LABORATORY EQUIPMENT/APPLICATION NOTE

Eliminating DNA loss & denaturation during storage in plastic microtubes

CLAIRE GAILLARD & FRANÇOIS STRAUSS

The preparation of biological samples often requires days or weeks of expensive experiments, and the choice of tubes in which such samples are stored cannot be made without some caution. Price is not the only factor; the quality of the tubes is also very important. Some defects are obvious and can be detected easily: tubes that leak, are too difficult to open, or do not withstand centrifugation do not remain on the market for very long. Other defects are more subtle and are difficult to detect, for example, contamination of plastics with chemicals used during the manufacturing process.[1] Another problem is caused by the loss of sample on the tube walls. Although this is a problem that has long been seen with proteins, the situation with DNA, however, is somewhat different. The DNA double helix is a highly charged, very hydrophilic molecule, whereas polypropylene, which is generally used for making plastic microtubes, is a very hydrophobic polymer. These characteristics are supposed to reduce the interactions of DNA with tube walls and to prevent or minimise losses of DNA on tube surfaces. It has been observed, however, that DNA can bind to polypropylene,[2] and that the interaction of DNA with tube walls induces a change of conformation, which can go as far as complete denaturation with strand separation.[3–6] This phenomenon, which is particularly noticeable with short DNA fragments and at high ionic strength, can be of interest as the conformations adopted by DNA upon binding lead to the formation of interesting alternative structures.[7] For most applications, however, it is extremely important not to lose DNA on tube walls and to keep DNA in its native, perfectly double-stranded state.

The authors observed previously that some tubes are more suitable than others for storage of DNA, with polyallomer tubes in particular, not showing the two defects of adsorption and denaturation observed with polypropylene tubes.[2] The price of polyallomer tubes, however, is more than five-fold higher than the average price of polypropylene tubes, which can be a problem given the large number of microtubes used in a typical laboratory.

This led the authors to test a large number of different kinds of commercially available tubes, looking for tubes of moderate price that would show neither DNA adsorption nor DNA denaturation on tube walls. Nine different kinds of tubes were purchased and arbitrarily labelled *a–i*. Of all the tubes, the only type with declared surface treatment were tubes *g* (siliconised polypropylene tubes). Since interactions of DNA with polypropylene are particularly strong at high ionic strength, the first tests were done in 2.5 M NaCl. A given amount of a DNA fragment that had been end-labelled with ^{32}P was deposited at the bottom of the tubes in 2.5 M NaCl, 10 mM Tris-HCl, 1 mM EDTA, pH 7.5. The tubes were incubated for 16 hr at 37 °C to prevent any condensation of liquid in the tube caps. The liquid was then removed and radioactivity bound to the tube walls was determined by counting, allowing the authors to determine the percentage of DNA adsorbed to the surface. As the results often showed important variability, many tubes of each kind were tested, and histograms were drawn in which the number of tubes was represented as a function of the percentage of DNA adsorbed, by intervals of 5%.

Figure 1 shows that, although the results for a given kind of tube can be highly variable, definite trends can be observed. With some tubes, the percentage of binding is most often high; with other tubes, the percentage of binding is usually low but still significant; and with other tubes, any DNA binding is hardly detectable. Some general conclusions can be deduced from these experiments: All ordinary polypropylene tubes tested showed, fairly reproducibly, a high percentage of DNA adsorption under the conditions used (high ionic strength). Polyethylene tubes bind DNA even more strongly.

With the commercial siliconised polypropylene tubes tested, DNA adsorption is not significantly lower than with ordinary polypropylene tubes. Adsorption is not as systematic as with ordinary tubes, but still reaches high values. Since the authors obtained similar results when they siliconised polypropylene tubes themselves, they determined that the role of siliconising tubes to prevent DNA losses remains to be proven.

