J Biochem Tech (2010) 2(5):S89-S90
ISSN: 0974-2328

Single strand conformation polymorphism of oligonucleotides in their isolation by means of magnetic micro particles

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Received: 25 October 2010 / Received in revised form: 13 August 2011, Accepted: 25 August 2011, Published: 25 October 2011

Abstract

Studies of biomolecules depend strongly on efficiency of their isolation from biological materials. For these purposes the application of paramagnetic particles (MPs) represents a new method. This method, faster and less laborious than commonly used phenol chloroform extraction, is based on the hybridization of target molecules on a surface of magnetic particles. For the isolation of nucleic acids MPs are modified by certain sequences of thymine or other nucleotides. Using MPs we studied the isolation of ODNs (a) with different sequences of adenine and cytosine and (b)with different chain lengths. It was found that single–strand conformation polymorphism (SSCP) of ODNs is crucial phenomena for their isolation.

Keywords: Paramagnetic particles, oligonucleotides, electrochemical detection

Introduction

Study of biomolecules depends on the efficiency of their isolation from biological materials. There is permanent effort to find new procedures and technologies which will make this process more effective. The main requirements are rapidity and also quality and quantity of isolated, often very expensive, material. For isolation of nucleic acids (NA) there were developed various technologies and approaches based on elimination of interacting proteins and subsequent precipitation with alcohol. These methods have relatively high yields but they are very time consuming and need high cost laboratory instruments. Paramagnetic beads (MPs–magnetic particles) belong to the one of the new and promising methods for NA isolation. This method has a great advantage of designing of MPs size and surface, by which we can effectively isolate various molecules. Moreover, there is no complicated and laborious pre–treatment of a sample which significantly reduces the time needed to obtain the target molecules and reduced the risk of samples contamination. The method is non–invasive and is based on the physicochemical properties of paramagnetic particles. It consists of three steps: binding of nucleic acids on the surface of MPs, washing and releasing the target molecule and its detection. We focused on the study of isolation of oligonucleotides (ODNs) using magnetic particles according to the nucleotide sequence and the ODN chain length. ODN were detected electrochemically (Biyani and Nishigaki 2005; Cantor, Warshaw et al. 1970).

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Materials and methods

Samples of oligonucleotides (Thermo Fisher Scientific, Ulm, Germany; Table 1) were prepared from lyophilized oligonucleotides (ODN), concentration was 50 $\mu g.ml^{-1}$ (content of adenine was the same for all ODNs).

Their concentration was determined spectrophotometrically (Spectronic Unicam, England). The surface of paramagnetic particles (Invitrogen Dynal AS, Norway) was modified sequences dT25. The size of the particles was 2.80 ± 0.2 μm. MPs were washed 3 times with buffer I (0.1 M NaCl, 50 mM Na_2HPO_4, 50 mM NaH_2PO_4). The washing was followed with hybridization. The MPs was added to hybridization solution, phosphate buffer and sample ODN. This mixture was placed on the centrifuge (Multi–spin MSC–3000, Biosan, Lithuania) with occasionally shaking and centrifugation. The hybridization occurred on the base pairing between adenine and the ODN sequences dT25 on the surface of MPs. The MPs with immobilized ODN were concentrated by using a magnetic stand; the hybridization solution was removed and followed by washing. Then the MPs were added to phosphate buffer II, and samples were placed on thermoblock (Thermo mixer 5355 Comfort/Compact, Eppendorf, Germany). The releasing of ODN

from the magnetic particles occurred at 85°C. The last step was to re–concentration of MPs and pipetting the sample with an isolated ODN away. For the electrochemical detection a cell with three electrodes (the reference electrode – Ag/AgCl/3M KCl, auxiliary electrode – carbon , working electrode – a hanging mercury drop electrode with an area of 0.4 mm^2 -HMDE) was used.

