Proc. Nat. Acad. Sci. USA
Vol. 71, No. 2, pp. 469–473, February 1974

Fluorotyrosine Alkaline Phosphatase from *Escherichia coli*: Preparation, Properties, and Fluorine-19 Nuclear Magnetic Resonance Spectrum

(kinetics/renaturation/ultraviolet spectra/conformational changes)

BRIAN D. SYKES*, HAROLD I. WEINGARTEN†, AND MILTON J. SCHLESINGER‡

* Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138; † Corporate Research Department, Monsanto Company, St. Louis, Missouri 63166; and ‡ Department of Microbiology, Washington University School of Medicine, St. Louis, Missouri 63110

Communicated by S. I. Weissman, September 17, 1973

ABSTRACT **Alkaline phosphatase (EC 3.1.3.1) containing *m*-fluorotyrosine has been prepared from *E. coli* grown in the presence of *m*-fluorotyrosine. The kinetic properties of the *m*-fluorotyrosine enzyme measured with *p*-nitrophenylphosphate at pH 8.0 and dinitrophenylphosphate at pH 5.5 are essentially the same as those of normal alkaline phosphatase. However, the ability of the *m*-fluorotyrosine protein to refold active enzyme after acid denaturation, while unchanged at pH 5.8, was markedly decreased at pH 7.6. This result implies that the tyrosines must be in their protonated form for the protein to refold, reassociate, and take on zinc. The ^{19}F nuclear magnetic resonance spectrum of *m*-fluorotyrosine alkaline phosphatase contains resolved resonances corresponding to different chemical environments for each *m*-fluorotyrosine in the folded protein. This demonstrates that ^{19}F nuclear magnetic resonance spectroscopy of enzymes specifically labeled with ^{19}F, even with enzymes as large as alkaline phosphatase (molecular weight, 86,000), will provide a very valuable probe for conformational changes in proteins.**

High resolution nuclear magnetic resonance (NMR) methods offer a powerful tool for the elucidation of the structure and function of proteins (1). When resonances in a protein NMR spectrum can be resolved and assigned to individual nuclei in the protein, these resonances then serve as probes of the structure, chemical state, and dynamic properties (in terms of reaction and molecular motion) of the protein in solution. To date, most applications of NMR have used ^1H NMR, since ^1H is the most sensitive nucleus with respect to signal intensity, and ^1H chemical shifts, coupling constants, and relaxation times are the best understood. However, ^1H chemical shifts are confined to a narrow 10-ppm range so that even at high frequencies (100–300 MHz), the ^1H NMR spectra of all but the smaller proteins are unresolved envelopes.

Several approaches have been used to simplify protein ^1H NMR spectra, including specific proton insertion of the protein (2), difference NMR methods (3), and the use of NMR "shift reagents" (4). The usefulness of these methods, however, is still limited by the intrinsic narrow chemical shift range of ^1H NMR, especially for larger proteins where the NMR linewidths are correspondingly larger.

One alternative approach is to prepare a protein specifically labeled with another nucleus (5). The advantages of ^{19}F as a label are severalfold. First, the sensitivity of ^{19}F NMR is only slightly lower than that of ^1H NMR. This sensitivity loss ($\approx 17\%$) is offset, in part, by the elimination of the dynamic range problems associated with ^1H Fourier transform NMR that are caused by the residual HDO resonance from the solvent (6). Second, ^{19}F NMR chemical shifts are much larger than ^1H NMR chemical shifts, reflecting the much greater sensitivity of ^{19}F chemical shifts to the chemical environment of the nucleus (7). Third, ^{19}F NMR can be obtained in H_2O rather than in D_2O. The ^{19}F NMR spectrum of a specifically labeled protein will therefore be simpler in terms of the number of resonances present, more spread out in chemical shift, and a more sentitive indicator of conformational changes in the protein.

