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Avoiding adsorption of DNA to polypropylene tubes and denaturation of short DNA fragments.

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Two problems can arise when working with small quantities of DNA in polypropylene tubes: first, significant amounts of DNA can become lost by sticking to the tube walls; second, short DNA fragments tend to denature when binding to polypropylene. In addition, DNA also tends to denature upon dehydration. We have found that a simple way to solve these problems is by using polyallomer tubes instead of polypropylene and by avoiding certain salts, such as sodium acetate, when drying DNA.

DNA is usually stored in polypropylene tubes, which have become widely used for their resistance to solvents, their strength, their ease of use and their low price. Polypropylene is a very hydrophobic material, whereas DNA is a highly charged macromolecule, two characteristics that minimize the interactions of DNA with tube walls and tend to avoid the adsorption problems often found with other macromolecules, especially proteins.

But DNA can, in fact, bind to polypropylene tubes. This is particularly striking at high ionic strength: (Fig. 1) shows that DNA fragments bind quickly to polypropylene tube walls in 2.5 M NaCl, with 75% of the material adsorbed after 1 h and 90% after 3 h in this experiment. Under such conditions, the amount of adsorbed DNA can be as high as 5 ng /mm^2 of tube wall. Tests performed at ionic conditions varying from TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) to TE plus 0.5 M NaCl, showed that DNA also sticks to tube walls at lower ionic strength, but with important variations between different batches of tubes. Tests were performed with 1 ng of radioactively labelled DNA per tube in 10 µl. With some batches of tubes, the percentage of adsorption was always high (between 80% and 95%; such tubes actually had a binding capacity as high as >10 ng for a volume of 10 µl). With other tube batches from the same manufacturer (Eppendorf) the percentage of adsorption varied apparently at random between 5% to 95%, all conditions being kept identical. Finally, other batches always presented little adsorption (<10%) at low ionic strength. In all those tests, using single-stranded instead of double-stranded DNA made no significant difference.

The problems resulting from this adsorption, loss of material and denaturation of adsorbed fragments (see below), led us to search for different kinds of tubes in which DNA would not adsorb to the tube walls. Upon testing the three different kinds of 1.5 ml plastic tubes sold by (Beckman), which are made of polypropylene, polyethylene, or polyallomer, respectively, we observed the same adsorption of DNA to polypropylene as before, and a similar adsorption to polyethylene. In strong contrast, no adsorption of DNA to polyallomer tubes was observed (Fig. 1), irrespective of the ionic strength. We have used polyallomer tubes for DNA storage since this finding, and although such tubes are reported to be slightly less resistant to aromatic and halogenated hydrocarbons than polypropylene tubes, the difference is not obvious and we have had no technical problems using them. Their only disadvantage is their price, which is currently about ten times higher than that of polypropylene tubes.

Another problem with polypropylene tubes comes from their tendency to denature DNA fragments. Storing short DNA fragments in polypropylene tubes often induces a significant amount of strand separation, a phenomenon that has been well documented by Belotserkovskii and Johnston (Ref. 1, 2, 3), and also observed by us (Ref. 4). Instead of stabilizing the double helix as expected, the high ionic strength stimulates the strand dissociation as well as the interaction of DNA with polypropylene, as shown in (Fig. 2). Incidently, such conditions of high salt concentration also lead to the formation of very interesting multistranded complexes, a detailed study of which will be presented elsewhere (Ref. 3, 5; Gaillard et al., unpublished; see the ladder of bands in Fig. 2a, lane 1).

As in the case of adsorption of DNA to tube walls, we have found that using polyallomer tubes is the most simple and

straightforward way to avoid this DNA denaturation.

Svaren and Chalkley (Ref. 6) also showed the tendency of short DNA fragments to denature upon dehydration, in particular during the dehydration step that usually follows ethanol precipitation. We have observed that this dehydration-induced denaturation depends strongly on the nature of the salt. (Fig. 2b) shows the same DNA fragment dissolved in two different salt solutions, sodium chloride and sodium acetate, and vacuum dried. No denaturation is observed with the fragment in sodium chloride, whereas an important percentage of denaturation is observed with the fragment in sodium acetate. We do not know the explanation for this difference. The precipitation efficiency being identical, we now use sodium chloride instead of sodium acetate for ethanol precipitation of DNA. Also, note again on (Fig. 2b) the superiority of polyallomer tubes, compared with polypropylene, for storage of DNA in its native state.

We now always use polyallomer tubes for DNA storage and strongly recommend them for storage of small amounts of short DNA fragments. On the other hand, it is possible to prevent DNA from interacting with polypropylene, the best way being by addition of 0.1% of a non-ionic detergent such as Triton X-100. Siliconizing tubes is an effective measure against DNA adsorption, but does not entirely suppress DNA denaturation on tube walls. Some manufacturers sell 'low-binding' tubes; indeed, we observed little adsorption of DNA with the ones we tested. However, as long as the exact nature of these tubes is kept a secret by manufacturers, the risk exists that some chemical added to polypropylene or to the tube surfaces will contaminate DNA. Examples can be found in the literature where a contamination by a substance released from the tubes has been well documented (Ref. 3, 7). In addition, we do not know whether DNA binds to polypropylene itself, or to some minor component of the plastic.

