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Proton Nuclear Magnetic Resonance Assignments and Surface Accessibility of Tryptophan Residues in Lysozyme Using Photochemically Induced Dynamic Nuclear Polarization Spectroscopy†

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ABSTRACT: Tryptophan resonances in the 360-MHz ^1H photochemically induced dynamic nuclear polarization spectrum of hen egg white lysozyme are investigated in detail. All resonances of one tryptophan and six of another are identified and assigned to their respective protons. The methods employed, all involving nuclear spin polarization, include the study of cross-relaxation effects and the use of selective radio-frequency irradiation, Gd^{3+} as a paramagnetic probe, and riboflavin as the chemically induced dynamic nuclear polarization generating dye. From a comparison of the experimental results with the known X-ray structure of lysozyme, second-stage assignments of the two tryptophan residues (Trp-62 and Trp-123) are proposed. A number of other resonances are characterized, among them Trp-63 C(2)H and four indirectly polarized methyl groups.

An essential prerequisite for a nuclear magnetic resonance (NMR)[1] investigation of the structure and properties of a molecule is the assignment of its NMR spectrum, that is, the identification of each spectral line with the nucleus responsible for it. In large molecules such as proteins, this task can be quite formidable, and one has often to settle for a partial assignment of the total spectrum. Among the many methods developed to tackle this problem (Campbell et al., 1975; Dobson, 1977; Jardetzky & Roberts, 1981; Wüthrich et al., 1982; Wagner & Wüthrich, 1982; Wider et al., 1982), an approach which is specific for certain aromatic amino acid residues on the surface of a protein is that of photochemically induced dynamic nuclear polarization (photo-CIDNP) (Kaptein, 1978, 1982). The method depends on reversible photochemical reactions of the protein with a dye to generate nuclear spin polarization, and hence NMR intensity enhancements, in the side chains of reactive residues. If accessible to the photoexcited dye (usually a flavin), tyrosine, histidine, and tryptophan can be polarized in this way. Subtraction of "light" and "dark" protein spectra gives a vastly simplified spectrum containing only polarized resonances (Kaptein, 1978).

The three residues have characteristic photo-CIDNP spectra which are easily distinguished. First-stage assignment (to a specific type of proton) of His and Tyr resonances is straightforward, owing to the simplicity of the spin systems, while Trp residues, in which all five aromatic protons become polarized, can be more awkward especially when, as in lysozyme, more than one Trp is accessible to the dye.

In a previous photo-CIDNP investigation of hen egg white lysozyme (Kaptein, 1978), two polarized Trp residues were found, and some tentative assignments were made, which are now shown to be partially incorrect. In what follows, we describe new experimental work designed to arrive at a more convincing set of assignments.

Materials and Methods

Photo-CIDNP Spectra. All experiments were performed at 360 MHz on a Bruker HX-360 NMR spectrometer controlled by a BNC 12 or an Aspect 2000 computer. The photo-CIDNP technique has been described previously (Kaptein, 1978; Kaptein et al., 1978), the only modification being the use of an optical fiber in some of the measurements to couple the argon laser beam into the NMR probe. As before, light pulses are controlled from the computer by means of a mechanical shutter.

"Light" and "dark" spectra are recorded alternately and subsequently subtracted to give a photo-CIDNP difference spectrum in which unpolarized resonances do not appear. Typically, five scans were necessary to obtain an acceptable signal to noise ratio; a CIDNP spectrum of 1 mM protein could thus be acquired in less than 2 min.

All spectra, except those required for chemical shift measurements, were recorded at 25 °C to ensure reasonable

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[1] Abbreviations: NMR, nuclear magnetic resonance; CIDNP, chemically induced dynamic nuclear polarization; NOE, nuclear Overhauser effect; rf, radio frequency; ppm, parts per million; flavin I, 7,8,10-trimethyl-3-carboxymethylisoalloxazine; DSS, sodium 4,4-dimethyl-4-silapentane-1-sulfonate.

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Table I: Expected Polarizations for Tryptophan Protons

proton	multiplicity[a]	polarization
C(2)H	S	direct
C(4)H	D	direct
C(5)H	T	indirect
C(6)H	T	direct
C(7)H	D	indirect

[a] S, singlet; D, doublet; T, triplet.

cross-relaxation rates within the polarized residues. Resolution enhancement was achieved with the Lorentzian to Gaussian transformation (Ferrige & Lindon, 1978; Lindon & Ferrige, 1979).

