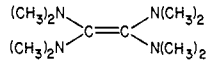
1,8-naphthoquinodimethane[7] (**5**) and diphenylcarbene[8] (**4**), respectively. The intensity of the diphenylcarbene EPR spectrum is much weaker than that obtained for the biradicals. These species were most likely produced by dissociative electron capture of **6–10**. Although **11** is the preferred reagent for matrix electron transfer and EPR spectroscopy, it is not useful in fluorescence spectroscopy due to its own strong emission.[9]



11

These preliminary results indicate that readily available dihalo compounds may be general precursors to biradicals, biradicaloids, and carbenes under matrix-isolation electron-transfer conditions. Applications of this approach to presently uncharacterized neutral intermediates and the utilization of other spectroscopic techniques are in progress.

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Delineation of α-Helical Domains in Deuteriated Staphylococcal Nuclease by 2D NOE NMR Spectroscopy

Dennis A. Torchia,*† Steven W. Sparks,† and Ad Bax‡

Bone Research Branch, National Institute of Dental Research and Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Disease National Institutes of Health Bethesda, Maryland 20892

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Two-dimensional proton NMR spectroscopy is a powerful means of elucidating the three-dimensional structures of small proteins in solution.[1] However, because the number of proton signals and their line widths increase with molecular weight, 2D NMR spectra are difficult to interpret when the protein molecular weight exceeds 10000. Deuteriation of nonexchangeable protons diminishes these problems.[2,3a,b] It also prevents diffusion of magnetization from NH to CH protons, suggesting that resolution and sensitivity of d_{NN} connectivities will be significantly enhanced by protein deuteriation. We demonstrate that this is the case in an application of the deuteriation approach to liganded Staphylococcal nuclease,[4] an enzyme ternary complex having a MW of 18 kD. Three long sequences of d_{NN} connectivities, signatures

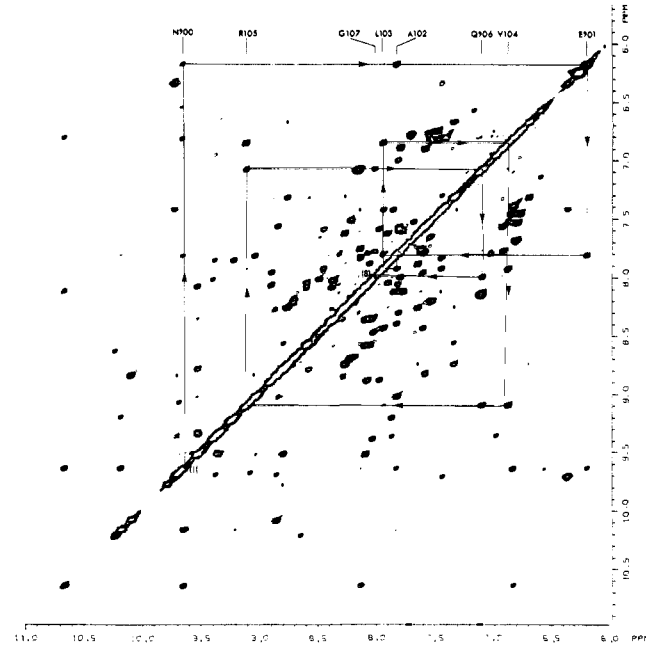

Figure 1. Downfield region of the 500 MHz absorption mode NOESY spectrum of deuteriated Nase. The sequence of d_{NN} connectivities traced by the solid line links the eight residues assigned at the top of the spectrum. The solution composition was as follows: H_2O, 90%; ^2H_2O, 10%; NaCl, 100 mM; borate buffer 50 mM, pH 7.7; Nase, 1.5 mM; pdTp, 5 mM; $CaCl_2$, 10 mM. NT 500 spectrometer settings were as follows: 90° pulse, 27 μs; recycle delay, 3 s; spectral window, 7400 Hz; mixing time, 0.15 s; 64 scans per t_1 values; 350 t_1 values; 36.5 °C. Water signal was suppressed by presaturation. Chemical shifts are referenced to HDO at 4.67 ppm.

of α-helices,[1,5-7] are clearly observed in the NOESY spectrum of the deuteriated protein.

