90 W). Water was used for the DLS measurement and DMF for the reaction. The SWNT dispersion was subjected to a series of centrifugations starting from the largest force. Each time, a supernatant solution was separated from the heavier residue, which was redispersed for further centrifugation. The concentration of each supernatant solution was adjusted to 1.0 $\mu g\,mL^{-1}$. DLS measurements were performed with a wavelength of 632.8 nm and a scattering angle of 90° at 30 °C and the data were analyzed with a cumulant method. Measurements have been repeated several times using different batches of the acid treatments. Subsequent experiments showed that the measurements were performed on SWNTs in the concentration-independent region.

The SWNT fractions in the plateau region were treated with thionyl chloride to produce SWNTs derivatized with acid chloride groups, as reported by others.[8, 10] In the present case, we used centrifugation and decantation cycles to wash the acid chloride-derivitized SWNTs with dry THF and DMF to minimize coagulation. A dispersion of acid chloride-derivatized SWNTs in DMF (10 μL) was added to the dendrimer (G10) dissolved in 10 mM NaOH. The concentration of SWNTs was so low that the solution hardly had any color. This was necessary, since amine-terminated dendrimers act as a glue to stick SWNTs together and coagulate the SWNTs immediately.[14] The mixture was stirred at room temperature for 12 h and the reaction mixture was directly cast on mica or silicon for AFM and SEM studies.

AFM was performed in a noncontact mode in air at room temperature. SEM images were taken with an acceleration voltage of 5.0 kV for Pt/Pd coated samples and 1.0 kV for uncoated samples.

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Direct Microscopic Observation of the Time Course of Single-Molecule DNA Restriction Reactions**

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While biophysical studies, such as force measurements[1–4] or laser cutting[5] of single DNA molecules can be found in the literature in a large variety of applications, direct light-microscopic detection of DNA single molecule reactions is rare. In most of the latter cases, unstained DNA molecules were coupled to microspheres (beads) and held with optical or magnetic tweezers. The reaction was then observed indirectly by the change of some distance or force, for example, displacement of the sphere reflected the reaction progress.[6–10] In this sense, single DNA-molecule reactions are usually detected in "blind" experiments. One of the exceptions is the direct microscopic observation of the reaction of fluorescently stained *E.-coli*-RNA-polymerase on unstained DNA.[11] One particularly interesting class of reactions are those of restriction endonucleases, since they allow individual DNA molecules to be characterized and distinguished. As many as 3000 different restriction endonucleases of one subclass (type II) are known.[12] The observation of restriction reactions is, however, difficult, since the restriction step is an all-or-none process, that is, cut or non-cut, of the DNA molecule at a given restriction site. To date, single-molecule restriction has been observed by blind cutting of individual fluorescently marked DNA molecules and analyzing the fluorescence bursts of the restriction fragments.[13]

We have already reported the cutting of a single, bead-coupled DNA molecule by the restriction endonuclease Apa 1, which has one cutting site in the target molecule,[14] and the restriction by Sma I and Eco RI with three and five cutting sites.[15] Herein, we introduce temporal resolution and thus information on the kinetics of single DNA-molecule restriction.

Figure 1 shows the restriction of a single DNA molecule by the enzyme Eco RI. In part (g) of Figure 1, the cutting pattern expected from the 48502 base pairs (bp) long sequence of Lambda phage DNA is shown. The molecule is stretched by hydrodynamic flow from bottom right to top left. In the example shown the DNA end with the longest restriction fragment (21226 bp) is attached to the bead. Binding of the short fragment (3530 bp) is also observed, but then usually only one or two cuts can be detected since some restriction sites are in the part of the DNA molecule which is wrapped around the bead. Such wrapping causes the bead fluorescence seen in Figure 1.

