Nucleic Acids Research, 1999, Vol. 27, No. 15 **3035–3041**

Oligonucleotide dendrimers: stable nano-structures

Mikhail S. Shchepinov*, Kalim U. Mir, John K. Elder, Maxim D. Frank-Kamenetskii[1] and Edwin M. Southern

Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK and [1]Center for Advanced Biotechnology and Department of Biomedical Engineering, Boston University, 36 Cummington Street, Boston, MA 02215, USA

Received April 28, 1999; Revised and Accepted June 15, 1999

ABSTRACT

DNA dendrimers with two, three, six, nine or 27 arms were reassociated as complementary pairs in solution or with an array of complementary oligonucleotides on a solid support. In all cases, duplex stabilities were greater than those of unbranched molecules of equal length. A theoretical treatment for the process of dissociation of dendrimers explains the major properties of the complexes. The favourable features of DNA dendrimers—their enhanced stability and the simple predictability of their association behaviour—makes them promising as building blocks for the 'bottom up' approach to nano-assembly. These features also suggest applications in oligonucleotide array/DNA chip technology when higher hybridisation temperatures are required, for example, to melt secondary structure in the target.

INTRODUCTION

Controllable formation of nano-scale architectures in solution and on solid supports is central to a range of activities in the emerging field of nanotechnology (1–4). DNA molecules are well suited for these purposes because of their unique molecular recognition features. Linear DNA chains can assemble into a range of non-linear structures: branching of the double helix is induced by breaking the run of the complementarity of the component strands (5,6). More versatile building blocks could be designed by linking DNA chains through components which take no part in the double helix. For example, dendrimers (7) with arms terminating in oligonucleotides of the same (8) or of different (9) sequences could be used to build cages, cryptands, tubes, nets, scaffolds and other more complex three-dimensional (3-D) structures. Here we report the synthesis of a range of dendritic DNA molecules and their assembly into base-paired structures. We also describe their interactions with oligonucleotides tethered to the surface of a solid support in the form of an array (10).

An important characteristic of nucleic acids is the sharp melting transition of the base-paired double strand. It is essential to know how dendrimerisation affects this behaviour in order to understand how branched nucleic acids may be used as molecular building blocks. We find that these assemblies have unexpectedly high thermal stabilities; the melting temperature is substantially higher than that of linear counterparts. A theoretical treatment explains this extra stability. It is an effect of combining the binding forces of individual duplexes by tying their ends together in the dendritic structure. The theory allows prediction of the effects of varying the length, number and composition of the branches and will be useful in designing the building blocks needed to assemble a particular architecture.