Preparation of Samples. Henn egg white lysozyme (Sigma Chemical Co., 3 times crystallized, dialyzed, and lyophilized) was heated to 70 °C for 10 min in D_2O to exchange labile protons and subsequently freeze-dried. Removal of acetate from the lysozyme solutions had no noticeable effect on the CIDNP spectra: this purification step was therefore not employed in the present work. Flavin I (7,8,10-trimethyl-3-carboxymethylisoalloxazine) was the generous gift of Dr. F. Müller (Wageningen); riboflavin (Sigma) was used as supplied.

NMR samples of volume 300 μL consisted of 1 mM protein and 0.1–0.4 mM flavin in 99.75% D_2O. A fresh sample was used for each measurement. pH values were not corrected for the deuterium isotope effect. Chemical shifts, measured by using dioxane as a reference (3.743 ppm from DSS at 54 °C, pH 5.3), are quoted in ppm downfield from DSS.

Assignment Methods. First-stage assignment (Dobson, 1977) of Trp resonances in photo-CIDNP difference spectra is in principle straightforward. Two types of CIDNP can be distinguished: direct polarization created in the radical pair intermediates of the photoreaction and indirect or cross-polarization which is derived from it by cross-relaxation (Closs & Czeropski, 1977; de Kanter & Kaptein, 1979; Hore et al., 1982). As shown in Table I, knowledge of the multiplicity of a resonance together with the nature of its polarization is sufficient to assign all five aromatic Trp protons unambiguously.

The two kinds of polarization are recognized by their characteristic dependence on either the duration the light pulse or the delay which follows it prior to the observing rf pulse (Egmond et al., 1980; Berliner & Kaptein, 1981; Hore et al., 1982). The clearest effect occurs on increasing this waiting period after a short (≤0.1 s) pulse of light.

To reveal which nuclei enjoy a mutual dipolar interaction, we selectively saturated chosen resonances during the illumination period to suppress the formation of any cross-polarization arising from them (Closs & Czeropski, 1977). The difference between photo-CIDNP spectra with and without selective rf irradiation is therefore a spectrum containing only resonances from the saturated spin and those protons physically close to it.

Further evidence that given resonances arise from the same Trp residue was obtained by using a dye (riboflavin) with selectivity different from that of flavin I and by performing the experiment in the presence of a lanthanide relaxation agent (Gd^{3+}) with a well-defined binding site (Campbell et al., 1973). Such observations also provide clues to second-stage assignments.

Results

Resolution of Resonances. A photo-CIDNP difference spectrum of 1 mM lysozyme in the presence of 0.1 mM flavin I is shown in Figure 1 together with the corresponding dark

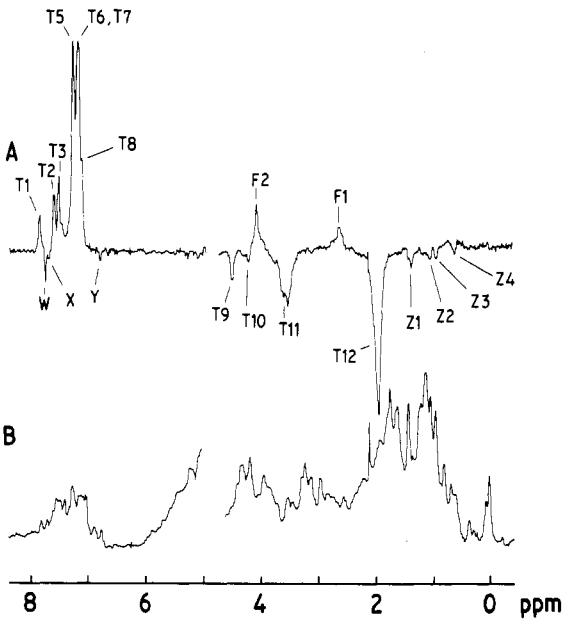

FIGURE 1: (A) Photo-CIDNP difference spectrum of 1 mM lysozyme with 0.1 mM flavin I in D_2O. The following conditions were employed: pH 4.5, 25 °C, 0.6-s light, 0.05-s delay, five scans, 5-W argon laser power. (B) The corresponding dark NMR spectrum. The HDO resonance at 4.8 ppm has been omitted from both spectra.

