Functional Materials

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Self-Immolative Polymers**

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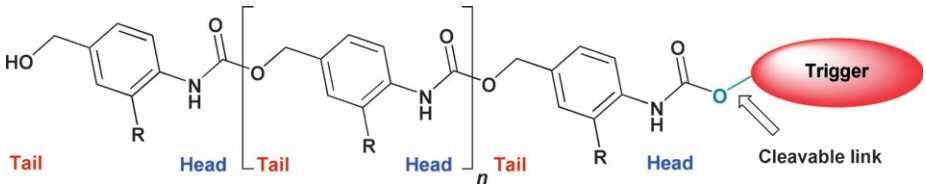
The investigation of synthetic polymers for biological applications is increasing as assembly techniques for generating well-defined macromolecules from artificial building blocks become more sophisticated. Synthesis techniques have now evolved such that a wide variety of functional groups can be tolerated by polymerization catalysts, and a fascinating and diverse range of macromolecular and polymeric materials has resulted.[1–3] These synthetic polymers are now beginning to resemble natural counterparts in terms of molecular architectures, suprastructures, and functions.[4,5] However, although there has been enormous progress on synthesis and assembly, there has been much less emphasis on the controlled depolymerization or disassembly of polymers. The limited interest in depolymerization is perhaps surprising when one considers that natural polymers are put together, modified, and dismantled with equal ease. Indeed, living systems show extraordinary abilities to move forwards and backwards along reaction pathways, and are incredibly atom-efficient in doing so. The repeated generation, processing, and hydrolysis of spider silk proteins is but one example amongst many in nature of this ability to assemble and disassemble polymers.[6] The search is on, therefore, for wholly artificial functional materials that are assembled easily yet broken down in an equally facile manner to switch between states of differing (biological) activity. One step along this road is to make polymers that are programmed through their synthesis to disassemble in ways that might be triggered environmentally to yield products that are biologically important.

In recent years, the research group headed by Doron Shabat at Tel Aviv University has made significant strides in this direction, with a series of publications describing "self-immolative" systems. Of particular interest is a study published earlier this year by Sagi et al.,[7] who described the sequential disassembly of a linear main-chain polymer by a single triggering reaction (Scheme 1).



Scheme 1. General structure of a main-chain self-immolating polymer.

The self-immolation system is based on an ingenious design philosophy: Polymers are prepared with architectures that enable the exploitation of neighboring-group interactions, 1,6-elimination, and decarboxylation reactions. The blocked isocyanate used for the polymer-assembly reactions underwent homopolymerization in the presence of a catalyst to generate polyurethanes, which were finally capped with a trigger group. By connecting up the constituent repeat units, or monomer fragments, by the urethane linkage through *para* positions of an aromatic ring and with a benzylic-carbon-atom spacer, the polymers are, in effect, set up to collapse the moment the end group is removed. This triggering effect is analogous to the removal of a keystone from an arch, whereby the whole structure is destabilized and the arch collapses, except that in this case the "keystone" group is at the end of the polymer "arch" rather than in the middle.

What is especially exciting about the recent research by Sagi et al. is the demonstration of cleavage cascade reactions with applications which extend well beyond programmed polymer degradation. In the first example, the single reaction to cleave the polymer chain end was used for enhanced-sensitivity protein detection. By capping the polymer with 4-hydroxy-2-butanone, a substrate for β elimination by the common protein bovine serum albumin (BSA), a protein sensor was installed at the head of the polymer chain. Careful monomer design enabled a fluorogenic group to be installed in the main chain. This unit exhibited low fluorescence-emission intensity when present in the carbamate form (i.e. in the polyurethane chain), but high emission intensity when released as the free amine. The incubation of the butanone-capped polymer with BSA resulted in the removal of the polymer head group, liberation of the terminal amine, and subsequent unzipping of the polymer to release the substituted 4-aminobenzyl alcohol, which in turn reported the reaction cascade through enhanced fluorescence (Scheme 2).

In essence, an amplification event occurs, in that a single signal, that is, hydrolysis of an end group, gives rise to multiple outputs, in this case the release of fluorescent reporter