Table 1: Characterization of ODNs (A – adenine, C- cytosine)

ODN	Number of A or ODN sequence	λ[cm^2 $mmol^{-1}$]	Molecular weight [$g.mol^{-1}$]	Melting point [°C]
dA9	9	12378	2757.9	18.0
dA12	12	12283	3697.5	24.0
dA18	18	12189	5576.8	37.6
dA36	36	12094	11214.6	60.1
Poly(dA)	400	12009	400000	73.2
H9 double	ACC CAC CCA ACC CAC CCA	9078	5288.5	57.7
H10 double	CCC CCC AAA CCC CCC AAA	8933	5288.5	60.9
H11 double	CCA CAC ACC CCA CAC ACC	8944	5288.5	51.8

Supporting electrolyte was acetate buffer with pH 4.5 (0.2 M CH_3COOH, 0.2 M CH_3COONa). The procedure of adsorptive transfer stripping was chosen. Voltammetric measurements were carried out with AUTOLAB analyzer (EcoChemie, the Netherlands) in connection with VA–Stand 663, (Metrohm, Switzerland). ODNs were also determined with increasing accumulation time (5, 10, 30, 60, 120, 240 and 360 s).

Results and discussion

Reduction signals of adenine (A) and cytosine (C) in ODNs were detected by linear sweep voltammetry associated with adsorptive transfer stripping (AdTS) technique (Huska Adam et al. 2009; Huska, Hubalek et al. 2009). The dependence of heights of reduction peaks on the accumulation time was investigated. ODNs were accumulated on the mercury electrode from 5 s to 360 s. We studied the signals of A, respectively A + C before and after isolation of ODNs by magnetic beads. The values of reduction peaks were measured with a polarization rate 100 $mV.s^{-1}$ at pH 4.5. The interesting observation is that reduction signals of C and A depend strongly on chain length and sequence of nucleobases for ODNs before and after isolation by MPs. Therefore, the isolation is affected by single–strand conformation polymorphism SSCP (Biyani and Nishigaki, 2005; Cantor et al., 1970; Warshaw and Tinoco, 1966). The highest value of the reduction peak was detected at accumulation time of 120 s. According to reduction signals it can be suggested a similar structure of dA9 and dA12 compared to dA18 and dA36 (Fig 1). ODN H10 with double sequence (Table 1) provides significantly low reduction signal. This phenomenon can be justified by the sequence of six Cs and three As which may non–covalently interact and create the i–motif. Bases are therefore less accessible to the reduction onto the electrode surface. Sequence H9 double and H11 double are mirror arranged. Higher signal for H9 can be explained by this ODN sequence ends with adenine, which is protonized at pH 4.5 and better interact with the negatively charged electrode.

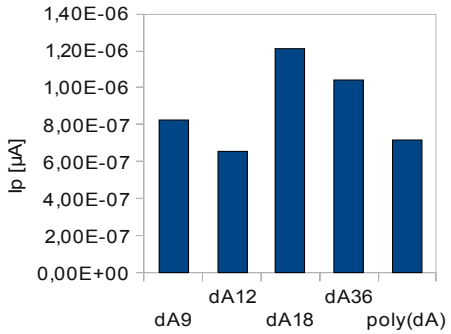

Figure 1: Reduction peak heights of adenine [A] for homo–ODNs isolated by MPs (pH 4.5, scan rate 800 mV.s–1, potential step 2mV)

Conclusion

The interesting study of single–strand conformation polymorphism (SSCP) and its effect on the isolation by MPs is novel. It was found that a chain length and a single nucleotide difference in ODNs can affect the change in their:

- Structure in buffer solutions
- Interaction with mps modified by dt25
- Dynamics on electrically charged interface
- Electrochemical signals
- Electrophoretical mobility

The effectiveness of isolation is influenced by MPs and ODN structure and possibly changing the structure during the isolation process. Electrochemical experiments revealed dynamic structures of ODN molecules in the dependence of their lengths and nucleotide sequences.

Acknowledgement

This work was supported by the following grants: INCHEMBIOL MSM0021622412, MSM0021630503 (MICROSYN), BIO–ANAL–MED LC06035 from the Ministry of Education, Youth and Sports of the Czech Republic and GAAV grant KAN208130801 (NANOSEMED).

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