At a given time, usually 5 – 20 seconds after injection of the enzyme, the first cut is seen (Figure 1b); this point is then

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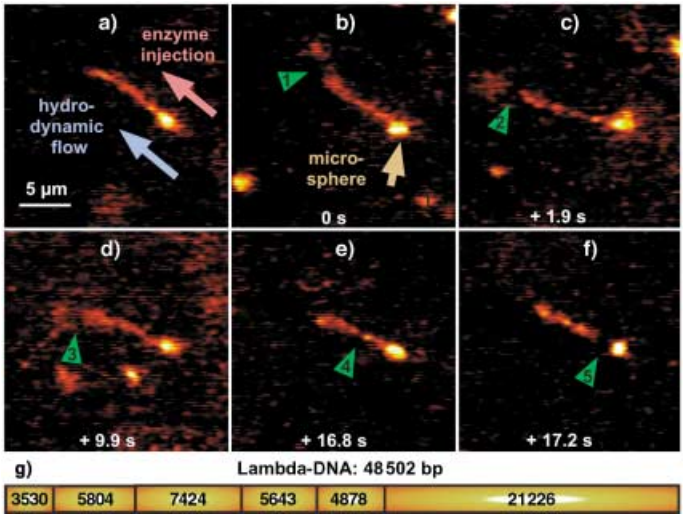

Figure 1. Example of the complete enzymatic digestion of a single hydrodynamically stretched lambda-DNA molecule with the restriction endonuclease EcoR I (a–f). All five expected restriction sites are observed. g) Expected cutting pattern (restriction map) of EcoR I on Lambda phage DNA

defined as zero time. After being cut from the main molecule, the fragment quickly collapses into a globular structure (as any DNA molecule does so when no force is applied) and is transported away with the flow. In steps of several seconds (for the exact times see Figure 1) the other expected cuts can be seen. Two details are important: the first cut occurs at the end of the DNA molecule which is distant from the bead, the second cut is closer to the bead and so on. Obviously, the reaction proceeds against the hydrodynamic flow. Furthermore, the cuts are consecutive, that is, no expected cutting site is omitted. These observations can be most simply explained by the binding of a single enzyme molecule to the distant end and subsequent motion along the DNA molecule towards the bead; this will be discussed in some detail below.

An equivalent observation has been made with the restriction endonuclease Sma I, which has three expected cutting sites in the Lambda phage DNA molecule. In total, ten molecules were investigated with Sma I and ten molecules with Eco RI, this corresponds to a total of 80 restriction events. The features outlined above were observed for more than 50 % of these molecules, in another 30 % restriction sites at one end are omitted, but consecutive cutting is still preserved. Only in four of the 20 molecules the features described above do not hold. This regularity indicates that indeed enzymatic cuts and not just mechanical DNA breaks are seen. A more detailed proof is given by the fact that the fluorescence intensity of the single restriction fragments divided by the total fluorescence of DNA molecules reflects the expected restriction fragment size (see ref.[15]).

From the times at which the cuts are observed, time courses of the reactions can be generated. Figure 2a shows such time courses of six complete Sma I restriction reactions, Figure 2b the corresponding results for five Eco RI reactions. Each curve was measured for an individual DNA molecule.

The observed result becomes even clearer when the reactions between two adjacent cutting sites are represented

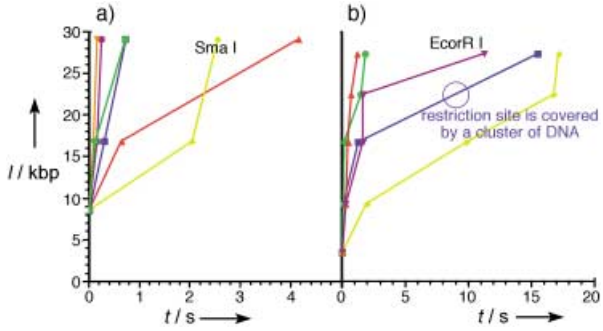

Figure 2. Restriction time courses of Sma I (a) and EcoR I (b) for 11 different molecules. Each curve represents the restriction of one single DNA molecule. Note that the time-axis scales are different for Sma I and Eco RI.

separately (Figure 3). For each enzyme two groups of data are found. Since the slope of each of the straight lines is a measure of the reaction rate, clearly each of the enzymes has a slow and a fast reaction rate and for Sma I this rate is generally faster than for Eco RI. The dotted lines correspond to free diffusion of the enzyme through the solution. Essentially, all rates are slower than the diffusion rate, which indicates that motion along the DNA molecule can, on the one hand, be thermally driven, but, on the other hand, is obviously reduced as if there were friction.

