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Dynamic Protein Adsorption onto Dendritic Polyglycerol Sulfate Self-Assembled Monolayers

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KEYWORDS

Vroman effect, anionic surface coatings, protein rearrangement, ionic interactions

Materials

All chemicals and solvents were purchased from Sigma Aldrich (Steinheim, Germany) and used without further purification unless stated otherwise. Glycidol (96%), epichlorohydrin (ECH, 99%), potassium phthalimide (99%), 2-ethyl-2-(hydroxymethyl)-1,3-propanediol (TMP, 98%), potassium *tert.*-butoxide (KO*t*Bu, 1 M in THF), N-methyl-2-pyrrolidinon (NMP, 99.5%), sulfamic acid (100%), DL-thioctic acid (TA, 98%), N,N-dimethylformamide (DMF, 99,8%), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCl, 98%) and CaH_2 (93%) were purchased from Acros Organics (Geel, Belgium). Glycidol was dried and distilled over CaH_2 and stored over 3 Å molecular sieve purchased from Carl Roth GmbH + Co. KG (Karlsruhe, Germany). Ethyl isothiocyanate (EtNCS, 97%) was purchased from Sigma Aldrich (Steinheim, Germany). Sodium sulfate (Na_2SO_4, 99%), sodium hydroxide (NaOH, 99%), sodium chloride (NaCl, 99.9%) and pre-wetted regenerated cellulose dialysis tubes (molecular weight cut-off (MWCO) 1000 g mol^{-1}, Spectra/Por® 6 Dialysis membrane) from SpectrumLabs were supplied by Carl Roth GmbH Co. KG (Karlsruhe, Germany). Diethyl ether (Et_2O) was supplied by VWR Chemicals (Fontenay-sous-Bois, France or Leuven, Belgium) and distilled before use to remove the stabilizer (BHT). Acetone and ethanol were distilled under reduced pressure before use to remove impurities. QCM-D measurements were performed with gold sensor chips from Biolin Scientific Holding AB (Stockholm, Sweden).

Methods

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 3 operating at 700 MHz or on a Jeol ECX at 400 and 500 MHz, respectively, and processed with the software MestReNova (version 7.1.2). Chemical shifts were reported in δ (ppm) and referenced to the respective deuterated solvent peak. Infrared (IR) measurements were conducted on a Jasco FT/IR-4100 LE (Jasco

GmbH, Groß-Umstadt, Germany) and processed with the software Spectra Manager 2. Mass spectral data were obtained on an Agilent 6210 ESI-TOF (Agilent Technologies, Santa Clara, CA, USA) spectrometer at flow rates of 4 mL min^{-1} and a spray voltage of 4 kV. Molecular weight distributions were determined by size exclusion chromatography (SEC) equipped with a refractive index detector (operated at 50 °C) providing the parameters M_n, M_p, M_w, and PDI. Measurements were performed under diluted conditions (10 mg mL^{-1}, injected volume 20 µL) using an Agilent 1100 solvent delivery system with an ISOpump, manual injector, and an Agilent 1100 differential refractometer. Three 30 cm columns in row (PSS SUPREMA, 5 µm particle size) were used at room temperature with a flow rate of 1.0 mL min^{-1} using an aqueous 0.1 M $NaNO_3$ solution as mobile phase. DIN certified Pullulan provided by PSS was used as polymeric standard. Samples with M_n = 342, 1080, 5500, 9200, 20 k, 100 k, 188.5 k, 358 k, and 636 kDa and PDI = 1.00−1.30 were used for regression. WinGPC Unity from PSS was used for data acquisition and interpretation. Static water contact angles were measured with an OCA contact angle system from DataPhysics Instruments GmbH (Filderstadt, Germany) and fitted with the software package SCA202 (version 3.12.11) using the sessile drop method. Contact angles were determined before and after surface functionalization. A drop of Milli-Q (MQ) water (2 µL) was placed onto the respective surface and contact angles were determined with an elliptical fitting model. For each substrate, contact angles were measured on at least three different spots to test for the homogeneity of the sample and independent substrates to test for reproducibility. The dry layer thickness of the polymer coatings was determined by multi-angle spectroscopic ellipsometry at incident angles of 50°, 60° and 70° with a SENpro spectroscopic ellipsometer from SENTECH Instruments GmbH (Berlin, Germany). The parameters of the gold layer before SAM formation was determined separately and respective average values of at least

