

Insights into the Structure of Invisible Conformations of Large Methyl Group Labeled Molecular Machines from High Pressure NMR

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Most proteins are highly flexible and can adopt conformations that deviate from the energetically most favorable ground state. Structural information on these lowly populated, alternative conformations is often lacking, despite the functional importance of these states. Here, we study the pathway by which the Dcp1:Dcp2 mRNA decapping complex exchanges between an autoinhibited closed and an open conformation. We make use of methyl Carr-Purcell-Meiboom-Gill (NMR) relaxation dispersion (RD) experiments that report on the population of the sparsely populated open conformation as well as on the exchange rate between the two conformations. To obtain volumetric information on the open conformation as well as on the transition state structure we made use of RD measurements at elevated pressures. We found that the open Dcp1:Dcp2 conformation has a lower molecular volume than the closed conformation and that the transition state is close in volume to the closed state. In the presence of ATP the volume change upon opening of the complex increases and the volume of the transition state lies in-between the volumes of the closed and open state. These findings show that ATP has an effect on the volume changes that are associated with the opening-closing pathway of the complex. Our results highlight the strength of pressure dependent NMR methods to obtain insights into structural features of protein conformations that are not directly observable. As our work makes use of methyl groups as NMR probes we conclude that the applied methodology is also applicable to high molecular weight complexes.