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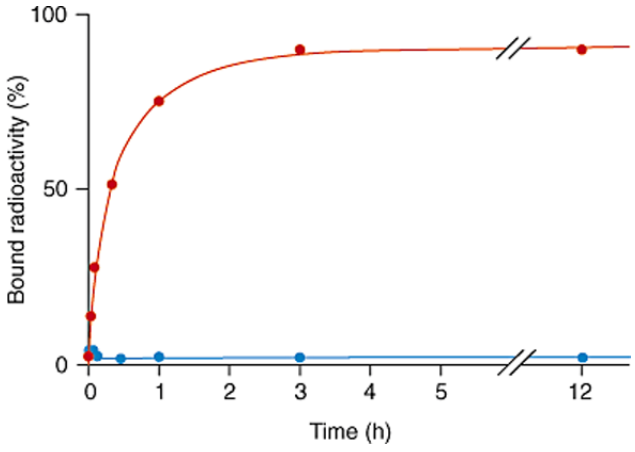

Figure 1. Binding of DNA to polypropylene and polyallomer (red and blue lines, respectively) tubes at high ionic strength. A 180 bp DNA fragment from pBR322 was radioactively labelled and incubated in 6 µl of 2.5 M NaCl, 10 mM Tris-HCl pH 7.5 and 1 mM EDTA at 37°C for the times indicated. After incubation, the supernatant was extracted with a micropipette and the amount of radioactivity left adsorbed to the tubes was counted.

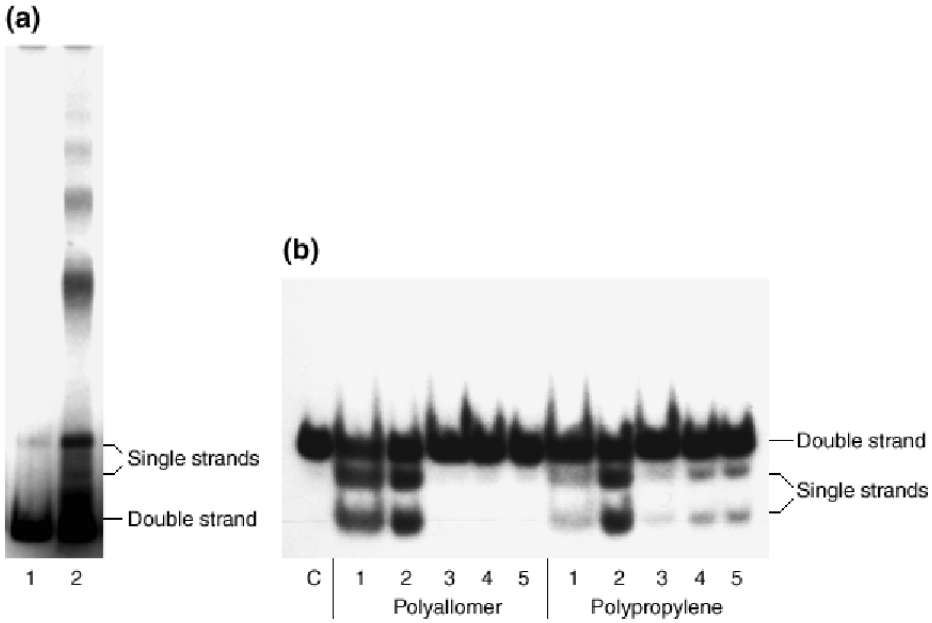

Figure 2. Denaturation of short DNA fragments. (a) Denaturation by adsorption to polypropylene. A labelled 120 bp fragment containing a 60 bp tract of poly(CA).poly(TG) (Ref. 5) was incubated in 2.5 M NaCl at 37°C in a polypropylene tube. After adsorption to the tube surface, the fragment was redissolved in TE + 0.1% Triton-X100 and analyzed on a 4% polyacrylamide gel (lane 2) along with the starting fragment which had been stored in a polyallomer tube (lane 1). Note the large amount of single strands that appear after adsorption of DNA to polypropylene, and the ladder of multistranded complexes (Ref. 5). (b) Denaturation by dehydration. A 123 bp fragment from plasmid pBR322, radioactively labelled, was placed in polyallomer or polypropylene tubes as indicated, in 10 µl of the following salt solutions: lanes 1, 100 mM sodium acetate; lanes 2, 25 mM sodium acetate; lanes 3, 100 mM sodium chloride; lanes 4, 25 mM sodium chloride; lanes 5, TE. After vacuum drying, the DNA was redissolved in TE and analyzed on a 4% polyacrylamide gel; lane C, control DNA fragment stored in solution in a polyallomer tube. Note the high percentage of denaturation for DNA in sodium acetate, but not in sodium chloride.

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ARTICLE COMMENTARY

H. Chaim Birnboim 18/1/99

Avoiding adsorption and denaturation

I am wondering what the pH is of the sodium acetate you used. We usually use sodium acetate that has been acidified with acetic acid to about pH 6.0 to provide a counter-ion during alcohol precipitation. Is it possible that unbuffered sodium acetate (usually above pH 7) becomes more alkaline owing to the volatility of the acetic acid during drying and that this promotes denaturation of small fragments? NaCl tends to be slightly acidic and would not be expected to increase in pH.

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Author response - Francois Strauss

Usually we use unbuffered sodium acetate. Its pH is 8.9, which is not so high as to denature DNA. Like you, we considered that denaturation might be due to a pH increase during evaporation. However, this does not appear to be the case:

- we obtain the same results when we use sodium acetate that has been acidified to pH 7 with acetic acid
- when DNA is in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5), the pH remains close to 7.5 upon addition of sodium acetate. But we still observe the denaturation effect
- actually, the pH of a sodium acetate solution (unbuffered) does not change significantly during evaporation. No acetic acid evaporates, in the same way as no chlorhydric acid evaporates from a solution of sodium chloride.

Therefore, our present hypothesis is that denaturation occurs at the very last moment of evaporation, when two phases - liquid and solid - coexist in the bottom of the tube.

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