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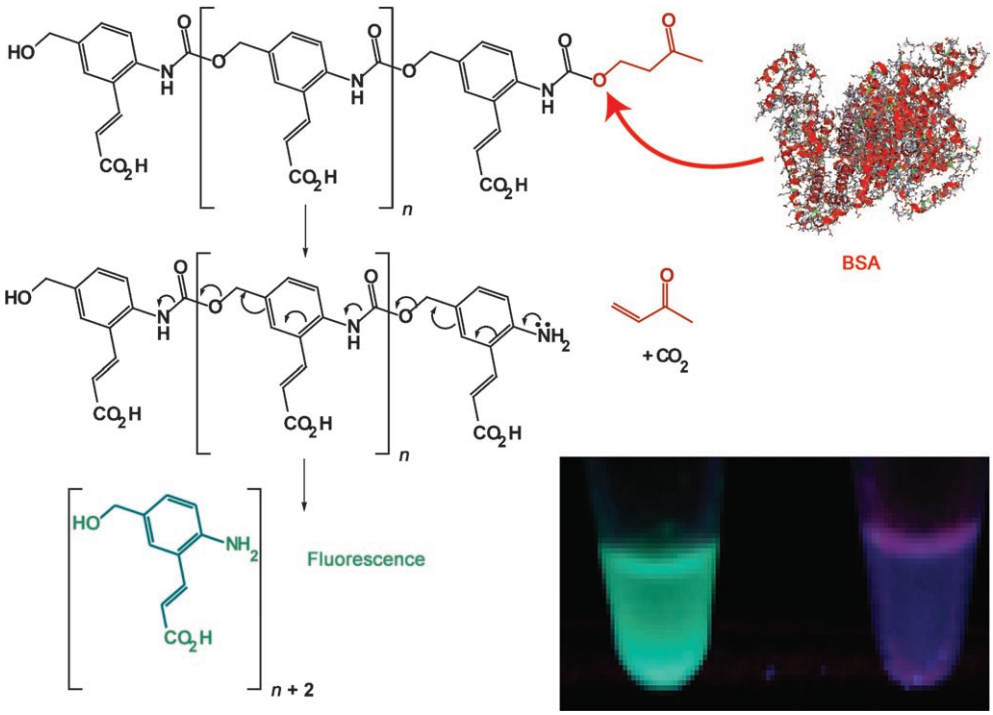

Scheme 2. BSA-induced cleavage of a self-immolative polymer composed of potentially fluorogenic units. The reaction cascade following removal of the protecting group on the head-group amine is shown and results in the release of fluorescent reporter molecules. Increased fluorescence ($\lambda_{ex} = 270$ nm, $\lambda_{em} = 510$ nm) is observed (left-hand Eppendorf tube) relative to that of the polymer in the absence of BSA (right-hand tube).

molecules. One could thus envisage a very powerful detection technology, the signal-to-output ratio of which could in principle be tuned simply by altering the degree of polymerization, as the higher the number of repeat units in the polymer, the higher the output from the initial chain-cleavage event. Furthermore, as the reporter output is independent of the type of event that leads to the uncaging of the polymer, a platform of sensor materials can be generated from a single polymer by using a range of end groups that are substrates for different enzymes. There is, of course, the usual caveat when it comes to the detection of enzymes and enzymatic activity. A single active enzyme molecule amongst a pool of inactive species is still capable of initiating multiple signaling events, and this method, like any others that rely on reactive species, can not distinguish between the active and inactive constituents. In most cases, it is not necessary to make this distinction, as for biochemically important enzymes, it is nearly always the specific activity of the enzyme that one needs to detect. However, there are some specialized cases (e.g. immune response) in which the absolute protein concentration is required, and very low levels of an immunogenic component would not be picked up by this and other methods of protein detection.

The recent study by Sagi et al. also represents an important extension to the self-immolation strategy.[8–17] Previously, the Shabat research group have investigated drug-release applications, again by using chemical reactions of the type that can be triggered by various biochemical stimuli (Figure 1). The mechanisms by which therapeutic agents are released have included nucleophilic intramolecular cyclizations that lead to stable cyclic species, quinone methide rearrangement, and self-elimination reactions. The key factor underlying all these systems is that the triggering group can be varied such that it is activated by a very wide range of stimuli. Because of the polymer design, the type of end group that can be installed is highly flexible, yet the reactivity profile can be tuned to be very substrate specific. The overriding requirement is that the triggering reaction caused by a biochemical or other trigger results in the generation of an active nucleophile, such as an aromatic amine or activated phenol. To date, the end groups have been designed to undergo activation upon the action of acids or bases, catalytic antibodies, amidases, and now esterases. However, in all cases, the stimulus and trigger reaction serve to expose a caged nucleophile, which then commences the cascade reactions that cause the polymers to disassemble sequentially.

