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Photochemical Coupling of Aryl Azides to Poly(ether urethane) Surfaces: Studies with a Fluorescent Model Compound

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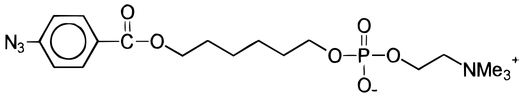
ABSTRACT: A fluorescent aryl azide [*N*-methyl-*N*-(2-hydroxyethyl)-5-(dimethylamino)naphthalene-1-sulfonamide 4-azidobenzoate] (**2**) was synthesized as a model compound for aryl azides that are used for the photochemical surface modification of poly(ether urethanes) (PEUs). We are using this method of surface modification in our search for artificial surfaces with optimized blood compatibility. As a part of this work, two pivotal questions are now addressed: (i) what is the surface density of coupled molecules that can be reached by this method and (ii) is binding to the surface really covalent? After the photochemical coupling of **2** to a PEU surface, the ester bonds of the coupled molecules were selectively hydrolyzed with 0.1 M NaOMe/MeOH and the concentration of fluorescent molecules in solution determined spectrofluorometrically. Since not all of the fluorescent molecules were removed by this procedure, an additional experiment was performed (in which the polymer was completely dissolved). It was found that 25 nmol/cm^2 had coupled to the outermost polymer layers and 7.7 nmol/cm^2 to polymer layers more within the material (unreachable for the NaOMe/MeOH reagent). In another experiment a modified PEU sheet was dissolved in THF, after which MeOH was added to precipitate it again. From the fact that no fluorescence was detected in solution it could be concluded that all of the fluorescent molecules were indeed covalently coupled to the polymer surface.

Introduction

Biomaterials are designed to perform their function in contact with living tissue. They have found widespread applications in all kinds of medical devices.[1] They can be made of polymers, ceramics, and metals. For applications like artificial blood vessels polymeric materials are needed with better blood compatibility. Surface modification of existing polymeric materials can improve the materials' interaction with the biological environment while the mechanical (bulk) properties remain essentially unchanged.[2]

Several research groups are using surface modification techniques that are based on the photochemical reactivity of aryl azides.[3] In our research group aryl azides have been used that carry a dipyridamole[4] or theophylline group[5] (which are both platelet inhibitors). At present we are studying aryl azides that carry a phosphorylcholine group. The rationale for this approach lies in the fact that phosphorylcholine is the polar head group of phosphatidylcholine, the most abundant phospholipid in the outer surface of the membrane bilayer of blood platelets. It was argued that phosphorylcholine could "passivate" an artificial surface in such a way that no platelet activation or contact activation can take place and initiate blood coagulation.[6]

Poly(ether urethanes) (PEUs) are already being used in blood-contacting devices, because of their excellent mechanical properties, but the blood compatibility has to be improved for long-term implants.[7] We have synthesized molecules like **1** that contain a photoactivatable 4-azidobenzoyl group at one end of the molecule and a phosphorylcholine group at the other end. After coupling with a PEU surface the phosphorylcholine group rendered the polymer more blood compatible, as will be reported elsewhere. This report deals with two important questions: (i) do aryl azides like **1** and **2** indeed couple covalently with reactive groups at a PEU surface and (ii) what surface density can be reached?



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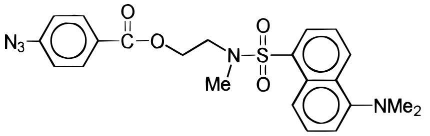

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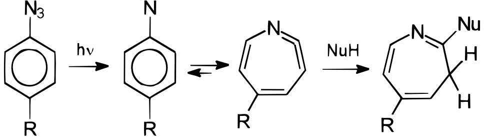
Upon photoactivation an aryl azide splits off nitrogen (N_2) and a highly reactive intermediate—a singlet nitrene—is formed. The chemistry of nitrenes is complex and not fully understood.[8] Reaction pathways depend on temperature, the presence of ring substituents, and the availability of nucleophiles.[9] The major pathway in the presence of nucleophiles is shown in Scheme 1. Ring expansion of the nitrene leads to a 1,2-didehydroazepine that can react with various nucleophiles. The electron-withdrawing para-carboxyl substituent increases the reactivity of the 1,2-didehydroazepine intermediate as a result of which it can react not only with amines but also with alcohols.[9c] We suggest that even urethane NH groups are nucleophilic enough to react with the didehydroazepine (vide infra). Didehydroazepine molecules that do not react with the polymer surface will form byproducts that can be washed off.

