Talanta 148 (2016) 247–256

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Study of nucleic acid–ligand interactions by capillary electrophoretic techniques: A review

I.O. Neaga [a,b], E. Bodoki [a,*], S. Hambye [b], B. Blankert [b], R. Oprean [a]

[a] Analytical Chemistry Department, "Iuliu Haţieganu" University of Medicine and Pharmacy, 4, Louis Pasteur St., 400349 Cluj-Napoca, Romania
[b] Laboratory of Pharmaceutical Analysis, Faculty of Medicine and Pharmacy, Research Institute for Health Sciences and Technology, University of Mons–UMONS, Place du Parc 20, 7000 Mons, Belgium

ARTICLE INFO

Article history:
Received 3 August 2015
Received in revised form
21 October 2015
Accepted 25 October 2015
Available online 28 October 2015

Keywords:
Nucleic acid–ligand interactions
Nucleic acid
Capillary electrophoresis
Bioaffinity studies
Data analysis

ABSTRACT

The understanding of nucleic acids–ligand (proteins, nucleic acids or various xenobiotics) interactions is of fundamental value, representing the basis of complex mechanisms that govern life. The development of improved therapeutic strategies, as well as the much expected breakthroughs in case of currently untreatable diseases often relies on the elucidation of such biomolecular interactions.

Capillary electrophoresis (CE) is becoming an indispensable analytical tool in this field of study due to its high versatility, ease of method development, high separation efficiency, but most importantly due to its low sample and buffer volume requirements. Most often the availability of the compounds of interest is severely limited either by the complexity of the purification procedures or by the cost of their synthesis.

Several reviews covering the investigation of protein–protein and protein–xenobiotics interactions by CE have been published in the recent literature; however none of them promotes the use of these techniques in the study of nucleic acid interactions. Therefore, various CE techniques applicable for such interaction studies are discussed in detail in the present review. The paper points out the particular features of these techniques with respect the estimation of the binding parameters, in analytical signal acquisition and data processing, as well as their current shortcomings and limitations.

Contents

1. Introduction . . . 248
2. Methodology . . . 249
   2.1. Capillary type . . . 249
   2.2. Internal standard . . . 249
   2.3. CE techniques for bioaffinity studies . . . 249
      2.3.1. Capillary zone electrophoresis (CZE) . . . 250
      2.3.2. Affinity capillary electrophoresis (ACE) . . . 250
      2.3.3. Hummel–Dreyer (HD) technique . . . 251
      2.3.4. Frontal analysis (FA) technique . . . 251
      2.3.5. Vacancy peak (VP) technique . . . 251
      2.3.6. Vacancy affinity capillary electrophoresis (VACE) . . . 251
3. Types of interactions involving nucleic acids analyzed by CE . . . 251
   3.1. a) Nucleic acid–protein interactions . . . 251
   3.2. b) Nucleic acid-small ligand (small molecules) interactions . . . 252
   3.3. c) Nucleic acid–nucleic acid interactions . . . 253
4. Data analysis . . . 254
5. Conclusions . . . 254

* Corresponding author.
E-mail address: bodokie@umfcluj.ro (E. Bodoki).

http://dx.doi.org/10.1016/j.talanta.2015.10.077
0039-9140/

Acknowledgments . . . 255
References . . . 255

1. Introduction

Whether it gets to the interactions between the elements of the biosphere or down to the interactions within cells, bio-interactions are one of the driving forces of life. Some of the most common types of such interactions include: antibody–antigen reactions, receptor activation by an agonist, DNA translation or the activation or inactivation of different genes.

Nucleic acids are large biomolecules contained in the chromosomes of the living organisms and viruses. Nucleotides are the building blocks of nucleic acids, each one having three components: a pentose, a phosphate group and a nitrogenous base. Deoxyribose, the pentoze in DNA is replaced by ribose in RNA. The nucleic acids have different purposes within the cells and viruses, from encoding and storing the genetic or other type of information with very high efficiency (1 g of DNA can code up to 2.2 petabytes of data [1]), to transmitting and expressing this encoded information. Unfortunately, along with genetic information, a number of diseases can be also transmitted [2].

A mutation within nucleic acids, usually within DNA, is related to a change in the nucleotide sequence, caused either by base deletion, addition or base change. If natural DNA repair mechanisms are not activated, the damage will propagate further, potentially leading to abnormalities in the encoded protein [3]. When a specific protein indispensable for the functionality of the organism is affected, the symptoms of a disease may appear [4].