The protein that we have chosen to study is the alkaline phosphatase (EC 3.1.3.1.) produced by *Escherichia coli*. This enzyme is a zinc metalloprotein consisting of two identical subunits, and an extensive body of information has accumulated over the past 10 years concerning its structure and function (see ref. 8). It offers the additional advantage that the bacterial system can be manipulated to allow for almost exclusive incorporation of a fluorine-containing amino acid into protein and for the production of enzyme primarily during that portion of the cells' growth when the analogue is present. In previous studies with *E. coli* alkaline phosphatase, analogues of arginine (canavanine), histidine (2-methylhistidine and triazolealanine), proline (azetidine-2-carboxylate), phenylalanine (fluorophenylalanine), and tryptophan (azatryptophan and tryptazan) have been inserted into the enzyme with varying results. Only analogues of the latter two natural amino acids allowed active enzyme to be formed (9, 10). All others produced aberrant, inactive subunits (10). In the current study, we prepared enzyme from bacteria grown in the presence of fluorotyrosine. We wished to determine whether the fluorotyrosine residues in a protein as large as *E. coli* alkaline phosphatase (molecular weight = 86,000) would be useful as NMR probes for analyzing conformational changes in proteins.

MATERIALS AND METHODS

Fluorotyrosine alkaline phosphatase was isolated from a tyrosine auxotroph of *E. coli* W3747 (American Type Culture Collection No. 27256) (11). Cells were transferred from agar plates containing a Tris-buffered minimal salts medium (12) supplemented with L-tyrosine (20 μg/ml) and L-methionine (20 μg/ml), 0.2% glucose, and P_i (6×10^{-4} M) to flasks containing the same media, except that the tyrosine and P_i were replaced with pepticase (Sheffield Scientific Co., at 5 mg/ml). This amount of pepticase provided a level of P_i equal to 1.5 $\times$

Abbreviations: NMR, nuclear magnetic resonance; UV, ultraviolet; NOE, nuclear Overhauser enhancement.

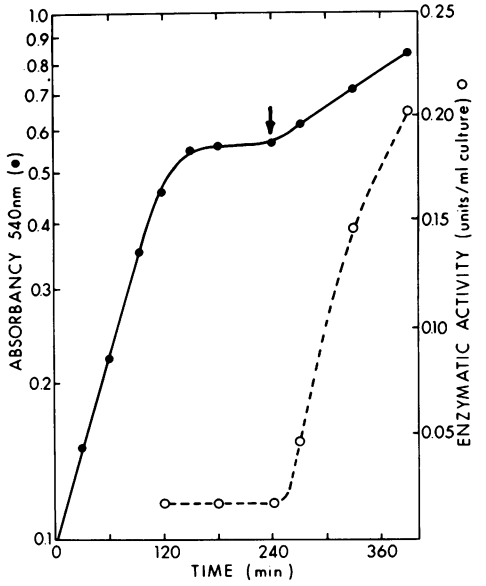

FIG. 1. Cell growth and alkaline phosphatase formation in presence of fluorotyrosine. Refer to *Methods* for details of culture growth and composition of medium. The *arrow* indicates time of addition of the analogue. ●——●, cell density; ○- - -○, enzymatic activity.

10^{-4} M and L-tyrosine at 2×10^{-5} M determined from bacterial growth experiments. (The value for tyrosine is much lower than the mM value reported by the supplier.) Flasks were incubated at 37° and growth was recorded as absorbancy at 540 nm. At approximately 2–4 hr after the end of the exponential growth phase, *m*-fluoro DL-tyrosine (Aldrich Chem. Co.) was added to a final concentration of 7.5×10^{-5} M, and 2 hr later the culture was harvested. For large scale preparation of enzyme, flasks containing 200- to 500-ml cultures in exponential growth were added to 50 liters of the media described above in a Fermacel (New Brunswick); fluorotyrosine was added under the same conditions as described above. Enzymatic assays for alkaline phosphatase activity (13) were measured on sonically-disrupted 1-ml samples taken at intervals during growth of the culture. Alkaline phosphatase was purified according to published procedures starting with formation of spheroplasts prepared with lysozyme–EDTA followed by column chromatography on DEAE–cellulose and gel filtration on Sephadex G-100 (12). The final preparations were judged greater than 90% pure enzyme on the basis of acrylamide-gel electrophoresis and amino-acid analysis.

Amino-acid analyses were determined on samples that were hydrolyzed with 6 N HCl under reduced pressure for 28 hr. Trifluoroacetyl-*n*-butyl ester derivatives of the amino-acid hydrolysate were prepared and analyzed by gas chromatography under conditions that allowed for good separation of tyrosine and fluorotyrosine, according to procedures developed by Dr. Craig Warren (unpublished experiments). Separate analyses were performed on identical protein samples with a Spinco 120 C amino-acid analyzer according to established procedures (14), but fluorotyrosine did not show a distinctive peak with the latter method.

