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Short communication

Stabilization of polymer-based gene delivery systems

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Abstract

To preserve the size and transfection potential of polymer–plasmid complexes, freeze-drying and freeze-thawing were used for stabilization of these complexes. The concentration of the sugars is an important factor affecting both the size and transfection capability of the complexes after freeze-drying and freeze-thawing. However, the type of lyoprotectant (sugar) used is of minor importance. It is also shown that when damage to polymer–plasmid complexes occurs, it results from the drying process but is not due to the freezing step. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Plasmid; Poly(2-dimethylamino)ethyl methacrylate; Stabilization; Freeze-drying; Freeze-thawing; Gene therapy

1. Introduction

The use of poly(2-dimethylamino)ethyl methacrylate (PDMAEMA) was reported as an efficient non-viral transfectant (Cherng et al., 1996; Wetering et al., 1998). This polymer is able to bind electrostatically to plasmids yielding polymer–plasmid complexes. It was found that the size of the complexes formed was a dominant factor for the transfection efficiency (as shown in Fig. 1). The highest transfection efficiency was observed at a polymer/plasmid ratio of 3 (w/w) and average molecular weight of the polymer above 250 kDa. Under these conditions particles with a size between 0.15 and 0.25 μm and a slightly positive ζ-potential were formed. However, the complexes have a limited stability in aqueous solution due to possible chemical and physical degradation processes.

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2. Methods

The complexes consist of pCMV-lacZ plasmid which contains a bacterial lacZ gene preceded by a nuclear location signal under control of the CMV promoter (Bout et al., 1993) and PDMAEMA. We selected a simple buffer solution (20 mM Hepes, pH 7.4) with or without a lyoprotectant to prepare the transfection complexes. In a control experiment, it was shown that 2.5% (w/v, concentration in transfection) sucrose in RPMI (medium of transfection) did not influence the transfection efficiency of freshly prepared polymer–plasmid complexes. At higher sucrose concentrations a slight increase in the number of transfected cells was observed as reported by Ciftei et al. (1996). Therefore, to exclude the effect of sucrose on transfection, we reconstituted the samples in RPMI (8 times dilution) yielding a sugar concentration not exceeding 2.5%, and a polymer and plasmid concentration of 15 and 5 μg/ml, respectively, which corresponds with conditions where the transfection showed an optimum.

3. Results and discussion

In the first series of experiments polymer–plasmid complexes were prepared with a varying concentration of sucrose (0–5%, concentration in

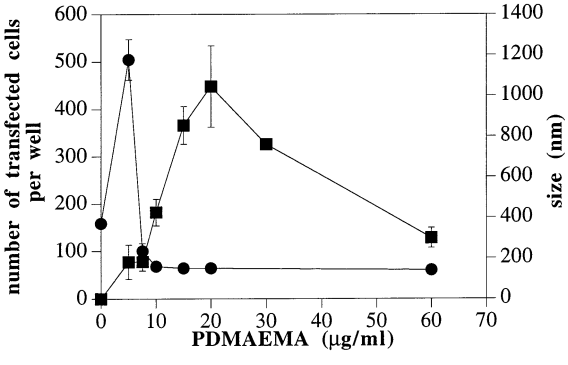

Fig. 1. Effect of polymer concentration at a fixed plasmid concentration (5 μg/ml) on the size (●) and the number of tranfected COS-7 cells (■). The results are expressed as means ± S.D. of 3–5 experiments.

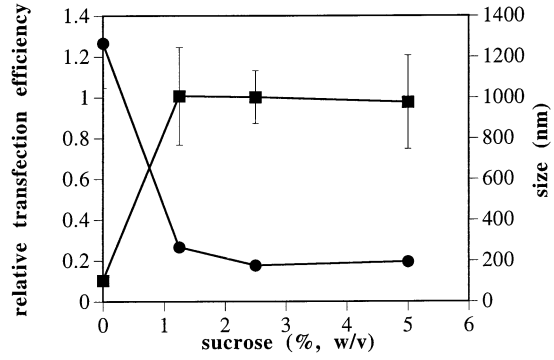

Fig. 2. Size (●) and relative transfection efficiency (■) of polymer–plasmid complexes after freeze-thawing. The percent sucrose refers to the concentration present in the aqueous polymer–plasmid solution used for the preparation. The results are expressed as means ± S.D. of 3–5 experiments. The transfection values were normalized to the number of transfected cells found after incubation of the cells with freshly prepared polymer–plasmid complexes in Hepes (relative transfection efficiency).

preparation). After freeze-thawing, the size and transfection efficiency of the complexes were evaluated. The results in Fig. 2 show that in the absence of sucrose, large particles were found which showed a reduced transfection efficiency. On the other hand, in the presence of sucrose both the particle size and the transfection potential of the complexes were preserved.