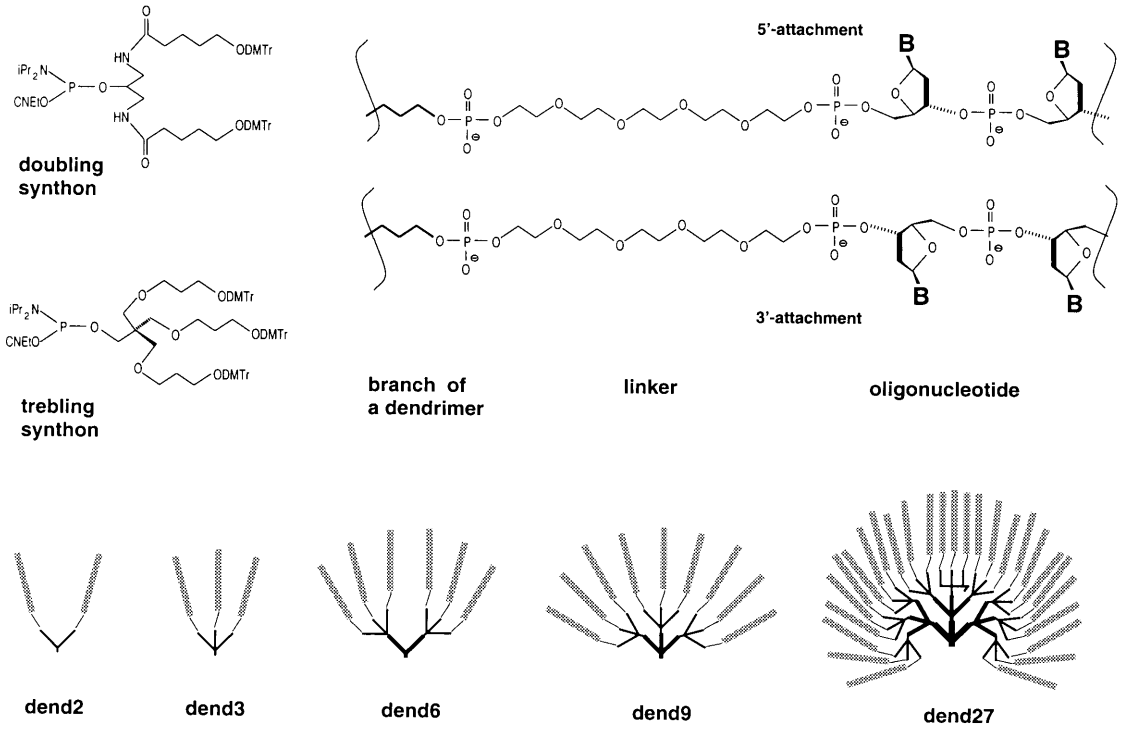
MATERIALS AND METHODS

Synthesis of dendrimers

Different generations (7) of dendritic cores were synthesised on derivatised 2500 Å controlled pore glass (CPG) supports by the divergent approach using doubling and trebling reagents (8,9; Fig. 1A), with subsequent 17-atom extension of the branches, using pentaethyleneglycol phosphoramidite (Cruachem), before synthesis of a set of paired structures based on a decanucleotide motif (Table 1) on the ends. The core of the dendrimers comprised branching chains formed by coupling a two- or three-branched synthon. Two, three, six, nine and 27 branches were generated in one, two and three couplings, respectively. Use of 3'- or 5'-nucleoside phosphoramidites yielded dendrimers with 3'- or 5'-attached ('reversed') oligonucleotides. Synthesis of a dendritic core can also be carried out atop of another oligonucleotide still connected to the CPG, thus giving an additional branch of any sequence. Increased supply of phosphoramidites and tetrazole and prolonged waiting steps during the synthesis ensure an average condensation yield for branching reagents of >98% [4,4'-dimethoxytrityl (DMTr) assay], and for 10–15mer oligonucleotide dendrimers the final yield was ~90%. All dendrimers and controls were deprotected by standard protocols and used without further purification. The set includes: a pair of linear 10mers (#1+#2); a pair of 30mers comprising three tandemly linked repeats of the 10mers (#3+#4); four pairs of dendrimers with three (#5+#6), six (#24+#25), nine (#8+#9) or 27 (#11+#12) branches and with the 10mers attached to each arm through their 3'-ends; three pairs of dendrimers with two (#22+#23), three (#5+#7) and nine (#8+#10) branches terminating in a 10mers linked through the 5'-ends. For each pair, the 10mers comprised the two complementary sequences. Dendrimers with six branches (#24+#25) were synthesised using condensation of doubling reagent and subsequent coupling with trebling reagent.

*To whom correspondence should be addressed. Tel: +44 1865 275226; Fax: +44 1865 275283; Email: misha@bioch.ox.ac.uk

A



Melting experiments

Base-paired structures were formed by allowing pairs of corresponding complementary uniplex structures to associate [uniplex structure: a non-duplexed, single-stranded structure (linear or dendrimeric) as opposed to duplex structure]. Their melting behaviour was analysed by UV spectrophotometry. Each combination of strands was mixed in 325 µl of an appropriate solvent [tetramethylammonium chloride (TMA) buffer: 0.01% SDS, 50 mM Tris, 3.5 M TMA, 0.002 M EDTA; 6× sodium citrate (SSC) buffer: 0.9 M NaCl, 0.03 M trisodium citrate] in a cuvette. This was placed into a DU 640 spectrophotometer (Beckman) and heated to 95°C for 5 min followed by slow (overnight) cooling to 20°C in order for annealing to occur. Melting curves were taken following annealing and degasing of solutions containing dendrimer combinations. Solutions were heated from 20 to 95°C and then back to 20°C using a ramp rate of 1°C/min. Readings were taken once every minute.

RESULTS AND DISCUSSION

Melting experiments

Dendrimeric uniplexes of different generations with 'arms' made of oligonucleotides attached through either 3' or 5' ends and polypropylene-bound oligonucleotides were synthesised according to previously described protocols (8–11). Different combinations of linear and dendrimerised uniplexes (Table 1A) were mixed together in equivalent amounts in TMA and SSC buffers, annealed and their melting behaviour analysed as described in Materials and Methods.

In solution, there were striking increases of 7.2 and 10.7°C in the T_m of the two (#22+#23; T_m = 51.2°C) and three (#5+#6; T_m = 54.7°C) branched complementary dendrimers as compared with the linear complementary 10mers (#1+#2; T_m = 44°C; Table 1B). Further increases were seen for the nine-branched (#8+#9; T_m = 62.5°C) and 27-branched (#11+#12; T_m = 67.9°C) dendrimers. The T_m of the three-branched dendrimer lies between the T_m of the linear 10mer (#1+#2; T_m = 44°C) and that of a linear 30mer (#3+#4; T_m = 77°C) comprising three copies of the 10mer linked in tandem. Selected melting curves are shown on Figure 2. Melting occurs over a narrow temperature range, slightly broader than that of linear duplexes. The interval of the melting transition (ΔT_m) and the hyperchromic shift vary for different structures. The latter is generally lower than that of a linear duplex, suggesting incomplete duplex formation, though we cannot rule out the possibility that branched structures have different absorbances than their linear counterparts.