We have devised a method that enables us to measure the number of aryl azide molecules that have reacted with a PEU surface.[10] For this purpose we have synthesized model compound **2** (Scheme 2). The compound can be physically adsorbed to a PEU surface and

Scheme 1



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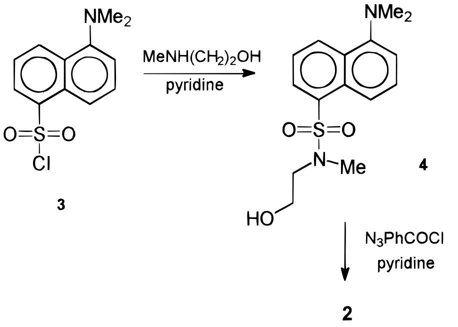
Scheme 2



irradiated with UV light, which leads to coupling to the surface. The ester bonds in the coupled molecules can then be hydrolyzed, allowing analysis of the resulting solution. The intense fluorescence of the [5-(dimethylamino)naphthyl-1]sulfonyl (dansyl) group[11] is used to determine the surface density of coupled molecules.

Results and Discussion

Dansyl chloride **3** was converted into the dansyl alcohol **4** by reacting it with an excess of 2-(methylamino)ethanol in pyridine (62% yield). 4-Azidobenzoyl chloride was obtained in two steps (67% overall yield) from 4-aminobenzoic acid by standard reactions. Coupling of **4** with 4-azidobenzoyl chloride in pyridine yielded (81%) azide **2**.

A 5 mM solution of **2** in 2-propanol (in which PEU hardly swells) was sprayed onto a PEU (Pellethane 2363-55D, Figure 1) surface followed by drying. The polymer sheet with the adsorbed aryl azide was irradiated with UV light (300−400 nm), resulting in coupling of **2** with the polymer surface. After extensive washing, the polymer sheet was viewed under UV light (365 nm). Judging from the (green) fluorescence distribution, the material was homogeneously coated. A piece of the polymer was dissolved in THF, after which MeOH was added to precipitate it again. The precipitate showed fluorescence, while the solution did not (Figure 2). It follows that the dansyl derivative **2** is indeed covalently bound to the PEU surface. The nature of the chemical bond is uncertain. Probably, the urethane NH groups have reacted with the electrophilic didehydroazepine molecules.[12] Alternatively, NH or CH insertion of the singlet (or triplet) nitrene intermediate may have occurred (while insertion reactions of aryl azides in solution are rare, when immobilized on a surface the insertion efficiency might be higher).[13] We found no indication for the formation of insoluble high molecular weight polyazepines (often called "tar"),[14] since all byproducts could easily be washed off, leaving a transparent polymer sheet.

To determine the surface density of coupled molecules, a piece of the modified polymer was treated with 0.1 M NaOMe/MeOH (14 h) in order to hydrolyze the ester bonds.[15] Note that the urethane groups of the polymer are resistant to this treatment.[16] The concentration of dansyl derivative **4** in solution was determined spectrofluorometrically. It was calculated that 25 nmol/cm^2 (7.9 μg/cm^2) had been present on the polymer surface. Because the polymer sheet still showed some fluorescence (which was not affected by fresh NaOMe/MeOH solution), the material was completely dissolved in THF and 0.1 M NaOMe/MeOH was added in order to hydrolyze the ester bonds. The amount of added solution (ca. 20% v/v) was such that no polymer precipitated. After 14 h excess MeOH was added to precipitate the polymer. After centrifugation the solution was isolated (the precipitate showed no fluorescence) and evaporated to dryness. The residue was dissolved in MeOH, and the concentration of dansyl derivative **4** was determined spectrofluorometrically. It was calculated that 7.7 nmol/cm^2 (2.3 μg/cm^2) had been present in the polymer sheet, unreachable for NaOMe/MeOH. So, in total, 32.7 nmol/cm^2 (10.2 μg/cm^2 or 2.0 $\times$ 10^{16} molecules/cm^2) of **2** had coupled to the PEU surface, of which 25 nmol/cm^2 to the outermost polymer layers (hydrolyzable by NaOMe/MeOH) and 7.7 nmol/cm^2 to polymer layers more within the material (not directly hydrolyzable by NaOMe/MeOH).[17]

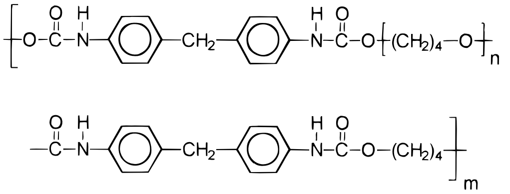


Figure 1. Structural formula of Pellethane 2363-55D. It is made of 4,4′-methylenebis(phenyl isocyanate), poly(tetramethylene oxide), and 1,4-butanediol, although its exact composition is proprietary.[7b]

