Modification of Protein with Polymer-Bisphosphonate Ligand to Increase Bone Affinity

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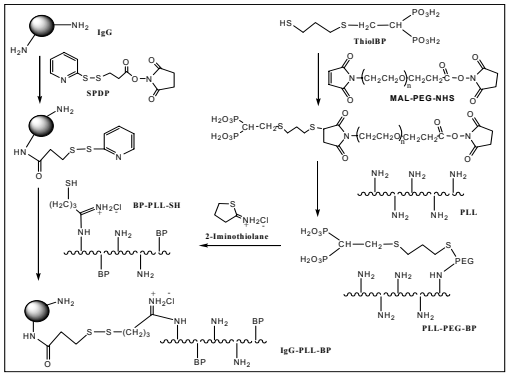
Introduction: Bisphosphonates (BPs) have an exceptionally high affinity for bone mineral hydroxyapatite (HA) because the P-C-P motif can chelate calcium ions by bidentate coordination to allow the rapid and selective targeting of BPs to bone mineral surfaces *in vivo* [1]. Previous studies have shown that chemical derivatization of proteins with BPs is an effective means to enhance the mineral affinity of a wide-range of proteins [2]. The main objective of this study is to develop a new approach to conjugate BPs to proteins. Instead of linking BPs directly on proteins (as previously attempted [2]), BPs were linked to a polymeric molecule, after which polymer-BP conjugates were linked to the proteins. We hypothesized that conjugating proteins with a polymer-BP ligand could enhance the mineral affinity and, meanwhile, minimize the possibility of the protein modification that might adversely affect a protein's bioactivity. Polyethylenimine (PEI) and poly-*L*-lysine (PLL) were chosen to link an in-house synthesized BP, 2-(3-mercaptopropylsulfonyl)-ethyl-1,1-bisphosphonic acid (thiolBP), to the polymers. Bovine IgG (150 KDa) was used as a model protein since several therapeutic antibodies are currently pursued for treatment of bone diseases, such as osteoarthritis.

Methods: The synthesis of thiolBP was described as before [3]. ThiolBP was conjugated to PEI and PLL by using succinimidyl-4-(*N*-maleimidomethyl)-cyclohexane-1-carboxylate (SMCC) and maleimide-polyethylene-glycol-succinimide (MAL-PEG-NHS), separately. The extent of BP substitution per polymer (no. of thiolBP/polymer) was determined by a phosphate assay [4] and TNBS (picrylsulfonic acid solution) assay. HA binding assay was performed to determine the mineral affinity of the conjugates. The polymer-BP ligand was thiolated by 2-iminothiolane. The thiolated polymer ligand was conjugated to bovine IgG through the linker N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP) (**Scheme 1**). A spectroscopic method was used to quantify the dithiopyridine (DTP) content of IgG-SPDP. The IgG-SPDP conjugate was incubated with thiolated polymer-BP ligand for 1 hour at room temperature, and then analyzed by SDS-PAGE.



Scheme 1. Polymer-BPs conjugation to the antibody IgG

Results/Discussion: The extent of thiolBP substitution on polymer can be controlled by varying the concentration of linker SMCC or MAL-PEG-NHS (**Figure 1A**). With the increase of SPDP concentration, the DTP content released from the IgG-SPDP conjugates increased accordingly (**Figure 1B**). The SDS-PAGE analysis showed the difference between before and after reacting IgG-SPDP with BP-PLL-SH **(Figure 1C)**. The HA binding assay was carried out for IgG-PLL-BP conjugates *in vitro*, and 4~5 fold higher binding were obtained by the conjugate samples than the control (**Figure 1D**).

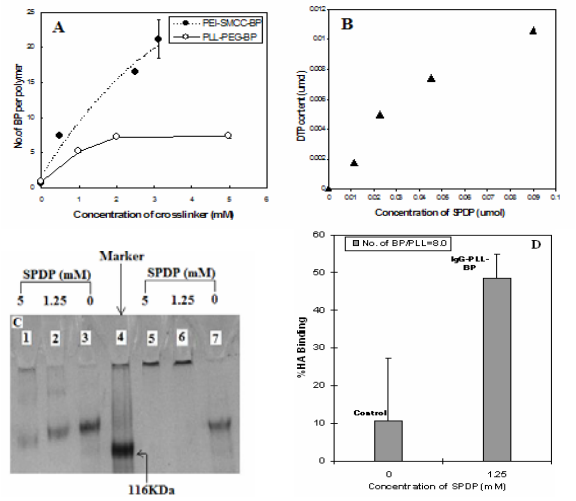


Figure 1. (**A**) Conjugation efficiency of PEI-SMCC-BP and PLL-PEG-BP. (**B**) The detection of DTP content in IgG-SPDP conjugates. (**C**) The SDS-PAGE analysis of the protein-BP conjugates. Lane 4 indicated the lane of the known molecular weight markers. Lane 1, 2 and 3 were IgG-SPDP, and lane 5, 6 and 7 were (IgG-SPDP) + (BP-PLL-SH). (**D**) *In vitro* HA binding of IgG-BP conjugates vs. control.

Conclusions: The polymer-BP ligand can be synthesized with various extent of BP substitution, and it is possible to conjugate this ligand to IgG to enhance the mineral affinity. These studies are helpful in engineering biomaterial carriers for delivery of therapeutic proteins to improve their efficacy in bone diseases.

References: [1] Russell R.G. G., et al, Calcif. Tissue Res., 1970; 6:183. [2] Zhang S. et al. Biomacromolecules. 2005; 6: 2800. [3] Bansal G. et al. JBMR: A, 2005; 74: 618. [4] Bai J. et al. J. Polym. Sci. *A*, 2004; 42: 6143.