In addition, we made 15mers tethered to a flat polypropylene support (11) through the 5' (#14 and #17) or the 3'-end (#13 and #16) in the form of small arrays in which sequences of the oligonucleotides were different but the base compositions were the same. These arrays were hybridised to mixtures of complementary linear and dendrimeric oligonucleotides (bottom part of Table 1B) at different temperatures and then washed at different temperatures as shown in Figure 3. Again T_m increased from the linear 15mer (#13+#15, #14+#15; T_m = 45°C), through the nine-branched (#16+#18; T_m = 57.5°C), to the 27-branched (#16+#19; T_m = 58°C) dendrimer.

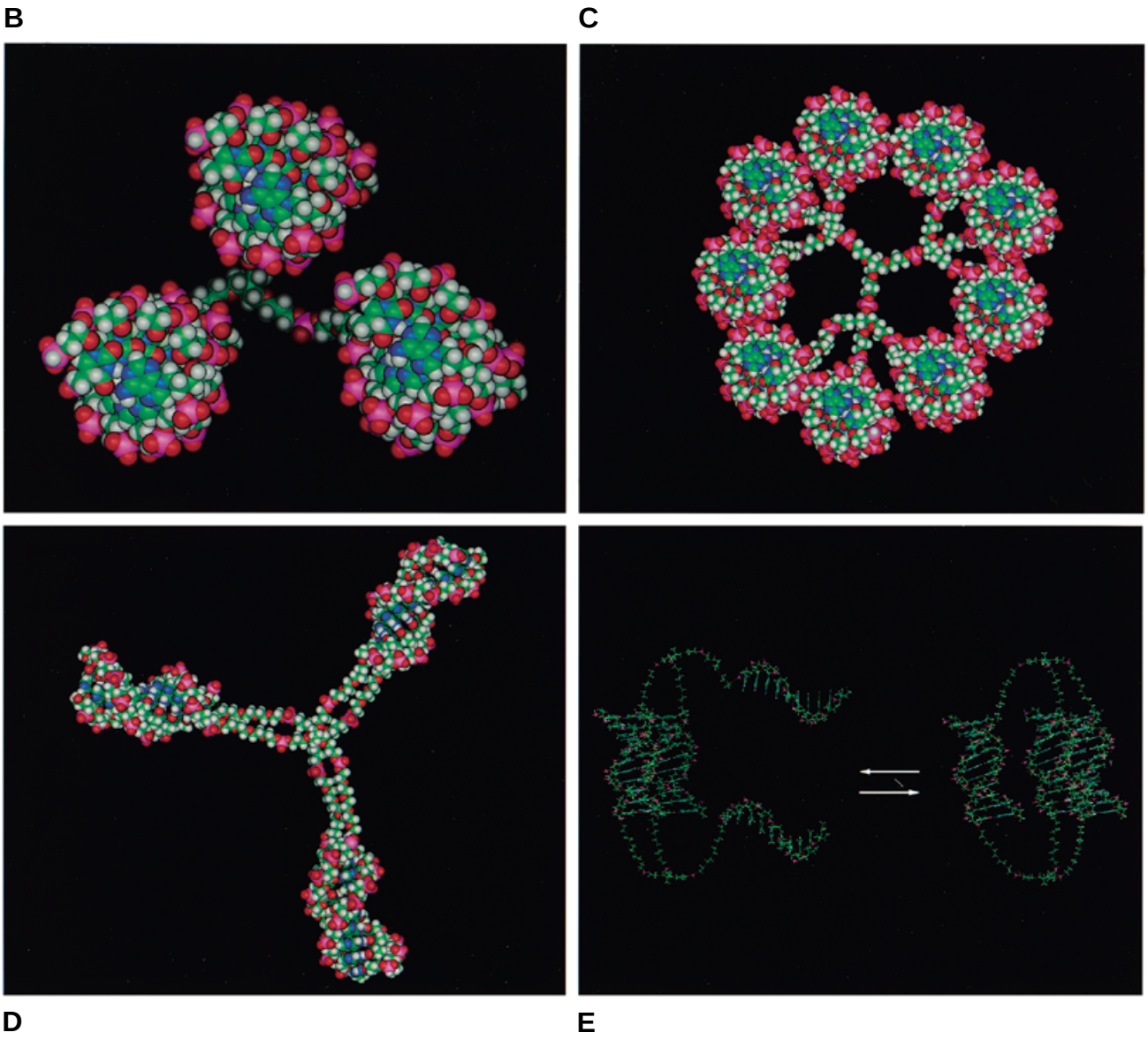


Figure 1. (Opposite and above) (**A**) The chemical structure of the doubling and trebling synthons (8,9) used in this work, the ways of attachment of oligonucleotide 'arms' to a dendrimer, and schematic representation of different generations of dendrimeric uniplexes. (**B**) Top view of a three-branched cage-like structure of (#5+#6). The branching part on top of the complex is not shown. (**C**) Top view of a nine-branched cage-like structure of (#8+#9). The branching part on top of the complex is not shown. (**D**) Side view of a three-branched structure of (#5+#7). (**E**) An equilibrium between two complementary dend3 parts (#5+#6). Optimised molecular models (B–E) were created using 'Insight-II® 95.00' molecular modelling software (MSI). In all cases, phosphate groups at the branching centers of dendrimers are also omitted.

