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Synthesis and Characterization of α,ω-bi[2,4-dinitrophenyl (DNP)] poly(2-methoxystyrene) Functional Polymers. Initial Evaluation of the Interaction of the Functional Polymers with RBL Mast Cells

Biswajit Sannigrahi[a]; Dwaipayan Sil[b]; Barbara Baird[b]; Xiao-Qian Wang[c]; Ishrat M. Khan[a]
[a] Department of Chemistry, Clark Atlanta University, Atlanta, GA [b] Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY [c] Department of Physics, Clark Atlanta University, Atlanta, GA

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Synthesis and Characterization of α,ω-bi[2,4-dinitrophenyl (DNP)] poly(2-methoxystyrene) Functional Polymers. Initial Evaluation of the Interaction of the Functional Polymers with RBL Mast Cells

BISWAJIT SANNIGRAHI,[1] DWAIPAYAN SIL,[2] BARBARA BAIRD,[2] XIAO-QIAN WANG,[3] and ISHRAT M. KHAN[1]

[1]*Department of Chemistry, Clark Atlanta University, Atlanta, GA*
[2]*Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY*
[3]*Department of Physics, Clark Atlanta University, Atlanta, GA*

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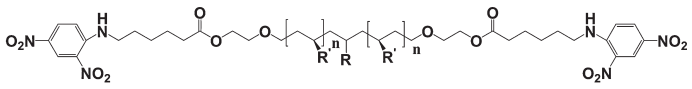
Two series of functional polymers, α,ω-bi[2,4-dinitrophenyl][poly(ethylene oxide)-b-poly(2-methoxystyrene)-b-poly(ethylene oxide)] (DNP-PEO-P2MS-PEO-DNP) and α,ω-bi[2,4-dinitrophenyl caproic][poly(ethylene oxide)-b-poly(2-methoxystyrene)-b-poly(ethylene oxide)] (CDNP-PEO-P2MS-PEO-CDNP), were synthesized by anionic living polymerization. The polymers were characterized by FT-IR, ^{1}H-NMR and Gel Permeation Chromatography (GPC). The molecular weight distributions for the lower molecular weight functional polymers were slightly broad (1.3–1.5). However, the molecular weight distributions for higher molecular weight polymers were narrower (1.1–1.2). Differential scanning calorimetry (DSC) studies showed thermal transitions indicative of the presence of microphases in the polymer solid state. The polymers were white powders and soluble in tetrahydrofuran. The binding affinity of DNP-PEO-P2MS-PEO-DNP ligands towards anti DNP IgE was determined by titrations with fluorescently labeled FITC-IgE. A water soluble CDNP-PEO-P2MS-PEO-CDNP/DMEG (dimethoxyethylene glycol) complex binds and achieves steady state binding with solution IgE within a few seconds. This strongly suggests that CDNP functional polymers with improved water solubility have potential in therapeutics. Higher molecular weight (water insoluble) CDNP-PEO-P2MS-PEO-CDNP polymers were electrosprayed as fibers (500 nm) on silicon surface. Fluorescence spectroscopy clearly showed that RBL mast cells were interacting with the fibers suggesting that the cell-surface receptors were clustered along the fiber surface. These observations suggest that the functional polymers hold promise for developing an antibody detection device.

Keywords: functional polymers; differential scanning calorimetry (DSC); gel permeation chromatography (GPC); fluorescence; binding assay; mast cell; electrospinning; biosensor

1 Introduction

An area of considerable interest is the design and synthesis of new functional polymers capable of selective interaction with biomarkers e.g. proteins. The goal of such studies is the eventual development of novel therapeutic agents and tools for biodiagnostics (1–3). In this communication, we report the synthesis, characterization and initial evaluation of the biofunctional properties of α,ω-bi[2,4-dinitrophenyl (DNP)] polymer systems of the general structure shown in Scheme 1. The reason we selected to work with DNP functionalized polymers is because 2,4-dinitrophenyl (DNP) conjugates of bovine serum albumin are commonly used as effective stimulators of anti-DNP IgE-FcεRI receptor complexes. However, the binding and crosslinking of these complexes via multivalent protein structures is complex in nature. Furthermore, the intrinsic heterogeneity of the protein conjugates renders these ligands inappropriate for studies aimed at discerning information about receptor spacing and orientation involved during cell activation (4–7). Small, synthetic ligands had been extensively used to study the various receptor mediated signaling pathways in several cellular systems such as the mast cells as well as in B and T cells (8, 9). Previous studies had demonstrated that structurally well-defined ligands can regulate the magnitude of IgE-FcεRI receptor mediated cellular responses in mast cells (10–12). In this context, bivalent DNP functionalized ligands with relatively short rigid spacers made with double stranded DNA (dsDNA) were previously used to investigate

Address correspondence to: Ishrat M. Khan, Department of Chemistry, Clark Atlanta University, Atlanta, GA 30314. E-mail: ikhan@cau.edu



R= phenyl and R'= 2-methoxyphenyl

Sch. 1. Structure of bivalent α,ω-bi[2,4-dinitrophenyl(DNP)]-poly(2-methoxystyrene).

the structural aspects of receptor spacing. Overall these ligands were mostly ineffective stimulators of cell activation, although the bivalent ligands with rigid spacer length of 4–5 nm were relatively more effective than the longer ones with similar structure (13). Earlier studies with a series of DNP functionalized poly(ethylene glycol), a hydrophilic polymer, of different molecular weights demonstrated that these could be very effective inhibitors of mast cell activation particularly those that could intramolecularly crosslink individual IgE-receptor complexes and block subsequent cellular responses (14). The present study was, therefore, undertaken to examine the possibility of utilizing DNP functionalized hydrophobic polymers as stimulators of mast cell activation. Poly(2-methoxystyrene) was selected as the hydrophobic (or very slightly soluble in aqueous solution) polymer because it has been demonstrated to be reasonably biocompatible i.e. supports the attachment and growth of HeLa Ovarian cancer cells (15). Water solubility of these hydrophobic systems may be improved by non-covalent conjugation with PEO (16).

