AAP Chemistry FACCE

Use of continuous front-end capillary electrophoresis (CFCE) for the characterisation of interactions between transcription factors and target DNA sequences, a step towards the development of an analytical microsystem.

ABSTRACT

The growth and development of living organisms are complex and dynamic processes that require the harmonious expression of many genes. The spatio-temporal control of gene expression is therefore a process that is central to all living organisms, from the most elementary (e.g. bacteria) to the most complex (e.g. yeast, mammals, plants). Dysfunctions in these gene regulations are responsible for several diseases in humans, and can alter the productivity and quality of crop products.
The regulation of gene expression is mainly orchestrated by the activity of proteins called transcription factors (TFs), which have the ability to interact directly and specifically with certain DNA sequences (cis-regulatory elements).

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Project leader : Christian DUBOS/Joseph Chamieh
Project leader's institution : INRA-INRAE
Project leader's RU : BPMP

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Funding : Labex

GOAL

The objective of this project is to address the detailed study of interactions between FTs and their DNA targets by developing a method to characterise these interactions. In this context, the FACCE approach (continuous front-end capillary electrophoresis) offers a very promising approach. Indeed, FACCE is a free medium electrophoretic technique (absence of gel) allowing the quantification of free ligand in pre-equilibrated substrate-ligand mixtures, and has the advantage of using small sample volumes (a few nL). The quantification of the free ligand, in mixtures with varying ligand concentrations, allows the establishment of an interaction isotherm that allows the determination of the interaction parameters between the ligand and the substrate, notably the stoichiometry and the association constant. This technique has already been successfully applied to the study of protein/protein or biopolymer/protein interactions for which affinity constants have been measured

RESULTS

We studied the interaction between the transcription factor ILR3/bHLH105, recently identified (by the BPMP partner) as playing a central role in the control of iron homeostasis in Arabidopsis thaliana (a plant model organism), and one of its target sequences. This interaction was previously validated in vivo

(single hybrid in yeast) and in vitro by gel delay (EMSA: electromobility shift assay). Analyses conducted by capillary electrophoresis in continuous frontal mode, at physiological pH, made it possible to determine the interaction parameters between the ILR3 protein and the target DNA. A dissociation constant kd = 2.49E-7 M-1 and a complex stoichiometry n = 1.3 were found. Although there is a large uncertainty in the value of n due to the difficulty of determining the exact concentration of pure protein, the value obtained suggests a 1:1 complex in agreement with what is expected.