three different spots on the surface were taken as fixed values for the subsequent modeling of the adsorbed dPGS and dPG layers. The layer thickness was measured at wavelengths from 370 nm to 1050 nm and was fitted using a model consisting of the previously measured gold layer with fixed parameters, a Cauchy layer with a fixed refractive index of n=1.5 and air as the surrounding medium. QCM-D measurements were performed on Q-Sense E1 system from Biolin Scientific Holding AB (Stockholm, Sweden) with a standard flow module and a Reglo Digital peristaltic pump from Ismatec (Wertheim, Germany). The software QSoft401 version 2.5.22 was used for data acquisition and QTools 3 version 3.1.25 from Biolin Scientific AB 2000-2014 (Stockholm, Sweden) was used for data analysis.

Synthesis of N-(2,3-epoxypropyl)phthalimide (1)

Potassium phthalimide (PPI, 130.0 g, 702 mmol, 1 eq.) was dried under high vacuum in a 1 L Schlenk flask. An excess of epichlorohydrin (ECH, 325.0 g, 3.51 mol, 5 eq.) was added under rapid stirring and the mixture was refluxed at 120 °C for 5 hours. After stirring at rt overnight, the precipitated potassium chloride was filtered off, washed with THF (100 mL) and residual ECH was removed under reduced pressure. The crude product was recrystallized in methanol (500 mL) and dried under high vacuum to yield a white crystalline powder (112.9 g, yield: 79.2%). ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) = 7.86 - 7.79 (m, 2 H, Ar), 7.74 - 7.68 (m, Ar, 2 H), 3.92 (dd, *J* = 14.4 Hz, 5.1 Hz, 1 H), 3.77 (dd, *J* = 14.4 Hz, 5.0 Hz, 1 H), 3.21 (ddd, *J* = 8.9 Hz, 5.0 Hz, 2.5 Hz, 1 H), 2.81 - 2.57 (m, 2 H); ^{13}C NMR (101 MHz, $CDCl_3$): δ(ppm): 168.1, 134.2, 132.0, 123.5, 49.1, 46.2, 39.7; ESI-MS: m/z = 204.2 $[M+H]^+$, 160.3 $[M - C_2H_3O]^+$, 133.2 $[M - C_3H_5O]^+$, 104.1 $[M - C_4H_5O]^+$, 76.0 $[M - C_5H_5NO_3]^+$.

Synthesis of dPGS-amine (5)