In biomedical terms, this strategy is of considerable utility, although fully biocompatible self-immolative polymers and monomers have yet to be prepared. Nevertheless, the ability

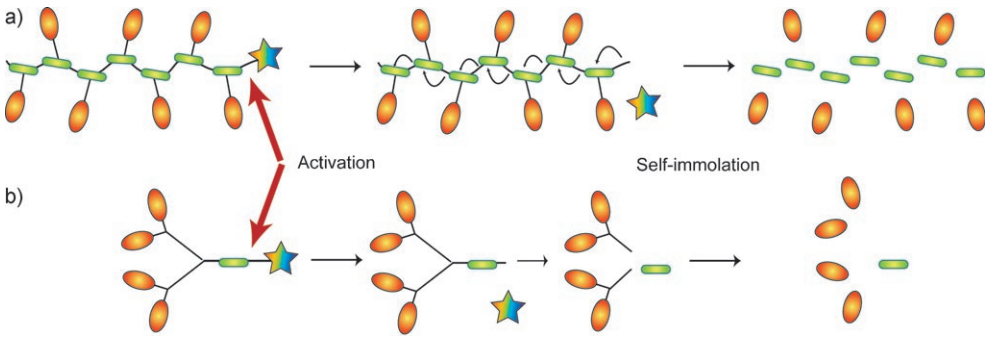

Figure 1. Self-immolation strategy for a) linear polymers and b) dendrimers. A single activation event induces a cascade of self-elimination reactions that lead to the complete dissociation of the linear polymer or dendrimer into its separate building blocks and the release of side chains or end groups.

of a specific biological trigger to promote a wide range and large number of cleavage reactions leads to some important therapeutic advantages. If the cleavage products are drug compounds, they can be held on the caged polymer to await a site-specific stimulus. For anticancer compounds, which are typically highly cytotoxic, the ability to hold the drugs on a polymer backbone until they reach a tumor prevents their release into nondiseased cells and thus leads to greatly reduced side effects. The strategy of self-immolative polymers has been used previously to release the DNA topoisomerase inhibitor, doxorubicin, in leukaemia cell lines, with either the catalytic antibody 38C2 or penicillin G amidase as the trigger.[11,12] Cell-growth assays with the dual-triggered polymer–doxorubicin conjugates showed that dose-dependent growth inhibition and complete suppression of growth occurred when the self-immolative polymers were used at a prodrug concentration of 10–100 nM. The new main-chain-cleavage methodology described by Sagi et al. could be even more advantageous in this context, for the amplification inherent in the polymer design and disassembly should enable very high drug loading. For some anticancer compounds, the major challenge is to deliver enough of the cytotoxic agent to the cell that tumor-cell kill is guaranteed. Conventional polymer therapeutics often suffer from poor overall drug payload, as the drug molecules are typically conjugated to a small proportion of the polymer side chains. If the self-immolative polymers can be engineered to contain solubilizing groups as well as drug compounds in their main chains, it may be possible to reach the high level of cytotoxic payload needed for resistant tumors.

Another factor that can be exploited in medical applications of this class of polymer is that the release mechanisms can be tuned to be complementary or orthogonal to existing processes already developed for drug release. Biochemical triggers used in drug-delivery systems to date include acid-labile polymer–drug conjugates that become reactive at the comparatively low pH values (between 7.4 and approximately 5.6) in endosomal compartments,[18–20] and reducing agents, such as glutathione, present in the cytosol to degrade polydisulfides.[21] Although not demonstrated in the current study, the installation of acetals, ketals, and hydrazones as triggering groups would generate polymers that should be effective in biostimulated release.

A further intriguing possible application of the new materials could result from the sequential nature of the disassembly process. The head-to-tail unzipping of the polymer was shown elegantly by the installation of a 4-nitroaniline reporter at the tail end of the polymer to enable the monitoring of total polymer degradation by reversed-phase HPLC. The evolution of 4-nitroaniline took place over 10 h in the presence of BSA. The results indicated that the reaction proceeded by the progressive sequence of amine formation, 1,6-elimination, and decarboxylation along the chain. It is not difficult to foresee polymer sequencing analysis by such a process by using different reporter groups at varying points along a chain.

In summary, the recent study by the Shabat research group points the way towards a new family of polymers, the assembly and disassembly of which are inextricably linked. The significance of this research is wide-reaching. First, the way in which the polymers are assembled, that is, by urethane synthesis, is amenable to modification; thus, many different types of substitution in the main and side chains are possible. Second, the degradation of the polymers is sequential and again could be tuned by the appropriate choice of chemical reactions to occur at different rates and thus enable delayed-release profiles if required. Finally, the triggering step is very versatile, so that the activation event can be fine-tuned to implicate any of a wide range of physical, chemical, or biological stimuli. Therefore, the range of possible applications is very broad.

By using a detailed knowledge of physical organic chemistry and exploiting efficient nucleophilic and cascade reactions, Sagi et al. have developed a novel class of materials that might function in areas as diverse as analytical probes and diagnostics through to drug-delivery vehicles and medical devices. Advances in organic chemistry can truly lead to some fascinating and useful materials.

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