^{19}F NMR spectra were obtained on a Varian XL-100-15 NMR spectrometer operating at 94.1 MHz for ^{19}F, and locked on the ^{2}H resonance of the solvent (D_2O). The ^{19}F chemical shifts were determined relative to an external capillary of CF_3-COOH, and have not been corrected for bulk diamagnetic susceptibility changes between samples. The 90° pulse length for ^{19}C Fourier transform NMR was 14 μsec, corresponding to $\gamma H_1 \simeq 18{,}000$ Hz, which is much greater than the spectral range observed. For large numbers of transients, spectra were accumulated by "block averaging." Typically, blocks of 128 transients were collected, weighted by an exponential ("sensitivity enhancement") filter function, Fourier transformed, and then averaged using double-precision arithmetic. The ambient temperature in the probe was 31°C. ^{1}H decoupling experiments were performed with a Varian Gyrocode decoupler. In this configuration, the transmitter coil is double-tuned to 94.1 and 100 MHz and the 90° pulse length for ^{19}F-Fourier transform experiments is slightly lengthened. However, the receiver section remains narrow-banded for 94.1 MHz and no noise is introduced into the ^{19}F spectrum from the ^{1}H decoupling.

pHs in D_2O were measured with a Beckman Expanomatic pH meter; pD is equal to meter reading plus 0.4 units (15). D_2O was used to facilitate our initial proton decoupling experiments and to remove intermolecular dipole–dipole line broadening that would occur with the solvent H_2O.

TABLE 1. *Comparison of amino-acid compositions of normal and fluorotyrosine alkaline phosphatases*

	Normal	Fluorotyrosine	Ratio
	nmol*		N/F†
Alanine	0.0709	0.0837	0.85
Valine	0.0138	0.0205	0.67
Glycine	0.0709	0.0838	0.85
Isoleucine	0.0116	0.0129	0.89
Leucine	0.0487	0.0534	0.91
Proline	0.0277	0.0297	0.93
Threonine	0.0384	—	
Serine	0.0435	0.0461	0.94
Methionine	0.0066	0.0048	1.37
Phenylalanine	0.0154	0.0189	0.81
Aspartic	0.0760	0.0868	0.87
Glutamic	0.0720	0.0727	1.00
Lysine	0.0415	0.0401	1.03
Tyrosine	0.0171	0.0051	0.90§
Fluorotyrosine	0.0226‡	0.0189	

* Determined by gas chromatography of trifluoroacetyl-*n*-butyl ester derivatives (see *Materials and Methods*).

† Ratio of the weight of Normal (N): Fluorotyrosine (F) enzyme analyzed = 0.90.

‡ This amount was added as free fluorotyrosine (prior to hydrolysis) to a sample of normal enzyme.

§ Amount of tyrosine + fluorotyrosine was used to calculate this ratio. 73% of total (tyrosine + fluorotyrosine) was fluorotyrosine.

RESULTS

Preparation

The effective incorporation of fluorotyrosine into bacterial proteins and the formation of an enzyme in which most tyrosines were replaced with fluorotyrosine required, first, the isolation of a tyrosine auxotroph. Calender and Berg (16) showed that fluorotyrosine could substitute for tyrosine and be incorporated into protein, but the K_m of the tyrosyl-tRNA synthetase for *m*-fluoro-DL-tyrosine (1.3×10^{-4}M) was some 20-fold higher than that for L-tyrosine (6.1×10^{-6}M). Thus,

