Journal of Chromatography B, 1008 (2016) 156–163

Contents lists available at ScienceDirect

Journal of Chromatography B

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

A capillary zone electrophoresis method for adenine nucleotides analysis in *Saccharomyces cerevisiae*

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ARTICLE INFO

Article history:
Received 30 June 2015
Received in revised form
19 November 2015
Accepted 21 November 2015
Available online 25 November 2015

Keywords:
Saccharomyces cerevisiae
Capillary zone electrophoresis
Adenosine triphosphate
Adenosine diphosphate
Adenosine monophosphate
Cyclic adenosine monophosphate

ABSTRACT

Adenosine triphosphate and its metabolites are involved in the cellular metabolism process in *Saccharomyces cerevisiae*. It is very important to simultaneously determine the relative contents of ATP and its metabolites in yeast. In this study, an effective capillary zone electrophoresis method with high selectivity was established. The calibration curves were linear in the concentration range from 1 to 20 mg/L (ATP and cAMP) and 2 to 40 mg/L (ADP and AMP) with excellent correlation coefficients (r^2) > 0.999. The recovery of ATP, ADP, AMP, and cAMP were 99.4%, 94.7%, 100.3% and 99.6%, respectively. Simple sample preparation and easy detection of ATP and its metabolites make this method suitable for the study of changes in the four adenine nucleotides levels caused by caloric restriction in yeast. It is expected that the current method may contribute to further energy metabolism and related investigations of yeast.

1. Introduction

Adenine nucleotides play important role in biological systems since they serve as units for DNA and RNA synthesis and allosteric regulators of enzymes [1]. In addition, adenine nucleotides are also associated with bioprocesses in combination with cellular development and proliferation [2], metabolism [3], energy transfer [4] and immune responses [5]. For example, ATP is known as a universal energy carrier in most organisms [6]. AMP, ADP and ATP can all potentially act as markers of cellular energy status [7], and cAMP fuctions as a second messenger in the adenylate cyclase cascade [8]. In recent years, *Saccharomyces cerevisiae* has been widely used in biochemical, medical, pharmacology studies and food industry as well-studied model organisms [9]. Several previous findings indicated that adenine nucleotides are involved in caloric restriction [10] and molecular regulation of drugs in yeast model [11,12]. However, the mode of actions of these adenine nucleotides in cellular metabolism is still unclear. The simultaneous determination of their levels with good sensitivity and accuracy is vital in yeast.

Several methods have been reported to measure adenylate levels in biological samples including gas chromatography (GC) [13,14], ^{31}P nuclear magnetic resonance spectroscopy (NMR) [15,16] anion-exchange chromatography [17] ion-pair reversed-phase HPLC [18–20] nanotechnology [21,22], capillary electrophoresis (CE) [23–25] and hilic and ion-pairing based LC–MS [26–28]. Among them, capillary electrophoresis is particularly powerful and well-suited for analyzing adenylate levels for its high separation efficiency and rapid analysis, inexpensive buffer salts and smaller quantities of both buffer and sample [29,30]. CE is usually classified as capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC or MECC), capillary electrochromatography (CEC), capillary isoelectric focusing (CIEF), capillary gel electrophoresis (CGE), and capillary isotachophoresis (CITP) and so on. Above all, CZE is the most common used and easiest mode. Previously, Tseng et al. once used capillary electrophoresis with laser induced fluorescence detection to accomplish the simultaneous analysis of ATP, ADP, AMP and cAMP [31]. So far, few methods of capillary electrophoresis, especially CZE have been reported to determine adenine nucleotides present in yeast.

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http://dx.doi.org/10.1016/j.jchromb.2015.11.040
1570-0232/