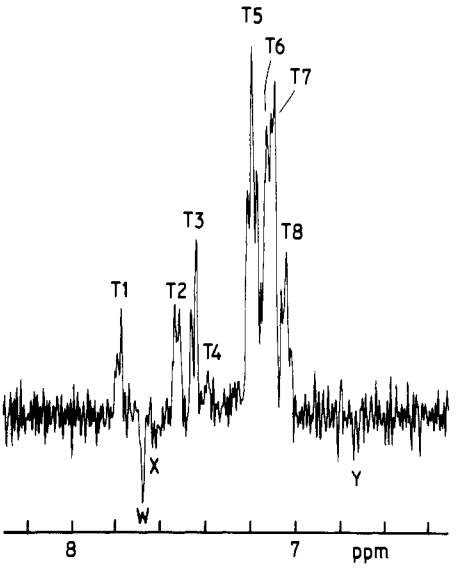

FIGURE 2: Resolution-enhanced aromatic region of the photo-CIDNP spectrum shown in Figure 1A.

NMR spectrum. The main features of Figure 1A are a group of absorptively polarized lines, labeled T1–T8, in the region of 7–8 ppm and four emissive peaks, T9–T12, between 1.5 and 4.5 ppm. Most of these have been observed previously (Kaptein, 1978) and identified as tryptophan resonances on the basis of their polarizations (absorption or emission) and the insensitivity of their chemical shifts to changes in pH. In addition, the two broad absorption lines (F1 and F2) arise respectively from the C(10) and C(8) methyls of the flavin dye. Other, weaker, signals are to be found in the aromatic region (W, X, and Y) and at high field around 1 ppm (Z1–Z4); all are emissively polarized.

The excellent signal to noise ratio in Figure 1A permits resolution enhancement, as shown in Figure 2 for the aromatic part of the CIDNP spectrum. The multiplicities of many of the lines are thus revealed as is the presence of an extra small resonance (T4) observable as a shoulder on the flank of T3

Table II: Resonances Observed in the Photo-CIDNP Spectrum of Lysozyme[a]

resonance[b]	polarization[c]	multiplicity[d]	chemical shift (ppm)
T1	A	D	7.75
T2	A	D	7.51
T3	A	D	7.44
T4	A	D	7.35
T5	A	T	7.18
T6	A	D	7.09
T7	A	S	7.06
T8	A	T	7.02
T9	E		4.40
T10	E		4.12
T11	E		3.52, 3.38
T12	E		1.84
T13	A	S	7.54
W	E	S	7.65
X	E		7.61[e]
Y	E	D	6.70
Z1	E		1.25
Z2	E		0.94
Z3	E		0.83
Z4	E		0.54
F1	A	S	2.48
F2	A	S	4.02
F3	E	S	7.87

[a] Chemical shifts, quoted in ppm downfield from DSS, were measured at pH 5.3, 54 °C, with 1 mM lysozyme and 0.1 mM flavin I. [b] See Figures 1 and 2. [c] A, enhanced absorption; E, emission. [d] S, singlet; D, doublet; T, triplet. [e] Chemical shift at 25 °C; this resonance was not observable at 54 °C.

in Figure 1A. Moreover, a peak earlier assigned to the Trp-62 C(6) proton (Kaptein, 1978) in fact consists of two close, overlapping resonances, a doublet (T6) and a singlet (T7).

A considerable improvement in resolution of the aromatic resonances was found on reduction of the amount of dye used to produce CIDNP. For example, in a spectrum recorded with 0.4 mM flavin I, the low-field doublet (T1) had a total line width of ~20 Hz which, in the presence of 0.1 mM flavin, fell to ~15 Hz. Lowering the concentration to 0.05 mM produced no further narrowing although slightly better resolution was apparent elsewhere in the aromatic region. The resonances observed in the photo-CIDNP spectra of lysozyme are summarized in Table II together with their chemical shifts (at 54 °C and pH 5.3), polarizations, and multiplicities.