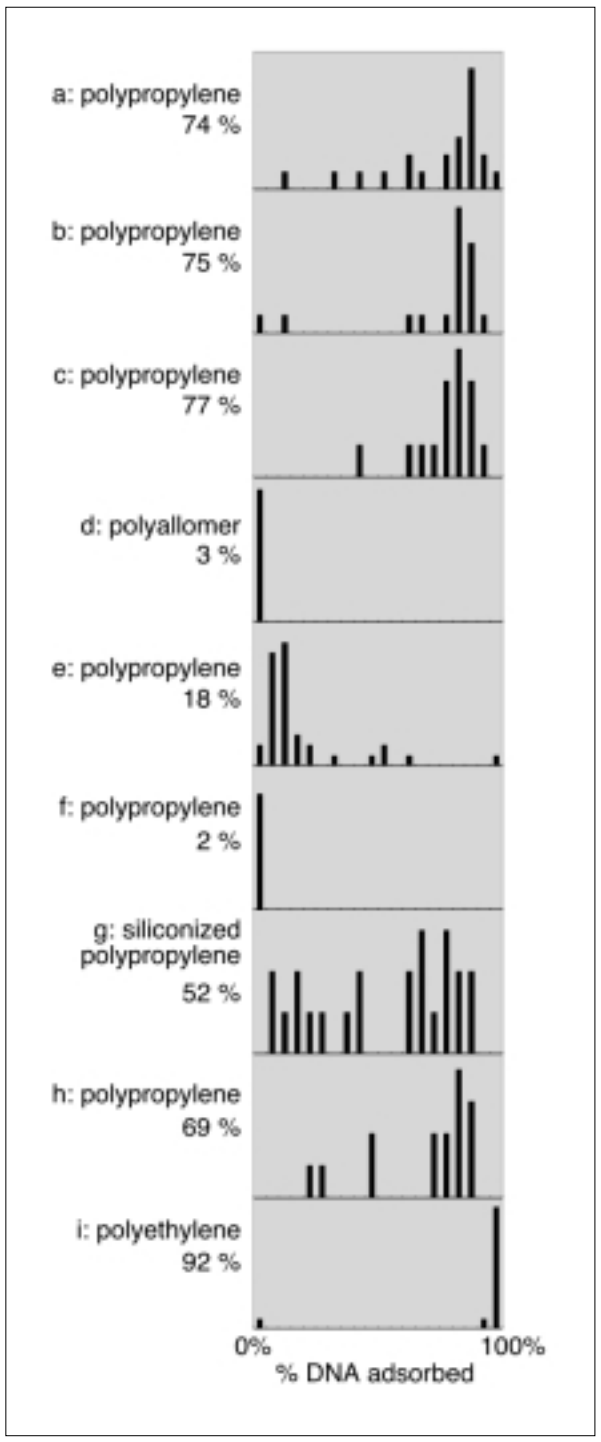

*Fig. 1 DNA adsorption to tube walls in different kinds of microtubes (where **e** is the Axygen Standard and **f** is the Axygen MAXYMum Recovery).*

Again, as observed in a previous study,[2] polyallomer tubes show no DNA adsorption. Interestingly, however, it was also observed that some kinds of polypropylene tubes did not show DNA adsorption.

DNA adsorption to tube surfaces is particularly striking at high ionic strength, but DNA is more often stored in solutions of low or moderate ionic strength. Therefore, tests were also performed with ionic conditions nearer to physiological, in 0.1 M NaCl, 10 mM Tris, 1 mM EDTA, pH 7.5. Under such conditions, DNA adsorption is usually much lower than in high salt (less than 5%) except for tubes *a* and *b*, which showed an average of approx. 25% binding (data not shown). Since the authors had previously observed a change of conformation of DNA upon storage in polypropylene tubes, they also studied the conformation of DNA after storage in different kinds of tubes. After a 16-hr incubation at 37°C, the samples were analysed by electrophoresis on polyacrylamide gels to separate the double-stranded and single-stranded forms of the fragment. Each sample was made in triplicate (results shown in *Figure 2*). A significant percentage of denaturation is observed with some kinds of tubes (polypropylene tubes *b* and *c*, siliconised polypropylene tubes *g*), the percentage of denaturation is very low but still detectable with tubes *e* (polypropylene), while no denaturation at all can be seen with tubes *d* and *f* (polyallomer and polypropylene, respectively).

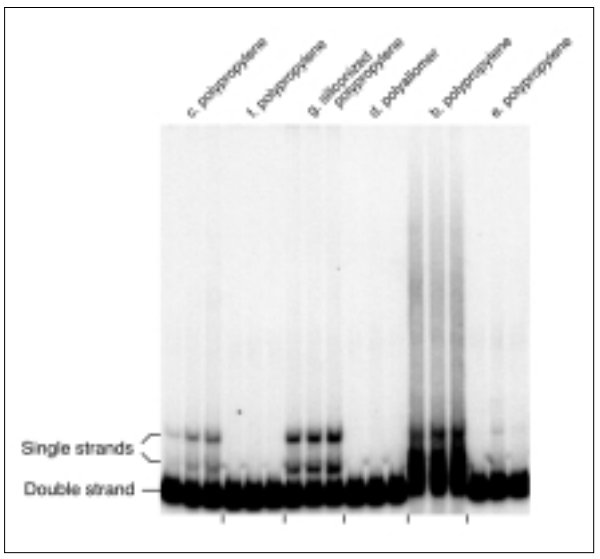

Fig. 2 Denaturation of DNA fragments as a function of the microtubes used. A given amount of labelled DNA was stored in 0.1 m NaCl, 10 mm Tris-HCl, 1 mm EDTA, pH 7.5, in different kinds of 1.5-mL microtubes. After a 16-hr incubation at 37 °C Triton X-100 (Sigma, St Louis, MO) was added to 0.1% to release any DNA bound to tube walls. Glycerol was then added to 2% and samples were loaded onto a 4% polyacrylamide gel in 6.7 mM Tris-acetate, 3.3 mM Na acetate, 1 mM EDTA, pH 7.8 at 4 °C with buffer recirculation. On this kind of gel, the single stands of the fragment can be separated from the double-stranded native form of DNA. The three DNA species are indicated on the figure.

In conclusion, when it is important to store lesser amounts of DNA in its native double-stranded state without losses by adsorption to tube walls, the choice of tubes can be very important. Regarding the tubes tested, ordinary polypropylene tubes cannot be recommended. In contrast, polyallomer tubes and some specially designed polypropylene tubes show none of the problems mentioned. Other factors can be important in the choice, one of them being the high price of polyallomer tubes as compared to polypropylene. The purity of the polymer used and the absence of surface treatment with chemicals that might remain in the tubes and interfere with further experiments are two other important parameters. Unfortunately, most manufacturers do not seem to be willing to divulge their secrets, often making it very difficult to know how the tubes are made, and to determine the additives to the polymer and the surface treatments involved in the manufacturing process.

The authors are with the Institute Jacques Monod, 2 Place Jussieu, 75251 Paris 05, France.

References

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