The conformational properties of ribosomal protein S1

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ABSTRACT

The proton NMR spectrum of S1 reveals that S1 has considerable tertiary structure in physiological buffers, but more structural flexibility than normal for globular proteins. S1's NMR spectrum is independent of the method of preparation.

INTRODUCTION

Protein S1, by far the largest protein in the ribosome of E. coli, is an essential component in the protein synthesizing apparatus of that organism. In its absence, synthesis directed by natural mRNAs does not take place\(^{(1)}\). S1 plays a significant role in the initiation of synthesis\(^{(2,3,4,5)}\).

There are a number of reasons for being interested in the properties of S1 as a free protein in solution. It is the only ribosomal protein known to have a physiological function independent of the rest of the ribosome; S1 is one of the host contributed factors in the replicase of bacteriophage Q\(\beta\)\(^{(6,7,8,9)}\). Furthermore, S1's association with the ribosome is the weakest of all ribosomal proteins. It dissociates at an appreciable rate under physiological conditions and, unlike many ribosomal proteins, is quite soluble\(^{(10,11)}\).

S1's molecular weight is 65,000-70,000. It exists as a monomer in solution at neutral pH and moderate ionic strength. Its hydrodynamic properties indicate that it is highly elongated, a conclusion strongly supported by small angle x-ray scattering data. S1 behaves as though it were a 10:1 prolate ellipsoid, which implies a major axis 200-250 Å in length\(^{(10,12)}\). The circular dichroism spectrum of S1 has been measured and indicates that S1 has appreciable secondary structure in solution. It contains roughly 13% α helix and 37% β structure\(^{(10)}\).
Below we present the results of a proton nuclear magnetic resonance study on SI. This work was directed at two questions. First, does SI have a well defined tertiary structure in solution? Second, is the tertiary structure displayed by SI dependent on its method of preparation? The importance of this latter point has been underlined by the recent findings of Morrison et al. They used NMR methods to show that some ribosomal proteins prepared in urea have a denatured, random coil configuration when returned to physiological buffers, but that many of the same proteins, prepared by milder means, have well-defined tertiary structures in the same buffers.

The NMR spectrum given by a globular protein with a well defined tertiary structure differs from that of the same protein under denaturing conditions in two respects. First, the reduction in mobility of residues when the protein folds into a stable tertiary structure produces a broadening of resonances. Second, alterations in chemical shifts arise due to the stable placement of specific protons in unique chemical environments which leads to the appearance of resonances in new positions.

The spectra obtained in this study show that SI is certainly not a random coil in solution. Its spectrum suggests, however, a greater degree of structural flexibility than is normal in globular proteins of its molecular weight. The spectrum of SI prepared in urea is indistinguishable from that given by SI prepared by "non-denaturing" techniques. Apparently SI, denatured in urea, is able to reestablish its normal conformation spontaneously upon return to physiological buffers.

MATERIALS AND METHODS

Proteins. Egg white lysozyme (3x crystallized) and carbonic anhydrase (bovine) were obtained from Sigma Chemical Corp. Bovine serum albumin (BSA) (crystallized) was purchased from Cal Biochem.

SI was prepared from E. coli PR13. Two methods were used: one involving exposure of the protein to 6 M urea at pH 5.6 to pH 8.0 ("denaturing"), and the second ("non-denaturing") avoiding exposure to urea and pHs outside the range of 7.0 to 8.0.

Preparation of Samples for NMR. Solutions of protein at 15 to 25 mg/ml were dialyzed into 0.1 M KCl, 0.01 M potassium phosphate, pH 7.5 in H₂O. 0.6 ml aliquots were lyophilized to dryness and taken up in the same volume of D₂O three times. The D₂O solutions produced were stored at -80°C until spectra were to be taken. Samples to be examined in the denatured state.
were dialyzed against H$_2$O, lyophilized and cycled through D$_2$O two times. After the third lyophilization the samples were stored dry at -80°C. Just before the spectra of these samples were to be taken, they were dissolved in deuterated trifluoroacetic acid (dTFA) (Aldrich).

Data Collection. Data were collected on a Bruker HX-270 NMR spectrometer operating in the Fourier Transform mode. Samples were placed in 5 mm sample tubes and maintained at 20°C during the run. 15 μsec pulses were used at intervals of 1.3 to 1.5 seconds. 200 transients were collected on each specimen.