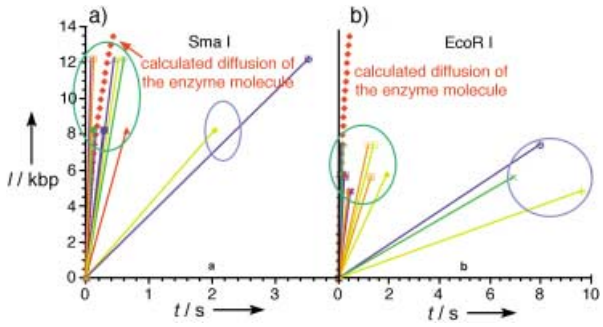

Figure 3. Restriction rates of Sma I (a) and Eco RI (b). The slope of each line reflects the restriction rate between subsequent cuts. Two different groups of restriction rates can be distinguished for each enzyme and are indicated by circles.

Diffusion could also explain why the recognition process between enzyme and DNA cannot require exact binding to the five (Eco RI) or three (Sma I) restriction sites on the 48502 bp long Lambda phage DNA. Both enzymes have six bp long recognition sites. It would require more than 1500 (EcoR I) or 3000 (Sma I) contacts between one enzyme molecule and the DNA molecule to statistically hit one specific site. The concentration of the microinjected enzyme surrounding the DNA is approximately 6×10^4 molecules per pL. The mean rate of contacts between one enzyme molecule and the DNA follows from this concentration to be around 1 contact per second. Thus, 1500 contacts would require half an hour for EcoR I and 3000 contacts for Sma I one hour. To find one restriction site solely by spatial diffusion of the enzyme in the observed time of about one second is impossible.

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Together with the above mentioned fact that most of the cuts are consecutive, this suggests that the enzyme molecule binds randomly (statistically) to some site on the DNA molecule, searches for its six-bp restriction site and cuts. Since in all the observed cases where the reaction continues, it does so in the forward direction, often with the same speed, we suggest that the enzyme cuts at the rear (lagging) side of its recognition sequence while already being attached to the forward (leading) side. For Eco RI this is easily conceivable, since its restriction site is 5′..G ↓ AATTC..3′ where the arrow indicates the cut. For Sma I the restriction site is highly symmetric (Sma I: 5′..CCC ↓ GGG..3′) and thus a simple explanation is not possible. Here a differential affinity of the enzyme for G and C tracts has to be invoked. A final answer will probably only be possible once the structure of Sma I is solved.

While the explanation given for the observations may appear plausible, an alternative possibility has to be excluded: that not a single, but several individual enzyme molecules are attached to the DNA molecule and cooperate. However, in this case one would have to invoke a hidden mechanism, which orchestrates these different molecules so that the cuts are consecutive. Such a mechanism is at least much less probable than the mechanism postulated above. In the case of Eco RI, with its five restriction sites, such a multimolecule process is highly improbable.

The observed speeds of enzyme progression along the DNA appear to be high, but can be explained by guided diffusion. Values observed in our experiments are 50 kbp s^{-1} (17 μm s^{-1}) for Sma I and 30 kbp s^{-1}(10 μm s^{-1}) for EcoR I. Other enzyme reaction rates are known for DNA polymerase I (10 bp s^{-1}), T7-DNA polymerase (300 bp s^{-1}) and the DNA polymerase holoenzyme (1.000 bp s^{-1}). Furthermore the reaction rate of RecBCD, a DNA helicase and exonuclease is estimated to be 1.000 bp s^{-1}.[16] All these reaction rates are slower than our measured reaction rates. Polymerases execute a reaction on every base pair and can gain energy from bond cleavage (for example by ATP consumption). In contrast, the linear motion of restriction endonucleases along DNA observed in the present work can only occasionally receive energy from bond cleavage and thus has to take its energy from thermal motion. It was already suggested above that this is possible on the basis of diffusion and thus the mechanism can be explained.

