Evaluation of the Resistance of DNA Immobilized on Ferrimagnetic Particles of Cobalt Ferrite Nanopowder against Nuclease Cleavage

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DNA was immobilized on ferrimagnetic particles of cobalt ferrite nanopowder ($CoFe_2O_4$) and its resistance to endonuclease (DNase I) hydrolysis was studied. Immobilization on cobalt ferrite nanoparticles prevented enzymatic cleavage of DNA. This process was not associated with enzyme inactivation under the effect of nanosize cobalt ferrite and was presumably determined by lesser availability of the DNA molecule as a result of its interaction with nanoparticles.

Key Words: *DNA; DNase I; nanoparticles; cobalt ferrite ($CoFe_2O_4$)*

Progress in molecular biology resulted in the development of new methods for disease diagnosis and treatment and creation of high-technology instruments of molecular genetic studies, based on the use of specific fragments of nucleic acids (NA) [3]. The key problem of effective practical introduction of these instruments is NA protection from adverse environmental factors, including enzymatic degradation [5]. A promising approach to stabilization of NA can be their conjugation with nanoparticles. Their well-developed active surface and small size create prerequisites for effective immobilization of a biomolecule at the individual level and do not prevent further manipulations with it. The use of magnetic particles as carriers is an attractive possibility, because it will lead to realization of highly sensitive detection and effective control of constructions on this base *in vitro* and *in vivo* by means of applying external magnetic field and modulation of its tension and direction [8]. It was shown not once that immobilization on nanoparticles can prevent nuclease cleavage of DNA molecules [4,7]. However, the protective properties of oxide nanoferrimagnetics obtained by mechanochemical synthesis towards nucleic acids have in fact never been studied.

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We studied the resistance of DNA immobilized on cobalt ferrite ($CoFe_2O_4$) supermagnetic nanopowder to endonuclease DNase I hydrolysis.

MATERIALS AND METHODS

Supermagnetic particles of cobalt ferrite ($CoFe_2O_4$) nanopowder (6-12 nm) were obtained at Department of Structural Macrokinetics, Tomsk Research Center, by mechanochemical synthesis from saline systems [1]. Ultrasound-fragmented salmon milt DNA (Medigen) was used in the study. The concentration of DNA was measured spectrophotometrically at λ=260 nm (Juniko 2800). The complex was formed by adding $CoFe_2O_4$ particles to a final concentration of 0.5 mg/ml to DNA solution (0.1 mg/ml; 0.308 mM phosphates) in 10 mM Tris-HCl. After ultrasonic treatment (Microson XL2005), the mixture was incubated for 24 h. The DNA–$CoFe_2O_4$ complex was separated using a permanent magnet with specific magnetic power of 0.2 T and washed twice in ddH_2O. The content of DNA immobilized on the particles (A) was calculated by the formula:

$$A=(C_i-C_o)/m,$$

where C_i is the initial concentration of DNA in the solution (in moles), C_o is the concentration of free DNA

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in the supernatant (in moles), and *m* is the weight of nanopowder in solution (in g).

Hydrolysis of DNA/DNA–$CoFe_2O_4$ by DNase I (Promega) was carried out at 37°C for 1 h in 20 μl reaction mixture containing 40 mM Tris (pH 8.0), 1 mM $CaCl_2$, 10 mM $MgSO_4$, 2 U enzyme, and 2 μg analyzed DNA. The reaction was stopped by adding EGTA in a final concentration of 2 mM and warmed at 65°C for 10 min. Electrophoretic analysis was carried out in 1.5% agarose gel (Tris-acetate buffer, pH 8.0) with ethidium bromide in a constant electric field at 150 V. DNA was visualized in UV light. The image was fixed using Vitran-Photo image processing system (Biocom).

RESULTS

Spectrophotometry showed that DNA incubation with dissolved $CoFe_2O_4$ nanopowder led to binding of biomolecules with cobalt ferrite nanoparticles ($5.93\pm0.01\times10^{-4}$ mol/g). Conjugation of DNA molecules and $CoFe_2O_4$ nanopowder particles was confirmed by gel electrophoresis. Two groups of DNA bands were discernible on electrophoregram of magnetically separated DNA and $CoFe_2O_4$ nanoparticles precipitate. They differed by their migration rate in gel (Fig. 1, track *3*). The appearance of characteristic DNA bands with lowered electrophoretic mobility (Fig. 1, track *3*, *I*) in comparison with control DNA (Fig. 1, track *1*) attests to the formation of DNA composite with cobalt ferrite nanoparticles. Changed rate of migration in gel of DNA conjugated with nanoparticles could be due to increased size and weight of the forming complex and/or changes in its charge in comparison with free DNA [5]. The presence of $CoFe_2O_4$-bound DNA fraction in electrophoretic profile (Fig. 1, track *3*, *II*), corresponding to the control by mobility, can be explained by separation of some DNA molecules from nanoparticles under the effect of electric field. The difference in the stability of bonds between DNA and $CoFe_2O_4$ nanoparticles can be explained by two mechanisms of binding of polynucleotide fragments to cobalt ferrite nanoparticles. Strong binding of DNA molecules can be realized due to the formation of coordination bonds between electron acceptor Fe and Co atoms of cobalt ferrite having a free d-orbital and DNA atoms with an unshared electron pair: phosphate group and bases (guanine N7 and O6, adenine N7 and N1, and pyrimidines N3) [2]. Participation of DNA base groups in binding to nanoparticles would lead to denaturation of DNA double strand. However, free reaction of ethidium bromide (intercalation dye) with $CoFe_2O_4$–DNA can be regarded as an evidence of retention of the double strand structure of the polynucleotide component of the composite with nanoparticles [6]. Hence, it is more likely that mainly PO_2 groups of the DNA sugar-phosphate backbone are involved in the formation of the bond. After saturation of type one bonds DNA can presumably less intensely nonspecifically react with cobalt ferrite nanoparticles.

