Abstract

We have exploited the large number of known allosteric crystal structures to systematically characterize local conformational changes in allosteric proteins toward the goal of increasing the theoretical understanding of the structural basis of protein allostericity. We have compiled a set of 51 pairs of known inactive and active allosteric protein structures from the Protein Data Bank. We have measured changes in dihedral angles and Ca atom displacements for backbone and side chain movements in residues making contacts for each protein. Several examples show that these automated calculations reveal unexpectedly large conformational changes that were previously observed manually by crystallographers. In addition, statistical analysis of the calculated motions shows that on average, 20% of residues affected significantly by the two crystal structures of an allosteric protein in addition to possible changes in dynamics. Allosteric motion is more probable in weakly constrained local structural environments like loops and solvent-exposed regions than in strongly constrained environments like helices, strands, and buried regions. Backbone and contact motions are correlated at separations of up to 20 residues in sequence space and up to 2 A in Ca space. Together, these observations suggest structural tools for designing allosteric protein systems.

Importance and applications

- Major mechanism of control and regulation in biology
- Improved high-resolution understanding of allostery will aid in understanding, treating diseases caused by malfunctioning allosteric proteins
- Designing novel allosteric proteins as biological control devices

Previous work in allostery

- KN backbone structural transition model
- Low-resolution structural models
- Manually compare A and I states → qualitative mechanistic models

Precise, diverse motion calculations

- Discriminating true motions from crystallographic noise
- Precise subset of residues moved
- Set thresholds to exclude ~99% of background motion in controls
- Thresholds are intuitively reasonable cutoffs for large motions
- Smaller motions may be functionally significant in some allosteric proteins

Statistics of allosteric transitions

- Allosteric transitions comprise 10-20% of protein residues
- Most of protein is structurally conserved
- Contact changes, backbone motions cluster strongly in space
- Backbone displacements, dihedral changes, and contact motions localize to similar regions of structures
- PPF: motions localize between catalytic and allosteric sites (exp. contact changes) → possible allosteric pathway

Theoretical implications

- Calculate motions in three types of degrees of freedom important to protein structure
- Calculate motions do not in themselves constitute comprehensive mechanistic models
- Statistical analyses reveal basic insights into structural basis of protein allostericity
  - Significant changes in average structure (~20%) are common in allosteric proteins → not just a dynamic phenomenon
  - Protein structures use constraints to control location of motion, possibly to direct signal propagation between allosteric and functional sites
  - Local motions are correlated up to 20Å distance, enough to bridge two spatially distinct sites over several residues
  - Mechanical communication is an important, general phenomenon in protein allostericity
- Possible test resource for flexibility prediction algorithms such as COREX (Chen & Honig 1999), elastic network models (several of 1999), FIRST (Havel et al 1999) and statistical coupling analysis (Bartels et al 2001)

Results

- Three functional classes of allosteric proteins
- A wide variety of targets
- DNA-binding proteins
- Signaling proteins
- Enzymes
- Three-dimensional space
- Local structural environment influences allosteric motion
- Switch 1 and 2 previously identified by Milburn et al.
- Most of protein is rigid
- Motions tend to occur in contiguous segments (especially backbone and contact)
- Strong consensus between measures in most flexible regions

Goals and new contributions

- Precisely identify local motions in known allosteric protein structures
- Statistically investigate amount and distribution of motions
- Dataset of 51 Allosteric Proteins
- Three functional classes
- Signaling proteins
- DNA-binding proteins
- Enzymes
- Allosteric motions in protein space
- Sequence space
- Conserved motions
- Switch and 2 previously identified by Milburn et al.
- Most of protein is rigid
- Motions tend to occur in contiguous segments (especially backbone and contact)
- Strong consensus between measures in most flexible regions

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