A flame dried batch reactor (3 L) was loaded with TMP (8.37 g, 62.4 mmol, 1 eq.). TMP was melted at under high vacuum at 60 °C until a colorless liquid was obtained. The reactor was flushed with Ar, KO*t*Bu (1 M in THF, 18.7 mL, 18.7 mmol) was added and the obtained potassium salt was immediately dissolved in dry NMP (31.2 mL). The temperature was raised to 120 °C and freshly distilled glycidol (280.0 mL, 312.0 g, 67.5 eq.) in dry THF (700 mL) was added under rapid stirring (stainless steel stirrer) over a period of 18 h using a precision dosing pump (54.44 mL h^{-1}). A small aliquot of dPG **2** was removed from the mixture for characterization. ^{1}H NMR (700 MHz, CD_3OD): δ(ppm) = 4.37-3.40 (dPG backbone), 0.91 (s, 3H, CH_3, TMP). Reference CD_3OD quintuplet at 3.3 ppm. ^{13}C NMR (176 MHz, CD_3OD): δ(ppm) = 81.5, 80.0, 73.9, 73.3-71.9 (m), 70.8, 64.4, 62.8. Reference CD_3OD septuplet at 49.15 ppm. Subsequently, N-(2,3-epoxypropyl)phthalimide (85.54 g, 421 mmol, 6.75 eq.) in a mixture of anhydrous NMP/THF (500 mL, v/v = 1) was added over two hours and the mixture was stirred overnight. Again, a small aliquot of dPG-phthalimide **3** was removed for characterization, phthalimide deprotection and functionalization with thioctic acid. ^{1}H NMR (700 MHz, CD_3OD): δ(ppm) = 8.25-7.61 (m, 4 H, phthalimide), 4.37-3.40 (m, dPG backbone), 0.91 (s, 3 H). Reference CD_3OD quintuplet at 3.31 ppm. ^{13}C NMR (176 MHz, CD_3OD): δ(ppm) = 169.9, 135.3, 133.4, 124.1 (phthalimide), 81.5, 80.0, 73.9, 73.3-71.93 (m, dPG backbone), 70.8, 64.37, 62.8. Reference CD_3OD septuplet at 49.15 ppm. The reaction mixture was diluted with 250 mL anhydrous NMP and cooled to 90 °C. The sulfating agent sulfamic acid (409 g, 4.21 mol, 67.5 eq.) was added in bulk and the mixture was stirred for another 2 days at 150 rpm. The mixture was diluted with 500 mL of distilled water and removed from the reactor. The polymer solution was precipitated in a solution of sodium ethylate in ethanol (955 g, 30 wt.-%, NaOEt/EtOH 1:10). The excess solvent was decanted, and the solid product powder was filtered and washed

excessively with diethyl ether to remove residual NMP. The solvent was evaporated *in vacuo* and the crude intermediate dPGS-phthalimide **4** was dissolved in H_2O and freeze-dried. The product was dissolved in water (1.5 L) and sodium borohydride (63.6 g, 1.68 mol, 4 eq.) was slowly added to the solution of dPGS-phthalimide at rt over a period of 30 min and the reaction was stirred for 2 d at 50 °C. Acetic acid (434 mL, 7.58 mon, 18 eq.) was added dropwise, the mixture was stirred for another 3 d at 80 °C and precipitated in a solution of sodium ethylate in ethanol (955 g, 30 wt.-%, NaOEt/EtOH 1:10). The excess solvent was decanted, and the solid product powder was filtered and washed excessively with diethyl ether. The solvent was evaporated *in vacuo* and the crude product subjected to ultrafiltration (MWCO 1000 g mol^{-1}) using a half-concentrated sodium chloride solution followed by water until no residual salt was observed in the filtrate. The polymer fraction was freeze-dried to yield the title compound as a colorless salt with a calculated molecular weight of M_n = 12,050 g mol^{-1} in 71% yield. ^{1}H NMR (700 MHz, D_2O): δ(ppm) = 8.25-7.61 (m, phthalimide), 4.89-4.65 ($C_{sec}H$-OSO_3K), 4.48-4.17 ($C_{prim}H_2$-OSO_3K), 4.15-3.30 (m, dPG backbone), 1.53 (s, 2 H, $C\underline{H_2}CH_3$, TMP), 1.00 (s, 3 H, $CH_2C\underline{H_3}$, TMP). Reference: D_2O singlet at 4.79 ppm. ^{13}C NMR (176 MHz, D_2O): δ(ppm) = 169.9, 135.3, 133.4, 124.1 (phthalimide), 78.5, 77.3, 76.1, 75.9, 73.8, 71.1, 70.3, 69.7, 69.5, 68.9, 68.7, 68.3, 67.6, 66.9, 41.2 ($C(CH_2)_4$, TMP), 22.0 ($\underline{C}H_2CH_3$, TMP), 7.0 ($CH_2\underline{C}H_3$, TMP). IR (bulk): ν_{max} = 3460, 2952, 2884, 1640, 1460, 1220, 1071, 1027, 1006, 931, 770 cm^{-1}. GPC: M_n = 11,300 g mol^{-1}, PDI = 1.63. Sulfur content from elemental analysis: 17.9% corresponding to a degree of sulfation of 90%.

