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Triplet State of Tryptophan in Proteins: The Nature of the Optically Detected Magnetic Resonance Lines†

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ABSTRACT: Optical detection of magnetic resonance (ODMR) has been employed to examine the homogeneity of the tryptophan environment, both of the isolated residue in solvent, and of tryptophan in glucagon and lysozyme and azurin B (*Pseudomonas aeruginosa*). From the shifts in the zero-field splittings, we can safely conclude that tryptophan in lysozyme, azurin B, or glucagon does not have the same type of solvent interaction as the free residue. However, by "burning holes" in the ODMR lines, it is evident that the lines *in these cases* are inhomogeneously broadened. From the relative line widths and hole widths, it appears that ODMR can be used to examine the relative diversity of interactions for a luminescent amino acid in a protein. We have followed the ODMR line characteristics in a progression from free *N*-acetyl-L-tryptophanamide, to tryptophan in lysozyme, to "denatured" lysozyme, and present evidence that the line widths *narrow* as the tryptophan residues become less solvent accessible.

In the recent half decade, optical detection of magnetic resonance (ODMR)[1] (Sharnoff, 1967; Kwiram, 1967) has been introduced as a method for investigating the triplet state properties of tryptophan and tyrosine in proteins. Early studies in this field were carried out by Yamanashi & Kwiram (1970), and by Zuclich et al. (1972, 1973, 1974). The latter investigators reported that ODMR could be used to resolve tryptophan sites in proteins, particularly in lysozyme and horse liver alcohol dehydrogenase (HLAD).

HLAD is rare in that the two tryptophan sites have unique phosphorescence which allows them to be optically resolved (Purkey & Galley, 1970). von Schütz et al. (1974) noted a discontinuity in the zero-field-splitting parameters, $|D|$ and $|E|$, at the precise region of overlap of the two tryptophan phosphorescence peaks. In the case of lysozyme, there are six tryptophan residues but the optical spectrum shows no evidence for resolved sites. Yet, again discontinuities in $|D|$ and $|E|$ were found at a particular phosphorescence wavelength; specifically, a distinct ODMR doublet was observed. von Schütz et al. interpreted these results as evidence for at least two classes of tryptophan sites in both enzymes. However, they reported that the 100-MHz wide ODMR lines in lysozyme—in contrast to HLAD—were *homogeneously* broadened. This is rather surprising since one would expect magnetic resonance lines observed in condensed media to be *inhomogeneously* broadened as a result of a quasi-static distribution of interactions between the chromophores and their respective environments. (Unless, of course, there are unusual dynamic processes which facilitate spectral diffusion.) Moreover, if one assumes that the ODMR lines in lysozyme reflect the contribution of more than one distinct tryptophan site, then the

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[1] Abbreviations used: ODMR, optical detection of magnetic resonance; zfs, zero-field splitting, ELDOR, electron double resonance; CW, continuous wave; HLAD, horse liver alcohol dehydrogenase; Gdn·HCl, guanidine hydrochloride.

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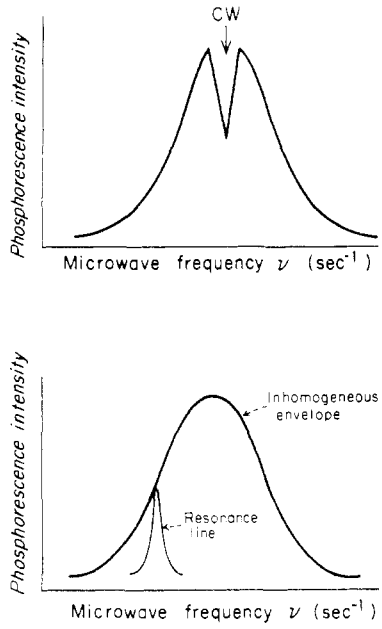

FIGURE 1: (Top) Simulated "hole-burning" in an inhomogeneous line. (Bottom) Superposition of smaller resonance "homogeneous lines" to form inhomogeneous envelope (schematic).

resonance envelope will have contributions from all *emitting* tryptophan residues each in turn broadened by the variations in its local environment. Thus one would certainly expect the ODMR lines to be inhomogeneously broadened.