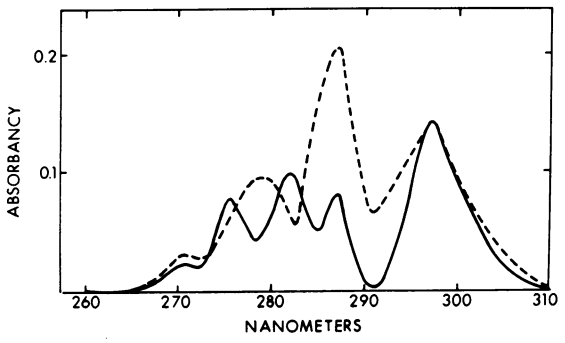

FIG. 2. UV Difference Spectra between metal-free and zinc alkaline phosphatase. The reaction was carried out in matched double cell cuvettes in which one chamber of each cuvette contained 1 ml of 1 mg/ml of enzyme in 0.05 M Tris·HCl, pH 7.4 buffer and the other chamber contained only buffer. To the chamber containing enzyme in one cuvette and chamber containing buffer in the other cuvette were added 10 μl of 1 M nitrilotriacetic acid in 0.05 *M* Tris·HCl, pH 7.4 buffer. To the other two chambers were added 10 μl of buffer. The scans recorded were made on the Cary 14 UV spectrophotometer, wavelength between 310 and 260 nm, 60 min after addition of chelator. - - -, normal enzyme; ——, fluorotyrosine enzyme.

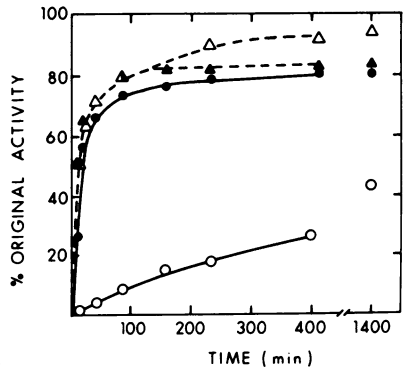

FIG. 3. Effect of pH on reactivation of acid-denatured alkaline phosphatase. For renaturation at pH 7.6, 0.1 ml of enzyme solution (8 mg/ml) was added to 0.9 ml of HCl (0.01 N) at 23°. All measurable activity was lost after 10–15 min. To this solution was added 0.5 ml of 1 M Tris·HCl, pH 8.0, to give a final pH of 7.6. The solution was incubated at 37° and samples were assayed for activity. For renaturation at pH 5.8, the above procedure was repeated except that 1 M 2-(*N*-morpholino) ethanesulfonic acid (MES, Nutritional Biochemical Corp.) pH 6.25, was used in the renaturation reaction. O——O, fluorotyrosine enzyme, pH 7.6; ●——●, fluorotyrosine enzyme, pH 5.8; Δ- - -Δ, normal enzyme, pH 7.6; ▲- - -▲, normal enzyme, pH 5.8.

fluorotyrosine added to the media of a prototrophic strain would compete poorly with endogenous tyrosine for the activating enzyme and would not be effectively incorporated into protein.

To insure that the alkaline phosphatase isolated from the bacterial cell contained mostly fluorotyrosine, we established conditions such that synthesis of this protein began only when the fluorotyrosine was in the culture medium. Fig. 1 shows the bacterial cell-growth pattern and alkaline phosphatase formation under the conditions found to maximize fluorotyrosine incorporation. Levels of P_i and tyrosine were adjusted so that cells starved for tyrosine at a concentration of P_i low enough to insure induction of enzyme on subsequent addition of fluorotyrosine. Under normal conditions, the P_i level of the culture media must be reduced to the order of 10^{-5} M to induce enzyme synthesis (17).

Purification of enzyme obtained from 50-liter batches of cells was carried out according to procedures established for the normal tyrosine enzyme. The final yield of pure enzyme was on the order of 50 mg, a value that is only 10% that obtained for normal enzyme. The low yield reflects the limited capacity of the bacterial cell to make enzyme in the presence of fluorotyrosine as the only source of tyrosine. The amino-acid composition of purified fluorotyrosine alkaline phosphatase very closely resembled that of the purified normal enzyme and showed that about 70% of the tyrosine had been substituted (Table 1).

