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A Polymer Scaffold for Protein Oligomerization

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Protein clustering is critical for information transfer in biology. For example, it is implicated in growth factor-mediated cell signaling[1,2] and integrin-facilitated cell adhesion.[3] Few approaches exist, however, for testing the consequences of protein clustering. Here we report the design, synthesis, and biological activity of a well-defined polymer for the oligomerization of proteins. The reported scaffold uses the interaction between a Ni^{2+} complex and a sequence of histidine residues (i.e., a "His tag").[4] We present a general method for the oligomerization of a protein bearing a His tag.

Our studies build on observations that saccharide-functionalized polymers can oligomerize concanavalin A.[5,6] Given the importance of protein oligomerization in biology, we sought a general, modular method to assemble multiple copies of a protein. Proteins that possess a His tag can readily be produced, and the Ni^{2+} complex−His tag interaction has been widely applied.[7−11] We envisioned that a polymer possessing multiple copies of a Ni^{2+} complex could be used to oligomerize proteins.

Our polymer targets were designed to possess several key characteristics. Specifically, we sought to generate polymers of various lengths and narrow polydispersity indices (PDIs). Such compounds would allow examination of how the extent of protein oligomerization influences a response. Second, we wanted a means to determine the relative loading of metal chelator on the polymer backbone. Atom-transfer radical polymerization (ATRP) can be used to generate modifiable scaffolds (e.g., **1**) of various lengths (Figure 1).[12] Moreover, ATRP can afford materials with narrow PDIs.[13] Finally, polymers bearing a unique end group can be generated. Thus, we investigated the suitability of ATRP for generating polymers capable of assembling proteins.

To append a moiety that would bind to proteins bearing an oligohistidine tag, we synthesized nitriloacetic acid (NTA) derivative **2** as the Ni^{2+}-chelating group (Figure 1). Benzyl protecting groups on the NTA derivative provide a resolved NMR signal for the determination of relative coupling efficiencies. A diglycine linker was added to the NTA-containing nucleophile to facilitate efficient coupling of the metal chelating unit to the polymer backbone.

To generate the target polymers, compound **1** was end-functionalized using TBSCl.[14] The protected NTA derivative was conjugated to the backbone under standard conditions. To vary the "density" of chelator groups, either 0.75 or 2.0 equiv of **2** were added to the coupling reaction. Excess ethanolamine was added to quench any remaining reactive sites (Figure 2). The ester protecting groups were removed via hydrolysis. Ni^{2+} was added under basic conditions, and the polymers were subjected to extensive dialysis to afford the final products, **4a** and **4b**.

The ability of multivalent displays to cluster a protein of interest, fibroblast growth factor 8b (FGF-8b), was assessed. FGFs are

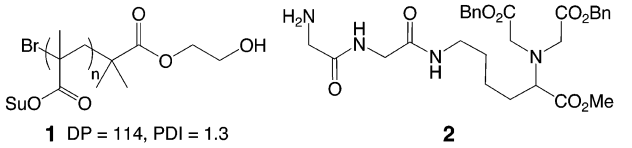

Figure 1. (Left) Succinimide ester-substituted polymer generated by ATRP has a degree of polymerization (DP) of 114 and a polydispersity index (PDI) of 1.3. Su is defined as succinimide. (Right) NTA derivative possessing an amino group and diglycine linker that was employed in the conjugation reaction.

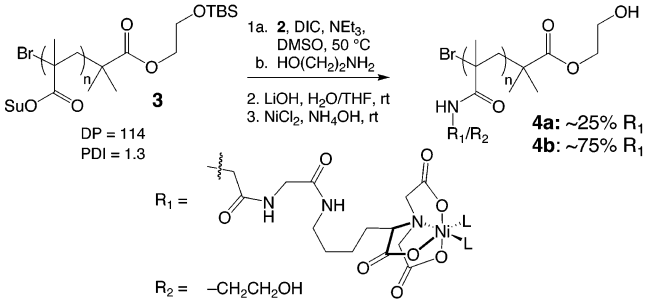

Figure 2. Scheme for synthesis of polymers presenting Ni^{2+}-chelating groups. L = water or His. DIC = diisopropylcarbodiimide. R_1/R_2 represents a random distribution of R_1 and R_2.

critical for many signaling processes including morphogenesis and tumor angiogenesis.[1,2,15] The oligosaccharide heparan sulfate chains of proteoglycans (or the polysaccharide heparin) are critical,[16] as they can oligomerize FGFs and present multiple copies of them to FGF receptors (FGFRs).[17,18] We sought to determine whether our synthetic polymer would activate FGF-mediated signaling.