In our investigations (Rousslang, 1976; Ross, 1976; Ross et al., 1977), we have found that the ODMR lines in tryptophan and in a variety of tryptophan containing peptides and proteins are inhomogeneously broadened. Therefore we decided to reinvestigate the nature of the ODMR lines in lysozyme in order to evaluate the degree of inhomogeneity. We wish to present here results which suggest that the ODMR lines in lysozyme are also inhomogeneously broadened. Moreover, we find a correlation between the total ODMR line widths and the line widths of the holes that can be burned in these transitions. We shall also discuss the relationship of the line widths, both homogeneous and inhomogeneous, to the degree of local disorder in the molecular environment of proteins, smaller polypeptides, and selected amino acid models.

Experimental Section

The "Hole-Burning" Experiment. A homogeneous magnetic resonance line (Portis, 1953) is one for which each observed molecule is indistinguishable from another, and the line width reflects one class of spins broadened by interactions or mechanisms external to the spin system, but fluctuating rapidly compared with the time required for a magnetic transition. The homogeneous line represents the minimum intrinsic line width that the system can exhibit. In fact, the molecules in a macroscopic sample are rarely indistinguishable since the environment will vary from one site to the next. Thus an inhomogeneous line will be the superposition of many homogeneously broadened resonance lines (Figure 1), *each* arising from molecules experiencing a slightly different local environment.[2] Experimentally, one distinguishes between homogeneous and inhomogeneous lines by carrying out so-called "hole-burning" experiments.

In a hole-burning experiment, continuous wave (CW) rf power at frequency ω is applied near the center of the ODMR line at a power level sufficient to cause saturation. Microwaves from a second source are then swept slowly through the line. If the entire line is uniformly diminished in height, the line is said to be *homogeneously* broadened. If, however, the spins affected by the fixed frequency constitute only an isolated portion (a spin packet) of the total unresolved line width, then the normal response of the spin system, monitored with low level (nonsaturating) rf, will reveal a dip in the intensity at the frequency ω (Figure 1). This "hole-burning" is taken as evidence that the line is *inhomogeneously* broadened.

Usually, the actual hole width observed does not reflect anything but an upper limit on the homogeneous line width, because the width of the hole depends not only on the power and band width of the rf, but on spin diffusion as well. For example, an inhomogeneously broadened line may *appear* to be homogeneously broadened if spin diffusion (Dalton et al., 1972) allows energy to be transferred between nonidentical triplets at a rate fast compared with the experimental observation time. To distinguish between the *intrinsic* homogeneous line width of a spin packet and the line width of the dip *observed* in the hole-burning experiment, we shall refer to the latter as the "homogeneous" line width (i.e., we shall use quotation marks to indicate that this is an apparent line width—not necessarily a minimum line width).

Method of Measurement. The general optical and microwave arrangements for the steady-state and delayed ODMR experiments have been given in a preceding paper (Ross et al., 1977). The two microwave sources for the ELDOR (electron double resonance) experiment (Kuan et al., 1970) were simultaneously coupled to a rigid coaxial cable which terminated in a helix surrounding the sample. Model 8620 B and 8690 B microwave oscillators (Hewlett-Packard, Inc., Palo Alto, Calif.) were used as the swept microwave sources, while a Model 624 BK Alfred backward wave oscillator (Singer, Palo Alto, Calif.) provided the fixed-frequency microwaves. The bandwidth typically was 50 kHz (roughly three orders of magnitude narrower than the smallest hole width observed). The swept microwaves were generally not amplified and power levels were maintained between 10 and 25 mW. The CW power levels necessary to burn holes rarely exceeded 5 mW, and power levels exceeding 40 mW produced both wider and deeper holes, adding no new information.

Materials. Glucagon, purchased from Elanco products, was purified by ion-exchange chromatography (Ross, 1976). Lysozyme was obtained from both Worthington Biochemical (salt-free preparation 2× recrystallized) and Sigma, and the disulfide bridges were reduced and S-aminoethylated as described by Cole (1967). Azurin B (*Pseudomonas aeruginosa*) was a gift from Professor J. Herriott, University of Washington, Seattle, Wash. *N*-Acetyltryptophanamide was purchased from Fox Chemical. Ethylene glycol, from Matheson Coleman and Bell, was chromatoquality (99+ mol %). All other chemicals were reagent grade.