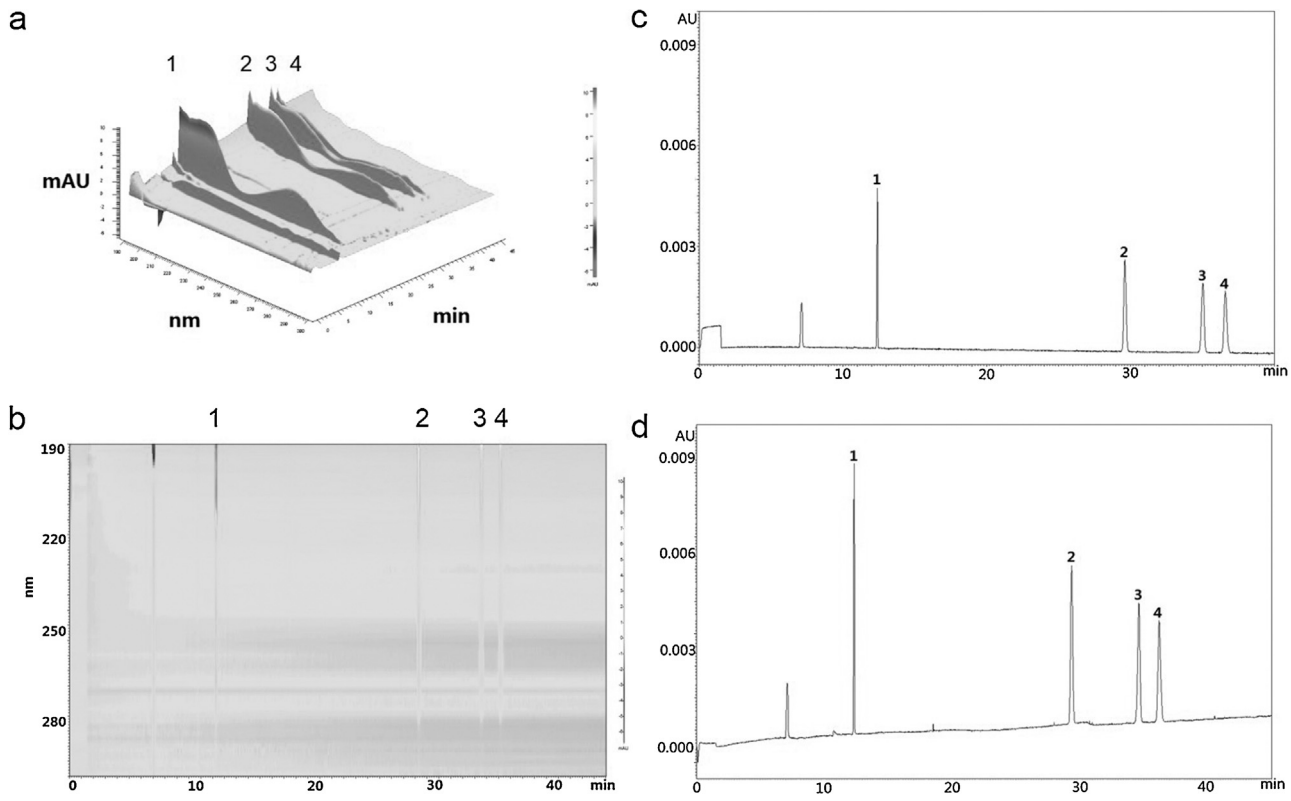

Fig. 1. Effects of detection wavelength on adenine nucleotides analysis. (a) The three-dimensional graphs of four adenine nucleotides ranging from 190 nm to 300 nm; (b) A contour graph of four adenine nucleotides under 210 nm; (c) CE separation of a mixture of nucleosides standards under 260 nm (d) CE separation of a mixture of nucleosides standards under 210 nm. In each picture, (1) cAMP, (2) AMP, (3) ADP, (4) ATP.

In this study, an alternative method for analysis of ATP and its metabolites (ADP, AMP and cAMP) in yeast by CZE was developed. To demonstrate the easy and rapid analysis of yeast samples, ATP and its metabolites (ADP, AMP and cAMP) present in different energetic state of yeast was determined.

2. Materials and methods

2.1. Materials

The adenosine 5-triphosphate disodium salt (ATP, 98%), adenosine 5-monophosphate sodium salt (AMP, 99%) were purchased from Aladdin Industrial Corporation, China. Adenosine 5-diphosphate disodium salt (ADP, 98%) was obtained from Tokyo Chemical Industry., Ltd., Japan. Adenosine 3,5-cyclic monophosphate (cAMP, 95%) was purchased from Ruibio, Germany. Polyethylene glycol (PEG, MW = 20,000) were purchased from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. D-Glucose and sodium tetraborate were obtained from Shanghai Lingfeng Chemical Reagent Co., Ltd., Yeast extract and Tryptdne were obtained from Oxoid Ltd,. Basingstoke, Hampshire, England.

2.2. Metabolite extraction procedure

The seeds of yeast strain *S. cerevisiae*, BY4741 were inoculated at 0.1 of OD600 after rejuvenation, and grown at 30 °C in 2% or 0.5% glucose-containing YPD media respectively [10]. The adenosine triphosphate and its metabolites were extracted according to a formerly described method with some modifications [32]. For each extraction, 1×10^8(10 OD) yeast cells were harvested to determine ATP levels. The harvested yeast cells were washed three to four times by distilled water. The supernatant were removed. The cell pellets were stored at −80 °C. In order to extract ATP and its derivatives from the frozen yeast, we added 200 μL of distilled water to the sample and lysed yeast by boiling for at least 10 min. Lysed samples were centrifuged at 4 °C and 13,000 rpm for 10 min. The supernatant was collected and stored at −80 °C.

2.3. CE separation method

The CE separation process was performed on the a Beckman PA800 Plus CE system (Beckman Coulter Instruments, Fullerton, CA, USA) equipped with a photodiode array (PDA) detection system. The instrument was controlled by 32 Krarat Station Software. Total lengths of untreated fused silica capillary and inner diameter were 44 cm and 50 μm, respectively. The parameters used for optimization of the CE separation conditions were: (a) concentration of Borate buffer (range 30–60 mM) and Borate buffer pH (range 8.5–10.5), (b) detection wavelength, (c) injection volume and (d) separation voltage. In addition, the tetraborate buffers contains 1% (w/v) polyethylene glycol (PEG) at each pH adjusted by using 1 M NaOH and 1 M HCl. The separation temperature was set at 22 °C. Before each runs, the capillary was rinsed for 2 min with 1 M NaOH, 2 min with water, and 2 min with running buffer. Under the optimized condition, linearity of the method was determined using calibration curves. The curves were constructed by plotting the peak areas against the concentration of the standards (ATP, ADP, AMP and cAMP). The limit of detection (LOD) and limit of quantification (LOQ) were determined from the ratio of peak signal and baseline noise level (S/N) as 3 and 10 folds, respectively. Between- and within-day variations were chosen to determine the precision of the method. The within-day variability test was determined by repeated analysis of a single volume of spike standard compounds for six times. For between-day variability test, a single volume of standard compounds was examined among six different days. The recovery was performed by adding spike standard