Properties

One effect of the presence of fluorotyrosine was to change the extinction coefficient of the enzyme, measured at 280 nm; fluorotyrosine exhibited an $A_{280}^{0.1\%} = 0.90$ based on amino-acid analysis of the measured sample compared to the $A_{280}^{0.1\%} = 0.77$ recorded for the normal enzyme (18). The kinetic properties of the fluorotyrosine enzyme measured with *p*-nitrophenylphosphate at pH 8.0 and dinitrophenylphosphate at pH 5.5 using stopped-flow measurements were essentially the same as those recorded for normal alkaline phosphatase. Turnover numbers of 1400 mol of *p*-nitrophenylphosphate per mol of enzyme per min were recorded for both species of enzyme. Both fluorotyrosine and tyrosine alkaline phosphatases were stable to heating at 90° for 30 min in the presence of 10 mM Mg^{++}, and both forms of the enzyme could be reversibly denatured by treatment with nitrilotriacetic acid (19) or by acidifying the protein to pH 2.0 (20).

The fluorotyrosine eyzyme, however, exhibited some properties that were distinct from those of the tyrosine enzyme. When normal alkaline phosphatase is treated with the chelator nitrilotriacetic acid, zinc is removed from the enzyme, destroying its catalytic activity (19), and an ultraviolet (UV) difference spectrum is generated between active and Zn^{++}-free enzyme that shows three major peaks, one at 280 nm, one at 286 nm and one at 298 nm (Fig. 2). The fluorotyrosine enzyme treated with nitrilotriacetic acid shows a difference spectrum with four peaks, at 276, 282, 287, and 298 nm, with differences in extinction coefficients compared to the normal enzyme. The difference spectrum noted with tyrosine enzyme represents changes in the hydrophobic environment of six to eight tyrosines per enzyme and probably one tryptophan (the 298-nm peak) as the metal is removed. Some of this change may reflect the interaction of Zn^{++} with a tyrosine group in the active enzyme (19).

Another important difference was the decreased ability of the fluorotyrosine protein to reform active enzyme after acid denaturation when the renaturation process was carried out at pH 7.6 (Fig. 3); normal enzyme rapidly reforms active enzyme at this pH value. Lowering the pH to 5.8, however, allows for total recovery of fluorotyrosine enzyme activity. The most likely interpretation of this result is that tyrosine residues must be in their protonated form in order for the alkaline phosphatase subunits to fold, reassociate, and take on zinc. Data from previous experiments on renaturation of active alkaline phosphatase from unfolded subunits indicated that the environment of eight of the eleven tyrosines in each of the subunits became more hydrophobic during the process

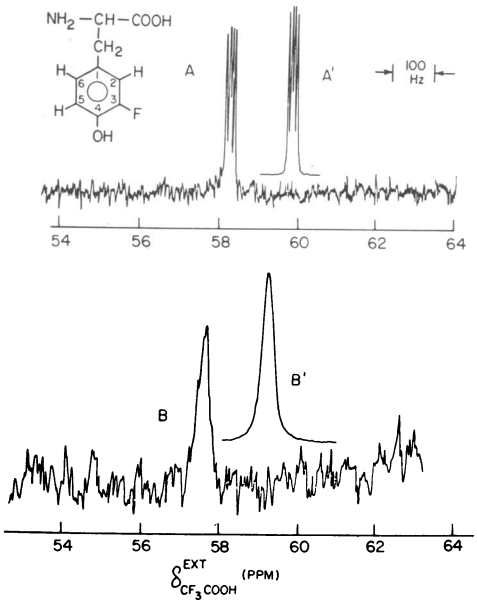

FIG. 4. (*A*) ^{19}F NMR spectrum of 1.7×10^{-2} M *m*-fluoro-DL tyrosine in 0.3 M Tris·HCl buffer (in D_2O), pD 9.9; 256 transients, acquisition time = 0.8 sec. (*A'*) Simulated ^{19}F NMR spectrum of *m*-fluoro-DL-tyrosine, $^3J(F_3,H_2) = 13$ Hz, $^4J(F_3,H_5) = 8$ Hz, $\Delta\nu = 3$ Hz. (*B*) ^{19}F NMR spectrum of 6 mg/ml of fluorotyrosine alkaline phosphatase in 6 M guanidine·HCl, pD = 7.3; 8633 transients, acquisition time = 0.3 sec, sensitivity enhancement filter function = 0.1 sec. (*B'*) simulated spectrum of denatured fluorotyrosine alkaline phosphatase, parameters are the same as (*A'*) except $\Delta\nu = 20$ Hz.