Experimental Section

Biotinylation and staining: Lambda DNA (48,502 bp or 16.5 μm in its stretched form, New England Biolabs, Schwalbach, Germany) was biotinylated by using biotin-16-ddUTP (Roche Diagnostics, Mannheim, Germany). The lambda-DNA molecules in a concentration of 2.5×10^8 molecules per μL together with a 10-fold excess of biotin-16-ddUTP were incubated with terminal transferase (Boehringer Mannheim, Germany) in the recommended buffer (5 × the concentration recomended by the supplier: 1M potassium cacodylate, 125 mM Tris-HCl (Tris = tris(hydroxymethyl)aminomethane), 1.25 mg/ml bovine serum albumin, pH 6.6, Boehringer Mannheim) and 1.5 mM $CoCl_2$ (Boehringer Mannheim) for 20 min at 37 °C. The transferase reaction was terminated by adding EDTA (50 mM; EDTA = ethylendiaminetetraacetate). The biotinylated DNA was diluted at a ratio of 1:20 with H_2O and stained by mixing it with the fluorescent intercalating dye SYBRGreen (Molecular Probes, Oregon, USA). SYBRGreen is not the optimal dye in terms of stability, but, besides DAPI, is the only dye where we could observe an restriction endonuclease activity on stained DNA, so far.[14] Since Molecular Probes does not provide the exact dye concentration in the stock solution, a dilution of 0.08 % was determined empirically to be best suited for the staining procedure

Coupling of DNA to microspheres: In the following step, the stained and biotinylated molecules were coupled to streptavidin coated microspheres (diameter 1 μm, Polysciences, Eppelheim, Germany).[15] DNA preparation still containing residual material from the biotinylation and staining procedures was mixed with the microspheres (10^7 spheres per μL in H_2O) at a ratio of 5:1 and incubated for 15 min in the dark at room temperature. The final solution contained 3×10^5 molecules of DNA per μL in H_2O. Under these conditions, only one or at most a few DNA molecules are expected to bind to one microsphere, and the low ion concentration (for all dilution steps water was used) makes DNA stretching easily possible. For coupling single DNA molecules to polystyrene microbeads, the DNA molecules were end labeled at the 5′position with biotin, while the beads were covered with streptavidin. Because of the high binding constant of streptavidin with biotin, DNA bead binding occurs immediately upon mixing. For details of this procedure see ref. [15]

Handling of DNA molecules by optical tweezers and hydrodynamic flow: For handling of a single DNA molecule the sphere/DNA suspension (5 μL) was dropped onto a microscope cover slide and investigated in the fluorescence microscope. To retard fast photobleaching, an antifade kit (Appligene Oncor, Heidelberg, Germany) was used in a dilution of 2:5. Observation in the bright field shows the microspheres and in the fluorescence mode the DNA. Switching between both modes allowed a microsphere with an attached DNA molecule to be identified and then trapped with optical tweezers.[17, 18] The DNA molecule was stretched by the hydrodynamic force between the DNA molecule and the surrounding buffer which is generated by moving the cover slide while holding the microsphere with the tweezers. When the motion of the slide was stopped, the stretched DNA molecule coiled up within one or two seconds.

Enzymatic Reaction: The restriction endonucleases Sma I and EcoR I (New England Biolabs, Schwalbach, Germany) were used. The enzymes were suspended in a 5 × higher concentration than recommended by the supplier, for the recommended reaction buffers (Sma I: 250 mM potassium acetate, 100 mM Tris acetate, 50 mM magnesium acetate, 5 mM DTT (DTT = 1,4-dithiothreitol), pH 7.9; EcoR I: 250 mM NaCl, 500 mM Tris-HCl, 50 mM $MgCl_2$, 0.125 % Triton X-100, pH 7.5) to provide the necessary ion concentration for the restriction reaction after enzyme injection. Purification requires filtration with 0.22 μm centrifugal filter units (Ultrafree-MC, Millipore, Eschborn, Germany). If a higher concentrated reaction buffer is used, the DNA molecule on the microsphere tends to collapse as a result of the high ionic strength of the mixture even in hydrodynamic flow. A micromanipulator and microinjector device (Eppendorf, Hamburg, Germany) was used to inject the endonuclease and reaction buffer mixture.