The advantage of using a DNP functionalized water insoluble (hydrophobic) system raises the possibility of preparing fibers by electrospinning. Electrospun fibers decorated with DNP functional groups will permit studying mast cell-surface interaction and thus, the possibility of developing biosensors. Conventional electrospinning technique, which uses a syringe source, has been used for the rapid fabrication of randomly oriented fibers with diameters around 50–300 nm (17–20). Electrospun fibers have the potential for use in a wide range of applications such as high performance filters, drug delivery, scaffolds for tissue engineering and biosensors (21–24). In this communication, we also report the preparation of electrospun fibers using the DNP functionalized polymers. The fibers prepared were evaluated for their capacity to localize IgE-receptors on RBL mast cell surfaces.

2 Experimental

2.1 Materials

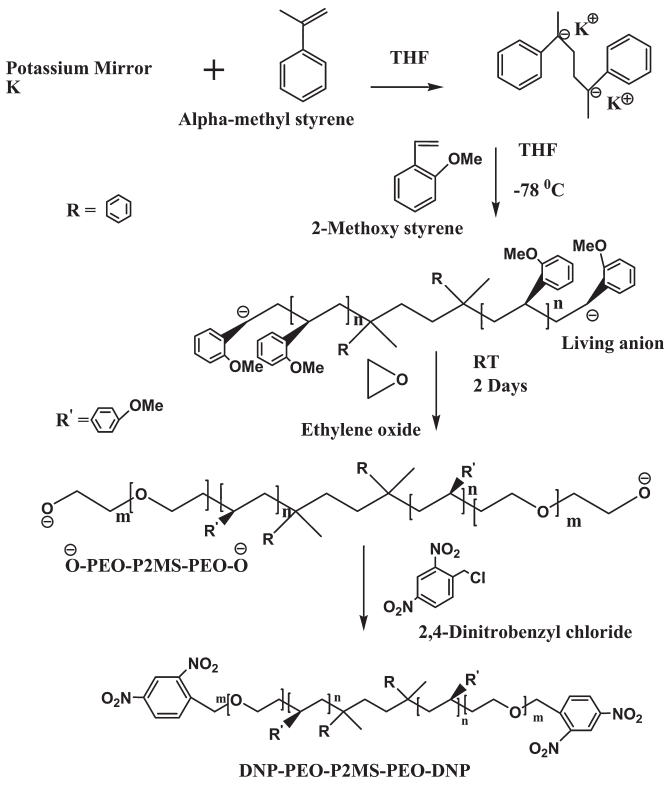
Sodium metal, potassium metal, calcium hydride, benzophenone, α-methylstyrene, 2-methoxystyrene (2-MS), ethylene oxide, 2,4-dinitrophenyl benzylchloride, N-2,4-DNP-∈-aminocaproic acid, DCC, DMAP were procured from Aldrich Chemical Company, USA. Monomers were dried over CaH_2 and distilled prior to polymerization, and other chemicals were used as received. Tetrahydrofuran (Fischer Scientific, USA) was purified by refluxing over fresh sodium benzophenone complex.

2.2 Preparation of Diinitiator

The dicarbanion initiator (α-methylstyrene dimer dianion, see structure in Scheme 2) used for the polymer synthesis was prepared by reacting potassium (5 g) mirror with α-methylstyrene (6 ml) in 200 ml THF. The experiment was conducted under high vacuum techniques. The solution turned red after shaking, indicating the formation of the diinitiator. The diinitiator was stored under high vacuum in ampoules equipped with breakseals.

2.3 Synthesis of α,ω-bi[2,4-dinitrophenyl][poly(ethylene oxide)-b-poly(2-methoxy styrene)-b-poly(ethylene oxide)], abbreviated as DNP-PEO-P2MS-PEO-DNP, (Scheme 2)

Polymerizations were carried out in all-glass, sealed reactors using standard high vacuum techniques. The reactors were cleaned and annealed; the reactor was equipped with monomers (2-methoxystyrene and ethylene oxide) and initiator ampoules (with breakseals). The reactor was attached to the vacuum line, and 10 ml THF was distilled into the flask. The reactor was next detached from the vacuum line by heat-sealing with a hand torch and transferred to a



Sch. 2. Synthesis of α,ω-bi[2,4-dinitrophenyl][poly(ethylene oxide)-b-poly(2-methoxystyrene)-b-poly(ethylene oxide)], DNP-PEO-P2MS-PEO-DNP.