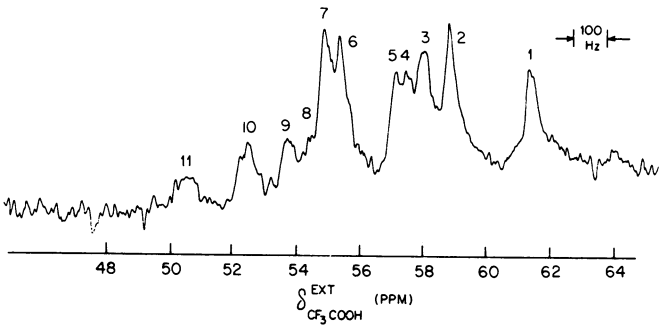

FIG. 5. ^{19}F NMR spectrum of 25 mg/ml of fluorotyrosine alkaline phosphatase, 0.3 M Tris·HCl buffer (in 250 μl of D_2O), pD = 8.3; 352 blocks of 128 transients each, acquisition time = 0.2 sec, pulse delay between transients = 1.3 sec, sensitivity enhancement filter function = 0.1 sec.

of refolding, reassociation, and zinc activation of the apodimer (21). The fluorine atom shifts the pK of the hydroxyl group in fluorotyrosine to about two pH units below that in tyrosine (S. E. Halford, private communication); thus, at pH 7.6 many of the fluorotyrosines in the subunit would be negatively charged and possibly unable to fold into a hydrophobic region of the protein.

NMR spectrum

The ^{19}F NMR spectrum of *m*-fluoro-DL-tyrosine is shown in Fig. 4*A*. The ^{19}F resonance appears as a quartet due to coupling to hydrogens on the ring; this quartet pattern collapses upon double irradiation at the ^1H NMR resonance frequency of the ring protons. The coupling constants used in the simulated spectrum (Fig. 4*A'*) were $J(F_3,H_2) = 13$ Hz, $J(F_3,H_5) = 8$ Hz. The linewidth used in the simulated spectrum (3 Hz) indicates the presence of a small unresolved splitting from H_6.

The ^{19}F NMR spectrum of fluorotyrosine alkaline phosphatase in 6 M guanidine·HCl (in D_2O, pH $\simeq$ 7) is shown in Fig. 4*B*. The single ^{19}F resonance observed indicates that the protein is denatured to the extent that all 11 fluorotyrosines per monomer (molecular weight 43,000) have the same chemical environment. The linewidth of this resonance narrows to 16 ± 4 Hz upon double irradiation at the ^1H NMR resonance frequency of the ring protons. This is the same, within experimental error, as the linewidth (about 20 Hz) determined from the simulated spectrum (Fig. 4*B'*) for the individual resonances of the overlapping quartet in the spectrum without ^1H double irradiation.

The ^{19}F NMR spectrum of fluorotyrosine alkaline phosphatase in 0.3 M Tris·HCl (in D_2O) at pH 7.9 is shown in Fig. 5. In contrast to the spectrum of the denatured enzyme, the spectrum of the enzyme in its native state contains many resonances, corresponding to a different chemical environment for each fluorotyrosine in the folded structure. The chemical shifts for the fluorotyrosines are spread over a range of 1200 Hz. The areas of the peaks, normalized to the area of the peak labeled 1, are given in Table 2. All of the resonances may not have their full intensities, however, if the delay between transients (1.5 sec.) in the accumulated spectrum was not much longer than the T_1's of all of the protein resonances (see below). Nor is it necessary from a chemical viewpoint that there be 11 resonances of unit area corresponding to the 22 fluorotyrosines in the dimer; for example, the dimer may be asymmetric. The fact that there are at least 10 resolved resonances with a total area corresponding to on the order of 11 tyrosines per monomer is therefore very encouraging. The resonance labeled 8 is more clearly resolved in spectra with different intensities taken at shorter pulse delays (W. E. Hull, private communication).