Depending on the site of attachment of oligonucleotide to the core structure, there will be two types of paired products with two significantly different structures. If the complementary oligonucleotide arms of the two dendrimers are attached to the core through the same ends (attachment through 3' and 3' or through 5' and 5'), the cores will be at the opposite ends of a cage-shaped, cryptand-type structure (Fig. 1B, C and E); with different orientations of oligonucleotide arms of two complementary dendrimers (attachment through 3' and 5' ends) the cores of the dendrimers will be close to each other forming a hub from which the duplexes radiate, resembling a starfish (#5+#7, Fig. 1D). The 'starfish' duplexes were slightly less stable than the corresponding 'cages'. Dend3 in solution: #5+#6, T_m = 54.7°C, 3' + 3', and #5+#7, T_m = 49.5°C, 3' + 5'; dend9 in solution: #8+#9, T_m = 62.5°C, 3' + 3', and #8+#10, T_m = 62.1°C, 3' + 5'; dend9 on the solid support: #16+#18, T_m = 57.5°C, 3' + 3', and #17+#18, T_m = 55°C, 3' + 5'. Decreased stability in the case of the three-branched 'starfish' (#5+#7, Fig. 1D, T_m = 49.5°C), as compared to the corresponding 'cage' (#5+#6, T_m = 57.5°C), could be explained by

Table 1. (**A**) Oligonucleotide sequences used in this work. (**B**) Combinations of structures used for hybridisation experiments in solution and on the solid support

A

##	Uniplex Type	Sequence	Link
1	linear	5'-atg.gtt.agt.g	no
2	linear	5'-cac.taa.cca.t	no
3	linear	5'-(atg.gtt.agt.g)$_3$	no
4	linear	5'-(cac.taa.cca.t)$_3$	no
5	dend3	5'-atg.gtt.agt.g	3'
6	dend3	5'-cac.taa.cca.t	3'
7	dend3	5'-cac.taa.cca.t	5'
8	dend9	5'-atg.gtt.agt.g	3'
9	dend9	5'-cac.taa.cca.t	3'
10	dend9	5'-cac.taa.cca.t	5'
11	dend27	5'-atg.gtt.agt.g	3'
12	dend27	5'-cac.taa.cca.t	3'
13	linear	5'-gaa.gag.aaa.gag.aaa	3'
14	linear	5'-gaa.gag.aaa.gag.aaa	5'
15	linear	5'-ttt.ctc.ttt.ctc.ttc	no
16	linear	5'-aag.gaa.gag.aaa.gaa	3'
17	linear	5'-aag.gaa.gag.aaa.gaa	5'
18	dend9	5'-ttc.ttt.ctc.ttc.ctt	3'
19	dend27	5'-ttc.ttt.ctc.ttc.ctt	3'
20	linear	5'-(atg.gtt.agt.g)$_6$	no
21	linear	5'-(cac.taa.cca.t)$_6$	no
22	dend2	5'-atg.gtt.agt.g	5'
23	dend2	5'-cac.taa.cca.t	5'
24	dend6	5'-atg.gtt.agt.g	3'
25	dend6	5'-cac.taa.cca.t	3'

B

## from Table 1A	Duplex Type	Simplest Pairing	Tm,°C 3.5xTMA	Tm,°C 6xSSC	dimensions h,nm	dimensions d,nm
Solution						
1 + 2	linear	=	44.0	41.6		
3 + 4	linear concatemer	(=)$_3$	77.0	76.5		
6 + 1	dend3		46.0			
5 + 6	dend3		54.7	50.0		
5 + 7	dend3 'starfish'		49.5			
8 + 9	dend9		62.5	56.4		
8+ 10	dend9 'starfish'		62.1			
11+12	dend27		67.9	59.2		
20+21	linear concatemer	(=)$_6$	81.6	81.5		
22+23	dend2		51.2	47.2		
24+25	dend6		60.4	54.5		
Solid Support						
13+15	linear		45.0		5	2
14+15	linear		45.0		5	2
16+18	dend9, favourable		57.5		5	8
17+18	dend9, unfavourable		55.0			
16+19	dend27, favourable		58.0		5	10

(**A**) Compounds #22 and #23 were synthesised using 3'-nucleoside phosphoramidites and hexaethyleneglycol phosphoramidite (Cruachem), with subsequent synthesis of the second oligonucleotide atop of the first one using 5'-nucleoside phosphoramidites.
(**B**) Buffers: TMA, tetramethylammonium chloride; SSC, sodium citrate. For solution studies, the T_m values were determined automatically by integrating the curves (Beckman DU 640 software). The measurements were carried out three to five times and the melting temperatures were within 1°C (for non-smoothed curves) of the average indicated in the Table.

repulsion of the adjacent negatively charged dendritic cores. With nine branches the difference between 'starfish' (#8+#10) and 'cage' (#8+#9) is less marked. In this case, the negative charge in the dendritic core is more diffuse, and consequently, the charge repulsion of the two core structures in the 'starfish' will be weaker.

