초청강연 I

Rejection of DNA, Protein-DNA Complexes and Chromatin by Hollow Fiber Membranes

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1. Virus and DNA removal in bio-drug manufacturing processes has received a great deal of attention in recent years. Removing of a virus using a membrane process is a promising method, because inactivated virus can be removed from the bio-drug and the process can be used as an additional and security inactivation after the method of general heat-inactivation of the virus in the bio-drug. The FDA and the biopharmaceutical industry have recently announced strict guidelines for impurities of virus and DNA contamination. The regulatory guidelines on residual amounts of DNA in mammalian cell culture products require DNA contamination of less than 100 pg/dose[1]. Therefore, permeation and rejection of DNA through the porous membranes have become important in the application of DNA removal in bio-drug manufacturing using membrane technology. In this study, the permeation of DNA and chromatin through regenerated cellulose hollow fibers that have a mean pore diameter of 15 nm was investigated.

2. Dead-end type ultrafiltration was carried out under a constant transmembrane pressure of 200 mmHg at 25℃ with a filtration volume of 55 cm^3 or 70 cm^3. The membrane used in this study is a high performance regenerated cellulose hollow fiber (BMM) having a mean pore diameter of 15 nm (PLANOVA™ 15N, Asahi Chemical Industry Co., Ltd.).

Calf thymus DNA was dissolved in Tris/EDTA buffer and adjusted to a

pH of 7.4 - 8.0 by adding 0.1M acetic acid.

The histone-DNA complex was prepared according to the salt-urea method of Camerini-Otero et al.[2] Chromatin originated from HeLa S3 cells (RCB0271, Riken Cell Bank, Tsukuba, Japan) were prepared using modified procedures described in the literature[3].

The DNA solution, the histone-DNA complex solution and chromatin solution were ultrafiltered using the BMM membranes, and every 15 ml of the permeate solution was collected (Permeates No. 2 - No. 5) after initially sampling 10 ml (Permeate No. 1) as described previously[4]. Dead-end type ultrafiltration was carried out under a constant transmembrane pressure of 200 mmHg at 25℃ with a filtration volume of 55 cm^3. The permeation ratio (P) of the DNA is defined as $P\ (\%) = [C_p/C_f] \times 100$, where C_f is the concentration of the DNA in the feed solution and C_p is the concentration of the DNA in the permeate solution.

The fraction of single stranded DNA in the total DNA of feed, permeate and concentrate solution was estimated from the data that the absorbance at 260 nm of the solution denatured to single stranded DNA is 1.37 times higher than that of the native solution that contains only double stranded DNA.

3. A DNA solution of 50 ppm containing no protein and 0.4 M NaCl was filtered through BMM membranes at a pH of 7.8. These experiments were performed for comparison with the permeation of the histone-DNA complex through the membranes, because the histone-DNA complex is only soluble and its solution is transparent in the presence of more than 0.4 M NaCl. The permeation ratio, the flux and the fraction of single stranded DNA in the feed and permeate solutions during several permeation (sampling) times are summarized in Table 1. It is found that DNA in 0.4 M NaCl can be rejected to some extent by the BMM membranes, because the permeation ratio is 14-23 % in the table. It is summarized that no significant influence on the permeation (rejection) of DNA was found in the presence of 0 - 0.4 M NaCl in the DNA solution.

The fraction of single stranded DNA, $R_{SS\text{-}DNA}$, in the permeate solution was found to be higher than the $R_{SS\text{-}DNA}$ in the feed solution from Table 1. This result leads to the fact that the single stranded DNA preferentially permeates through the BMM membranes. This is mainly explained by the higher flexibility of the single stranded DNA over the double stranded DNA.

The histone-DNA complex in the buffer containing 10 mM Tris-HCl, 1.0 mM EDTA and 0.4 M NaCl was filtered through the BMM membranes at a pH of 7.8. Typical UV spectra of the feed and permeate solutions are shown in Fig. 1. Because the absorbance of the permeate solution is less than that of the feed solution, it is found that DNA can be rejected to some extent by the BMM membranes.

It is well known that chromatin is composed of a 30-nm fiber, and the AFM shows that the image of apparent width of chromatin is 20 - 30 nm. The BMM membranes having a mean pore diameter of 15 nm should reject the chromatin-like structure of the histone-DNA complex completely. The AFM measurements of the histone-DNA complex in the feed and permeate solutions were performed to determine whether the BMM membranes can reject the fibers of the histone-DNA complex completely. Fig. 2a shows a typical AFM image of the histone-DNA complex in the feed solution. The apparent width of these structures was found to be 25-30 nm. Fig. 2b shows a typical AFM image of the permeate solution. No chromatin-like structures or fibers were found in Fig. 2b. It is suggested from the AFM measurements that the histone-DNA complex can be rejected by the BMM membranes.