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cold bath maintained at −78°C. First, 4 ml (0.27 mmol/ml) initiator was added by breaking the breakseal of the initiator ampoule. This was followed by the introduction of 2-methoxystyrene (1.2 ml). The solution turned deep red, indicating the formation of poly(2-methoxystyrene) potassium anion. The reaction was allowed to proceed for 40 min. and was followed by the addition of ethylene oxide (1 ml). After the mixture was stirred for a few minutes the color disappeared completely indicating the initiation of second monomer. Polymerization of second monomer was continued for two days at room temperature. Finally, the polymerization was deactivated by adding 2,4-dinitrophenylbenzylchloride to obtain the α,ω-bi[2,4-dinitrophenyl][poly(ethylene oxide)-b-poly(2-methoxystyrene)-b-poly(ethylene oxide)], (DNP-PEO-P2MS-PEO-DNP). The functional polymers were purified by precipitation into hexanes. Yields were >90%.

2.4 Preparation of α,ω-bi[2,4-dinitrophenyl caproic][poly(ethylene oxide)-b-poly(2-methoxystyrene)-b-poly(ethylene oxide)], abbreviated as CDNP-PEO-P2MS-PEO-CDNP, (Scheme 3)

The dihydroxyl terminated polymer was prepared by rapidly deactivating the living polymers with HCl (6 M)/methanol (1/20, vol/vol). The dihydroxyl polymers were purified by precipitation into hexanes. The hydroxyl functionalized polymers were reacted with N-2,4-DNP-∈-amino caproic acid. To a three-necked flask, 0.5 g, 0.03 mmol of the dihydroxyl terminated polymer ($\bar{M}_n = 13.9$), 0.043 g, 0.15 mmol of N-2,4-DNP-∈-amino caproic acid, 0.018 g, (0.09 mmol) DCC and 0.005 mmol DMAP were added and dried on a vacuum line for 4 h. Next dry dichloromethane (15 ml) was distilled into the flask. The vacuum was released under nitrogen, and the reaction was stirred for 12 h. Reaction mixture was filtered and pure polymer was recovered by precipitating twice into hexanes and methanol.

2.5 Equilibrium Binding of Ligands to FITC-modified IgE

Equilibrium binding experiments with FITC-modified IgE were conducted using a reported procedure (25). The fluorescence measurements were made on a SLM 8000 fluorimeter in time based acquisition mode. Excitation and emission wavelengths were 490 nm and 520 nm, respectively. Equilibrium titrations with soluble FITC-IgE were conducted at room temperature (25°C) with continuous stirring in the cuvette. The experiments were carried out in BSS buffer (20.0 nM HEPES pH 7.4, 135 mM NaCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 5.6 mM glucose) containing 1 mg/ml of 5% gelatin.

2.6 Electrospinning of Polymers

Polymers were electrospun on to silicon substrates using the method described previously (21). Polymers were dissolved in chlorobenzene at a concentration of 30% by weight along with polystyrene ($\bar{M}_w = 100{,}000$) at a concentration of 25% by weight. The solution was added to a silicon tip and a voltage of about 10 kV was applied to the tip. The polymeric solution releases as it jets towards the ground electrode and is deposited as fibers on silicon substrate attached to the ground electrode.

2.7 Confocal Microscopic Studies with Nanofibers

The confocal system used for the study was a Bio-Rad MRC-1024 equipped with an Argon-Krypton laser and attached to Olympus IX70 inverted microscope. The excitation wavelength was 488 nm, and the emission wavelength was 520 nm. Silicon substrates containing fibers were incubated with Alexa488-IgE in BSS for about 20 min, washed with BSS buffer and visualized under the microscope.

To study fiber interaction with cells, RBL-2H3 cells were sensitized with excess Alexa488-IgE for about 1 h, washed and resuspended in BSS containing 1 mg/ml of BSA. The cells were incubated with the silicon substrate containing polymeric fibers for about 10–15 min, after which they were further washed and observed under the microscope.

2.8 Analysis

Size exclusion chromatography (SEC) was performed using a Perkin-Elmer Binary LC pump 250, a 2792 injector and a Perkin-Elmer LC-30 RI detector. Three columns, HR3, HR 4E, HR 5E, were used in conjunction with a 2 μm pre-column filter. The columns were housed in an oven maintained at 30°C. Tetrahydrofuran was used as the eluent at a flow rate of 1 ml/min. Molecular weights were calculated relative to polystyrene (Aldrich) molecular weight standards.

2.9 Measurements

^{1}H-NMR spectra were obtained using a Bruker ARX 400 NMR spectrometer in $CDCl_3$ and THF-d_8. Trimethylsiloxane was used as an internal standard. FTIR spectra were recorded on a Nicolet 510P FT-IR spectrometer with an accuracy band of $\pm 2\ cm^{-1}$. Differential scanning calorimetry was performed on a Seiko DSC220 at a heating rate of 5°C per minute, and the reported values were obtained from the second heating after quench cooling the sample. The T_g's were taken at the midpoints of the heat capacity changes, the T_m's were taken at the maximum of the enthalpy endothermic peaks. The DSC was calibrated for temperature and enthalpy using an Indium standard under nitrogen gas atmosphere.