RESULTS

The NMR spectrum of SI. Spectra were taken of lysozyme, carbonic anhydrase, SI, BSA and BSA in dTFA as described in Materials and Methods, with the results shown in Figure 1. BSA in dTFA is fully denatured. The spectrum it gives is sharp and well resolved, and distinguishable from that given by SI in dTFA in only minor respects. Lysozyme, carbonic anhydrase and BSA are globular proteins of 14,333$^{(15)}$, 30,000$^{(16)}$ and 69,000$^{(17)}$ molecular weight respectively. In the lysozyme spectrum one sees several resonances occurring at frequencies not represented in the denatured spectrum due to the placement of residues in unique environments within the protein. These are particularly conspicuous in the region upfield of the terminal methyl peak at +1000 Hz. As one proceeds up in molecular weight the combination of peak splitting and peak broadening due to rotational immobilization of protons within the globular structures combine to smear out the spectrum. The spectrum of BSA is hardly more than an averaged envelope.

SI is a protein whose molecular weight is about the same as BSA. Its rotational correlation time should be no less than BSA's, given its elongated shape, assuming both are rigid structures$^{(18)}$. Thus if SI were as rigid as an ordinary globular protein, its spectrum should resemble that of native BSA. Its spectrum, in fact, appears better resolved than that of BSA, resembling more the spectrum of carbonic anhydrase, whose molecular weight is half SI's. SI's spectrum bears no resemblance to that of a denatured protein.

From this observation two qualitative conclusions may be drawn. First, SI is far from a random coil when in solution in low ionic strength buffers at neutral pH. It must have appreciable tertiary structure. Second, SI's tertiary structure must allow some degree of flexibility. Flexibility has
Figure 1. Proton NMR Spectra of BSA, SI, Carbonic Anhydrase, and Lysozyme.

Spectra of protein samples were taken as described in Materials and Methods. The spectra of samples b-e were obtained in D$_2$O. The region around the HDO peak at 0 Hz is omitted for clarity of presentation. This region is included in spectrum a for which dTFA was the solvent.

(a) BSA in dTFA
(b) Lysozyme in D$_2$O
(c) Carbonic anhydrase in D$_2$O
(d) SI in D$_2$O
(e) BSA in D$_2$O

to be postulated to account for the fact that its spectrum is better resolved than expected for a protein of its molecular weight.

The Effect of Preparative Technique on the Structure of SI. Figure 2 shows spectra obtained from SI prepared by denaturing techniques (Fig. 2a) and methods avoiding exposure to high concentrations of urea (Fig. 2b). The spectra are indistinguishable. One must conclude that at the limited resolution afforded by this technique, the structure SI attains under the
Figure 2. Proton NMR Spectra of S1 prepared under non-denaturing and denaturing conditions.

Spectra were taken as described in Materials and Methods. S1 was prepared both by a method involving exposure to urea and by a technique avoiding urea exposure. Equal amounts of both kinds of S1 were examined in D$_2$O:

(a) Spectrum of urea prepared S1
(b) Spectrum of "native" S1.

conditions of measurement is independent of preparation technique.

DISCUSSION

One could imagine obtaining an NMR spectrum from S1 similar to that observed if S1 were organized into two roughly equal lobes of stable tertiary structure connected by a flexible "hinge". The data presented here do not establish the validity of this model; other hypothesis could be proposed to account for the fact that S1's spectrum is well resolved relative to its molecular weight. However, there are two independent pieces of evidence which makes this model for S1 an attractive one. First, Chu and Cantor (19) have shown that fluorescent chromophores attached to S1 show polarization of fluorescence decay with a half time too small to be accounted for by rotational diffusion of the whole S1 molecule. Second, when S1 is exposed to proteolytic enzymes under mild conditions, some large fragments can be isolated, just as can be done with myosin and immunoglobulins, to name two well established examples of segmentally flexible proteins (20) (A. P. Subramanian, personal communication; Laughrea, unpub-
lished observations). All of these observations can be accounted for by the hypothesis that S1 also is segmentally flexible.

We earlier demonstrated that S1's hydrodynamic properties and circular dichroism spectrum are independent of the method of protein preparation. The NMR results strengthen the conclusion draw from these previous data, namely that the changes urea induces in S1's structure are readily reversible. Judging by the results of Morrison and coworkers (13), S1 may be exceptional among the ribosomal proteins in this respect, as in so many others. Their basic finding was that many ribosomal proteins do not regain their "normal" tertiary structures upon return to physiological buffers from urea.

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