Theoretical treatment

We believe that the dendrimers are more stable than the corresponding monomer because multiple interactions of the arms allow for stable, partially melted intermediates. Consider the position at the T_m, when half of the strands are melted. In the case of the linear monomers, half of the strands will have dissociated and diffused away from their complement. By contrast, when half of the strands of any dendrimer are dissociated, the other half may still hold the molecule to its complement, allowing the dissociated strands to come together again and reform a duplex.

When both reactants are in solution at relatively high concentration, forward and reverse reactions are both significant and T_m (observed melting temperature) is concentration dependent. The theoretical treatment of the system is complex. By contrast, it is relatively easy to formulate a theory to describe the melting of strands from the oligonucleotide complements tethered to a surface. In this case, the melting reaction is carried out in a large volume with a negligible concentration

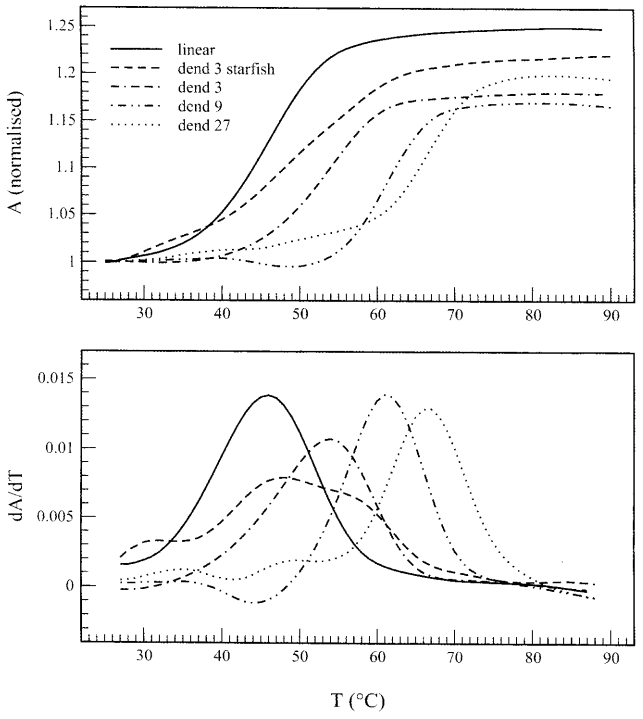


Figure 2. Selected melting curves for linear and dendrimeric duplexes in 3.5 M TMA buffer, for the samples annealed overnight. Shown below are the first derivatives of the melting curves.

of the displaced strand in the solution above the support. We need not consider the reverse reaction. For the support-bound duplex, the kinetics are strongly temperature-dependent, so that at low temperature the complex lives virtually infinitely long whereas at high temperature it dissociates in microseconds. The 'melting point' is the intermediate temperature at which the complex dissociates within a few seconds. Therefore, if τ is the dissociation time of the complex, the kinetic melting temperature is determined from the equation:

$$\tau(T_m) = t_{exp} \quad \mathbf{1}$$

where t_{exp} is the characteristic time of the experiment. The τ value is determined by the equilibrium stability constant s for elongation of the helix by 1 bp. For a helix consisting of N bp, the lifetime is:

$$\tau = \frac{\tau_o}{N} s^N \quad \mathbf{2}$$

where $\tau_o = 10^{-6}$ s. This equation describes the time over which the two complementary strands consisting of N bp separate (12).

Let the dendrimer-array complex consist of n duplexes, m bp each. To dissociate from the array, the dendrimer must lose all its links with the array, so in the exponent of equation **2**, $N = nm$.

But in contrast to the nm-bp-long continuous duplex, the dendrimer-array complex does not melt sequentially from only two ends. After a branch is melted, the next branch may be any of the remaining bound branches. Therefore, the melting of a dendrimer will proceed $(n - 1)!$ times faster than the corresponding nm-bp-long continuous duplex, so

$$\tau_{dend} = \frac{1}{mn!} \tau_o s^{mn} \quad \mathbf{3}$$

The s value is expressed via DNA thermodynamic parameters:

$$s = \exp(-\Delta G/RT) = \exp(\Delta S(T_o - T)/RT) \quad \mathbf{4}$$

where ΔG is the Gibbs free energy of melting of 1 bp, ΔS is the melting entropy [ΔS = 25 eu (cal/mol × K)], R is the gas constant, T is the temperature in Kelvin, and T_o is the equilibrium melting temperature of infinitely long DNA (Table 1, #20+#21, $T_m = T_o$ = 82°C) with the same GC-content as our m-bp-long complexes. Substituting equation **4** in equation **3** and the result in equation **1**, we have:

