed BGE it was not possible to determine an accurate $K_b$ of the $Na–R^+$ complex. The mobility of R remained constant despite the increasing concentrations (0-30 mM) of NaCl in the Tris–chloroacetate BGE. From these results it could be concluded that $Na^+$ ion does not interact with R. Nevertheless, additional experiments with R in the sodium chloroacetate BGE (25 mM $ClCH_2COONa$–50 mM $ClCH_2COOH$) showed that in this BGE R migrated slightly before the EOF peak, which proved the weak binding of $Na^+$ ion to R. Based on the results obtained in sodium chloroacetate BGE, the approximate $K_b$ of $Na–R^+$ complex was evaluated as $\log K_b \cong -0.70$.

## 4. Conclusion

The employed ACE method was found to be an effective tool for investigation of $K^+/Na^+$ selectivity of hexaarylbenzene-based receptor R. It was shown that R forms strong complex with $K^+$ ion and only very weakly interacts with $Na^+$ ion. The strengths of the R complexes with $K^+$ and $Na^+$ ions in methanolic medium were quantitatively characterized by the apparent binding constants $K_b$ as $\log K_b = 3.20 \pm 0.22$ for $K–R^+$ complex, and $\log K_b \cong -0.7$ for $Na–R^+$ complex.

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