Upon double irradiation at the ^1H NMR resonance frequency of the ring protons, the ^{19}F NMR spectrum of the native enzyme disappears. This is the result of a negative nuclear Overhauser enhancement (NOE) (22) and indicates that the fluorotyrosines in the native enzyme have a correlation time characterizing their rotational motion that is much greater than the reciprocal of their NMR resonance frequency; that is, they are tumbling at a rate appropriate for a molecule of the size of alkaline phosphatase. Assuming that the ^{19}F relaxation is dominated by intramolecular dipole–dipole interaction with the adjacent ring proton modulated by the rotational tumbling of the enzyme, the fractional NOE can be written for unlike spins (I = ^{19}F, S = ^1H) as:

$$\frac{\text{area (with } ^1\text{H irradiation)} - \text{area (without } ^1\text{H irradiation)}}{\text{area (without } ^1\text{H irradiation)}} = \frac{\gamma_H}{\gamma_F}\left[\frac{5 + 5\omega_I^2\tau^2 + 6(\omega_I - \omega_S)^2\tau^2 - (\omega_I + \omega_S)^2\tau^2 + 6\omega_I^2(\omega_I - \omega_S)^2\tau^4 - \omega_I^2(\omega_I + \omega_S)^2\tau^4}{10 + 7\omega_I^2\tau^2 + 4(\omega_I + \omega_S)^2\tau^2 + 9(\omega_I - \omega_S)^2\tau^2 + 6\omega_I^2(\omega_I - \omega_S)^2\tau^4 + \omega_I^2(\omega_I + \omega_S)^2\tau^4 + 3(\omega_I - \omega_S)^2(\omega_I + \omega_S)^2\tau^4}\right]$$

TABLE 2. *Relative areas of the resonances in the ^{19}F NMR spectrum of fluorotyrosine alkaline phosphatase in 0.3 M Tris·HCl (in D_2O), pH = 7.9 (See Fig. 5.)*

Peak	Relative Area
1	1.0 ± 0.3*
2–5	3.7 ± 0.3
6–9	3.4 ± 0.3
10	0.8 ± 0.3
11	0.7 ± 0.3

* Estimated experimental errors.

where γ_H and γ_F are the gyromagnetic ratios for ^1H and ^{19}F, respectively; $\omega_I = \gamma_F H_0 = 2\pi \cdot 94.1 \cdot 10^6$ cycles, $\omega_S = \gamma_H H_0 = 2\pi \cdot 100 \cdot 10^6$ cycles; and τ is the rotational correlation time for the intramolecular dipole–dipole interaction. A value for τ of the order of 10^{-8} sec is calculated for a protein of this size from the Stokes–Einstein–Debye equation. For $\omega_I^2(\omega_I + \omega_S)^2\tau^4 \gg 1$, the fractional NOE becomes $\simeq -1$, corresponding to loss of the ^{19}F NMR signal. Given the conclusion that $\omega_I^2\tau^2 \gg 1$, the T_1's of the protein ^{19}F NMR resonances can become long (23) ($T_1 \approx 0.1 - 1.0$ sec, W. E. Hull, private communication), indicating the necessity of sufficiently long-pulse delays during spectrum acquisition if area measurements in the final spectrum are desired.

Even without assignment of the peaks in the ^{19}F NMR spectrum of the fluorotyrosine enzyme, there are several interesting features. The most obvious is that the resonances that are shifted the most downfield also have the greatest linewidths. One explanation is that these resonances correspond to tyrosines that are buried inside the protein and, therefore, experience a hydrophobic environment, resulting in their downfield chemical shifts, and are the least mobile. In addition, these tyrosines may be making the most contacts with other protein ^1H nuclei.

There seems no question therefore that the ^{19}F spectrum of this enzyme will provide a very valuable probe for conformational changes in the enzyme, particularly when these data are correlated with information about the three-dimensional structure of the protein, currently being obtained by x-ray crystallographic analyses (24), and with the knowledge of the complete amino-acid sequence that is also currently under investigation (12). For *E. coli* alkaline phosphatase two kinds of conformation changes are of interest: (*1*) those involved in the catalytic mechanisms and the role of Zn^{++}, and (*2*) those involved in subunit interactions. Preliminary results with fluorotyrosine alkaline phosphatase treated with chelator to remove metal show that the ^{19}F NMR spectrum is distinctly different from the spectra of the fully denatured protein and the fully active enzyme that are reported here. Future communications will describe these differences as well as those expected to be generated from other conformations of this enzyme.