3 Results and Discussion

3.1 Synthesis of the Functional Polymers, DNP-PEO-P2MS-PEO-DNP and CDNP-PEO-P2MS-PEO-CDNP

Two slightly structurally different series of functional polymers, α,ω-bi[2,4-dinitrophenyl][poly(ethylene oxide)-b-

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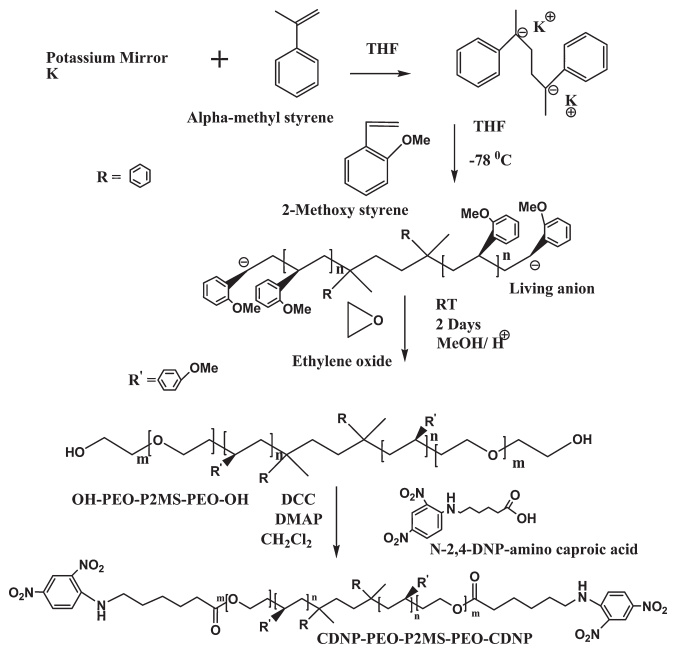
poly(2-methoxystyrene)-b-poly(ethylene oxide)] (DNP-PEO-P2MS-PEO-DNP) and α,ω-bi[2,4-dinitrophenyl caproic][poly(ethylene oxide)-b-poly(2-methoxystyrene)-b-poly(ethylene oxide)] (CDNP-PEO-P2MS-PEO-CDNP), were prepared by anionic living polymerization. The synthetic methods are shown in Schemes 2 and 3. All polymerizations were carried out using high vacuum break seal techniques (26). The difunctional initiator was prepared by electron transfer reaction of α-methylstyrene and potassium (mirror) metal (27). As shown in Scheme 2, the 2-methoxystyrene monomer was added first, followed by the addition of the second monomer, ethylene oxide, and finally the living polymer was terminated by 2,4-dinitrophenylbenzylchloride. This resulted in the preparation of α,ω-bi[2,4-dinitrophenyl][poly(ethylene oxide)-b-poly(2-methoxystyrene)-b-poly(ethylene oxide)] (DNP-PEO-P2MS-PEO-DNP). It should be noted that the polymerization of the first monomer, 2-methoxystyrene, was carried out for 40 min at −78°C; whereas, the second monomer, ethylene oxide, was polymerized at room temperature for 2 days. The functional polymers were obtained in yields of 90% or higher. The polymers were white powders. Table 1 list the composition and molecular weight of polymers prepared by Scheme 2.

The α,ω-bi[2,4-dinitrophenyl caproic][poly(ethylene oxide)-b-poly(2-methoxystyrene)-b-poly(ethylene oxide)] (CDNP-PEO-P2MS-PEO-CDNP) was prepared as shown in Scheme 3. In this approach, the α,ω-dialoxide living polymer was terminated with methanol to produce the α,ω-dihydroxyl polymer [HO-PEO-P2MS-PEO-OH]. Reaction of the α,ω-dihydroxyl polymer with N-2,4-DNP-∈-amino caproic acid, by DCC coupling, results in the formation of α,ω-bi[2,4-dinitrophenyl caproic][poly(ethylene oxide)-b-poly(2-methoxystyrene)-b-poly(ethylene oxide)] (CDNP-PEO-P2MS-PEO-CDNP). The structural difference between DNP-PEO-P2MS-PEO-DNP series and the CDNP-PEO-P2MS-PEO-CDNP series is caproate together with a C-6 spacer preceding the DNP groups in the second series of polymers. The CDNP-PEO-P2MS-PEO-CDNP functional polymers are listed in Table 2. The polymers were white powders and were recovered by precipitation into hexanes.



Sch. 3. Synthesis of α,ω-bi[2,4-dinitrophenyl caproic][poly(ethylene oxide)-b-poly(2-methoxystyrene)-b-poly(ethylene oxide)], CDNP-PEO-P2MS-PEO-CDNP.

Table 1. Composition and molecular weights of α,ω-bi[2,4-dinitrophenyl][poly(ethylene oxide)-b-poly(2-methoxystyrene)-b-poly(ethylene oxide)], DNP-PEO-P2MS-PEO-DNP

Sample Number	[a]M_1/M_2 (^{1}H-NMR)	$\bar{M}_n$ (GPC)	$\bar{M}_w/\bar{M}_n$ (GPC)
1	98/02	2.5 k	1.3
2	63/37	6.3 k	1.5
3	7/93	6.4 k	1.3
4	11/89	7.2 k	1.5
5	14/86	38 k	1.2
6	19/81	42.4 k	1.2

[a]Weight fraction of monomers 2-methoxystyrene (M_1) to ethyleneoxide (M_2).