$$\ln(t_{exp}/\tau_o) = mn\Delta S(T_o - T_m)/RT_m - \ln n! - \ln m$$

(where T_m is the observed temperature of dissociation, or the kinetic melting temperature). Or, introducing the value $\delta T = T_o/T_m - 1$ ($T_o > T_m$), we have:

$$mn(\Delta S/R)\delta T = \ln(t_{exp}/\tau_o) + \ln n! + \ln m,$$

or finally:

$$\delta T = \frac{R}{mn\Delta S}\left(\ln\frac{t_{exp}}{\tau_o} + \ln n! + \ln m\right) \quad \mathbf{5}$$

This equation gives the relation between the observed melting temperature T_m, the limiting equilibrium melting temperature T_o, and the values m and n. The $\ln(t_{exp}/\tau_o)$ value cannot be found precisely but in practice it cannot be varied widely, and if we take t_{exp} in the order of magnitude of 1 s, then $\alpha = \ln(t_{exp}/\tau_o) \approx 15$, and $\beta = R/\Delta S = 0.08$. Let us consider two limiting cases:

(i) Binding of the single-stranded (n = 1) oligonucleotide 15mer control to the array: $\delta T = 0.08 \times (15 + \ln m)/m = 0.1$ (for m = 15), and $T_o - T_m$ = 32°C.

(ii) Binding of the dendrimer (n >> 1). Using Stirling's formula ($\ln n! = n\ln n - n$), we have from equation **5**:

$$\delta T = \frac{\beta}{m}\left(\frac{\alpha + \ln m}{n} + \ln n - 1\right) \quad \mathbf{6}$$

As a function of n, the T_m value experiences a maximum at n_{max}, so that:

$$n_{max} = \alpha + \ln m \quad \mathbf{7}$$

A surprising conclusion from this is that for dendrimers with more than 18 branches, there is no further increase in the melting temperature. In fact, further increase of n leads to a decrease (although very slow due to the logarithmic form of