Nowadays, there are a number of diseases, ranking from cancer to viral infections, that are treated with drugs that act on the nucleic acids as target. [5]. These drugs include, but are not limited to: intercalating agents (doxorubicin, dactinomycin), alkylating agents (cisplatin, dacarbazin), chain cutters (calicheamicin), chain terminators antivirals (acyclovir, deoxyguanosine) and antisense oligonucleotides [5].

The screening stage in the development process of a new drug consists in the evaluation of the interaction of different synthetized molecules with the target receptor. The quantitative aspect of this interaction is usually described by the affinity constant and reaction stoichiometry.

The affinity constant, K_a is an equilibrium constant describing a system where an association–dissociation reaction takes place between and receptor (R) and a ligand (L) with the formation of a complex (C). The use of *ligand* and *receptor* is arbitrarily associated, without being a clear definition for each.

The general reaction can be summarized as follows:

$$R+L \rightleftharpoons C \tag{1}$$

The reaction is also characterized by the on-rate constant $k_{on}$and off-rate constant, k_{off}:

$$R+L \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} C \tag{2}$$

This can be converted to the following differential equation:

$$\frac{d[C]}{dt} = [R] \times [L] \times k_{on} - [C] \times k_{off} \tag{3}$$

At equilibrium, $\frac{d[C]}{dt} = 0$, so $[R] \times [L] \times k_{on} = [C] \times k_{off}$, thus by arranging the equation, K_b can be expressed as:

$$K_b = \frac{k_{on}}{k_{off}} = \frac{[C]}{[R] \times [L]} \tag{4}$$

The two reaction constants, the on-rate constant k_{on} and the off-rate constant k_{off}, have units of 1/(concentration × time) and 1/time, respectively.

The analytical techniques currently used for the study of biomolecular interactions in general and nucleic acids interactions in particular can be divided in two separate groups, namely mixture based and separation based techniques. In mixture based techniques, the affinity constants can be estimated by means of UV and Fourier transform infrared spectroscopy (FTIR) [6,7], nuclear magnetic resonance (NMR) [8], mass spectrometry [9,10], Raman spectroscopy [11–13], spectrofluorimetry [14], equilibrium (competition) dialysis [15–18], surface plasmon resonance [19,20] and ultracentrifugation [21]. The separation based techniques include techniques such as liquid chromatography (LC) and electrophoresis.

The use of liquid chromatography (i.e. HPLC techniques) exploiting biomolecular interactions was firstl described in the late 60′s for the separation and purification of enzymes and antibodies [22,23]. Currently, there are several chromatographic techniques available for the biomolecular interaction studies, such as frontal affinity chromatography, zonal affinity chromatography or Hummel–Dreyer analysis [24,25]. However, except for the case of preparative purposes, the volumes of sample (μL range) and mobile phase (hundreds of mL) required by the chromatographic techniques are most often too high. This can be even more critical in the case of minute amounts of pure ligand and/or receptor whose availability is limited, either due to tedious purification or synthesis procedures or to elevated costs of purchase. Therefore,, due to its inherently low sample (nL range) and buffer (few mL) volume requirements, CE represents a more appropriate alternative for the study of biomolecular interactions.

As compared to LC, CE is a relatively new separation technique, and, besides the low sample and running buffer consumption, it offers other numerous advantages that include high separation efficiency and ease of method development. All these features recommend its use for the study of nucleic acid–ligand interaction where one of the components is scarce or not available in a pure state.

So far, several reviews have dealt with the application of CE in the study of biomolecular interactions, yet none of them focused on the particularities of CE in nucleic acid studies.

Among these reviews, Busch et al. [26] were the first to compare different capillary electrophoresis techniques for the study of interaction between protein and ligand (warfarin and human serum albumin) pointing out the difference between them, as well as emphasizing that using different techniques might have an influence on the value of calculated affinity constant. Rundlett et al. [27] published another noticeable review describing several techniques (affinity capillary electrophoresis, Hummel–Dreyer, frontal and vacancy peak analysis) along with their advantages, limitations and practical applications.

Other publications are less focused, dealing in general with the topic of CE in the study of biomolecular interactions [28–39], however none of them covered its application in the study of nucleic acid–ligand interactions.