Synthesis of dPGS- and dPG-thioctic acid (6)

To a solution of dPGS-amine **5** (1 g, 0.084 mmol) in water (5 mL) was added a solution of thioctic acid (78.4 mg, 0.380 mmol, 4.5 eq.) and EDCl (94.7 mg, 0.494 mmol, 5.9 eq.) in DMF

(15 mL). The solution was stirred at 50°C for 4 h and overnight at rt. The solvent was evaporated under reduced pressure, the raw product dissolved in water (10 mL) and the excess of thioctic acid was centrifuged of. The solution was decanted and dialyzed against 1 M aqueous NaCl for 1 d and water for 3 d by exchanging the solvent at least 3 times a day. Water was removed by lyophilizing and the product was obtained as a white crystalline salt (867.3 mg, yield: 86%, conversion: >10%). ^{1}H NMR (700 MHz, D_2O): δ(ppm) = 8.25-7.61 (m, phthalimide), 4.89-4.65 ($C_{sec}H$-OSO_3Na), 4.48-4.17 ($C_{prim}H_2$-OSO_3Na), 4.15-3.30 (m, dPG backbone), 2.74, 2.52, 2.32, 2.08, 1.77, 1.66, 1.26 (m, thioctic acid), 1.53 (s, 2 H, $C\underline{H_2}CH_3$, TMP), 1.00 (s, 3 H, $CH_2C\underline{H_3}$, TMP). Reference: D_2O singlet at 4.79 ppm. ^{13}C NMR (176 MHz, D_2O): δ(ppm) = 169.9, 135.3, 133.4, 124.1 (phthalimide), 78.5, 77.3, 76.1, 75.9, 73.8, 71.1, 70.3, 69.7, 69.5, 68.9, 68.7, 68.3, 67.6, 66.9, 41.2 ($C(CH_2)_4$, TMP), 22.0 ($\underline{C}H_2CH_3$, TMP), 7.0 ($CH_2\underline{C}H_3$, TMP).

Quenching of remaining amines in dPGS-thioctic acid (dPGS-TA) with ethyl isothiocyanate (7)

To a solution of dPGS-TA **6** (500 mg, 0.042 mmol) in PBS (pH = 8,5 mL) and DMF (15 mL) was added EtNCS (0.17 mL, 1.875 mmol, 4.5 eq.). The solution was stirred at rt overnight. The solvent was evaporated under reduced pressure, the raw product was dissolved in water (10 mL) and dialyzed against 1 M aqueous NaCl for 1 d and water for 3 d by exchanging the solvent at least 3 times a day. Water was removed by lyophilizing and the product was obtained as a white crystalline salt (402.9 mg, yield: 79%, conversion: >40%). ^{1}H NMR (700 MHz, D_2O): δ(ppm) = 8.25-7.61 (m, phthalimide), 4.89-4.65 ($C_{sec}H$-OSO_3Na), 4.48-4.17 ($C_{prim}H_2$-OSO_3Na), 4.15-3.30 (m, dPG backbone), 2.74, 2.52, 2.32, 2.08, 1.77, 1.66, 1.26 (m, thioctic acid), 1.53 (s, 2 H, $C\underline{H_2}CH_3$, TMP), 1.28, 1.18 (m, EtNCS), 1.00 (s, 3 H, $CH_2C\underline{H_3}$, TMP). Reference: D_2O singlet at 4.79 ppm. ^{13}C NMR (176 MHz, D_2O): δ(ppm) = 169.9, 135.3, 133.4, 124.1 (phthalimide), 78.5,

77.3, 76.1, 75.9, 73.8, 71.1, 70.3, 69.7, 69.5, 68.9, 68.7, 68.3, 67.6, 66.9, 41.2 ($C(CH_2)_4$, TMP), 22.0 ($\underline{C}H_2CH_3$, TMP), 7.0 ($CH_2\underline{C}H_3$, TMP).