3.2 Characterization of DNP-PEO-P2MS-PEO-DNP and CDNP-PEO-P2MS-PEO-CDNP Polymers

The DNP functional groups in the polymers were observed by FT-IR spectroscopy. Figure 1 shows the FT-IR spectrum of DNP-PEO-P2MS-PEO-DNP # 2 listed in Table 1. Aromatic NO_2 shows symmetric and asymmetric stretching. These absorptions have been identified for low molecular weight polymers. For example, the asymmetric and symmetric stretching for -NO_2 observed at 1536 cm^{-1} and 1351 cm^{-1}, respectively.

^{1}H-NMR also confirms the presence of DNP functional groups in the polymer chain ends, ^{1}H-NMR spectrum of DNP-PEO-P2MS-PEO-DNP #2 is shown in Figure 2. Peaks at 7.37 (a), 7.87 (b) and 8.28 (c) are attributed to aromatic protons in DNP units, respectively. The extent of

Table 2. Composition and molecular weights of α,ω-bi [2,4-dinitrophenyl caproic][poly(ethylene oxide)-b-poly(2-methoxystyrene)-b-poly(ethylene oxide)], CDNP-PEO-P2MS-PEO-CDNP

Sample number	[a]M_1/M_2 (^{1}H NMR)	$\bar{M}_n$ (GPC)	$\bar{M}_w/\bar{M}_n$ (GPC)
1	11/89	29 k	1.3
2	22/78	23.4 k	1.3
3	42/58	13.9 k	1.2
4	19/81	50.4 k	1.1

[a]Weight fraction of monomers 2-methoxystyrene (M_1) to ethyleneoxide (M_2).

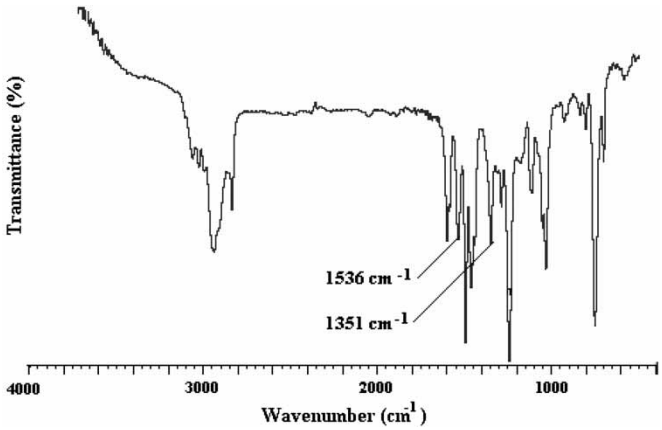


Fig. 1. FT-IR spectrum of DNP-PEO-P2MS-PEO-DNP # 2, Table 1.

functionalization has been determined for the lower (<8K) molecular weight polymers by ^{1}H-NMR and the degree of functionalization was found to be greater than 90%.

Figure 3 shows the IR spectra of (a) HO-PEO-P2MS-PEO-OH, precursor to CDNP-PEO-P2MS-PEO-CDNP # 3 and (b) CDNP-PEO-P2MS-PEO-CDNP # 3, Table 2. The dihydroxyl functional polymers show a broad absorption around 3500 cm^{-1} which is indicative of presence of the hydroxyl functional groups. After coupling with N-2,4-DNP-ε-amino-n-caproic acid, the disappearance of hydroxyl group was observed. Complete disappearance of hydroxyl group is indicative of quantitative functionalization.

The molecular weights of all polymers were determined by GPC relative to polystyrene standards. Polymers were soluble in tetrahydrofuran and thus molecular weights were determined using THF as eluent. The molecular weight distributions for the lower molecular weight polymers were slightly broad (1.3−1.5). However, the molecular weight distributions for higher molecular weight polymers were narrow (1.1−1.2). GPC of higher molecular weight polymers produced sharp peaks. The GPC chromatograms of CDNP-PEO-P2MS-PEO-CDNP # 3 and # 4 are shown in Figure 4.

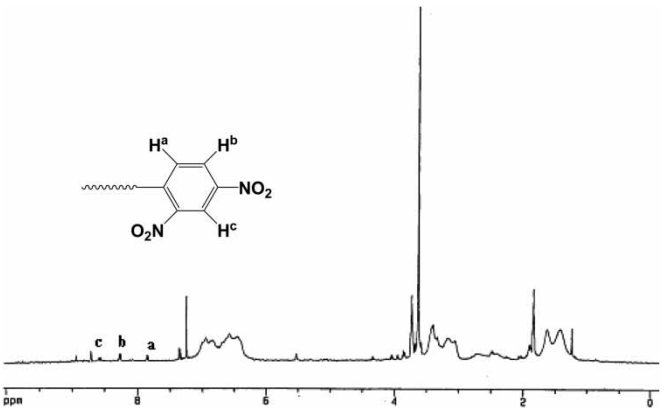


Fig. 2. ^{1}H-NMR spectrumDNP-PEO-P2MS-PEO-DNP #2, Table 1.