We employed an FGF-8b fusion protein that possesses a His tag. To determine whether multivalent **4a** can assemble multiple copies of FGF-8b, we carried out chemical cross-linking studies on a mixture of polymer **4a** and His-tagged FGF-8b.[14] The amount of FGF-8b dimer increases with increasing concentrations of polymer **4a** until a maximum value is reached. At higher concentrations of polymer **4a**, the amount of dimer decreases because each polymer binds to fewer copies of FGF-8b. These data indicate that functionalized polymers generated by ATRP can be used to cluster a target protein.

We next tested whether such polymers could promote FGF-8b signaling using a cell proliferation assay. A heparan sulfate-deficient cell line (BaF3 cells) transfected with a single FGF receptor (FGFR4) was employed. In the absence of FGF-8b and a sulfated polysaccharide such as heparin, these cells undergo apoptosis. They can be rescued by treatment with soluble heparin and FGF-8b, but both components are required. We hypothesized that if our polymer could oligomerize FGF-8b, it might promote cell proliferation in the absence of heparin. Cells treated with the polymer alone did not proliferate. When BaF3 cells were treated with polymer **4a** and the FGF-8b fusion protein, however, they proliferated (Figure 3). Similar results were obtained with polymer **4b**. This proliferation

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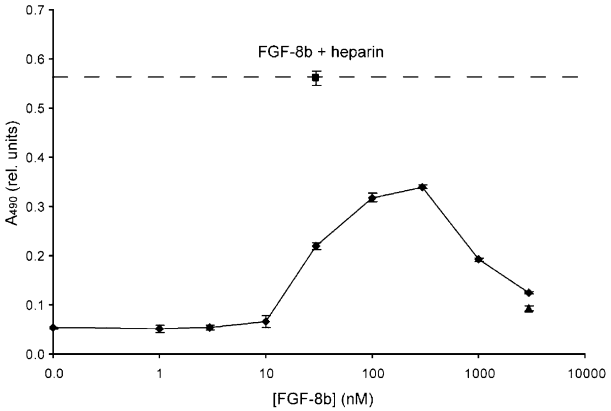

Figure 3. Proliferation of BaF3 cells. Proliferation was measured using a modified MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Cells were incubated at 37 °C for 48−72 h after treatment as follows: 30 nM polymer **4a** and FGF-8b (◆); 3.0 μM FGF-8b alone (▲). The value obtained for FGF-8b (30 nM) and heparin (100 nM) is also indicated (■). The errors represent the standard deviation of samples performed in quadruplicate. Experiments performed on 3 separate days gave similar results; the data shown are from experiments carried out on a single day.

was dependent on the presence of FGF-8b and the polymer (Figure 3), as cells treated with FGF-8b alone or polymer alone failed to proliferate to any significant extent.

We hypothesized that multiple copies of FGF-8b could assemble on the polymer and that it is the oligomerized FGF-8b that promotes proliferation. If such a model is correct, the extent of proliferation should depend on the relative concentrations of FGF-8b and the polymer. If the polymer concentration is held constant, proliferation activity should increase as the concentration of FGF-8b increases; more copies of FGF-8b should bind to each polymer. The extent of proliferation should reach a maximum when the polymer cannot accommodate additional copies of FGF-8b. At very high concentrations of FGF-8b, however, excess monomeric FGF-8b can begin to compete with the polymer-bound FGF-8b for the FGFR. If FGF-8b binds directly to the FGFR, the extent of proliferation will decrease because monomeric FGF-8b does not activate proliferation (Figure 3).

Using a fixed concentration (30 nM) of polymer **4a**, we tested the effect of FGF-8b concentration on proliferation. Activity begins at an FGF-8b-to-polymer ratio of ca. 1:1 and reaches a maximum when the ratio is 10:1. As is predicted for multivalent binding, once this maximum is reached, higher concentrations of FGF-8b do not afford increases in proliferation. These data indicate that polymer **4** may be used to explore the consequences of protein clustering. The decrease in proliferation at ratios of 30:1 or greater likely represents competition between the polymer-oligomerized FGF-8b and monomeric FGF-8b for the FGFR binding sites.

An alternative mechanism of action for polymers **4a** and **4b** is that they stabilize FGF-8b under cellular conditions, as has been reported for FGF-1.[19,20] We therefore measured the melting temperature (T_m) of FGF-8b.[14] The value determined, 60 °C, indicates that FGF-8b is stable under the assay conditions. Together the data suggest the polymers cluster FGF-8b and present it to the FGFR, thereby activating signaling.