The injection capillary was positioned 10 μm in front of the stretched DNA molecule. To start the enzymatic restriction, a volume of 0.1 – 0.2 pL of the prepared enzyme/buffer mixture was microinjected into the DNA solution on the cover slide at room temperature. The enzyme-buffer was thereby diluted by a factor of approximately 1:5 so that it reached its recommended concentration.

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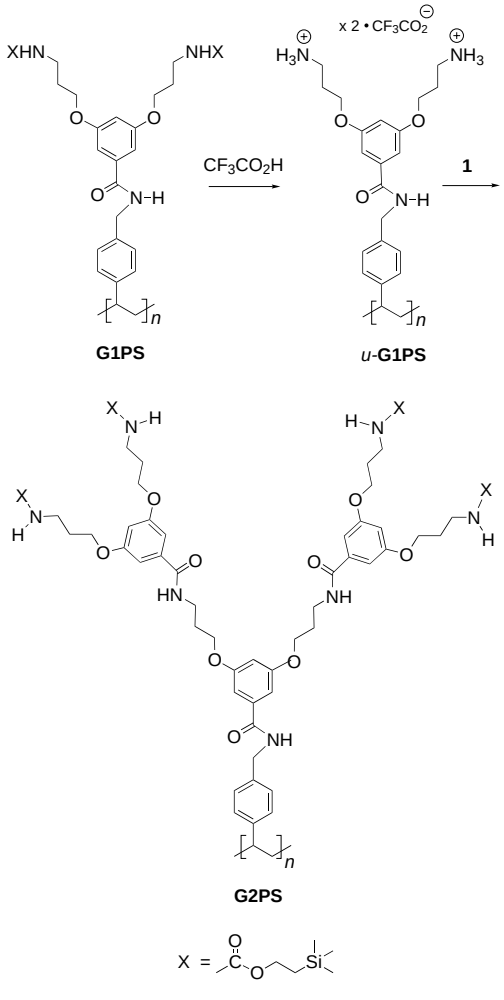
Extremely Long Dendronized Polymers: Synthesis, Quantification of Structure Perfection, Individualization, and SFM Manipulation**

Lijin Shu, A. Dieter Schlüter,* Christof Ecker, Nikolai Severin, and Jürgen P. Rabe*

The enormously successful top-down approach to nanostructured materials[1] increasingly faces its limitations. For example, it is virtually impossible to both control and determine nanostructures on the atomic scale by cutting and etching. In recent years the alternative bottom-up approach to the nano world has made considerable progress from single atom manipulation[2a] towards rather well defined, more complex structures,[2b] although not enough to make it technologically important at this stage. Complex molecules with predictable shapes on the nanometer-scale were constructed, handled, and characterized and it was possible to individualize and manipulate them[3] or to assemble even more complex functional arrays.[4] The bottom-up approach is closely related to the scanning-probe microscopies which provide particularly important analysis, manipulation, and construction tools. Their consequent application and further methodical development have provided indispensible knowledge about both individualized and assembled properties of molecules, the behavior of molecules at interfaces,[5] and thus laid the foundation for what may become competition for the top-down approach within the next 10 years or so. For resolution reasons, scanning force microscopy (SFM) is useful for larger objects (a few nm in diameter) than scanning tunneling microscopy (STM, a few Å). On the other hand, there are no structural limitations for SFM, whereas STM is restricted to sufficiently thin molecules on conducting substrates. Dendronized polymers have been developed over the last 10 years as nanoscaled molecular objects at the interface between the materials and bio sciences.[5f] Despite considerable effort in many laboratories to date it has not been possible to synthesize such polymers carrying fourth generation (G4) dendrons and having both high molar mass and a functionalized surface for chemical modifications. We here report the divergent synthesis of an extremely high molar mass, surface functionalized, G4 dendronized polystyrene (PS), its individualization and SFM visualization on graphite, and its manipulation with the SFM tip.

Radically initiated polymerization of dendronized vinyl-type macromonomers leads to polymers the number of repeat units of which decreases with increasing dendron genera-



Scheme 1.

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