The authors would like to acknowledge the financial support of the National Science Foundation (GB-27613 to MJS and GP-32317 for purchase of the NMR spectrometer) and the National Institutes of Health (GM-17190 to BDS), the assistance of W. E. Hull in obtaining the NMR spectra, and helpful discussions with W. E. Hull, Drs. S. E. Halford, H. Gutfreund, and J. Schaefer. We are indebted to Dr. Craig Warren, Corporate Research Department, Monsanto Co., for providing the analyses of fluorotyrosine alkaline phosphatase, and Dr. Will Bloch for performing the stopped-flow kinetic measurements on the fluorotyrosine enzyme.

1. Roberts, G. C. K. & Jardetzky, O. (1970) *Advan. Protein Chem.* **24**, 447–545.
2. Putler, I., Barreto, A., Markley, J. L. & Jardetzky, O. (1969) *Proc. Nat. Acad. Sci. USA* **64**, 1396–1403.
3. King, N. L. R. & Bradbury, J. H. (1971) *Nature* **229**, 404–406.
4. Campbell, I. D., Dobson, C. M., Williams, R. J. P. & Xavier, A. V. (1973) *Ann. N.Y. Acad. Sci.*, in press.
5. (*a*) Huestes, W. H. & Raftery, M. A. (1972) *Biochemistry* **11**, 1648–1654; (*b*) Huestes, W. H. & Raftery, M. A. (1971) *Biochemistry* **10**, 1181–1186; (*c*) Browne, D. T., Kenyon, G. L., Packer, E. L., Wilson, D. M. & Sternlicht, H. (1973) *Biochem. Biophys. Res. Commun.* **50**, 42–47.
6. Patt, S. L. & Sykes, B. D. (1972) *J. Chem. Phys.* **56**, 3182–3184.
7. Ensley, W., Feeney, J. & Sutcliffe, L. H. (1966) *High Resolution Nuclear Magnetic Spectroscopy* (Pergamon Press, New York), Vol. 2.
8. Reid, T. W. & Wilson, I. B. (1971) *The Enzymes*, ed. Boyer, P. (Academic Press, New York), Vol. IV, 3rd ed., pp. 373–415.
9. Richmond, M. H. (1963) *J. Mol. Biol.* **6**, 284–294.
10. Schlesinger, M. J., Reynolds, J. A. & Schlesinger, S. (1969) *Ann. N.Y. Acad. Sci.* **166**, Art. 2, 368–379.
11. Schlesinger, S. & Nester, E. W. (1969) *J. Bacteriol.* **100**, 167–175.
12. Kelley, P. M., Newmann, P. A., Schriefer, K., Cancedda, F., Schlesinger, M. J. & Bradshaw, R. A. (1973) *Biochemistry* **12**, 3499–3503.
13. Halford, S. E., Lennette, D. A., Kelley, P. M. & Schlesinger, M. (1972) *J. Biol. Chem.* **247**, 2087–2094.
14. Moore, S. & Stein, W. H. (1963) in *Methods in Enzymology*, eds. Colowick, S. P. & Kaplan, N. O. (Academic Press, New York) Vol. 8, pp. 819–831.
15. Covington, A. K., Paabo, M., Robinson, R. A. & Bates, R. G. (1968) *Anal. Chem.* **40**, 700–706.
16. Calender, R. & Berg, P. (1966) in *Procedures in Nuclei Acid Research*, eds. Cantoni, G. C. & Davies, D. R. (Harper & Row, New York), pp. 384–399.
17. Torriani, A. (1960) *Biochim. Biophys. Acta* **38**, 460–469.
18. Rothman, F. & Byrne, R. (1963) *J. Mol. Biol.* **6**, 330–340.
19. Reynolds, J. A. & Schlesinger, M. J. (1969) *Biochemistry* **8**, 588–593.
20. Schlesinger, M. J. & Barrett, K. (1965) *J. Biol. Chem.* **240**, 4284–4293.
21. Reynolds, J. A. & Schlesinger, M. J. (1967) *Biochemistry* **6**, 3552–3559.
22. Balaram, P., Bothner-By, A. A. & Dadok, J. (1972) *J. Amer. Chem. Soc.* **94**, 4015–4016.
23. Doddrell, D., Glushko, V. & Allerhand, A. (1972) *J. Chem. Phys.* **56**, 3683–3689.
24. Knox, J. R. & Wyckoff, H. W. (1973) *J. Mol. Biol.* **74**, 533–545.