Cross-Polarization. Several CIDNP spectra of the aromatic protons of lysozyme are presented in Figure 3. Spectra B–F show the effect of increasing the delay after a 0.1-s light pulse from 0.05 to 0.25 s while spectrum A is the spectrum obtained with a 0.6-s illumination and a 0.05-s delay. It is plain from spectra B–F that T3 (doublet) and T8 (triplet) are cross-polarized, becoming more intense with increasing delay. These resonances must therefore arise from C(7)H and C(5)H Trp protons, respectively (see Table I). Similarly, T2 and T5–T7 are clearly directly polarized and may therefore be assigned to C(4)H, C(6)H, C(4)H, and C(2)H protons, respectively, by using the information in Tables I and II.

The origin of T1, which shows little change in spectra B–F, is less certain. Like T3 and T8, it is more intense, relative to the directly polarized lines T5–T7, in spectrum A than in spectrum B although the effect is perhaps not so pronounced. Moreover, the intensity of T1 as a function of the illumination time exhibited features of both direct and indirect enhancement. Of the remaining lines, T11 and T12 [both Trp β-CH_2 protons (Kaptein, 1978)], T13 and Y are all directly polarized while T9, T10, W, and Z1–Z4 are the result of cross-polarization.

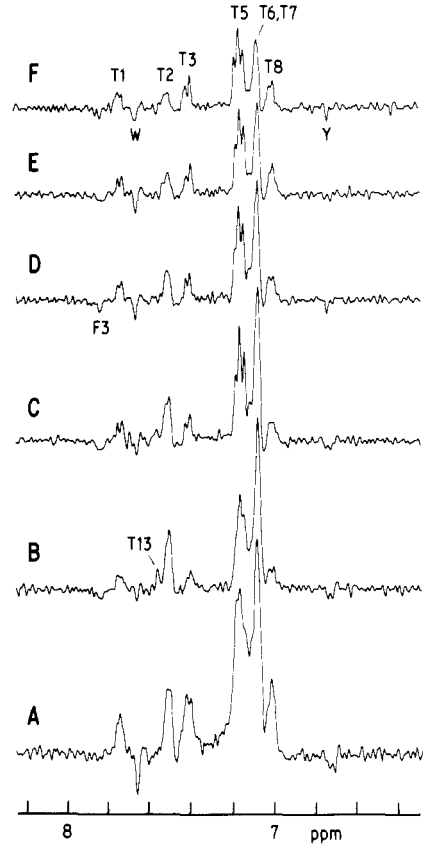

FIGURE 3: Resolution-enhanced aromatic region of photo-CIDNP spectra of 1 mM lysozyme with 0.4 mM flavin I in D_2O. The following conditions were employed: pH 4.5, 25 °C, five scans each, 5-W argon laser power. 0.6-s light and 0.05-s delay (A); 0.1-s light and 0.05-s delay (B); 0.1-s light and 0.10-s delay (C); 0.1-s light and 0.15-s delay (D); 0.1-s light and 0.20-s delay (E); 0.25-s delay (F).

Selective rf Irradiation. Figure 4 shows the aromatic region of the photo-CIDNP spectrum without rf irradiation (A) and *with* irradiation at the position of T12 (B) and T11 (C) during a 0.6-s light pulse. Spectra D and E of Figure 4 are the differences between spectra B and A and between spectra C and A, respectively.

From Figure 4D, it is clear that the cross-polarized line W receives its enhancement from T12. In agreement with this, a NOE from T12 to W has been observed. Similarly, either or both of T6 and T7 are close enough to these β-CH_2 protons to acquire some of their polarization. This is consistent with our assignment of T6 and T7 to Trp (C4)H and C(2)H, respectively, and establishes that one or both must be in the same residue as T12.

In Figure 4E, a broad line appears covering the positions of both X and T13. The absence of T13, therefore, from Figure 4A must be due to polarization transfer from T11 (Trp β-CH_2) which almost completely cancels T13's direct enhancement as well as causing the rapid disappearance of T13 apparent in Figure 3B–F. Taken with the narrowness of this resonance, these observations strongly suggest a Trp C(2)H proton.