In order to study the impact of DNA binding to $CoFe_2O_4$ nanoparticles for the biomolecule resistance to in enzymatic degradation, the composite was incubated with DNase I characterized by endonuclease activity towards single- and double-stranded NA. Electrophoretic analysis of hydrolysis products showed that 1-h incubation with DNase I resulted in complete degradation of the polynucleotide in the control sample (Fig. 1, track *2*). Endonuclease treatment of $CoFe_2O_4$–DNA led to cleavage of poorly bound fraction, while DNA immobilized on cobalt ferrite nanoparticles exhibited resistance against enzymatic hydrolysis, which was seen from the presence of characteristic bands on the electrophoregram (Fig. 1, track *4*). Several possible mechanisms of the protective effect of nanoparticles against enzymatic degradation of NA are discussed: inactivation of the enzyme or modification of the DNA substrate as a result of reaction with the particles

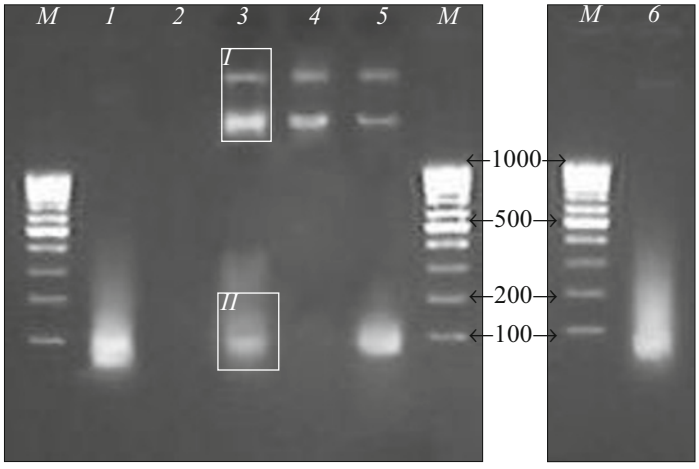

Fig. 1. Electrophoregram of DNA and DNA–$CoFe_2O_4$ complex before and after endonuclease cleavage (1.5% agarose gel). *M*: 100-1000 b. p. marker. *1*) DNA (control); *2*) DNA (control) after incubation with DNase I; *3*) DNA–$CoFe_2O_4$ composite; *4*) DNA–$CoFe_2O_4$ composite after incubation with DNase I; *5*) destruction of DNA–$CoFe_2O_4$ by 20 mM EDTA (30 min) after incubation with DNase I; *6*) destruction of DNA–$CoFe_2O_4$ by 20 mM EDTA (12 h) after incubation with DNase I.

[5,9]. The results showed that nuclease inhibition of $CoFe_2O_4$–DNA hydrolysis cannot be attributed to inhibition of enzyme activity, because DNA of the loosely bound fraction was easily cleaved in the presence of cobalt ferrite nanoparticles.

The formation of $CoFe_2O_4$–DNA composite could cause steric difficulties in inoculation of the enzyme and/or be associated with conformation changes in the DNA structure as a result of binding. In order to evaluate availability of the polynucleotide conjugated with nanoparticles for the enzyme molecules, the $CoFe_2O_4$–DNA composite after treatment with DNase I was destroyed by EDTA (chelating agent) in a final concentration of 20 mM and the degree of DNA fragmentation was analyzed. A DNA band of the control level appeared in the electrophoretic profile of the sample after 30-min incubation of the composite with EDTA, the fluorescence intensity of "poorly mobile" fragments characteristic of $CoFe_2O_4$–DNA decreased (Fig. 1, track *5*). Prolongation of incubation to 12 h led to disappearance of the bands corresponding to DNA immobilized on $CoFe_2O_4$ nanoparticles, this indicating complete destruction of the composite under the effect of EDTA (Fig. 1, track *6*). Comparison of electrophoretic mobility of the initial DNA fragments (Fig. 1, track *1*) and fragments of DNA separated from the nanopowder particles confirmed that the polynucleotide in the composite was not much fragmented by DNase I treatment. Hence, DMA immobilized on cobalt ferrite nanoparticles is unavailable for the enzyme throughout its entire length, which is in line with the hypothesis on the polynucleotide fixation at the expense of phosphate groups.

Hence, the study demonstrated the principal possibility of creating a magnetic sensitive bionanocomposite complex based on $CoFe_2O_4$ nanopowder and DNA molecules. It was shown that DNA in the bionanocomposite is resistant to endonuclease cleavage.

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