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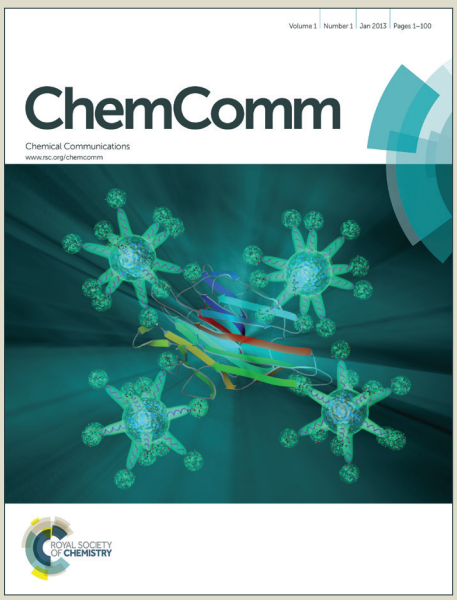

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Evidence for hydrophobic catalysis of DNA strand exchange

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B. Feng*[a], F. Westerlund[b] and B. Nordén[a]

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The catalytic role of hydrophobic co-solutes on DNA strand exchange is demonstrated by FRET kinetics. Two mechanisms contribute: base stacking destabilisation and nucleation-promoted DNA strand invasion. We propose that hydrophobic catalysis is involved in the strand-exchange activity of recombination enzymes.

DNA strand exchange, catalysed in vivo by gene recombination enzymes such as RecA and Rad51, is fundamental to homologous recombination and DNA repair. The detailed mechanisms of these enzymes are still poorly understood but, based on the structures of assembled protein-DNA complexes, stretching and destabilization of the DNA helix by close protein-DNA contacts facilitating search for homology and reject of mismatches are considered essential[1]. A recent discovery, that GC-rich DNA upon stretching undergoes a change into a stable elongated conformation, has added a new twist to the understanding of the strand exchange mechanism. It was proposed that the elongated conformation undergoes disproportionation into stacked base-pairs surrounded by larger gaps, like the heterogeneous structure found in complex with RecA, with triplets of stacked bases.[2] In addition, strand exchange is central to various biotechnical and nanotechnical applications, including the use of oligonucleotides to fuel DNA motors as well as in situ formation of self-assembled DNA nanotechnology.[3] Therefore, it is highly interesting to develop artificial DNA strand exchange model systems with catalytic ability, both in order to better understand the underlying mechanisms of biological enzymes as well as to facilitate the development of DNA-based nanotechnical devices.

We here demonstrate that DNA strand exchange in vitro can be catalysed by the presence of two hydrophobic and structurally related compounds, 1,2-dimethoxyethane (DME) and polyethylene glycol (PEG), but not by the more hydrophilic dextran or Ficoll. We shall denote this effect 'hydrophobic catalysis', as we can argue that the catalytic activity is caused mainly by hydrophobic close contact between the DNA and co-solute molecules.

We have earlier speculated that strand exchange between oligonucleotides in presence of PEG-6000 (average molecular weight 6000) may be accelerated by either of two possible mechanisms: hydrophobic interactions or volume exclusion.[4] As to the first putative effect, close PEG-DNA contacts may decrease water activity around DNA, thereby weakening the strength of nucleobase stacking. Consequently DNA breathing will be promoted which could provide nucleation sites for single strand invasion. The alternative possibility relates to the reduced effective reaction volume caused by the high molecular weight of PEG-6000. The volume excluded by PEG-6000 is much larger than the size of the PEG polymer itself[5], so the effective concentration of available single strands surrounding each unreacted DNA duplex is higher than the bulk concentration, which could accelerate strand exchange.

A FRET (fluorescence resonance energy transfer) assay is here used to monitor strand exchange, one strand of a 20-mer DNA duplex (Sequences in Fig S1, ESI) is labelled with FAM (carboxyfluorescein) at the 5' end and the other strand with TAMRA (carboxytetramethylrhodamine) at the 3' end. An unlabelled strand (sequence identical to the TAMRA-strand) is added five times in excess. Upon strand exchange, the unlabelled strand displaces the TAMRA strand, which dissociates the quenched FRET pair (see ESI for experimental details). The same assay has been used in several earlier DNA strand exchange studies.[4, 6]

The effects of hydrophobic interactions and volume exclusion have previously not been separated from each other as both increase with the molecular crowding of PEG polymers. We here varied the molecular weight of PEG, also including 1,2-dimethoxyethane (DME) to represent a PEG "monomer". DME can be regarded to have a negligible volume of exclusion, but due to its structural similarity to PEG it should provide a similar kind of hydrophobic environment. The influence of PEG-6000 and DME as co-solutes on strand exchange kinetics is shown in Figure 1.