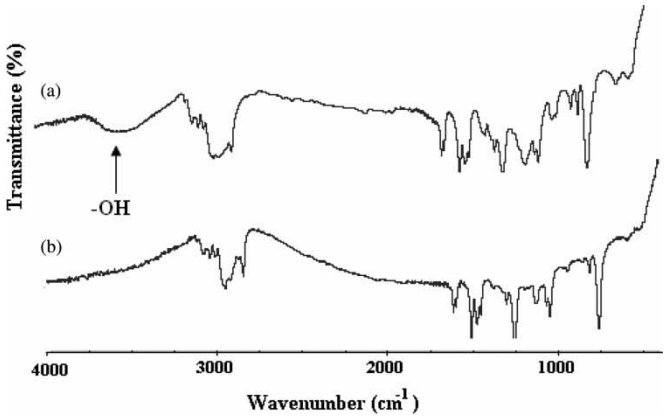


Fig. 3. FT-IR spectra of (a) OH-PEO-P2MS-PEO-OH, precursor to CDNP-PEO-P2MS-PEO-CDNP # 3, Table 2 and (b) CDNP-PEO-P2MS-PEO-CDNP # 3, Table 2.

The sharp functional block copolymer peak is always associated with a small peak that can be associated with the central poly(2-methoxystyrene) block. This suggests that a small fraction (∼2−4%) of the poly(2-methoxystyrene) potassium living polymer does not initiate the copolymerization and is terminated by residual impurities in the ethylene oxide monomer. Because the unfunctionalized polymer content is a small fraction of the overall functional polymer, this is not expected to make a difference in the polymer-antibody interaction studies.

To determine the morphology of the functional polymers, differential scanning calorimetry (DSC) studies were carried out over the temperature range of -50 to 200°C. Figure 5 shows the DSC thermograms of CDNP-PEO-P2MS-PEO-CDNP # 3, CDNP-PEO-P2MS-PEO-CDNP # 4 and the precursor hydroxyl functional polymer for CDNP-PEO-P2MS-PEO-CDNP # 3. The lower molecular weight CDNP-PEO-P2MS-PEO-CDNP # 3 polymer showed a glass

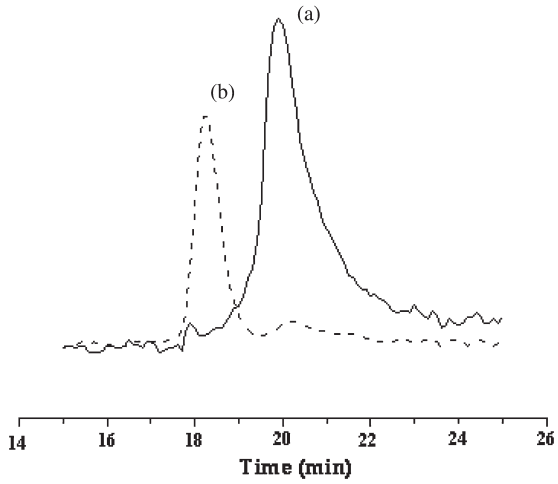


Fig. 4. (a) GPC chromatogram of CDNP-PEO-P2MS-PEO-CDNP # 3, Table 2 (b) GPC chromatogram of CDNP-PEO-P2MS-PEO-CDNP # 4, Table 2.

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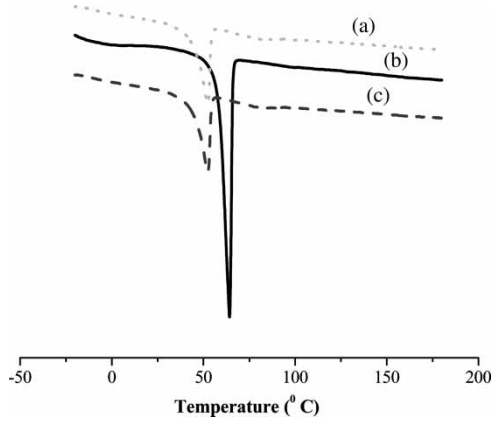

Fig. 5 (a) DSC thermogram of OH-PEO-P2MS-PEO-OH, precursor to CDNP-PEO-P2MS-PEO-CDNP # 3, Table 2, (b) DSC thermogram of CDNP-PEO-P2MS-PEO-CDNP # 4, Table 2 and (c) DSC thermogram of CDNP-PEO-P2MS-PEO-CDNP # 3, Table 2.

transition temperature (T_g) at 100°C and a melting temperature at 60°C for PEO; whereas, higher molecular weight polymer CDNP-PEO-P2MS-PEO-CDNP # 4 was characterized with only PEO melting at 67°C. This indicates that the ethylene oxide microphase in the higher molecular weight polymers are more crystalline than the lower molecular weight polymer. Furthermore, in the lower molecular weight polymers, where the segments lengths are short, mixing between the two components results in the formation of an interphase of the two components displaying a T_g at 100°C. Homopolymers of poly(2-methoxystyrene) display a melting temperature between 275–295°C, but displays no glass transition temperature (28). The DSC thermograms did not show any glass transition temperature (around −60°C) that may be attributed to the formation of PEG amorphous phase. The presence of DNP group at chain end did not make any difference in the melting temperature of the PEO phase, the CDNP-PEO-P2MS-PEO-CDNP # 3 and its precursor hydroxyl functional polymers show similar thermal behavior by DSC (Figure 5). The DSC studies suggest that in the solid state the higher molecular weight polymers were present in a microphase separated system with a PEO microphase and a poly(2-methoxystyrene) microphase. The lower molecular weight polymers contain a PEO microphase (mostly crystalline) and a mixed phase composed of the two (PEO and P2MS) components.

