Novel Method for DNA Isolation Using High-throughput Monolithic Silica Gel Column in Combination with Anti-chaotropic Effect

Hajime Karatani,*[1] Keiichiro Miyamoto,[1] Chihiro Katsukawa,[2] Tomoharu Kono,[3] and Hiroyoshi Minakuchi[3]
[1]*Department of Biomolecular Engineering, Graduate School of Science and Technology, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585*
[2]*Osaka Prefectural Institute of Public Health, Nakamichi, Higashinari-ku, Osaka 537-0025*
[3]*Kyoto Monotech Co., Kajii-cho, Kamigyo-ku, Kyoto 602-0841*

(Received September 25, 2006; CL-061112; E-mail: karatani@kit.ac.jp)

DNA molecules were completely adsorbed by the silica gel in the presence of saturated ammonium sulfate with a marked anti-chaotropic property, and the adsorbed DNAs were quantitatively desorbed with a low salt buffer. Based on such adsorption–desorption behavior of DNA, a novel isolation method for DNA was successfully developed.

Rapid isolation of DNA molecules at the sub-μg level using a high-throughput silica gel spin column became an essential element in the molecular biological experiments.[1] The DNA isolation on a high-throughput column consists of three steps: the adsorption of DNA onto the column, washing the column and the DNA desorption. Especially the DNA adsorption is pivotal to achieve high recovery. For this purpose, the chaotropic effect caused by guanidine (Gdn)•SCN at high concentration is widely employed.[2] The other chaotropic salts, such as NaI and NaSCN, are also applicable for the DNA adsorption.[2,3] The DNA adsorption on the silica gel is considered to be associated with the breakdown of water structure owing to the chaotropic effect, resulting in the disruption of the DNA secondary structure.[4]

In this study, we expected that by providing the hydrophobic environment free from organic solvent the formation of the hydrogen bond between DNA and silica gel would be facilitated. In light of this, we examined the anti-chaotropic effect on the adsorption–desorption of DNA and then applied the effect to develop a new method for the DNA isolation.

The high-throughput column was designed and made based on the widely used commercial column. The monolithic silica gel (MSG) with 5-μm macropore was employed for the column substrate because of not only ease in processing but also high permeability (Figure 1).[5–7]

The diameter (ϕ) of the MSG column was either 4.0 or 2.5 mm, and the thickness (t) was fixed at 1.5 mm. Smart Ladder (total DNA, 144 ng/μL, Nippon Gene) containing various sizes of linear double-stranded DNA fragments between 0.2 and 10 kbp was used as the standard. Ten μL of the standard DNA was dissolved in the 5 times volume of anti-chaotropic salt solution. Such a DNA amount (1.44 μg/sample) is a large excess for the usual DNA isolation. The DNA sample was loaded onto the column and centrifuged. After washing the column with a 500 μL of the ethanol–10 mM phosphate buffer (pH 6) (4:1 v/v). the adsorbed DNA was eluted with 50 or 30 μL of 10 mM tris(hydroxymethyl)aminomethane (Tris)-HCl buffer (pH 8.5), which is capable of eluting DNA adsorbed by the silica gel.[3] All centrifugations for adsorption, washing and desorption were done at 6500 *g* for 1 min, according to the commercial column operation. The filtrate obtained in the adsorption step and the eluate were subjected to the 1 w/v% agarose gel electrophoresis (agarose, grade S, Nippon Gene). The amount of individual DNA stained with ethidium bromide was determined based on the brightness of the standard DNA using a Gel-Pro Analyzer (ver. 4.5) (Media Cybernetics, MD, U.S.A.).

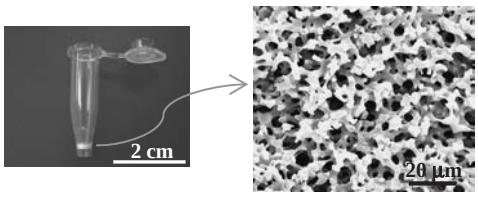

Figure 1. High-throughput monolithic silica gel column used for DNA isolation (A) and SEM image of the gel column (B).

