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Title:	Initial Proteomic Analysis of the Partially Purified Carboxyl-PTH Receptor(s) in Bone Cells
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We had previously reported the synthesis and characterization of synthetic CPTH peptides that bind and crosslink (Benzoylphenylalanine mediated photoaffinity crosslinking) to the putative CPTH receptors in bone cells. These peptides had a C-terminal biotin to affinity purify the CPTH-CPTHR complex and also to aid the detection of the complex throughout the purification procedure. We had reported that these CPTH fragments were associated with 220 and 80 kDa putative receptors, of which, the former was a transient complex comprising the 80 kDa entity. An additional 30 kDa minor band was also detected. Here, we report the preliminary data obtained from the purification and identification the putative CPTH receptor(s). We used [Bpa24, Tyr34]hPTH(23-84)-biotin ± 125I as the ligand on OC59 (PTH1R-/- osteocytic cell line) and ROS 17/2.8, both of which are known to express high levels of CPTHR. The experimental approach involved the binding of the ligand to the receptor followed by crosslinking with UV radiation. Upon lysis, one or two step affinity purification were done with immobilized anti-PTH antibody and/or CaptAvidin-Agarose or Streptavidin-Magnetic beads. The sample(s) were then analyzed by Mass Spectrometry after 2D gel separation or SDS-PAGE. In the first few attempts, we identified 78 kDa Glucose Regulatory Protein, GRP78 for the 80 kDa spot and Voltage Dependent Anion Channel (VDAC1) from the 30 kDa spot. Further studies demonstrated that GRP78 was very unlikely to be the receptor itself and being the most abundant protein in the 80 kDa molecular weight range, was actually masking the putative CPTH receptor through all our purification steps and in Mass Spectrometry. As an alternative approach, both GRP78 and VDAC1, cloned into mammalian expression vectors and expressed in COS7 cells, failed to enhance the binding of the ligand to the cell surface. From the same data, we also tried to find out peptide(s) with mass modifications introduced due to Bpa-crosslinking. The approximately 671 dalton modification was found on Bromodomain adjacent to zinc finger domain 1B (Baz1b), a transcription factor regulating the Vitamin-D receptor expression in cells. Further attempts to identify the CPTHR and characterization of the identified proteins are in progress.