Staphylococcal nuclease, Nase, is well characterized chemically and has been the subject of many structure-function studies.[8a-d] Recently, the Nase gene has been expressed in *Escherichia coli*,[9] a development that has given new impetus to these studies.[10a-f] We have prepared purified Nase from *E. coli*[10f,11] (provided by Professor John Gerlt) grown in defined media containing perdeuteriated amino acids (Merck). A comparison of ^1H NMR spectra of deuteriated and protiated Nase showed that 80–85% of the nonexchangeable hydrogens were deuteriated in the labeled protein. Spectrophotometric assays[12] showed that the protiated and deuteriated Nase samples had the same enzymatic activities.

The 6–11-ppm region of the NOESY spectrum of deuteriated Nase, Figure 1, shows many resolved intense cross-peaks. In

† Bone Research Branch.
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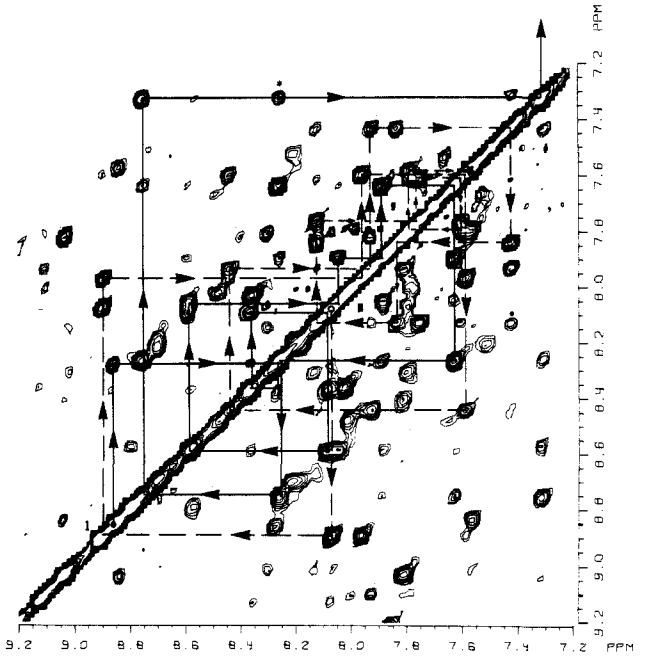

Figure 2. Two long sequences of d_{NN} connectivities in the 7.2–9.2-ppm region of the Nase NOESY spectrum. The sequence of d_{NN} connectivities traced by the solid line originates at the filled circle, terminates at 6.69 ppm, and links 12 residues. Two of the $d_{NN}(i,i+2)$ connectivities in this sequence are indicated by asterisks. The sequence of d_{NN} connectivities traced by the dashed line originates at the open circle, terminates at the open square, and links 13 residues. Experimental parameters as in Figure 1, except the mixing time was 0.3 s.

contrast the NOESY spectrum of the protiated protein obtained under the same conditions (not shown) shows strong cross-peaks mainly for the aromatic protons and for the nonequivalent sidechain amide protons. While the sidechain amide cross-peaks are seen in Figure 1, most of the cross-peaks in this spectrum are in sequences of d_{NN} connectivities. One such sequence, linking eight NH's, is indicated in the figure. The sequential assignments are given at the top of the figure.

Two longer sequences of d_{NN} connectivities are shown in the expanded NOESY spectrum, Figure 2, obtained by using a 0.3-s mixing time. The longer mixing time enables us to observe $d_{NN}(i,i+2)$ connectivities[1] as well. The observation of the $d_{NN}(i,i+2)$ connectivities removes possible ambiguities in a sequence of d_{NN} connectivities that can result from chemical shift degeneracy. Indeed, the $d_{NN}(i,i+2)$ connectivites permitted us to make an unambiguous assignment of the eight residue and thirteen residue d_{NN} sequences, in spite of a chemical shift degeneracy involving these sequences at 7.94 ppm. The $d_{NN}(i,i+2)$ connectivities are not normally found in the spectrum of a protiated protein because the large mixing time required to observe these signals generates many interfering second-order cross-peaks, and spin diffusion to nonexchangeable protons strongly attenuates the connectivity. We have not yet quantified the contributions of direct magnetization exchange and spin diffusion to the $d_{NN}(i,i+2)$ connectivities.