Results

The result of a hole-burning experiment in a frozen solution of native lysozyme is shown in Figure 2. The power level of the saturating microwave source was held at about 1 mW incident on the helix. The effective bandwidth was 5 kHz, approximately 0.1% of the minimum hole width we observed. Similar results are obtained for the other transitions. In other words, application of fixed frequency (CW) microwaves of sufficiently

[2] This can take the form of differences in the solvent (electrostatic) environment, or, as in the case of mixed crystals, differences in the spin (magnetic) environment, or, as in the case of macromolecules, differences in the intramolecular environment due to a distribution of local structure within the the macromolecule (due to the multiplicity of nearly degenerate potential minima).

TABLE I: ODMR Line Characteristics for Different Tryptophan Environments.

Sample		*N*-Acetyl-tryptophan-amide	Glucagon	Lysozyme	Azurin B
$\|D-E\|$	Frequency (GHz)[a]	1.767	1.682	1.577	1.630
	Line width (MHz)[b]	141	94	83	51
	Hole width (MHz)[b]	26	19	10	8
$\|2E\|$	Frequency (GHz)[a]	2.431	2.592	2.731	2.785
	Line width (MHz)[b]	206	156	136	70
	Hole width (MHz)[b]	15	11	9	10
	$\|D\|$ (cm^{-1})	0.0992	0.0993	0.0981	0.1008
	$\|E\|$ (cm^{-1})	0.0402	0.0432	0.0455	0.0465

[a] Average maximum position of magnetic resonance spectra at 1.3 K, monitored at 432 nm with excitation at 297 nm. Estimated precision of data is ±7 MHz. [b] The $|D-E|$ and $|2E|$ line widths full width at half-height are reported for scans of 7 and 14 MHz/s, respectively, and are precise within ±7% for the specified scan rates. The hole widths are reproducible within ±1 MHz.

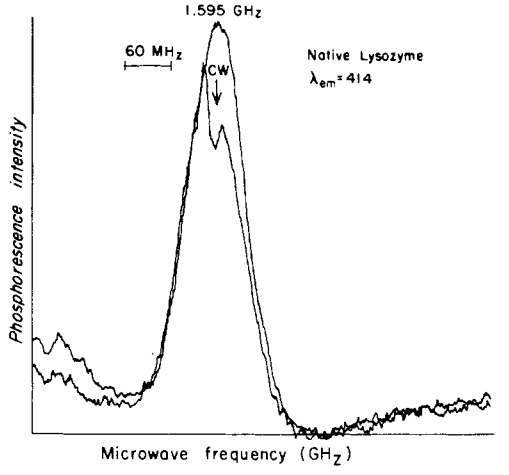

FIGURE 2: "Hole-burning" of tryptophan in native lysozyme solution. First trace represents normal slow passage ODMR signal (swept up in frequency). Second trace is identical with first except CW microwaves are applied at 1.595 ± 2.5 × 10^{-6} GHz; signal is not diminished in total intensity except in a narrow region ca. 10 MHz about 1.595 GHz.

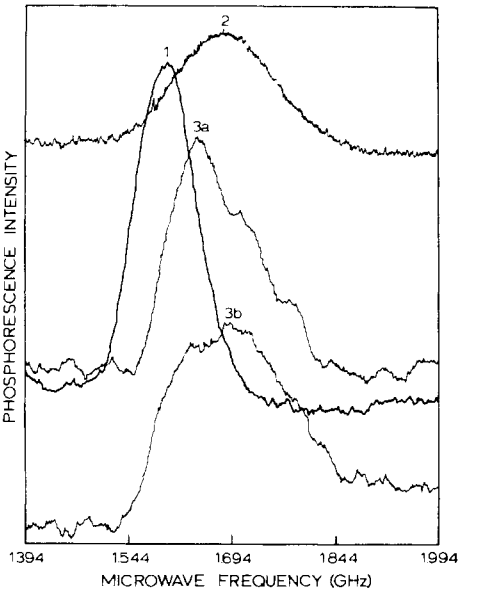

FIGURE 3: ODMR as a function of "denaturation". (1) Native lysozyme, λ_{em} = 437 nm. (2) Reduced and alkylated lysozyme in 6 M Gdn·HCl, λ_{em} = 443 nm. (3a) Lysozyme in 6 M Gdn·HCl, λ_{em} = 406 nm. (3b) Lysozyme in 6 M Gdn·HCl, λ_{em} = 435 nm. All signals are attributed to the $|D-E|$ transition of tryptophan in lysozyme. Each line is inhomogeneously broadened, and in the case of 3a and 3b each peak in the structured line exhibited "hole-burning". Note the relative intensity of the peaks as a function of emission wavelength in 3a and 3b. λ_{ex} = 297 nm in all cases.

narrow bandwidth at the center or any part of any transition resulted in "burning a hole" in the ODMR line; CW microwaves applied at any position in the lines communicated to only a portion of the spins in that line.