3.3 Evaluation of Interaction of the Functional Polymers with anti DNP-IgE

Two series of divalent ligands or functional polymers, DNP-PEO-P2MS-PEO-DNP and CDNP-PEO-P2MS-PEO-CDNP have been prepared. To understand the relationship between the structural constraints of the divalent ligands, initial biofunctional properties were determined. Our motivation here is to develop a correlation between the polymers structure with its effectiveness for interaction with anti DNP-IgE.

3.4 Equilibrium Binding Studies by DNP-PEO-P2MS-PEO-DNP Ligands

The initial study in this project involved the two polymers, DNP-PEO-P2MS-PEO-DNP # 1 (2.8 K), Table 1 and DNP-PEO-P2MS-PEO-DNP # 2 (6.3 K), Table 1. However, studies showed that the insolubility of these two polymers in aqueous medium limit their applications in cellular environments. Hence, the two polymers, DNP-PEO-P2MS-PEO-DNP # 3 (6.4 K), Table 1 and DNP-PEO-P2MS-PEO-DNP # 4 (7.2 K), Table 1 that are partially soluble in water and other aqueous buffer were selected for binding studies. It is clear that increasing ethylene oxide content improves the water solubility of the polymers. To test the binding affinity of these ligands towards anti DNP IgE, titrations with fluorescently labeled FITC-IgE in solution and bound to receptors on cell surfaces were performed. Fluorescence quenching due to binding was observed as a function of ligand concentration. The study suggested that the binding affinity for IgE in the case of both the ligands was low, differing by about two orders of magnitude as compared to a standard ligand of high affinity such as dinitrophenyl caproic tyrosine (DCT). Studies were conducted to determine if these polymers stimulate the RBL mast cell, and quantifying stimulated of cellular degranulation (measured as the release of an enzyme β-hexaminadase). No stimulated degranulation was detected, even at μM concentration as compared to multivalent antigen such as DNP-BSA. We also tested these polymers for their capacity to compete with DNP-BSA and thereby inhibit degranulation. Inhibition assays with these ligands were also negative. The reason that these ligands do not associate effectively with anti-DNP IgE may be because of the absence of a flexible linker at the end of polymer chain that connects the DNP group. Previous studies showed that a short hydrocarbon chain segment as a spacer enable non-covalent interactions between the DNP groups and the binding pockets of the anti-DNP IgE (6). In this context, the CDNP-PEO-P2MS-PEO-CDNP which does include a six carbon chain as flexible linker can serve as more effective ligands. The caproate group has also been shown previously to increase affinity to anti-DNP IgE (29).

3.5 IgE Binding Studies by CDNP-PEO-P2MS-PEO-CDNP Series Ligands

Structurally the CDNP-PEO-P2MS-PEO-CDNP polymers and the DNP-PEO-P2MS-PEO-DNP polymers are different because of the presence of the C6 linker and a caproate group in the CDNP-PEO-P2MS-PEO-CDNP functional polymers. The CDNP-PEO-P2MS-PEO-CDNP also had limited solubility in water and the water solubility can be improved through complexation with dimethoxypoly(ethylene glycol), DMEG, of molecular weight 550 (16). The functional CDNP-PEO-P2MS-PEO-CDNP # 3 (13.9 K), Table 2 was incubated with DMEG-550 in the weight ratio of 1:20 to form a complex (CDNP-PEO-P2MS-PEO-CDNP:DMEG).

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To evaluate the affinity of the DNP based ligands to anti-DNP IgE, a series of equilibrium titrations were performed with IgE in solution. The experimental equilibrium binding data were fitted to a simplified bivalent binding model as described previously by Erickson et al. (25) to obtain an apparent equilibrium dissociation constant, $K_d = 33\ \mu M$ (Figure 6). By comparison, the bivalent ligands having dsDNA as rigid spacers, showed an apparent affinity in the range between 12–16 nM. The apparent affinity of the polymers towards the antibody is thus found to be relatively low by a few orders of magnitude. This may be because the CDNP-PEO-P2MS-PEO-CDNP:DMEG water soluble complex is rather bulky (high DMEG content), and possibly the steric hindrance between the complex and the IgE is a significant problem to obtaining effective binding. To improve its apparent binding affinity in the cellular environments, our results suggest that the water solubility of the CDNP-PEO-P2MS-PEO-CDNP functional polymers should be improved by modifications that do not present steric problems. We are currently investigating new synthetic strategies to prepare water soluble ligands, and although these functionalized polymers have not yet been optimized but preliminary studies do show the promise of the water soluble CDNP-PEO-P2MS-PEO-CDNP polymers for interacting specifically with antibodies.

3.6 Electrospun Fiber Fabrication of CDNP-PEO-P2MS-PEO-CDNP Polymers

We also studied higher molecular weight (water insoluble) CDNP-PEO-P2MS-PEO-CDNP polymers to investigate cell-surface interaction and to develop biosensors for diagnostics purpose. Towards this goal, CDNP-PEO-P2MS-PEO-CDNP # 4 (50.4 K), Table 2 were electrosprayed as fibers on silicon surface using a technique as previously described (21). It is hypothesized that the terminal DNP groups will be spatially localized at the edge of the fiber surface and will be therefore accessible to the DNP specific antibody. Recent studies had demonstrated that DNP modified micron scale patterned lipid surfaces can provide information about micron scale cell-surface interaction (30–32). The described technique therefore presents an opportunity to understand the surface interactions at a nanometer level. The fibers deposited so far had a diameter of ~500 nm as determined by AFM studies. To investigate the binding of these fiber based antigen, they were incubated with the fluorescently labeled Alexa 488-IgE in BSS solution for 20 minutes. Confocal images obtained showed that the IgE binds with the DNP groups on the fiber surface (Figure 7).