When T12 is irradiated, T9 becomes weakly absorptive, and, similarly, when T11 is irradiated, T10 changes to absorption. Thus, T9 and T12 are respectively the α-H and β-H resonances of one Trp residue while T10 and T11 are those of another. Removal of cross-relaxation reveals the direct polarization of the α protons which, on the basis of their anticipated hyperfine couplings in the tryptophanyl radical, should indeed be small and absorptive.

Figure 5 shows the effects of irradiating T11 and T12 on

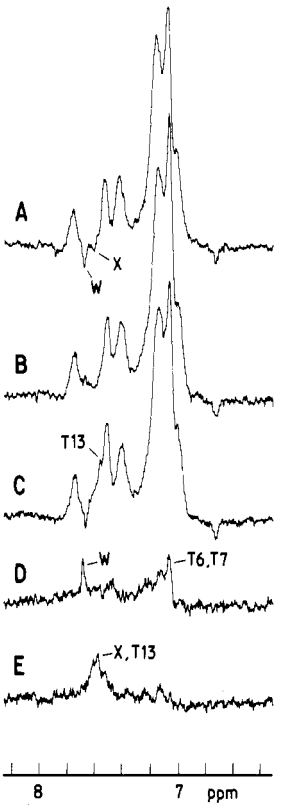

FIGURE 4: Aromatic region of photo-CIDNP spectra of 1 mM lysozyme with 0.4 mM flavin I in D_2O. The following conditions were used: pH 4.5, 25 °C, 0.6-s light, 0.05-s delay, five scans, 5-W argon laser power. (A) Without rf irradiation; (B) with rf irradiation of T12; (C) with rf irradiation of T11; (D) ≡ (B) − (A); (E) ≡ (C) − (A).

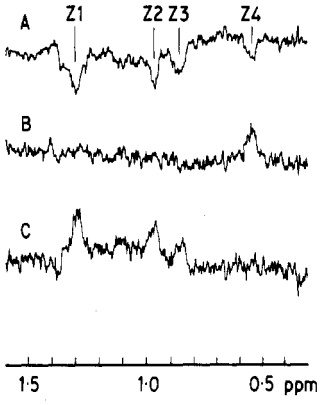

FIGURE 5: High-field region of photo-CIDNP spectra of 1 mM lysozyme with 0.4 mM flavin I in D_2O. The following conditions were used: pH 4.5, 25 °C, 0.6-s light, 0.05-s delay, five scans, 5-W argon laser power. (A) Without rf irradiation; (B) difference between a spectrum with irradiation of T12 and spectrum A; (C) difference between a spectrum with irradiation of T11 and spectrum A.

the four emissive resonances in the 0.5–1.5-ppm region. Saturation of T12 during a 0.6-s light pulse caused Z4 to change phase to enhanced absorption (shown as a difference spectrum in Figure 5B) while irradiation of T11 removes Z1–Z3 from the CIDNP spectrum (Figure 5C).

The observation that Z4 changes phase rather than simply disappearing indicates that it must receive cross-polarization not only from T12 but also from nearby, positively enhanced, aromatic protons, the latter only being revealed when the stronger contribution from T12 is removed. So that this could be confirmed, the experiment was repeated with irradiation of the T6 and T7 lines, which caused Z4 to be more strongly

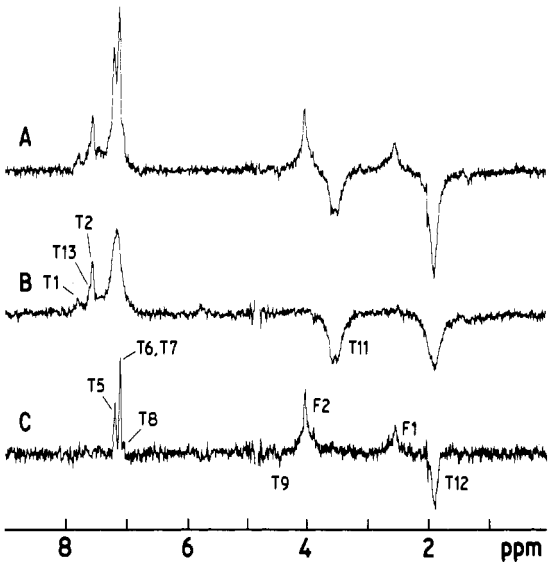

FIGURE 6: Photo-CIDNP spectra of 1 mM lysozyme with 0.4 mM flavin I in D_2O. The following conditions were used: pH 4.9, 25 °C, 0.1-s light, 0.05-s delay, 10 scans, 10-W argon laser power. (A) Without Gd^{3+}; (B) with 1 mM Gd^{3+}; (C) ≡ (A) − (B).

