

Biochemistry Cumulative – April, 2005

"Signal Transduction-Small G-Proteins"

Answer each of the following questions.

1. (WC) Membrane localization of ras proteins is essential for their cellular functions. Describe the mechanisms by which different ras proteins are localized in the membrane.

2. (GF) What would the effects of the following mutations be on the activity of Ras:
 - a) Mutant Ras that cannot bind GTP.
 - b) Mutant that cannot hydrolyze GTP.
 - c) Mutant that has an exceptionally high affinity for GDP.
 - d) Mutant that is missing its last 4 amino acid residues at the C-terminus.
 - e) Mutant that does not interact with a major Ras GAP.
 - f) Mutant that does not interact with a major Ras GEF.
 - g) Mutant that does not interact with a major Ras GDI.
 - h) Mutant that does not interact with Raf.

3. (LF) In a recent article, "Structure and Function of the GTP Binding Protein Gtr1 and Its Role in Phosphate Transport in *Saccharomyces cerevisiae*", by Jens O. Lagerstedt, Ian Reeve, John C. Voss, and Bengt L. Persson (Biochemistry 44, 511-517, 2005), the following EPR results were obtained. Please study the figure (see color figure on separate page) and give (1) your general conclusions from the EPR results (8 pts), and (2) specific residue-level information on Gtr1 (8 pts). These results are consistent with findings from other studies; please give one example (9 pts). No specific knowledge of EPR techniques is needed for the answers, though it may provide additional information.

4. (RJK) Describe small G-Proteins with respect to:
 - a) Protein structure, including monomeric or oligomeric nature, domains, etc.
 - b) Cellular location
 - c) Catalytic activity,
 - d) Activation and deactivation
 - e) Common type of signal transducer
 - f) Role in pathway

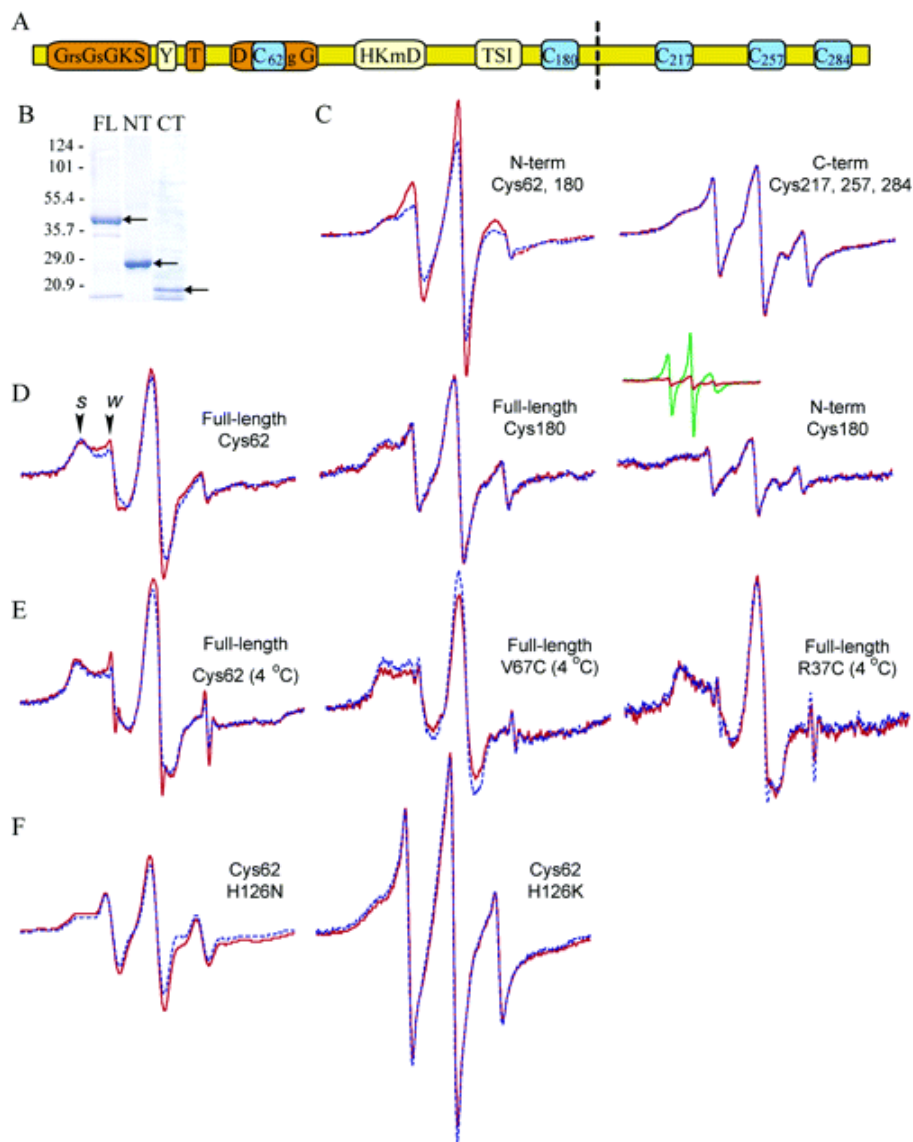


Figure 4 EPR spectroscopy of Gtr1 mutants. (A) Schematic presentation of the Gtr1 primary structure with phosphate/magnesium binding motifs (orange), guanine binding motifs (yellow), and native cysteines (blue). The dashed line in panel A indicates the site of separation between the N-terminal and C-terminal portions. The Coomassie Blue-stained SDS-polyacrylamide gel (B) shows the *E. coli*-produced and -purified full-length Gtr1 protein (FL, amino acids 1-310), the N-terminal portion of Gtr1 (NT, amino acids 1-185), and the C-terminal portion of Gtr1 (CT, amino acids 186-310). Site-directed mutants of the full-length and truncated forms of the Gtr1 protein were subjected to nitroxide spin labeling of cysteine residues and analyzed by EPR spectroscopy in the absence (dashed blue line) or presence (red line) of 1 mM GTP (C-F). The low-field line resolves two distinct populations in each sample, a broad line indicating strongly immobilized side chains (*s* in panel D) and a weakly immobilized population with greater motional freedom (*w* in panel D). GTP increases the motion of nitroxides attached to C62. In panel C, the spectra from spin-labels attached to the native cysteines in the N-terminal (C62 and C180, left) and C-terminal (C217, C257, and C284, right) halves of Gtr1. The individual cysteines C62 (full-length, left panel) and C180 (full-length form in the middle panel and N-terminal portion in the right panel) are analyzed in panel D. In panel E, the labels at C62 (left panel) and also at R67C (middle panel) and V37C (right panel) are analyzed at a low temperature (4 °C). The role of the histidine in the HKmD motif was analyzed in panel F, in a C62-labeled protein. The left panel shows the spectra of the H126N mutant, and the right panel shows the corresponding spectra for the H126K mutant. For all pairs, the CTP and GTP spectra were acquired on identical sample amounts and instrument settings. The protein concentrations were 16 μM (i.e., 62-fold excess of nucleotides) for all full-length Gtr1 mutants and 26 μM (i.e., 39-fold excess of nucleotides) and 35 μM (i.e., 28-fold excess of nucleotides) for the N- and C-terminal portions, respectively. Spectra are normalized to the amplitude of the unfolded protein (1% SDS; see the green line in panel D).