Bioactivity of PTH(1-34) Immobilized by its N-Terminus

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INTRODUCTION

A variety of approaches are being investigated for controlling tissue-biomaterial interactions. For example, proteins and bioactive peptides can be attached to biomaterial surfaces to affect the initial adhesion and/or subsequent responses of cells.

Parathyroid hormone (PTH) is an 84 residue peptide hormone, with the N-terminal 34 residues [PTH(1-34)] responsible for its biological activity. Binding of PTH(1-34) to its receptor induces adenylate cylcase activity and the subsequent production of cAMP.

A primary function of PTH is to regulate extracellular calcium homeostasis by acting on kidney, bone, and intestine. With respect to effects on bone, it can be either anabolic or catabolic. Short-term administration of PTH at low doses stimulates osteogenesis.

The objective of this study was to investigate the biological activity of PTH(1-34) immobilized in a controlled manner via its N-terminus.

MATERIALS AND METHODS

In these studies, coverslips were coated with non-encapped PLGA (5050 DL 2A, Alkermes). Dihydrazides of increasing length were attached using single step carbodiimide chemistry. The spacer arms were 2 (oxalic; C2), 4 (succinic; C4), 6 (adipic; C6), and 10 (sebacic; C10) carbon atoms long. Based on pilot studies, respective dihydrazide concentrations of 0.018, 0.057, 0.018, and 0.011 mM were used in an effort to have a similar number of hydrazide groups for each treatment. After attachment of the spacer molecule to carboxyl groups on PLGA, one of the hydrazide groups was available for binding to peptide. The N-terminal serine residue of PTH(1-34) was oxidized with periodate to create an aldehyde moiety, which reacts with hydrazide groups at neutral pH to form stable hydrazone bonds. For comparison, PTH was randomly adsorbed to the PLGA. As an indicator of availability of the peptide for interaction with cells, binding of polyclonal antibodies was investigated. One has a binding epitope at the N-terminus of the peptide (sc-9676) and the other at the C-terminus (sc-9677) (Santa Cruz). Also, 50,000 MC3T3-E1 cells (ATCC CRL-2593) were seeded following pretreatment with IBMX. After ten minutes of incubation with the surfaces, cells were lysed using sonication and a freeze-thaw cycle. The amount of cyclic AMP was measured using a cAMP kit (Endogen) and normalized by the amount of DNA in each well.

RESULTS AND DISCUSSION

Availability of the free C-terminus of PTH was statistically similar on all five surfaces, but with a trend of lower accessibility on the random surface (Figure 1). In contrast, the use of a spacer improved accessibility of the N-terminus, which was used for immobilization. Antibody binding to an N-terminal epitope was significantly greater on all four hydrazide-derivatized surfaces compared to the random surface.

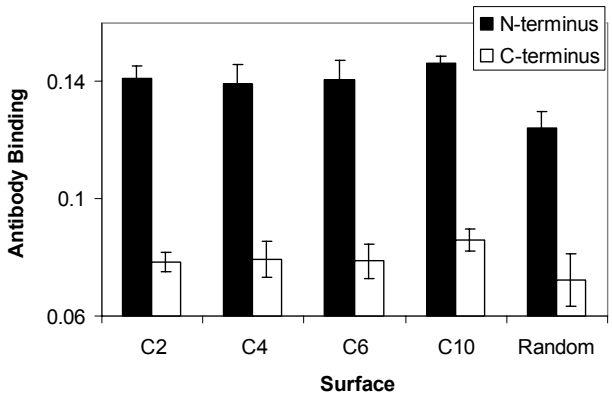

Figure 1: Amount of immobilized PTH(1-34).

Figure 2 shows cAMP production in response to immobilized PTH(1-34). Cellular response to the bound peptide was not significantly different between treatments, however there was a trend towards higher activity in the directed immobilization schemes compared to randomly adsorbed peptide. The site-directed immobilization appeared to make the PTH more easily accessible to cells interacting with it.

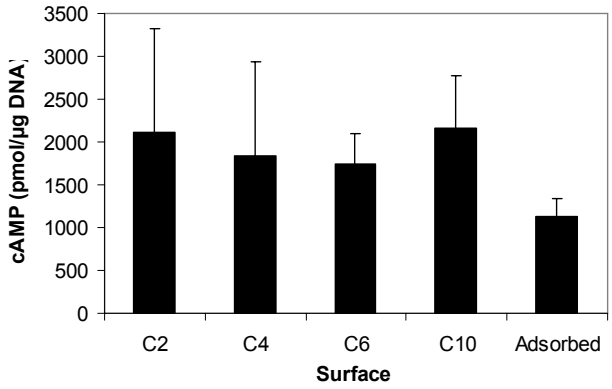

Figure 2. Celluar response to PTH(1-34).

CONCLUSIONS

Controlled immobilization of PTH(1-34) resulted in better cellular activity as measured by cAMP production. This directed binding of bioactive peptides to surfaces presents biomolecules for interaction with cells in a manner that enhances interaction with cell surface receptors.

ACKNOWLEDGEMENT

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