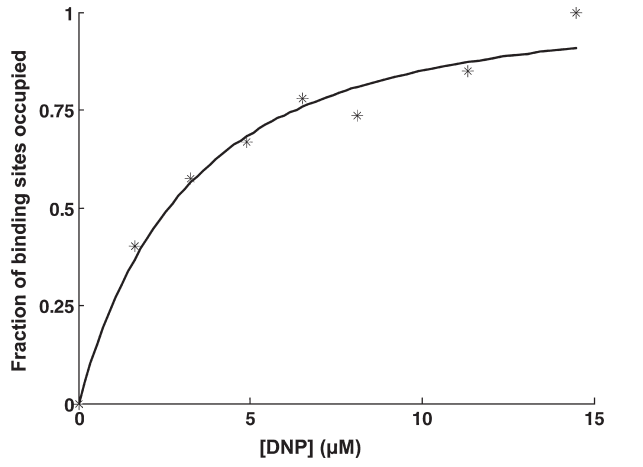

Fig. 6. Equilibrium binding of CDNP-PEO-P2MS-PEO-CDNP: DMEG complex with IgE in solution. The equilibrium binding data was fitted with a simplified binding model.

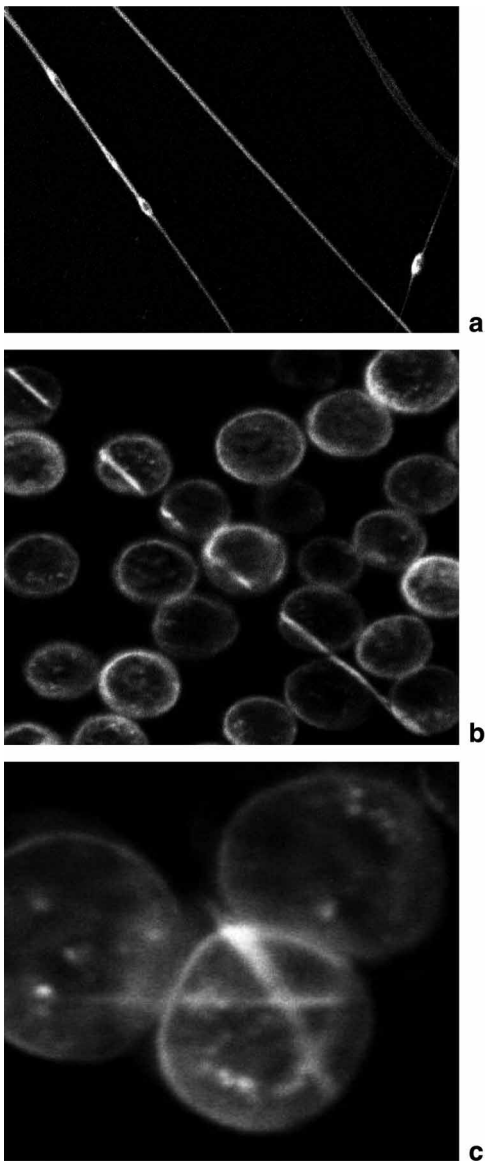

Fig. 7. Confocal images of polymeric fibers (a) incubated with Alexa 488-Ige in BSS solution; (b–c) localized clustering of the IgE-receptors on the RBL mastcell surface.

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In order to study polymer interaction on cells, RBL mast cells were sensitized with the Alexa-488 IgE. They were further incubated with the polymeric fibers for about 10–15 min and viewed under confocal microscope. Fluorescence micrograph images demonstrated that the cell-surface receptors were clustered along the fiber surfaces upon antigen-antibody binding. Unbound IgE-receptor complexes are able to diffuse freely, and they aggregate complexes upon binding with the DNP groups present on the fiber surfaces (Figure 7b, 7c). These functionalized fibers may provide an effective way of developing an antibody detection technique.

4 Conclusions

A synthetic method, based on anionic living polymerization, was developed to prepare functional polymers, α,ω-bi[2,4-dinitrophenyl caproic][poly(ethylene oxide)-b-poly(2-methoxystyrene)-b-poly(ethylene oxide)] (CDNP-PEO-P2MS-PEO-CDNP), capable of interaction with anti-DNP IgE receptors on RBL mast cells. In the solid state the functional polymers are present in a microphase separated system, and the microphase composition is dependent upon the molecular weight of the polymers. The binding affinity for these polymers at this stage of development is in the range of 33 μM. We expect that further improvement in the water solubility of these functional polymers will lead to candidates for possible therapeutic applications. Water insolubility; however, permit the preparation and evaluation of fibers for biosensors. Electrospun fibers of the higher molecular weight α,ω-bi[2,4-dinitrophenyl caproic][poly(ethylene oxide)-b-poly(2-methoxystyrene)-b-poly(ethylene oxide)] are effective in binding IgE and clustering IgE receptors on RBL mast cells. These results suggest that the functional polymers are promising materials for developing tools for biodiagnostics.

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