Table I lists the ODMR line characteristics for four contrasting tryptophan environments: *N*-acetyltryptophanamide, Trp-25 in glucagon, tryptophan in lysozyme, and tryptophan in azurin B.

There are several important features. Both the inhomogeneous ODMR line widths and the homogeneous line widths increase in the order: azurin B, lysozyme, glucagon, *N*-acetyltryptophanamide; paralleling this is a significant decrease in the zero-field-splitting parameter $|E|$. Furthermore, whereas $|D|$ remains the same for *N*-acetyltryptophanamide and glucagon, it decreases for lysozyme and increases for azurin B.

It has not been determined whether the phosphorescence arises from a single or several tryptophan residues in native lysozyme (which contains six tryptophans). In the case of the polypeptide hormone glucagon which contains a single tryptophan residue (Trp-25) that is at least partially exposed to the solvent, the line width is roughly 100 MHz ($|D-E|$). The same ODMR line width in lysozyme is 80 MHz. Even narrower lines of ~40 MHz are reported by Ugurbil et al. (1977) for the single fully buried tryptophan residue in azurin from *Pseudomonas aeruginosa*. However, they report that the line is *homogeneously* broadened. By contrast, we find that the lines in azurin (again, *Pseudomonas aeruginosa*) are inhomogeneously broadened with a line width of about 50 MHz (scan rate as in lysozyme, see Table I) and the holes burned in this transition are roughly 10 MHz wide as in lysozyme. These line widths, both homogeneous and inhomogeneous, may be characteristic of tryptophan residues buried in proteins. In order to explore this question further, we carried out a number of experiments in which we deliberately perturbed the conformation of lysozyme.

The results of denaturing lysozyme in 6 M Gdn·HCl are shown in Figure 3. In contrast to the single narrow line observed for native lysozyme, the denatured lysozyme lines are much broader and show structure when the disulfide bridges are still intact. The ODMR line obtained by monitoring the optical emission at 406 nm contains at least three distinct peaks. The relative intensity of these peaks changes if the

emission is monitored at 435 nm. Further, when the disulfide bridges are broken by reduction and alkylation, the ODMR line is very broad and exhibits no discernible structure regardless of the monitored phosphorescence wavelength.

Discussion

Inhomogeneous Broadening. Steady-state ODMR signals of isolated indole or tryptophan in frozen 50% (v/v) aqueous ethylene glycol are typically 150–300 MHz wide (von Schutz et al., 1974; Zuclich et al., 1974; Rousslang & Kwiram, 1976). The total line widths of tryptophan in glucagon and lysozyme are much narrower.

von Schütz et al. (1974) observed 100-MHz wide lines for the ODMR of tryptophan in lysozyme and reported that the lines are homogeneously broadened. They attributed this to "motional averaging of the zero-field parameters" and ruled that spin diffusion would probably not be effective over 100-MHz wide lines. Ugurbil et al. (1977) argued that the apparent homogeneous character of the ODMR line of tryptophan in azurin is a result of (low-temperature) proton tunneling.

The evidence from our hole burning experiments shows that, even in lysozyme, the ODMR lines are inhomogeneously broadened. In fact, in all of the tryptophan containing polypeptides and proteins that we have examined, the ODMR lines are inhomogeneously broadened. This is consistent with what we would expect, barring unusual dynamic processes. Our results would seem to obviate the need for postulating any such special processes at 1.2 K.

It is difficult to comment on the reasons for the discrepancy between our results on the inhomogeneous character of the lysozyme ODMR lines and the homogeneous properties reported by von Schütz et al. (1974), since these authors do not give full details regarding the experimental results.[3] It is possible that there are differences in the samples themselves. There is also the possibility that the microwave field distribution was sufficiently nonuniform so that substantial regions of the sample were not sufficiently saturated. Since the holes are not easy to observe, any portions of the sample which remain unsaturated would tend to mask the presence of a hole. We have encountered this problem in connection with studies to determine absolute values for the intersystem crossing rate constants associated with the triplet state (Leenstra & Kwiram, unpublished results). It might also be noted that sweep rates which are too rapid[4] will tend to fill in the hole as well. None of these factors may be relevant to the results reported earlier, but they are factors which must in general be carefully considered.