Table III: First-Stage Assignments of Trp Resonances

residue	C(2)H	C(4)H	C(5)H	C(6)H	C(7)H	α-H	β-H
Trp I	T7	T6	T8	T5	T3	T9	T12
Trp II	T13	T2	nr[a]	nr[a]	T1[b]	T10	T11

[a] Not resolved. [b] Tentative assignment.

emissive than in a spectrum with off-resonance irradiation.

In fact, there are probably more than four cross-polarized resonances in this high-field region. Some broad absorptive lines underlying Z1–Z4 are visible in Figure 1, and a fifth negative peak appears between Z1 and Z2 in spectra with long delays. These resonances, however, are too weak to be characterized properly.

Gd^{3+} Binding. Photo-CIDNP spectra of lysozyme in the absence (A) and presence (B) of 1 mM Gd^{3+} are shown in Figure 6 together with the difference spectrum of Spectra B minus spectrum A (Figure 6C). Resonances T5–T9 and T12 are clearly broadened while T1, T2, T11, and G13 are unaffected. It seems likely, therefore, that T5–T9 and T12 are all protons in a Trp residue which is closer to the lanthanide binding site than the tryptophan responsible for T1, T2, T11, and T13.

Riboflavin. A further method for distinguishing the resonances of the two tryptophans presented itself with the use of riboflavin as the CIDNP-producing dye. Compared to a flavin I spectrum, one obtained with riboflavin showed reduced intensity for T1, T2, and T13 relative to T3 and T5–T8 and T11 relative to T12 (Figure 7). It thus appears that riboflavin is more selective than flavin I in its reactions with lysozyme, presumably because of its larger size and/or lack of the negatively charged carboxylate group. Further investigation of the reactions of this and other dyes with lysozyme is in progress.

Discussion

Trp Resonances. The first-stage assignments deduced from the above results are summarized in Table III. Only two of the six tryptophan residues (positions 28, 62, 63, 108, 111, and 123) are substantially polarized: we shall refer to them as Trp I and Trp II, the former being the more strongly enhanced. All eight Trp I protons could be identified with their respective

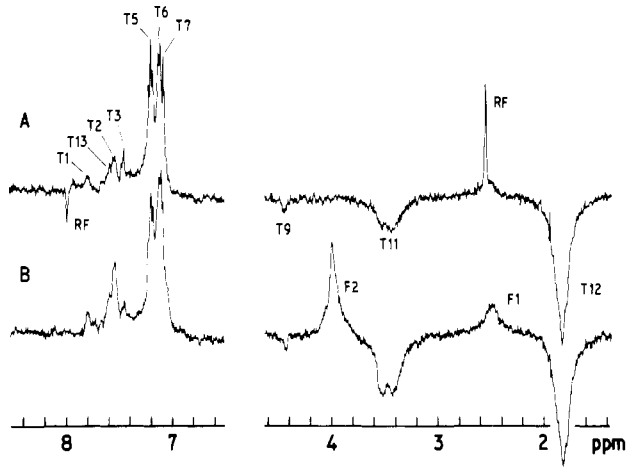

FIGURE 7: Photo-CIDNP spectra of 1 mM lysozyme in D_2O. The following conditions were used: pH 5.3, 25 °C, 0.1-s light, 0.05-s delay, five scans, 5-W argon laser power. (A) With 0.1 mM riboflavin; (B) with 0.1 mM flavin I. RF denotes riboflavin CIDNP peaks.

resonances while one probable and four definite assignments were made for Trp II. The C(5)H and C(6)H resonances of the latter were not resolved presumably because of overlap with the more intense Trp I lines. In fact, some partially resolved lines *are* visible in Figure 2 between T5 and T6. Occurring at ~7.12 ppm, they appear to be directly polarized and may thus arise from C(6)H or Trp II.

