Staphylococcal Nuclease: Sequential Assignments and Solution Structure

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CONDENSATION OF THE RESEARCH

PURPOSE OF THE STUDY

To obtain sequence-specific NMR assignments in staphylococcal nuclease as a basis for comparisons of the secondary structure of this protein in solution with the crystal structure, and for further studies by NMR

RESEARCHERS' APPROACH

With the use of recombinant techniques a variety of isotope-labeled forms of staphylococcal nuclease were produced in milligram quantities. This includes the fully ^{15}N-labeled protein, two types of deuteriated protein, and 16 protein preparations with ^{15}N and ^{13}C labeling of individual amino acid types. For the NMR measurements 1.5 to 2.0 mM solutions of these protein preparations were prepared with a 3-fold excess of pdTp and a 5-fold excess of $CaCl_2$, the sample temperature was in the range of 36°C to 38°C, and the pH was adjusted to 6.5–7.5. Using these samples almost complete ^{1}H and ^{15}N resonance assignments of the backbone atoms were obtained from homo- and heteronuclear 2D NMR experiments. About 30% of the side chain spin systems were also assigned, including Ala, Thr, Val, Tyr, Phe, and Trp. From sequential NOEs and longer range NOE connectivities between backbone hydrogens, secondary structure elements were identified that were in good correspondence with the structure found in the crystalline state by X-ray crystallography.[1] Many additional NOEs were found to correspond to proton pairs with an interproton distance of less than 4 Å in the X-ray structure. On the basis of these observations the authors concluded that the conformations of the polypeptide backbone are essentially the same in solution and in the crystal. This conclusion is further supported by amide proton exchange rates, since all amide protons found to exchange slowly in solution are hydrogen-bonded in the crystal structure. Additional observations on the amide proton exchange were used to investigate the relative rigidities of the structure in different regions of the protein molecule.

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WHAT RESEARCHERS ACCOMPLISHED

The researchers:

- Obtained 19 different isotopically enriched samples of staphylococcal nuclease.
- Recorded homo- and heteronuclear 2D NMR spectra of the aforementioned 19 isotope-labeled preparations of the protein and of the unlabeled form.
- Obtained sequence-specific NMR assignments for most backbone ^{15}N and ^{1}H atoms, and for about 30% of the side chain proton spin systems.
- Determined secondary structure elements of staphylococcal nuclease in solution.
- Compared the structure in the crystal and in solution on the basis of the NMR data and the known X-ray structure, and found extensive correspondence of the secondary structures in the two states of staphylococcal nuclease.

COMMENTARY ON THE RESEARCH

We have selected this work for *Chemtracts* mainly for two reasons: 1) The protein studied has long been a focus of interest for research on the protein folding problem, and the availability of sequence-specific NMR assignments promises to give new impetus for further studies; 2) The use of isotope labeling in support of NMR studies with proteins is extensively illustrated in this study of staphylococcal nuclease, and a large number of NMR spectra of the differently labeled protein are displayed.

RELATION TO EARLIER WORK

Staphylococcal nuclease is a well-characterized globular protein that does not contain disulfide bonds. Its crystal structure has been solved to 1.5 Å resolution.[1] Because of its relatively simple structure, ease of isolation and purification, and good thermal stability, numerous reports have been published on studies of folding and unfolding of this enzyme.[2-11] Although staphylococcal nuclease was studied by NMR more than 20 years ago and it was also one of the first proteins subjected to extensive isotope labeling for NMR studies,[3] applications of NMR methods have so far been limited by the small number of individual assignments available.

IMPLICATIONS

The great interest in staphylococcal nuclease is to a considerable part due to the availability of efficient expression systems. Over 80 point mutations have been obtained[5] and virtually all desired point mutations can be constructed. This makes the nuclease system amenable to systematic studies of the effects of point mutations on protein folding, structure, and function.[9,11] With the now available individual NMR assignments, the application of modern NMR methods can provide more detailed information about the solution structures of the parent protein and its mutants, where particular interest will foreseeably be focused on studies of internal mobility of the folded protein, and on studies relating to protein folding.

It is by now clearly established that in work with proteins with molecular weight above approximately 12,000, homonuclear ^{1}H NMR studies must in most cases be supported by isotope labeling and heteronuclear NMR experiments in order to obtain sequence-specific resonance assignments. These heteronuclear approaches are very nicely documented in this article. Nonetheless the results obtained are somewhat limited. Although the assignments for the polypeptide backbone will undoubtedly provide invaluable support of

studies on molecular mobility and investigations on the folding pathways of the protein, they are not sufficient as a basis for the determination of the three-dimensional structure in solution unless they are supplemented by resonance assignments for the majority of the amino acid side chains. It should also be considered that for many other proteins of biological interest it may be more difficult to obtain a large array of different isotope-labeled species. In such situations the present paper may be an important source of information from which to derive strategies for resonance assignments centered around the use of ^{1}H NMR with the unlabeled protein supported by heteronuclear experiments with a small number of optimally selected isotope-labeled species.

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