The Line Width of Native Proteins. As mentioned earlier, the inhomogeneous line width ($|D - E|$) decreases in going from *N*-acetyltryptophanamide (141 MHz) to glucagon (94 MHz) to lysozyme (83 MHz) to azurin B (51 MHz). This is paralleled by the decrease in the "homogeneous" line width (Table I). The width of the inhomogeneous ODMR lines is caused in general by the distribution of electric fields generated by the local environment at each of the emitting species. In proteins, the character of these fields is determined by two interrelated contributions: intermolecular or solvent interactions, and intramolecular interactions due to the effects of primary, secondary, and tertiary structure. Thus the inhomogeneous line width arises from the distribution of structural conformations and solvent effects. In other words, in solution (and probably to some extent in the crystal) the molecule undergoes fluctuations in structure and thereby samples a variety of conformations within a range of thermally accessible states defined by the multidimensional potential energy surface. Upon freezing a solution one obtains a distribution of conformations.[5] For small polypeptides containing only one tryptophan residue (e.g., glucagon), the solution conformation could provide a variety of similar but distinct local environments for the tryptophan residue as a consequence of structural fluctuations; a given residue therefore will experience a range of intramolecular interactions (the conformational distribution), and for each conformation a range of solvent interactions. Thus the ODMR line width should reflect the range of intramolecular and intermolecular interactions. For those proteins which contain several different tryptophan residues (e.g., lysozyme), one simply sums the contribution from each residue.

The experimental results suggest that the overall line width can be related to the degree of solvent availability (accessibility) of the phosphorescent residues. This is not unexpected since the solvent (water) with its rather large dipole moment would make a large contribution to the local field. The contributions from nearby residues which are either polar or charged might be smaller in general but the *range* of such contributions would be severely restricted by the intrinsic structure of the protein.[6] Obviously, as the range of accessible conformations decreases, the distribution of local fields experienced by a given tryptophan residue will also decrease. In other words, when the solvent is present we would expect it to dominate the line width. Hence, we would expect exposed tryptophan residues to experience a wide distribution of solvent interactions, and have a broad ODMR line. Buried residues will see a smaller distribution of solvent interactions and consequently a narrower ODMR line. We might expect that residues in a completely hydrophobic environment would exhibit the narrowest but nonetheless inhomogeneous ODMR line. As mentioned earlier, the tryptophan residue(s) responsible for *phosphorescence* in lysozyme are not known, although it has been experimentally determined that most of the *fluorescence* in lysozyme arises from Trp-62 and Trp-108 (Imoto et al., 1972). From the x-ray crystal structure of lysozyme (Blake et al., 1967), it is apparent that each tryptophan residue occupies a unique site. Trp-62 and Trp-63 are at the active site and close enough to interact with each other, although in solution only Trp-62 is largely exposed to solvent (Hinman et al., 1974). Based on the triclinic crystal structure model, it appears that Trp-108 is mostly buried in the tertiary structure and is only minimally exposed to solvent (Hodgson, J. C., & Jensen, L. A., personal communication). The remaining three tryptophans are completely buried and not freely accessible to solvent aside from bound water in the native protein. Consequently, based upon what is known about the conformation of lysozyme and the different tryptophan environments, there are at least two possible explanations for the narrow inhomogeneous ODMR lines in lysozyme: we are either observing wholly buried residues (solvent inaccessible), or both buried

[3] Their observation was not simply a negative result; they reported a uniform *decrease* of the entire 100-MHz wide ODMR line when they saturated a narrow region of the line.

[4] The sweep rates required to fill in the holes are nearly 50 MHz/s at which point the line shape is severely distorted by the life-time of the excited state.

[5] The process of cooling will, of course, change the range of accessible states. Moreover, the freezing process may of itself introduce new conformational distortions.

[6] There are other large dipoles to consider in a peptide. The dipole moment of the peptide bond is nearly twice that of water; consequently the dipolar field at a side chain such as tryptophan due to the peptide bond might be significant.

and *partially* exposed residues whose range of solvent environments is severely limited.