The one less certain assignments is that of T1 to the Trp II C(7)H. Since the other doublet belonging to Trp II (T2) is clearly directly polarized and therefore assigned to C(4)H, it is difficult to see what else T1 could be. However, its behavior after a short light flash (Figure 3) and its time dependence during a longer light pulse are not sufficient to exclude the possibility that a small directly polarized resonance underlies the cross-polarized C(7)H doublet.

Unequivocal *second-stage* assignments in protein NMR spectra normally require chemical modification or isotopic substitution (Jardetzky & Roberts, 1981). However, arguments based solely on photo-CIDNP effects and the known X-ray structure of lysozyme (Blake et al., 1976) *do* allow one to identify tryptophans I and II with resonable confidence. In view of the detailed work of Dobson and co-workers (Dobson, 1977; Cassels et al., 1978; Dobson et al., 1978; Poulsen et al., 1980) which demonstrates that the solution and crystal structures of lysozyme are substantially similar, this approach to second-stage assignment does not seem unreasonable. The following paragraphs present the evidence for our proposed assignments.

A fundamental prerequisite for an amino acid residue to be polarized is that it be physically accessible to a photoexcited flavin molecule. Estimates of the solvent exposure of the six tryptophan residues (Glickson et al., 1971) and inspection of a molecular model based on the X-ray coordinates (Blake et al., 1967) suggest that approach of the flavin to Trp-62 should be almost unhindered, that to Trp-63 and Trp-123 a little more difficult, and that to the remaining three very difficult or impossible.

The strong β-CH_2 resonance T12 (Trp I) has an upfield shift of 1.4 ppm from the random coil value (Bundi & Wüthrich, 1979) which must arise from the ring-current effect of a neighboring aromatic residue. Examination of the model of lysozyme reveals that only the β protons of Trp-62, lying just above the indole ring of Trp-63, could experience such a dramatic shift. Calculation supports this conclusion qualitatively although not quantitatively (Perkins & Dwek, 1980).

The broadening of Trp resonances induced by Gd^{3+} should decrease in the following order (Cassels et al., 1978): 108 > 62 ≃ 63 > 111 > 123 > 28. If, as seems probable, Trp I is Trp-62, then Trp II is unlikely to be Trp-63 or Trp-108 which should be affected to a similar or greater extent by Gd^{3+}.

The following assignments have appeared in the literature: all aromatic protons of Trp-28 and -108; all C(2)H and N(1)H; C(7)H or Trp-111 and -123 (Cassels et al., 1978; Poulsen et al., 1980). The chemical shifts of Trp-28 and -108 are incompatible with those given in Table II. Of the C(2) protons, the value of 7.55 ppm for Trp-123 agrees well with our measurement of 7.54 ppm for T13. T7 (7.06 ppm) is close to the shift for Trp-108 (7.08 ppm), but this residue can be rejected principally because of the absence of a C(5)H resonance at or near 6.49 ppm. The only other C(2)H resonances near to T7 belong to Trp-62 and -111 both at 7.03 ppm (Cassels et al., 1978).

On the basis of its accessibility and expected β-CH_2 chemical shift, we believe Trp-62 should be identified with the more strongly polarized residue, Trp I. Further, the line-broadening effect and the published assignments suggest that Trp II is Trp-123. The lack of direct polarization in Trp-63 is probably due to its proximity to the more accessible Trp-62 which would compete more effectively for flavin triplets.

Interestingly, resonance W is almost certainly the C(2) proton of Trp-63 indirectly polarized by the methylene group of Trp-62 (T12). According to the crystal structure, this proton lies 0.3 nm from one of the β-CH_2's of Trp-62, and its shift (Table II) agrees well with that found by Cassels et al. (1978).

Other Resonances. The origin of the two cross-polarized lines T4 and X is not clear: conceivably, they belong to Phe-34 which might be close enough to Trp-123 to allow polarization transfer.

Resonance Y, an emissive, directly polarized doublet, must arise from the (equivalent) C(3)H and C(5)H of a tyrosine residue: inspection of the work of Dobson et al. (1978) shows it to be Tyr-23. The less intense C(2)H, C(6)H doublet expected at 7.05 ppm (Dobson et al., 1978) is presumably obscured by T6–T8.

