experiment, our calculations suggest that in a very dilute DNA solution, <5 μg/ml for a molecular weight of 1×10^7, the collapsed form can be a stable configuration.

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LAC REPRESSOR

A Genetic and Nuclear Magnetic Resonance Study of Structure and Function

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The prototype gene control system, the *lac* operon of *E. coli* (1), has recently also become the best chemically characterized system to date (2). The complete primary sequence of both the gene (3) and the protein (4) responsible for the regulation of this operon, the repressor, is known, along with the DNA sequence of its site of action (5), the operator. The *lac* repressor is a tetrametic protein with four identical subunits of 360 amino acids each, giving a total molecular weight of 154,000. The *lac* operator sequence is about 25–30 base pairs long. With the wealth of information about the primary structure the next question is one of geometry. This leads to the application of either x-ray diffraction or nuclear magnetic resonance (NMR) methods, since these are the only approaches that yield information about the geometry and environment of specific groups and atoms in these molecules. Since we are interested in the interaction of repressor with a variety of small molecular weight inducers and anti-inducers, as well as the operator sequence in aqueous solution, we chose the NMR approach. As of this writing, no useful crystals of the *lac* repressor or the repressor and any of its ligands have been reported. Because of our extensive genetic work with this system, we have a unique advantage in taking this approach as well.

Using a comprehensive mutagenesis and mapping scheme, we have isolated 90 nonsense mutations in the gene coding for the repressor protein (2, 6). It is possible to examine the properties of each of these nonsense mutations in all of the known nonsense suppressor backgrounds. We have looked at the effect of over 400 individual single amino acid alterations on repressor function (6). This ability to vary specific amino acids in specific locations in the protein allows a direct method for the assignments of features in the spectrum. In the case under consideration here, we have used the added expedient of looking initially

only at the tyrosine residues, of which there are 8 per monomer (at positions 7, 12, 17, 47, 126, 204, 273, and 282 in the amino acid sequence). We chose this amino acid for our initial studies because: (*a*) we have been able to produce biosynthetically 3-fluorotyrosine substituted repressor with properties virtually identical with normal repressor (7); (*b*) among the nonsense mutations that we have isolated all of the 8 tyrosine locations are included, so that it is possible to remove each tyrosine and its resonance from the NMR spectrum, respectively; (*c*) the tyrosine aromatic protons yield resonances that are nearly isolated in proton magnetic resonanc spectra for most proteins; thus a comparison can be made between our 3-fluorotyrosine results with data from natural tyrosine; (*d*) tyrosines 17 and 47 have been implicated in our genetic experiments as being required for operator binding; (*e*) tyrosines have been utilized for DNA interactions in two proposed models of *lac* repressor structures (8, 9).

METHODS

The procedures for incorporating 3-fluorotyrosine (7), assaying ligand interactions with the *lac* repressor (10), and limited enzymatic digestion of the repressor have been published (11). All of the NMR spectra were taken at 141 MHz on a Nicolet NT 150 (Nicolet Instrument Corp., Madison, Wis.) equipped with a ^{19}F probe capable of taking sample tubes of 20 mm in diameter.

RESULTS

Using the procedure we mentioned above, we have been able to assign all of the eight resonances in the ^{19}F NMR spectrum of the 3-fluorotyrosine substituted *lac* repressor. It is immediately apparent that there are 4 narrow resonances and 4 broad resonances. The assignment leads to the conclusions that the narrow resonances are all in the N-terminal fragment, that can be removed by trypsin or chymotrypsin, which contains the first 59 or so amino acids. The broad resonances correspond to tyrosines 126 and those later in the sequence. A striking observation is that both in the presence and absence of inducer molecules, the spectrum of the tetrameric core portion is superimposable on the broad resonances in the spectrum of the intact repressor. This leads one to conclude that the tyrosine NMR signals in the N-terminal fragment are independent of the core of the protein.

This conclusion is reinforced by the observation that under conditions of broad band proton decoupling, the resonances corresponding to the core of the protein show the expected nuclear Overhauser enhancements of -1 for macromolecular systems of this type (12, 13). On the other hand, the 4 N-terminal tyrosine resonances in the intact repressor give enhancements of only -0.5 to -0.7. This result reflects significantly greater and independent mobility of the tyrosines for this part of the molecule. These results are complementary to the recent observation using proton NMR of the tyrosines in the isolated N-terminal fragment and cores (14, 15).

Examination of the changes in the ^{19}F NMR spectrum of the 3-fluorotyrosine substituted repressor upon inducer and anti-inducer binding show very different changes in the spectrum. Upon binding isopropyl-β-D-galactoside (an inducer), we observe a 2 ppm downfield shift of the resonance due to tyrosine 282 and a 0.5 ppm shift upfield for tyrosine 204. From our genetic data this residue must be a tyrosine or leucine for proper quaternary structure (6). The binding of o-nitro-phenyl-D-fucoside, an anti-inducer, results in a much smaller, if any, change in the resonance due to tyrosine 282 and a 0.5 ppm downfield shift for tyrosine 204. Does the binding of these molecules, which drastically modifies operator binding properties (2), affect the NMR observations of any of the N-terminal tyrosines? The interesting result is

the absence of any significant changes in the resonances corresponding to the N-terminal tyrosines when these allosteric effectors are bound.

Experiments are currently in progress to examine interactions with operator DNA, a variety of paramagnetic metals, and organic spin labels. These experiments with observations of ternary complexes of inducers and anti-inducer-repressor-operator DNA should shed more light on how this regulatory protein works.

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ION EFFECTS ON THE *LAC* REPRESSOR-OPERATOR INTERACTION

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The effects of ions on the binding of *lac* repressor protein and operator DNA have been studied using the membrane filter technique. The association and dissociation rate constants were measured, and the equilibrium association constants calculated, as a function of