All freeze-dried samples (except the formulation without lyoprotectant) formed good cakes which readily dissolved in water. Fig. 3 shows that after rehydration of the freeze-dried samples prepared at a low sucrose concentration (1.25%), large particles were present. Obviously, this amount of sucrose is not enough to prevent aggregation of the polymer–plasmid complexes. In agreement with previous studies (Cherng et al., 1996; Wetering et al., 1997), these large complexes possessed a relatively low transfection efficiency. By combining Figs. 2 and 3, it can be concluded that at this low sucrose concentration the damage to the complexes occurs during the drying process. Fig. 3 also shows that small particles were detected after rehydration of the freeze-dried cakes with 1 ml water, which were obtained after freeze-drying of the aqueous polymer–plasmid solutions containing a concentration of sucrose $\geq 2.5\%$. No

substantial change in particle size was observed after the addition of 7 ml RPMI. Moreover, the transfection efficiency of these particles was not significantly different from freshly prepared particles. Although the different cakes contained a varying amount of residual water, it was demonstrated by DSC analysis that the T_g value of the sucrose matrices was above 50°C. Moreover, it was shown that the residual water was uniformly distributed in the matrices.

Knowing that sucrose is an effective lyoprotectant for polymer–plasmid complexes, we compared its performance with two other frequently used lyoprotectants (trehalose and maltose). Fig. 4 shows that for the sugars investigated, freeze-drying and freeze-thawing gives the same transfection efficiency. DLS experiments demonstrated that the size of the transfection complexes only slightly increased from approximately 0.18 μm (directly after preparation) up to 0.22 μm after freeze-thawing and freeze-drying. This increase in particle size was associated with a slight increase in the polydispersity index, suggesting that some limited aggregation had occurred. Interestingly, the type of lyoprotectant had no effect on either the size or the transfection efficiency of the complexes after freeze-drying and freeze-thawing. Aggregation of the complexes in the sugar matrices might be prevented due to the glassy character of the matrices and/or specific interactions (e.g. H-bonding) between the sugars and the polymer–plasmid complexes. However, other factors like physical separation of the complexes in the sugar matrices also might contribute to the observed stability. This paper shows that freeze-drying is an excellent method to preserve the size and transfection potential of polymer–plasmid complexes. The type of lyoprotectant (sugar) used is of minor importance. However, the concentration of the sugars is an important factor affecting both the size and transfection capability of the complexes after freeze-drying and freeze-thawing. If there is damage to polymer–plasmid complexes during freeze-drying, it results from the drying process but not due to the freezing step.

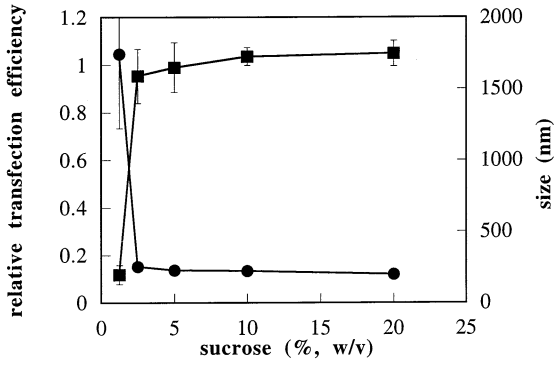

Fig. 3. Size (●) and relative transfection efficiency (■) of polymer–plasmid complexes after freeze-drying and rehydration. The percent sucrose refers to the concentration present in the aqueous polymer–plasmid solution used for the preparation of the cakes. The results are expressed as means ± S.D. of 3–5 experiments. The transfection values were normalized to the number of transfected cells found after incubation of the cells with freshly prepared polymer–plasmid complexes in Hepes (relative transfection efficiency).

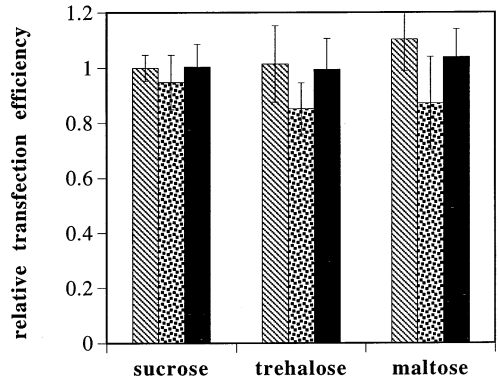

Fig. 4. Relative transfection efficiency of polymer–plasmid complexes prepared with different sugars (10%, w/v): freshly prepared (▨), after freeze-drying and rehydration (□), after freeze-thawing (■).

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