Our results indicate that ATRP can be used to synthesize biologically active polymers capable of clustering target proteins. We have demonstrated that polymers presenting Ni^{2+} complexes can cluster His-tagged FGF-8b via noncovalent interactions. This clustering results in FGF-mediated cell proliferation in the absence of heparin or heparan sulfate. Thus, these polymers can be used to dissect the role of FGF clustering in FGF signaling. Because our strategy is modular, polymers of this type can be used to examine the consequences of clustering any soluble protein bearing a His tag. We anticipate that polymers of different lengths can be employed to dissect how the extent of protein clustering influences a range of cellular responses.

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Supporting Information Available: Gel permeation chromatography data, synthetic procedures and spectroscopic data, results from FGF-8b cross-linking experiments, procedures used in the determination of the T_m of FGF-8b, and procedures for the proliferation assay (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Ornitz, D. M., *Bioessays* **2000**, *22*, 108−112.
(2) Taipale, J.; Keski-Oja, J. *FASEB J.* **1997**, *11*, 51−59.
(3) van der Flier, A.; Sonnenberg, A. *Cell Tissue Res.* **2001**, *305*, 285−298.
(4) Hochuli, E.; Bannwarth, W.; Dobeli, H.; Gentz, R.; Stuber, D. *Biotechnology* **1988**, *6*, 1321−1325.
(5) Cairo, C. W.; Gestwicki, J. E.; Kanai, M.; Kiessling, L. L. *J. Am. Chem. Soc.* **2002**, *124*, 1615−1619.
(6) Dimick, S. M.; Powell, S. C.; McMahon, S. A.; Moothoo, D. N.; Naismith, J. H.; Toone, E. J. *J. Am. Chem. Soc.* **1999**, *121*, 10286−10296.
(7) Hochuli, E.; Dobeli, H.; Schacher, A. *J. Chromatogr.* **1987**, *411*, 177−184.
(8) O'Shannessy, D. J.; O'Donnell, K. C.; Martin, J.; Brighamburke, M. *Anal. Biochem.* **1995**, *229*, 119−124.
(9) Sigal, G. B.; Bamdad, C.; Barberis, A.; Strominger, J.; Whitesides, G. M. *Anal. Chem.* **1996**, *68*, 490−497.
(10) Paborsky, L. R.; Dunn, K. E.; Gibbs, C. S.; Dougherty, J. P. *Anal. Biochem.* **1996**, *234*, 60−65.
(11) Dorn, I. T.; Neumaier, K. R.; Tampe, R. *J. Am. Chem. Soc.* **1998**, *120*, 2753−2763.
(12) Godwin, A.; Hartenstein, M.; Muller, A. H. E.; Brocchini, S. *Angew. Chem., Int. Ed.* **2001**, *40*, 594−597.
(13) Matyjaszewski, K.; Xia, J. *Chem. Rev.* **2001**, *101*, 2921−2990.
(14) See the Supporting Information.
(15) Loo, B. M.; Salmivirta, M. *J. Biol. Chem.* **2002**, *277*, 32616−32623.
(16) Rapraeger, A. C.; Krufka, A.; Olwin, B. B. *Science* **1991**, *252*, 1705−1708.
(17) Spivakkroizman, T.; Lemmon, M. A.; Dikic, I.; Ladbury, J. E.; Pinchasi, D.; Huang, J.; Jaye, M.; Crumley, G.; Schlessinger, J.; Lax, I. *Cell* **1994**, *79*, 1015−1024.
(18) Kwan, C. P.; Venkataraman, G.; Shriver, Z.; Raman, R.; Liu, D. F.; Qi, Y. W.; Varticovski, L.; Sasisekharan, R. *J. Biol. Chem.* **2001**, *276*, 23421−23429.
(19) Chi, Y. H.; Kumar, T. K. S.; Wang, H. M.; Ho, M. C.; Chiu, I. M.; Yu, C. *Biochemistry* **2001**, *40*, 7746−7753.
(20) Arunkumar, A. I.; Kumar, T. K. S.; Kathir, K. M.; Srisailam, S.; Wang, H. M.; Leena, P. S. T.; Chi, Y. H.; Chen, H. C.; Wu, C. H.; Wu, R. T.; Chang, G. G.; Chiu, I. M.; Yu, C. *Protein Sci.* **2002**, *11*, 1050−1061.

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