The Line Width of Denatured Protein. From the large shift in $|D|$ and $|E|$ between *N*-acetyltryptophanamide and Trp-25 in glucagon or tryptophan in native lysozyme (Table I), it appears obvious that the tryptophan residues in native lysozyme are not in the same type of environment as the fully solvated model compound or Trp-25 (see Ross et al., 1977). It is not surprising that the ODMR lines of *denatured* lysozyme are shifted with respect to that of the native molecule. The fact that at least three major ODMR peaks ($|D - E|$) are present after denaturation with Gdn·HCl is consistent with the observation by Coan et al. (1975) that Gdn·HCl does *not* completely unfold lysozyme. They find from charge transfer studies at room temperature that there is a correlation between the apparent degree of solvent exposure of tryptophan residues and their average position in the cross-linked polypeptide chain. Thus it is possible that even in denaturing solvents the ODMR data could be interpreted in terms of residual structure in the protein resulting in distinctly different exposure of aromatic residues. We take the structure in the denatured lysozyme ODMR line to be evidence that more than one class of tryptophans is emissive in partially unfolded lysozyme. However, it should be emphasized that there is *no* evidence for emission from different tryptophan residues in the phosphorescence spectrum. As seen in Figure 4, denaturation simply reduces the sharpness of the vibrational structure producing broadening of the optical line and a marked blue shift of the overall envelope.

The relative exposure of tryptophan in denatured lysozyme with intact vs. broken disulfide bridges has also been examined by Coan et al. (1975). They find that the closer an aromatic residue is to a cross-link, the more restricted its apparent exposure. The peaks observed in the $|D - E|$ transition of denatured lysozyme range between the frequency of *N*-acetyltryptophanamide and native lysozyme. Reduction and alkylation, which facilitate unfolding and relax the steric restriction on tryptophan side chains near the disulfide bridges, eliminate the structured ODMR line and produce a single broad peak. The frequency of this line is not identical with that of *N*-acetyltryptophanamide. This may reflect the fact that there are still interactions present which do not permit completely free access by solvent to those residues which contribute to the phosphorescence. Indeed the line width of the transition is greater than that of any tryptophan-containing polypeptide or model compound. This is most likely due to the overlap of several slightly differing zf transitions. These belong to residues which after alkylation and reduction of the disulfide bridges are now largely solvent available yet experiencing slightly different local environments (the intramolecular interactions contribution).

In conclusion, we would like to reiterate that, in glucagon, azurin B, and in all native and denatured lysozyme solutions, we observed hole-burning in each of the ODMR lines. Evidently, a distribution of tryptophan environments gives rise to inhomogeneously broadened lines in all these systems. Even for the structured ODMR lines in solutions of denatured lysozyme, hole-burning could be demonstrated at *any* position in the lines indicating that each tryptophan emitting site in lysozyme experiences a distribution of local environments, at least in the "unfolded" form. Finally, since the relative ODMR line widths in all the tryptophan-containing samples we studied reflect the degree of order in the local environment, we expect that the ODMR data such as line width and line position can be used as a relative measure of the distribution of conformations and the integrity of the protein structure. Further studies involving chemical modification of particular tryptophan residues in lysozyme are underway in an effort to establish more fully the structural characteristics we have outlined in this manuscript.

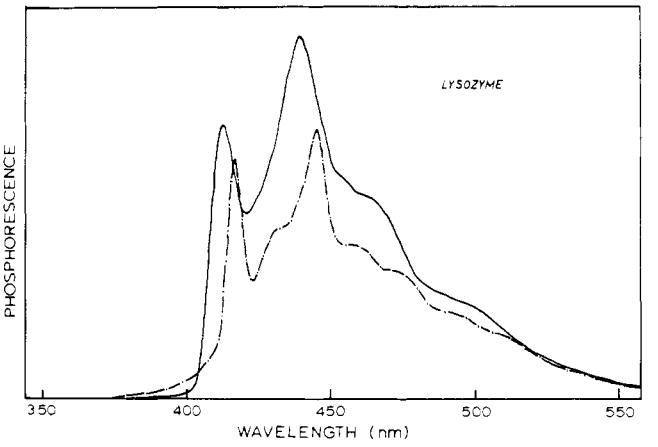

FIGURE 4: Phosphorescence, in arbitrary units, of native lysozyme (- - -) and lysozyme denatured in 6 M Gdn·HCl (—).