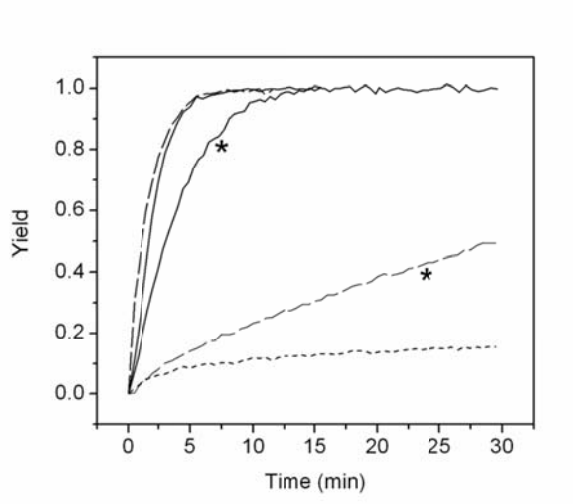

Figure 1. Representative kinetic traces of fluorescence intensity normalized with respect to DNA strand exchange yields, in 50 % PEG-6000 (solid lines) and 25 % DME (dashed lines) compared to in buffer only (dotted line). Asterisk (*) indicates that salt was added to increase melting temperature (T_m) of DNA (discussed below). Without extra salt DME and PEG have very similar catalytic activity, but DME is strongly suppressed by a T_m increase.

It can be seen from the kinetic traces in Figure 1 that 25 % (w/w) DME added to buffer catalyses DNA strand exchange to become slightly faster compared to 50 % (w/w) PEG-6000, both reaction rates being much faster than in buffer alone. In view of the small size of DME molecules we can immediately conclude that (at least with DME) volume exclusion effects cannot explain the increased DNA strand exchange rate. Also, on a per molecular weight basis, DME is roughly twice as effective compared to PEG-6000. We think this is reflecting a larger relative contact area of DME with DNA compared to the bulkier PEG polymer chains, which cannot make close contact with the hydrophobic interior of DNA due to sterical hindrance from the backbone.

To obtain the strand exchange rate constant k, the pseudo first order equation $I = I_\infty - \exp(-kt)$ was fitted to the fluorescence intensity I of the FRET assay according to previous standards[6]. In Figure 2, results for strand exchange catalysis in DME and PEG polymers of different lengths are shown in terms of k values. It can be concluded that for DME and all lengths of PEG investigated, the strand exchange reaction rate increases sharply with co-solute concentration, once a threshold concentration is exceeded. The clear trend that this threshold increases with PEG molecular weight supports our hypothesis that longer PEG chains cannot make effective contacts with the interior of DNA.

Table 1. Salt concentrations required to increase melting temperate of DNA, for each co-solute, to be equal to that in pure buffer with 50 mM Na^+.

Solution	T_m	Adjusted Na^+ concentration	T_m after adjustment
Buffer	50 °C	-	-
25 % DME	41 °C	325 mM	50 °C
30 % PEG-200	40 °C	400 mM	50 °C
45 % PEG-1000	41 °C	250 mM	50 °C
50 % PEG-6000	42 °C	250 mM	50 °C

As expected, while reduced water activity destabilizes nucleobase stacking, the presence of hydrophobic co-solutes also decreases T_m of DNA. To rule out that the fluorescence intensity increase is simply caused by the duplexes melting, and to further and more firmly differentiate between the effects of long and short PEG chains, we increased the sodium chloride concentration for each of the highest co-solute concentrations in Figure 2. Addition of salt increases the electrostatic stabilization and consequently raises T_m in presence of each co-solute to equal the melting temperature of DNA alone in buffer (see Table 1, and Figure S3 in ESI). After T_m matching, the strand exchange catalysing effect of DME is strongly suppressed, while longer PEG polymers were gradually less affected (Figure 3 and dashed lines in Figure 1). To verify that B-DNA conformation is preserved at 37 °C in the presence of hydrophobic co-solutes for the five samples without additional salt in Figure 3, CD spectra were measured (Figure S4, ESI).