We have assigned residues in all three long sequences of d_{NN} connectivities by using the NOESY spectra and ^1H detected shift correlation spectra[13a-d] of $^{15}N/^{13}C$ labeled samples. These assignments, which will be presented elsewhere, show that each d_{NN} sequence corresponds to an α-helical domain[8c] of the protein.

The perdeuteriation approach presented here opens the way to investigate the effects of important variables such as site mutations, ligand interaction, etc. upon the helical domains of many proteins. Considering the fact that excellent spectra were obtained by using a protein concentration of 1.5 mM and physiological conditions, it is likely that the experiment will be effective on proteins much larger than Nase, particularly when performed on the higher sensitivity instruments now available.

It is clear that the present methodology can be extended by incorporating protiated or selectively deuteriated amino acids into an otherwise deuteriated protein. In this way it should be possible to link the assigned α-helical peptide protons to various sidechain and backbone protons within and outside of the α-helical domains.

After this manuscript was submitted, the sequential assignment of randomly deuteriated thioredoxin, MW = 11.7 kD, was reported by LeMaster and Richards.[14] Their spectra clearly show the benefits of protein deuteriation in applications of 2D NMR spectroscopy to proteins having molecular weights above 10 kD.

Note Added in Proof. The Nase solution used herein had a pH meter reading of 6.97.

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"Solid-State" Voltammetry of a Protein in a Polymer Solvent

B. N. Oliver, J. O. Egekeze,† and Royce W. Murray*

Kenan Laboratories of Chemistry
University of North Carolina
Chapel Hill, North Carolina 27599-3290
Received December 11, 1987

We report the first example of diffusion-controlled voltammetry of a redox protein dissolved in a biocompatible, semirigid polymer film. The experiment[1] is based upon humidifying or adding liquid microdroplets to thin, ionically conductive films of polyethylene oxide (PEO) or polyacrylamide to form thin, semirigid gel solutions resting on a coplanar set of three electrodes (Figure 1). "Solid-state" voltammetry in this electrochemical "gel-cell" can be used to study how the electron transfer and associated chemistry of proteinaceous materials respond to structured, viscous media. The gel-cell also offers a novel approach to protein "immobilization"[4] in the design of bioanalytical sensors.

The direct (unmediated) voltammetry[5] of cytochrome *c* was

† Permanent address: Department of Chemistry and Physics, Augusta College, Augusta, GA 30910.

(1) The cell, Figure 1, consists of a 2.4-mm diameter EPG disk with concentric Pt foil (0.1 mm) ring auxiliary electrode and 0.35-mm diameter Ag wire tip reference electrode, all encapsulated in epoxy. The polymer films are cast from $PEO_{18}{\cdot}LiCF_3SO_3$ solution[2] or for polyacrylamide by laying a disk of swollen or dry (ca. 50 μm) gel on the surface of the electrode assembly. Cytochrome *c*, $CpFeCpCH_2N(CH_3)_3^+PF_6^-$, and electrolyte are sorbed as solution microdroplets into N_2-dried gels, or by premixing with the $PEO_{18}{\cdot}LiCF_3SO_3$ casting solution, or for polyacrylamide by gel-cell equilibration in a reagent solution. The polyethylene oxide and polyacrylamide contain $LiCF_3SO_3$ (or NaCl) and 0.1 M KCl electrolyte, respectively. Polyacrylamide gels (2% T, 2.6% C_{bis}, ca. 28% polymer by weight) were prepared[3] by room temperature, radical polymerization of 9.68 mL of a degassed, aqueous solution of acrylamide (9.68 mL) and *N,N'*-methylenebisacrylamide with 3-(dimethylamino)propionitrile (0.26 mL of 1% solution) and ammonium persulfate (0.1 mL of 1% solution) followed by thorough washing both before and after mounting on gel-cells to remove residual electroactive ammonium persulfate.

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