First, DNA adsorption on the silica gel in the presence of typical anti-chaotropic sulfates and desorption were evaluated. As expected, DNA was found to be adsorbed in the presence of high concentration sulfate (Figure 2). In particular, the aqueous 4.4 M $(NH_4)_2SO_4$ (pH 5.5), almost saturated at 25 °C, was very effective for the DNA adsorption. The DNA adsorption on the silica gel is carried out in weakly acidic conditions.[2] In the case of $(NH_4)_2SO_4$, the preferable pH was obtained by its hydrolysis, and the addition of any extra buffer is not required.

DNA was not detected in the washing solution, passed through the column, by the electrophoresis or by measuring A260/A280. The elution of DNA adsorbed in the presence of phosphate was less efficient than that in its absence. Oppositely, phosphate buffer was made use of the column washing step.

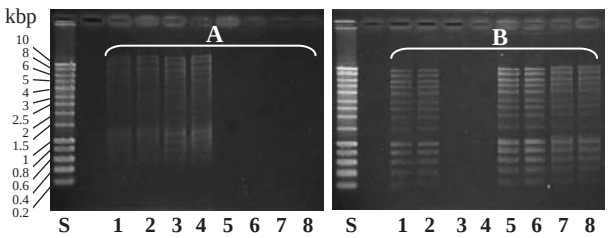

Figure 2. Agarose gel electrophoretic analyses of filtrates in the adsorption step (A) and eluates (B). Loaded volume, 5 μL/lane. Sulfate (50 μL), mixed with a standard sample containing 1.44 μg DNA (10 μL): 1 and 2, 2.0 M Na_2SO_4 in water (pH 5.7); 3 and 4, 2.0 M Na_2SO_4 in 0.1 M phosphate buffer (referred to as PB) (pH 6.0); 5 and 6, 4.4 M $(NH_4)_2SO_4$ in water (pH 5.5); 7 and 8, 4.4 M $(NH_4)_2SO_4$ in PB (pH 6.0). S, standard (720 ng/lane). Elution buffer, 50 μL. Note that DNA is diluted in filtrate (60 μL) and in eluate (50 μL). MSG column: ϕ, 4 mm; t, 1.5 mm.

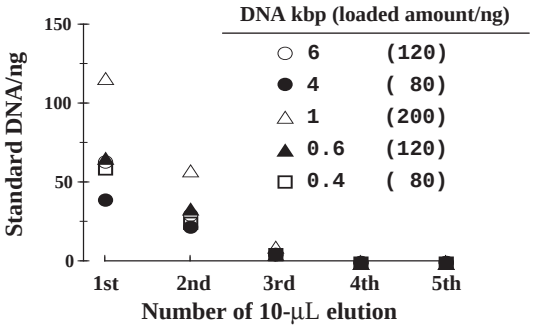

Figure 3. Plots of the amount of DNA desorbed against the successive 10-μL elution. MSG column: ϕ, 4 mm; *t*, 1.5 mm.

The other anti-chaotropic salts applicable for the DNA adsorption were the saturated $MgSO_4$ (≈2.7 M) and NH_4CH_3COO at higher than 8 M. However, larger volume of elution buffer was needed for the complete DNA desorption, as compared to the system with $(NH_4)_2SO_4$. At the present, $(NH_4)_2SO_4$ was shown to be the most potent anti-chaotropic salt for the DNA isolation. The adsorption efficiency for the standard DNAs was estimated to be approximately 100% when the $(NH_4)_2SO_4$ concentration was higher than 4 M.

DNA desorption efficiency was evaluated by the successive elution with a 10-μL aliquot of Tris-HCl buffer. DNA (1.44 μg) was mixed with 4.4 M $(NH_4)_2SO_4$ and then loaded onto a column. Assuming that the DNA is completely adsorbed by the silica gel, the desorbed DNA amount in each 10-μL eluted solution is plotted (Figure 3). About 60% of the adsorbed DNA was eluted at the first 10-μL elution and 100% desorption was attained at the 3rd elution, independent of DNA size. Thus, it was shown that DNA adsorbed by the silica gel in the presence of $(NH_4)_2SO_4$ was readily and quantitatively desorbed.

The reproducibility of the DNA adsorption–desorption was evaluated using the MSG column with 2.5-mm diameter. Using this column, the volume required for elution was able to be reduced to two thirds as compared to that for the 4-mm column. The identical sample containing 1.44 μg DNA was applied to the MSG column. The resultant filtrates and eluates were subjected to the electrophoresis (Figure 4).