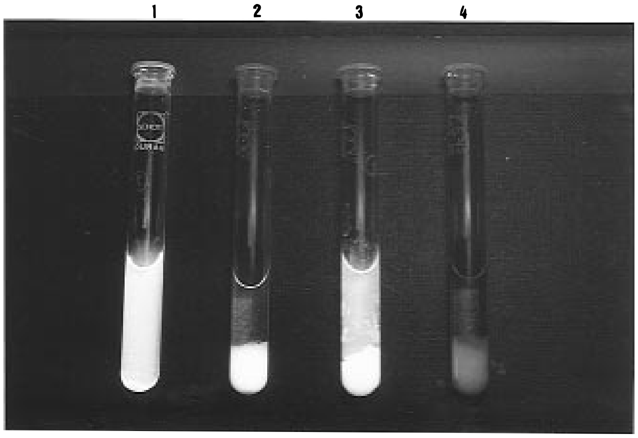


Figure 2. Glass test tubes viewed under UV light (365 nm). Tube 1 contains a solution of PEU modified with **2**, in THF. In tube 2, MeOH was added to a part of this solution, resulting in precipitation of the modified polymer. Because no fluorescence is detected in solution (only the precipitate fluoresces) it must be concluded that all of the fluorescent molecules are covalently bound to the polymer. In tube 3, 0.1 M NaOMe/MeOH was added instead of MeOH, resulting in hydrolysis of the ester bonds of coupled molecules **2**, so that the solution shows fluorescence again (not all of the coupled molecules were hydrolyzed in this experiment; see text). Tube 4 is a control: (untreated) PEU precipitated from a solution in THF by adding MeOH.

The found surface density (25 nmol/cm^2) is comparable to what was found in an earlier report for the coupling of an aryl azide carrying a dipyridamole group to a PEU surface (15 nmol/cm^2, measured by UV absorption).[4] In a determination (by a fluorescence assay) of the surface density of carboxylic groups on polyethylene a value of approximately 3.3 nmol/cm^2 was found.[18] In all of these cases the surface densities of functional groups are higher than the surface density of a compressed oriented stearic acid monolayer (0.8 nmol/cm^2)[19] or a phospholipid layer adsorbed and polymerized on a polyethylene surface (0.3 nmol/cm^2).[20] There can be two reasons for this. First, the true surface area of the polymers is much higher than the geometric surface area because of the surface roughness. Second, the surface modification schemes in the cited and our investigations undoubtedly result in the modification of a (unknown) number of polymer layers close to the surface.

Conclusions

It is now proven that the photochemical reaction of an aryl azide (containing a carboxyl group para to the azide group) with a poly(ether urethane) surface results in covalently coupled molecules. Furthermore, it appears that the surface density of coupled molecules can be increased to values of about 25 nmol/cm^2. With the described method of selective hydrolysis of a fluorescent model compound, we have been able to make a distinction between modified polymer layers closest to the surface and layers more deeply buried in the polymer. In our opinion these results contribute to the validation of photochemical surface modification techniques on the basis of aryl azides. We continue to follow this strategy in our attempts to prepare artificial surfaces with satisfactory blood compatibility *in vitro* and *in vivo*.

Experimental Section

NMR spectra were recorded on a Varian 400 MHz spectrometer (^{1}H: 399.9 MHz; ^{13}C: 100.6 MHz). Tetramethylsilane was used as the internal standard for ^{1}H and ^{13}C NMR. Column chromatography was done with Merck silica gel 60 (0.063−0.200 mm). TLC was performed with Merck silica gel 60 F_{254} on aluminum sheets. 2-(Methylamino)ethanol was distilled from CaH_2 under reduced pressure and stored under nitrogen.

***N*-Methyl-*N*-(2-hydroxyethyl)-5-(dimethylamino)naphthalene-1-sulfonamide (4).** A solution of dansyl chloride (1.89 g, 7.00 mmol) in 15 mL of pyridine was added to a solution of 2-(methylamino)ethanol (2.6 g, 35 mmol) in 15 mL of pyridine during 30 min at 0 °C. After stirring for another 30 min at 0 °C and 1 h at ambient temperature, the solution was evaporated. The residue was dissolved in EtOAc and washed with H_2O, 0.5 M citric acid, saturated $NaHCO_3$ solution, and brine. The organic layer was dried ($MgSO_4$) and evaporated. Silica gel chromatography (CH_2Cl_2/EtOAc 8:2 v/v, R_f 0.18) afforded **4** as a viscous yellow oil (1.36 g, 62%). ^{1}H NMR ($CDCl_3$): δ 2.04 (1 H, t, OH), 2.89 (6 H, s, NMe_2), 2.96 (3 H, s, NMe), 3.35 (2 H, t, CH_2N), 3.77 (2 H, q, CH_2O), 7.18, 7.56 (2 H), 8.20, 8.35, and 8.57 (6 H, naphthyl). ^{13}C NMR ($CDCl_3$): δ 35.2 (NMe), 45.4 (NMe_2), 51.8 (CH_2N), 60.0 (OCH_2), 115.2, 119.3, 123.1, 128.2, 130.0, 130.1, 130.2, 130.6, 133.7, and 151.8 (10 C, naphthyl). MS (EI): *m/z* 308 (M^+).