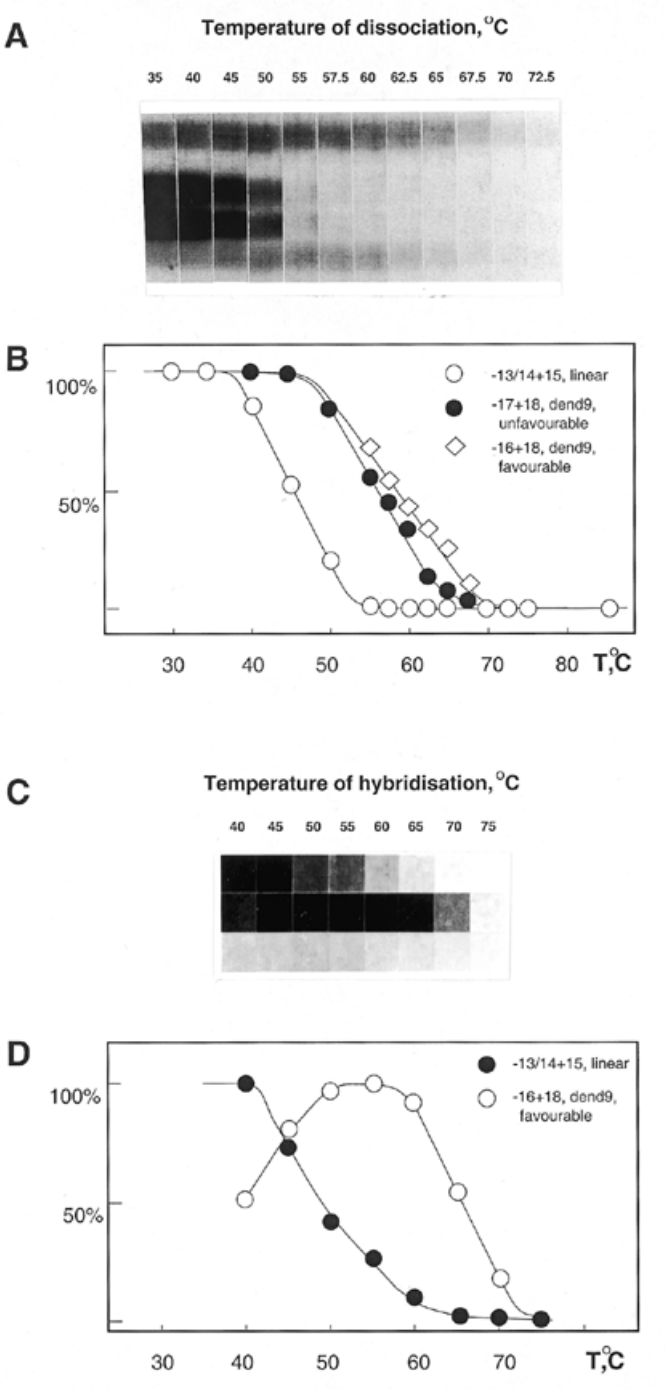


Figure 3. (**A**) Thermal dissociation from the array of an oligonucleotide dendrimer bearing nine 15mers and a monomeric 15mer, of the same base composition but of different sequence. The array was synthesised using a physical masking method (16) on the surface of aminated polypropylene and contained four stripes of 15mers, two complementary to the dendrimer (#18) [top (#16) and bottom (#17)] and two complementary to the control (#15) (two in the middle, #13 and #14). The oligonucleotides on the top two stripes are attached through their 3'-ends, whereas oligonucleotides on the bottom two stripes are attached through their 5'-ends. The linear 15mer (#15) and the dendrimer (#18) were ^{32}P-labelled, mixed together (~1 eq. of dend9 and 9 eq. of the control) and hybridised to the array at room temperature in 3.5 M TMA buffer for 3 h. The array was then washed with 5 ml portions of TMA buffer at temperatures increasing at 2.5°C intervals. Images were taken after each wash. PhosphorImages were obtained on a Molecular Dynamics Model 400A (90 min exposure). (**B**) Melting curves of the single-stranded control (#15 and #13/#14, white dots), of dend9 in the unfavourable orientation (#18 and #17, black dots), and dend9 in the favourable orientation (#18 and #16, white diamonds). PhosphorImages (A) were quantified using the ImageQuant program. (**C**) Thermal association experiment. The above array was cut in strips bearing patches complementary to dend9 and single-stranded linear control. The same mixture of ^{32}P-labelled dend9 (#18) and single-stranded linear control (#15) in the same buffer was hybridised to the strips at different temperatures for 1 h, the strips were washed for 5 min in the same buffer at the temperatures 5°C less than the hybridisation temperature, and then simultaneously imaged as described in (A). The composite picture shows the control 15mer (top), dend9 in the favourable orientation (middle) and aminated polypropylene hybridised to the mixture in the same conditions (bottom). (**D**) Association curves of the single-stranded control (#15 and #13, black dots) and of dend9 in the favourable orientation (#18 and #16, white dots). PhosphorImages (C) were quantified as described in (B).

equation **6**) of the kinetic melting temperature. At $n = n_{max}$, we have:

$$\delta T = \frac{\beta}{m} \ln(\alpha + \ln m)$$

therefore $T_o - T_m = 5$°C. Comparing this with the control case ($n = 1$), we see that the maximal increase of the melting temperature for dendrimers is ~27°C as compared with $n = 1$. The maximum difference in melting temperature achieved on the solid support (Table 1, Fig. 3) was 13°C, with only 0.5°C difference between Dend9 and Dend27. The smaller than predicted increase in T_m is probably due to the suboptimal arrangement of single-stranded oligonucleotide #16 on the polypropylene support, which leads to non-complete involvement of the branches of #18 and #19 in a duplex formation. Indeed, in the case of solution experiments, where the dendrimers with 10mer arms have an increased chance of forming a duplex with their complements (Fig. 1C), we see steady increase in melting temperature for all subsequent generations. For example, in solution, the T_m is 5.4°C higher for Dend27 (#11+#12) than for Dend9 (#8+#9).

This analysis considers only the 'OFF' reaction, which is entropy-independent, as opposed to the 'ON' reaction; that is why the melting temperature T_m does not depend on the dendrimer concentration. The theory explains why dendrimeric complexes are much more stable *kinetically* than the complexes of separate branches, which is most significant for applications where the complements are solid support-bound, for example, in oligonucleotide array/DNA chip technology.

Dissociation temperatures for array/dendrimer complexes were close to the corresponding temperatures in solution (Table 1). This indicates that, although it is difficult to know which condition (in terms of the 'ON' and 'OFF' rates) experiments in solution corresponded to, they probably can be analysed with the same equations.

CONCLUSIONS

Dendritic oligonucleotides can form many different base-paired structures: the pairing potential of the arms of the dendrimer

can be satisfied by interactions with all of the arms of a second with complementary arms; or they can interact with up to *n* molecules. The two-branched dendrimers could form linear concatemers; three-branched dendrimers could form a two-dimensional net; and higher generations could form three-dimensional networks. Bimolecular interactions would be favoured in dilute solution (13). Preliminary evidence, from gel electrophoresis, suggests that the dendrimers with arms bearing only one type of sequence initially form multimeric networks, which on longer incubation resolve into dimeric structures under the conditions used in our experiments. Additional evidence comes from the optical melting and reassociation behaviour of dendritic duplexes. When dissociated duplexes of dendrimers, which can form 'cages', are cooled rapidly through the reassociation temperature, the hypochromic shift is smaller than that observed when melted duplexes are cooled slowly. The same does not apply to linear duplexes or to those which can form 'starfishes'. We believe that single-stranded branches can become trapped within the cages formed by duplexed dendrimers, and that the trapped branches are released slowly, to form duplexes with other single-stranded branches. Such trapping can not occur in linear or 'starfish' dimers, and is less likely to occur in three-branched than higher dendrimers, in accordance with the observed behaviour (data not shown).