The native chromatin extracted from HeLa S3 cells was filtered through the BMM membranes at a pH of 7.8. Because the extracted amount of chromatin solution in one cycle of the experiments is only 0.4 ml having 0.5 absorbance at 260 nm, the chromatin was filtered through the BMM membrane with a filtration volume of 2 ml containing a DNA concentration of 5 ppm.

AFM measurements of chromatin in the feed and permeate solutions were also performed to determine whether the BMM membranes can reject the native chromatin completely. It is suggested from the AFM measurements that chromatin can be completely rejected by the BMM membranes.

1. J.C. Petricciani, Developments in Biological Standardization, 59 (1985) 149-153.
2. R.D. Camerini-Otero et al., Cell, 8, (1976) 333.
3. Y. Ishimi et al., J. Biochem., 94, (1983) 735-744.
4. A. Higuchi et al., J. Membrane Sci. (1996) in press.

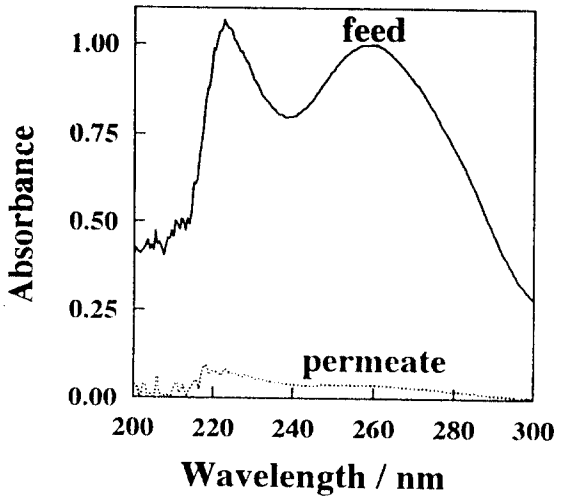

Fig. 1 UV spectra of feed and permeate solutions. The feed solution is the histone-DNA solution containing 0.4 M NaCl and the permeation experiments were performed at Cf=50 ppm, pH 7.8 and 25 ℃.

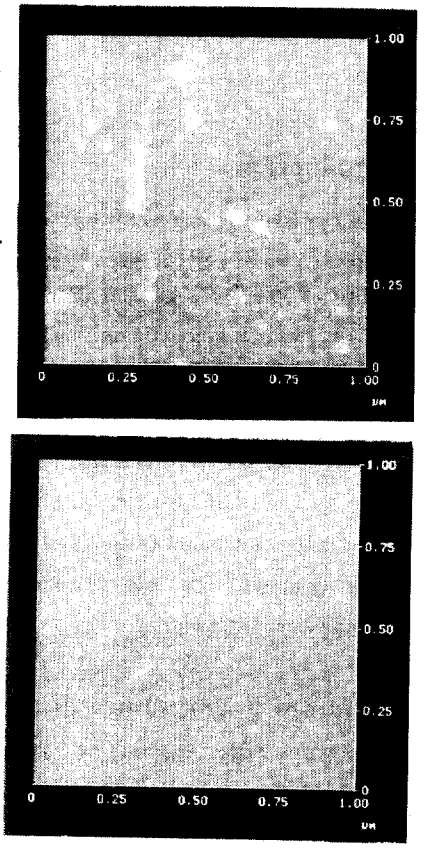

Fig. 2 AFM images of feed (a) and permeate (b) solutions. Feed solution is a chromatin solution extracted from HeLa S3 cells.

Table 1 Permeation of DNA in the presence of 0.4 M NaCl through BMM membranes at a transmembrane pressure=200 mmHg, 25 ℃, Cf=50 ppm and pH=7.8 (n=5).

Samples	Permeation time / min	Flux /$m^3m^{-2}day^{-1}$	Sieving coefficient/%	$R_{ss\text{-}DNA}$ / %
Feed				20.1±3.0
Permeate No. 1	0 - 33	0.147±0.26	22.1±3.0	70.7±8.2
Permeate No. 2	33 - 74	0.176±0.14	18.2±3.1	82.9±7.6
Permeate No. 3	74 - 113	0.197±0.09	16.6±3.3	83.4±5.6
Permeate No. 4	113 - 155	0.199±0.11	14.5±2.5	88.4±9.8