The observations that no band was detected in all the filtrates and that all the eluates gave clear bands corresponding to the standard DNAs showed that the adsorption–desorption of DNA was achieved in good reproducibility. From the analysis of the stained bands, the average recoveries for the standard DNA molecules between 0.2 and 2 kbp and for those larger than 2.5 kbp were almost 100 and 80%, respectively. The total recovery was at least 90%. At the moment, the performance of the proposed DNA isolation method can be regarded to be comparable to that of the most accepted commercial column, based on the evaluation under the same sample conditions.

In contrast to the chaotropic effect, the hydrophobic property is enhanced by the addition of anti-chaotropic salts at high concentrations because of the stabilization of the water structure.[8] Hence water molecules will be removed by the anti-chaotropic ions not only from the DNA molecule but also from the hydrated silica gel. Under such conditions, it is postulated that the formation of the hydrogen bond between hydroxy group on the silica gel and oxygen of the phosphodiester group in the DNA

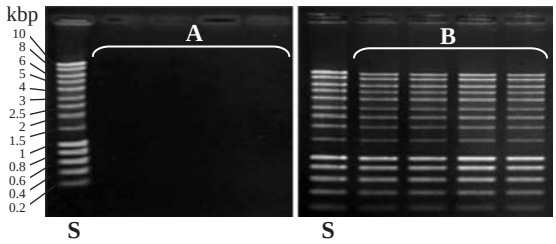

Figure 4. Agarose gel electrophoretic analyses of filtrates in the adsorption step (A) and eluates (B). Loaded volume, 5 μL/lane. S, the standard DNA (240 ng/lane). The standard DNA amount (=1.44 μg × (5/30)) was determined, assuming that both adsorption and desorption were attained at 100% efficiency. Elution buffer, 30 μL. MSG column: ϕ, 2.5 mm; *t*, 1.5 mm.

molecule is facilitated. The postulated hydrogen bond interaction between the DNA molecule and the silica gel seems to be, in part, analogous to that in the hydrogen bond chromatography for the protein isolation on the cellulose column, performed using the high concentration $(NH_4)_2SO_4$.[9]

It should be noted that no precipitation occurred by mixing 4.4 M $(NH_4)_2SO_4$ with the DNA samples, even from the supernatant of the microorganism cell lysates. The DNA fragments isolated by the proposed method with 4.4 M $(NH_4)_2SO_4$ were confirmed to be ready for the following polymerase chain reaction. Owing to the high permeability of the MSG column, the *g*-min for the centrifugation can be lowered to 1200 or less. Relating to this, the total time required for the DNA isolation will be much shortened. At the moment, the proposed method cannot be applied to the blood sample because of the protein precipitation. Not only the prevention of the protein precipitation in the blood sample, but also further characterization of the interaction between DNA and silica gel in the presence of the anti-chaotropic salts is our next goal.

This study was supported by a Grant for Technological Development by R&D Venture Businesses of the New Energy and Industrial Development Organization (NEDO).

References

1 K. A. Wolfe, M. C. Breadmore, J. P. Ferrance, M. E. Power, J. F. Conroy, P. M. Norris, J. P. Landers, *Electrophoresis* **2002**, *23*, 727.
2 W. R. Boom, H. M. A. Adriaanse, T. Kievits, P. F. Lens, U.S. Patent 5234809, **1993**.
3 B. Vogelstein, D. Gillespie, *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 615.
4 K. Hamaguchi, E. P. Geiduschek, *J. Am. Chem. Soc.* **1962**, *84*, 1329.
5 K. Nakanishi, N. Soga, *J. Am. Ceram. Soc.* **1991**, *74*, 2518.
6 H. Minakuchi, K. Nakanishi, N. Soga, N. Ishizuka, N. Tanaka, *Anal. Chem.* **1996**, *68*, 3498.
7 K. Nakanishi, H. Minakuchi, N. Soga, N. Tanaka, *J. Sol-Gel Sci. Technol.* **1997**, *8*, 547.
8 C. Tanford, *The Hydrophobic Effect: Formation of Micelles and Biological Membrane*, 2nd ed., Wiley-Interscience, New York, **1980**.
9 T. Fujita, Y. Suzuki, J. Yamauti, I. Takagahara, K. Fujii, J. Yamashita, T. Horio, *J. Biochem.* **1980**, *87*, 89.

Published on the web (Advance View) October 28, 2006; doi:10.1246/cl.2006.1354