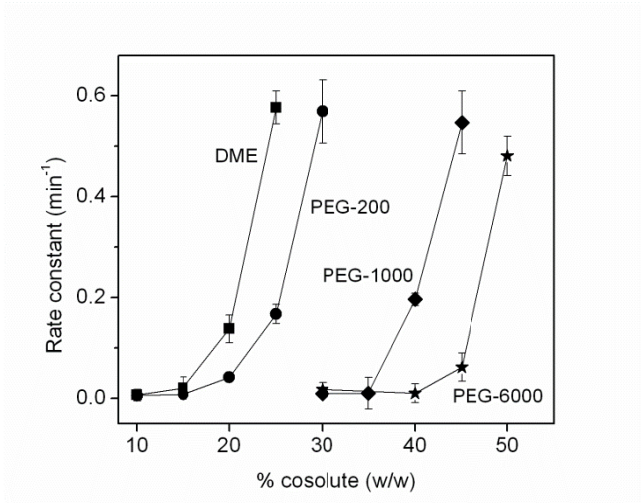

Figure 2. Strand exchange rate constants for DME and PEG of different molecular weights ([Na^+] = 50 mM). The rate constants increase sharply with increasing co-solute concentration but follows a similar curve profile for all co-solutes. Error bars indicate ± two standard deviations.

The difference between long PEG and short PEG/DME upon salt addition (asterisks in Fig 1, Fig 3) points to two separate catalytic mechanisms. Fast exchange rates are obtained in the presence of 25% DME or alternatively 30% PEG-200, while T_m is 10 °C lower than in pure buffer. However, most of this accelerating effect is lost upon a T_m increase to the melting temperature in pure buffer. Clearly the catalytic mechanism of DME and short PEG mostly depends on DNA destabilization in a hydrophobic environment, and is consistent with that decreasing the energy advantage of nucleobase stacking is followed by duplex breathing and strand invasion. At least for DME, the effect of volume exclusion can be ruled out. The additional Na^+ ions counteract the first proposed mechanism since ionic shielding of the phosphate backbones will decrease inter-strand electrostatic repulsion, therefore stabilizing the pre-exchange DNA duplex.

The longer PEG polymers also destabilize DNA due to their hydrophobic nature, and therefore some of their catalytic activity is suppressed by a T_m increase. However it is evident, especially for PEG-6000, that there is a second type of hydrophobic catalysis which does not depend on DNA destabilization. Trivially, this could be a volume exclusion effect, not present for shorter PEG molecules. However, non-hydrophobic macropolymers, such as dextran and Ficoll, do not exhibit any strand exchange catalysing effect. In the ESI (Figure S2), strand exchange kinetic traces for 50 % dextran and 50 % Ficoll are presented at the same experimental settings as with PEG, and we note that neither rate nor yield of exchange exceeds

that of using pure buffer. Therefore, volume exclusion cannot be an explanation for the salt-insensitivity of longer PEG polymers.

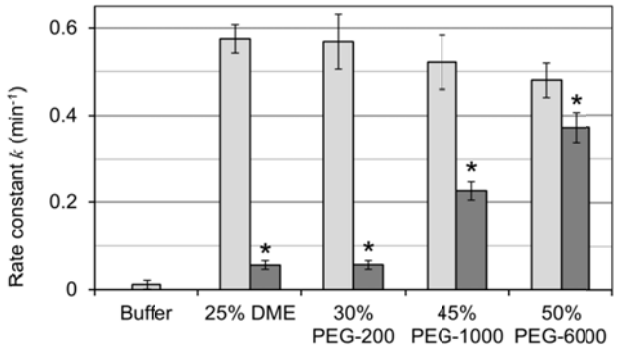

Figure 3. Effect of salt on rate constant *k*. Asterisk (*) indicates salt added to increase T_m of DNA in the presence of DME/PEG to equal T_m in buffer. After adjustment (asterisk) longer PEG polymers retain more catalytic ability than shorter PEG/DME. Error bars indicate ± two standard deviations.

The behaviour shown in Figure 3 can be explained when considering, as discussed above, that longer PEG chains cannot make optimal PEG-DNA (groove) contacts due to its large hydrodynamic radius. A potential explanation is that PEG-6000 can still make hydrophobic contact with DNA bases once strand separation has occurred and in this way thermodynamically stabilize the single strand and counteract recombination. This effect would prolong the window of opportunity for a third strand to invade and, consequentially, promote strand exchange. More importantly, since this kind of single strand stabilization does not depend on the pre-exchange DNA duplex stability, it is essentially salt insensitive and, therefore, this mechanism is compatible with the results presented in Figure 3.

Earlier model systems of DNA strand exchange have relied on increasing the effective DNA concentration using heavily charged cationic polymers[6b, 7], liposomes[8] and polypeptides[9]. However, concentrated cationic charges tend to distort DNA and do not truly reflect the crowded and hydrophobic environment inside a DNA-protein complex such as the assembled RecA filament.