Acknowledgments

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Primary Structure of the λ Repressor†

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ABSTRACT: The complete covalent structure of the bacteriophage λ repressor has been determined by sequential Edman degradation, gas chromatographic–mass spectrometric peptide sequencing, and DNA sequencing of the repressor gene *c*I. The repressor is a single-chain, acidic protein containing 236 amino acids. The amino terminal 40 residues are highly polar and basic. Lysines and arginines in the sequence tend to be clustered.

There are many proteins which recognize specific nucleotide sequences within double stranded DNA (for review, see von Hippel & McGhee, 1972; Jovin, 1976). The λ repressor is a particularly well-studied example of this class of molecules.

λ repressor, the product of the *c*I gene of bacteriophage λ, binds strongly to multiple sites in the phage DNA. The repressor binding sites are organized into two independent operators, several thousand base pairs apart. Each operator contains three sequences which have been identified as repressor contact sites (Maniatis et al., 1975a; Humayun et al., 1977). A site consists of a 17 base pair sequence, with an axis of approximate twofold rotational symmetry through the ninth base. The binding sites are similar but not identical in nucleotide sequence and vary in their affinity for repressor (Flashman, 1976).

Repressor bound to certain of the sites sterically prevents RNA polymerase from binding to promoter sequences in the DNA (Steinberg & Ptashne, 1971; Meyer et al., 1975; Walz & Pirrotta, 1975). In this way, repressor can prevent transcription of two major phage operons, as well as transcription of its own gene *c*I. Under conditions in which only the strongest repressor binding sites are occupied, repressor stimulates *c*I transcription (Meyer et al., 1975; Ptashne et al., 1976; Walz et al., 1976). Thus repressor is both a positive and a negative gene regulator, and its function depends upon which operator sites or combination of sites are occupied. Site occupation, in turn, is a function of the concentration of repressor in the cell.

The repressor molecule is a single chain acidic protein with a monomer molecular weight of 27 000. Monomers of repressor are in equilibrium with dimers at typical intracellular concentrations (Pirrotta et al., 1970). The dimer is the species which binds strongly to the operators (Chadwick et al., 1970). Higher oligomeric forms of repressor have been observed at concentrations 100-fold greater than that in a single λ lysogen (Brack & Pirrotta, 1975). However, the relevance of these oligomers to repressor action is uncertain.

Repressor allows phage λ to reside inactively in the chromosome of its host bacterium by preventing synthesis of phage proteins necessary for the development and growth of the phage. Treatment of the bacterium with ultraviolet light, or a number of other agents (for review, see Witkin, 1976) results in inactivation of repressor and thus lytic growth of the phage. Roberts & Roberts (1975) have shown that during this "induction" process the λ repressor is cleaved, and presumably inactivated, by a protease. Many phages related to λ are also inducible. At present it is not known whether a single protease can inactivate the repressors of these phages, but it is possible that phage repressors share a common structural element which allows them to be induced via the same pathway.

Both the λ and P22 repressors can be inactivated in a noncovalent manner by the antirepressor protein of phage P22 (Susskind & Botstein, 1975). This recognition of λ repressor by a protein from a different but related phage argues that at least the lambda and P22 repressors must share some common structure.

Repressors from phages 434 and 21 have been isolated and partially characterized (Pirrotta & Ptashne, 1969; Ballivet et al., 1977). These molecules are similar to λ repressor in their monomer molecular weights, and both appear to oligomerize. Furthermore, limited homology between the λ and 434 *c*I genes has been shown by heteroduplex mapping (Westmoreland et al., 1969).

We would like to understand more fully how the phage repressors are related and how their structures are involved in the molecular interactions discussed. As a first step in this process, we have determined the amino acid sequence of the λ repressor. To do this we have used two independent methods of protein sequencing: Edman degradation and the newer method of peptide sequencing by a gas chromatographic–mass spectrometric technique. In addition, we have sequenced a portion of the DNA of the repressor *c*I gene in order to complete the protein sequence.

Materials and Methods

Repressor Purification. The repressor used for these studies was purified from the *E. coli* strain 294/pKB252 (Backman et al., 1976). Cells were suspended in buffer containing 100

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