As demonstrated in Figure 5, Z1–Z4 are indirectly polarized by the β-CH_2 of Trp II (Z1–Z3) and Trp I (Z4). The X-ray coordinates of lysozyme reveal that three methyl groups (the γ-CH_3 group of Ile-124 and both γ-CH_3 groups of Val-29) are less than 0.4 nm from the Trp-123 β-CH_2, while a single methyl (Leu-75) is within this distance of the Trp-62 β-CH_2. This apparent agreement with our proposed assignments may be fortuitous. None of these methyls appears to have been previously assigned.

After the work described in this paper was completed, Dr. C. M. Dobson sent us his assignments of the whole aromatic region of the lysozyme NMR spectrum. With one exception, these are in complete agreement with our results (*including* our proposed second-stage assignments) with only minor differences in chemical shifts of 0.02 ppm or less. Our measurement of 7.06 ppm for the C(2) proton of Trp-62 does not agree so well with his value of 7.11 ppm although it is closer to an earlier measurement by Cassels et al. (1978) of 7.03 ppm. We note that his assignments support our identification of T1 with the C(7) proton of Trp-123 and also of T4 with Phe-34.

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Registry No. Lysozyme, 9001-63-2; Trp, 73-22-3; gadolinium, 7440-54-2.

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Limited Cleavage of Eucaryotic Elongation Factor Tu by Trypsin: Alignment of the Tryptic Fragments and Effect of Nucleic Acids on the Enzymatic Reaction†

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ABSTRACT: Treatment of eucaryotic elongation factor Tu (eEF-Tu; M_r 53 000) with trypsin in the presence of 25% (v/v) glycerol results in cleavage of the factor at two sites and generates a single polypeptide of 43 000 daltons termed eEF-Tu^t and low molecular weight peptide fragments [Slobin, L. I., Clark, R. V., & Olson, M. O. J. (1981) *Biochemistry 20*, 5761–5767]. Digestion of eEF-Tu with carboxypeptidase A for varying lengths of time indicated that the carboxyl-terminal sequence of the factor is Ala-Ser-COOH. The presence of serine at the carboxyl-terminal position was confirmed by hydrozinolysis. eEF-Tu^t was found to possess the same carboxyl-terminal sequence, proving that the low molecular weight peptide fragments released by trypsin, designated T1 and T2, originate from the amino-terminal end of the factor. Peptide T2 contains a blocked amino-terminal residue, as does the starting eEF-Tu, and has a M_r of ca. 4000 based on gel electrophoresis and amino acid composition. Peptide T1 has a M_r of 6000 based on gel electrophoresis and amino acid composition. Taken together, T1, T2 and eEF-Tu^t account for essentially all of the mass of eEF-Tu. Amino-terminal sequence analysis of T1 reveals as striking sequence homology with the amino-terminal sequence of EF-Tu. In particular, the sequence Glu-Lys-Phe-Glu occupies positions 3–6 in T2 and 3–6 in EF-Tu. Other sequence homologies are evident in the first 14 residues of T1 and EF-Tu. Treatment of eEF-Tu in the presence of 28S rRNA markedly accelerates the rate of trypsin cleavage of the factor, whereas treatment of eEF-Tu in the presence of 28S rRNA and in the absence of glycerol stabilizes eEF-Tu^t against trypsin cleavage. By contrast, cleavage of eEF-Tu by trypsin is strongly inhibited by the presence of aminoacyl-tRNA and GTP in reaction mixtures. These results, when combined with additional structural information, suggest that the aminoacyl-tRNA binding site of eEF-Tu is located at the amino-terminal end of the factor.

Recent work in this laboratory has revealed some structural homology between bacterial elongation factor Tu and its functional homologue in eucaryotic cells, eEF-Tu (Slobin et al., 1981).[1] It was found that treatment of eEF-Tu with trypsin resulted in cleavage of the factor at at least two sites, producing a single polypeptide of 43 000 daltons, eEF-Tu^t, and two or more unidentified peptides of M_r ca. 5000. Amino-terminal sequence analysis of eEF-Tu^t showed that the first

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[1] Abbreviations: EF-Tu, procaryotic elongation factor Tu; eEF-Tu, eucaryotic elongation factor Tu; aa-tRNA, aminoacyl-tRNA. eEF-Tu has been referred to by other investigators as EF-I_α or EF-1_L.

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