A different range of structures is possible when dendrimers interact with oligonucleotides bound to a solid support, which essentially represent a dendrimer with an infinite number of branches and a single core. In this case, the formation of an infinite network is not possible. When all oligonucleotides on the surface are involved in duplex formation, the complements will form a monolayer on the surface.

However, growth of structures from the surface can be seeded from dendrimers which have some branches which tie it to the surface and others which are not complementary to the oligonucleotides on the surface. Complex patterns of oligonucleotides with different sequences can readily be synthesised on surfaces, for example, by light directed methods (14,15) or a physical masking method (16,17). It is easy to imagine how complex 3-D structures could be created by exploiting the possibilities that are offered by the sequence specificity of DNA duplex formation.

We have shown here that the properties of dendritic DNA building blocks can be varied in a number of useful ways. The number of branches determines the nature and the number of structures which can form; the length and number of arms determines the stability and the dimensions of the unit structure; the base sequence determines the strength and the specificity of the interaction of the arms. If arms have different sequences, the number of possible interactions is expanded still further.

It would be desirable to create nano-structures to design. This is difficult with most self-assembling building blocks because the thermodynamic properties of the interacting moieties are complex and therefore difficult to control. By contrast, the interactions of complementary oligonucleotides are well characterised. In this work we have shown how thermodynamic parameters describing oligonucleotide interactions can be incorporated into a simple model to describe the behaviour of DNA dendrimers.

The increased melting temperatures of DNA dendrimers suggest applications for oligonucleotide array/DNA chip technologies where elevated hybridisation temperatures may be required to destroy secondary structure in the target.

ACKNOWLEDGEMENTS

We are grateful to J. E. Dahlberg, M. L. H. Green, C. R. Cantor and G. M. Whitesides for reading the manuscript and valuable comments. 5'-phosphoramidites of protected nucleosides and aminated polypropylene were a gift from Beckman Instruments.

REFERENCES

1. Whitesides,G.M., Mathias,J.P. and Seto,C.T. (1991) *Science*, **254**, 1312–1319.
2. Mirkin,C.A., Letsinger,R.L., Mucic,R.C. and Storhoff,J.J. (1996) *Nature*, **382**, 607–609.
3. Elghanian,R., Storhoff,J.J., Mucic,R.C., Letsinger,R.L. and Mirkin,C.A. (1997) *Science*, **277**, 1078–1081.
4. Alivisatos,A.P., Johnsson,K.P., Peng,X.G., Wilson,T.E., Loweth,C.J., Bruchez,M.P. and Schultz,P.G. (1996) *Nature*, **382**, 609–611.
5. Winfree,E., Liu,F.R., Wenzler,L.A. and Seeman,N.C. (1998) *Nature*, **394**, 539–544.
6. Seeman,N.C. (1998) *Annu. Rev. Biophys. Biomol.*, **27**, 225–248.
7. Newcome,G.R., Moorefield,C.N. and Vogtle,F. (1996) *Dendritic Molecules: Concepts, Synthesis, Perspectives*. VCH Publishers, New York.
8. Shchepinov,M.S., Udalova,I.A., Bridgman,A.J. and Southern,E.M. (1997) *Nucleic Acids Res.*, **25**, 4447–4454.
9. Shchepinov,M.S. and Southern,E.M. (1998) *Russ. J. Bioorg. Chem.*, **24**, 794–797.
10. Southern,E.M. and Maskos,U. (1994) *J. Biotechnol.*, **35**, 217–227.
11. Matson,R.S., Rampal,J.B. and Coassin,P.J. (1994) *Anal. Biochem.*, **217**, 306–310.
12. Anshelevich,V.V., Vologodskii,A.V., Lukashin,A.V. and Frank-Kamenetskii,M.D. (1984) *Biopolymers*, **23**, 39–58.
13. Mammen,M., Shakhnovich,E.I., Deutch,J.M. and Whitesides,G.M. (1998) *J. Org. Chem.*, **63**, 3821–3830.
14. Pease,A.C., Solas,D., Sullivan,E.J., Cronin,M.T., Holmes,C.P. and Fodor,S.P.A. (1994) *Proc. Natl Acad. Sci. USA*, **91**, 5022–5026.
15. Pirrung,M.C. (1997) *Chem. Rev.*, **97**, 473–488.
16. Southern,E.M., Case-Green,S.C., Elder,J.K., Johnson,M., Mir,K.U., Wang,L. and Williams,J.C. (1994) *Nucleic Acids Res.*, **22**, 1368–1373.
17. Southern,E.M., Maskos,U. and Elder,J.K. (1992) *Genomics*, **13**, 1008–1017.