Hydrophobic catalysis can have impact on a number of aspects of DNA interaction mechanisms. Generally the thermodynamics of nucleic acids can be regarded as based on mainly four components: electrostatic attractions and repulsions, dispersive attractions and hydrophobic effects. While the first ones are rather easy to assess quantitatively both experimentally and theoretically, the role of hydrophobicity is harder to accurately determine. Specifically, this is true for the hydrophobic influence in transition states, including those of gene recombination. In addition to the here presented evidence for the significance of hydrophobic catalysis for DNA strand exchange there are observations in the literature that might be explained in terms of transition states stabilized by hydrophobic interactions. They include anomalous behaviour observed for strand-invasion by non-ionic peptide nucleic acid (PNA)[10] and bulky thread-intercalating ruthenium compounds[11].

Conclusions

In conclusion, we have shown that hydrophobic catalysis of DNA strand exchange is a significant effect, in the case of polyethylene glycol potentially involving several mechanistic contributions: shorter PEG chains tend to destabilize the DNA duplex by decreasing the water activity while longer PEG chains might also topologically stabilize openings once breathing has occurred, and in this way promote strand invasion.

Notes and references

[a] Chalmers University of Technology, Department of Chemistry and Chemical Engineering
[b] Chalmers University of Technology, Department of Biology and Biological Engineering

† Electronic Supplementary Information (ESI) available: Experimental details and DNA sequences, strand exchange yields in the presence of Ficoll and dextran, melting curves and CD spectra in the presence of DME/PEG. See DOI: 10.1039/c000000x/

1 (a) Z. Chen, H. Yang and N. P. Pavletich, Nature, 2008, 453, 489; (b) A. Reymer, K. Frykholm, K. Morimatsu, M. Takahashi and B. Nordén, Proceedings of the National Academy of Sciences, 2009, 106, 13248; (c) S. C. Kowalczykowski and R. A. Krupp, Proceedings of the National Academy of Sciences, 1995, 92, 3478; (d) S. Atwell, L. Disseau, A. Z. Stasiak, A. Stasiak, A. Renodon-Corniere, M. Takahashi, J. L. Viovy and G. Cappello, Nucleic acids research, 2012, 40, 11769; (e) K. C. Millett, E. J. Rawdon, T. V. Tran and A. Stasiak, The Journal of chemical physics, 2010, 133, 154113; (f) J. Nomme, A. Renodon-Corniere, Y. Asanomi, K. Sakaguchi, A. Z. Stasiak, A. Stasiak, B. Norden, V. Tran and M. Takahashi, Journal of medicinal chemistry, 2010, 53, 5782.
2 (a) N. Bosaeus, A. H. El-Sagheer, T. Brown, B. Åkerman and B. Nordén, Nucleic acids research, 2014, gku441; (b) N. Bosaeus, A. H. El-Sagheer, T. Brown, S. B. Smith, B. Åkerman, C. Bustamante and B. Nordén, Proceedings of the National Academy of Sciences, 2012, 109, 15179.
3 (a) J. B. Lee, Y. H. Roh, S. H. Um, H. Funabashi, W. Cheng, J. J. Cha, P. Kiatwuthinon, D. A. Muller and D. Luo, Nat Nanotechnol, 2009, 4, 430; (b) A. J. Turberfield, J. Mitchell, B. Yurke, A. P. Mills Jr, M. Blakey and F. C. Simmel, Physical review letters, 2003, 90, 118102; (c) D. Y. Zhang and G. Seelig, Nature chemistry, 2011, 3, 103.
4 B. Feng, K. Frykholm, B. Nordén and F. Westerlund, Chemical Communications, 2010, 46, 8231.
5 R. J. Ellis, Trends in biochemical sciences, 2001, 26, 597.
6 (a) K. Frykholm, F. B. Bombelli, B. Nordén and F. Westerlund, Soft Matter, 2008, 4, 2500; (b) W. J. Kim, T. Akaike and A. Maruyama, Journal of the American Chemical Society, 2002, 124, 12676.
7 W. J. Kim, Y. Sato, T. Akaike and A. Maruyama, Nature materials, 2003, 2, 815.
8 K. Frykholm, B. Nordén and F. Westerlund, Langmuir : the ACS journal of surfaces and colloids, 2009, 25, 1606.
9 K. Tajima, W. J. Kim, Y. Sato, T. Akaike and A. Maruyama, Chemistry Letters, 2003, 32, 470.
10 (a) P. Wittung, P. Nielsen and B. Nordén, Journal of the American Chemical Society, 1996, 118, 7049; (b) K. K. Jensen, H. Ørum, P. E. Nielsen and B. Nordén, Biochemistry, 1997, 36, 5072.
11 (a) F. Westerlund, P. Nordell, B. Nordén and P. Lincoln, The Journal of Physical Chemistry B, 2007, 111, 9132; (b) P. Nordell, F. Westerlund, L. M. Wilhelmsson, B. Nordén and P. Lincoln, Angewandte Chemie, 2007, 119, 2253.