***N*-Methyl-*N*-(2-hydroxyethyl)-5-(dimethylamino)naphthalene-1-sulfonamide 4-Azidobenzoate (2).** 4-Azidobenzoic acid was prepared from 4-aminobenzoic acid by a standard diazotation reaction with $NaNO_2$ (1 equiv) in dilute hydrochloric acid. The resulting diazonium salt was converted into the azide by reaction with NaN_3 (1.1 equiv) (yield 83%; mp 183−185 °C dec, lit.[9d,21] mp 185 °C). The acid was converted into 4-azidobenzoyl chloride by refluxing with $SOCl_2$ in THF followed by crystallization from *n*-hexane (yield 81%; mp 54−56 °C, lit.[22] mp 57−58 °C). To a solution of **4** (0.72 g, 2.33 mmol) in 10 mL of pyridine was added 4-azidobenzoyl chloride (0.51 g, 2.8 mmol). After 14 h the solution was evaporated and the residue dissolved in EtOAc. This solution was washed with 0.5 M citric acid, saturated $NaHCO_3$, and brine. The organic layer was dried ($MgSO_4$) and evaporated. Silica gel chromatography (CH_2Cl_2/EtOAc 97:3 v/v, R_f 0.28) provided **2** as a viscous yellow oil (0.86 g, 81%). ^{1}H NMR ($CDCl_3$): δ 2.88 (6 H, s, NMe_2), 2.97 (3 H, s, NMe), 3.65 (2 H, t, CH_2N), 4.45 (2 H, t, OCH_2), 7.02 (2 H, d, Ph), 7.92 (2 H, d, Ph), 7.13, 7.48 (2 H), 8.20, 8.28, and 8.52 (6 H, naphthyl). ^{13}C NMR ($CDCl_3$): δ 35.0 (NMe), 45.4 (NMe_2), 48.4 (CH_2N), 62.0 (OCH_2), 115.1, 119.3, 123.0, 128.1, 129.9, 130.0, 130.1, 130.6, 133.9, and 151.7 (10 C, naphthyl), 118.8 (2 C), 126.1, 131.5 (2 C), and 144.9 (6 C, Ph), 165.4 (COO). MS (EI): *m/z* 453 (M^+). Anal. Calcd for $C_{22}H_{23}N_5O_4S$: C, 58.27; H, 5.11; N, 15.44. Found: C, 57.99; H, 5.24; N, 15.11.

Photochemical Surface Modification. A piece (6 × 2 cm) of poly(ether urethane) foil (Pellethane 2363-55D, Dow Chemical Inc., thickness 0.13 mm) was ultrasonically cleaned with 2-propanol, water, and ethanol (2 min in each solvent) and dried in vacuo. A 5 mM solution of azide **2** in 2-propanol was sprayed onto the surface 10 times, drying with hot air in between. It is estimated that about 0.5 mL of the solution is deposited on the surface. This corresponds with 2.5 μmol on 5.6 cm^2 of the polymer surface or 450 nmol/cm^2 (0.2 mg/cm^2; an obvious excess). UV irradiation was performed at a distance of 40 cm for 20 min with a medium-pressure metal halide lamp emitting radiation mainly between 300 and 400 nm. The foil was washed and dried as before (the efficiency of the washing steps could easily be verified by observing the solvents under UV light, 365 nm). The surfaces are now yellow (green fluorescent when viewed under UV) but still transparent.

Fluorescence Measurements. A piece of the modified PEU surface ($2.8 \times 2.0 = 5.6$ cm^2) was treated with 2.5 mL of 0.1 M NaOMe/MeOH (14 h) in order to hydrolyze the ester bonds of the coupled fluorescent molecules. The supernatant liquid was isolated and diluted to 10.9 mL (calculated from the weight measured) and the fluorescence intensity determined with a SLM/Aminco SPF-500C spectrofluorometer. The excitation wavelength was set at 340 nm, and emission was measured at 530 nm. This was compared to a reference solution of **4** in MeOH of about the same concentration (5.0×10^{-6} M). At these low concentrations there is a linear relationship between the fluorescence intensity and the concentration. From this the concentration in the sample was determined (1.3×10^{-5} M). It follows that 25 nmol/cm^2 had been present at the PEU surface.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **2** and **4** (4 pages). Ordering information is given on any current masthead page.

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