Journal of Magnetic Resonance **141,** 355–359 (1999)
Article ID jmre.1999.1940, available online at http://www.idealibrary.com on IDEAL®

Capillary Array Electrophoretic NMR of Proteins in Biological Buffer Solutions[1]

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Received June 23, 1999; revised September 9, 1999

The capillary array electrophoretic NMR (CA-ENMR) was developed to study protein mixtures in biological buffer solutions of high ionic strength. By enhancing the strength of the effective electric field across the sample, the technique permits the detection of the electrophoretic motion of 1 mM lysozyme in 50 mM NaH_2PO_4 aqueous solution, which was previously not achievable using conventional ENMR. Heat-induced convection was dramatically reduced by blocking convective current loops and by improving the efficiency of heat exchange. Thus, the capability of ENMR study of electrolyte solutions was extended from low to high ionic strength. In addition, capillary walls reduced rf-induced electrical eddy current, thereby maintaining good probe Q factors. Because of its parallel configuration to the static magnetic field, the capillary array chamber produced no susceptibility distortions of the ENMR signal. The technique offers great potential in characterizing multiple protein conformations and protein interactions in solution.

Key Words: **electrophoretic NMR; heating and convection; NMR in high-salt solutions; structure of mixed proteins; capillary array electrophoresis.**

NMR structure determination of multiple proteins and DNA molecules *in situ* in a biochemical reaction network provides fundamental understanding of biological processes (e.g., signal transduction, genetic control, and protein folding). However, such experiments are often hampered by severe signal overlap in the NMR spectra. Our recent results (*1*) suggest that this difficulty in resolving resonances from different macromolecules in solution can be overcome by multidimensional electrophoretic NMR (ENMR) (*1–17*). Without physical separation of the component molecules, the resonances from different proteins in a mixture can be sorted out by electrophoretic mobilities. Our ENMR study of a multicomponent protein solution containing Bovine Serum Albumin (BSA) and ubiquitin has demonstrated the feasibility of this approach (*1*). By applying a DC-electric field, the NMR resonances of the two proteins were modulated differently by their electrophoretic motion. In a two-dimensional ENMR experiment, the modulation frequencies were displayed in a new dimension of electrophoretic mobility. All resonances from BSA were grouped together and displayed at its characteristic frequency in the new dimension, different from that of ubiquitin. The technique has demonstrated potential to sort out protein resonances in mixtures.

When examining mixed proteins in biological buffer solutions of high ionic strength, however, we were challenged by heat-induced convection. In such systems, electric conductivity, κ, is high, and therefore, the electric field, $E_{dc} = I_e/\kappa A$, which drives electrophoretic motion of proteins, is low. Relatively large electric current, I_e, is needed in order to generate sufficient electric field strength, if the area of the sample tube, A, is fixed. Consequently, electric power deposition or resistive heating increases. In most ENMR experiments, heat is removed by cooling air outside the sample tube, and, therefore, heat removal at the edge of the tube is more efficient than that at the center. As a result, a temperature gradient is generated, which in turn produces a density gradient and bulk convective motion in the solution (Fig. 1a). Obviously, the electrophoretic flow superimposed on an irregular convective flow is very difficult to measure, if not impossible. In this communication, we report a new ENMR technology—the capillary array electrophoretic NMR (CA-ENMR)—as a solution to this problem. A capillary array sample chamber was designed to break the convective, as well as electrical eddy current loops (Fig. 1b), and to increase the strength of the electric field. CA-ENMR, furthermore, achieves excellent probe Q factors and spectral line shapes. These enhancements have allowed the first successful ENMR investigation of proteins in high salt biological buffer solutions.

In CA-ENMR, fused silica glass capillaries[3] (i.d. = 250 μm; o.d. = 450 μm) were flame-bent and bundled into U-shaped sample cells (Fig. 2). Two Teflon tubes of larger diameter (e.g., i.d. = 2.20 mm; o.d. = 3.30 mm) served as reservoirs to house two platinum electrodes at the ends of the bundled capillaries.

[1] Some of the preliminary results have been reported in the 39th Experimental Nuclear Magnetic Resonance Conference (ENC), Asilomar, California, March 22–27,1998.

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[3] Fused silica capillaries were purchased from Imprecision, Inc., with i.d. = 75, 100, 150, and 200 μm, respectively; Teflon tubes were also used.

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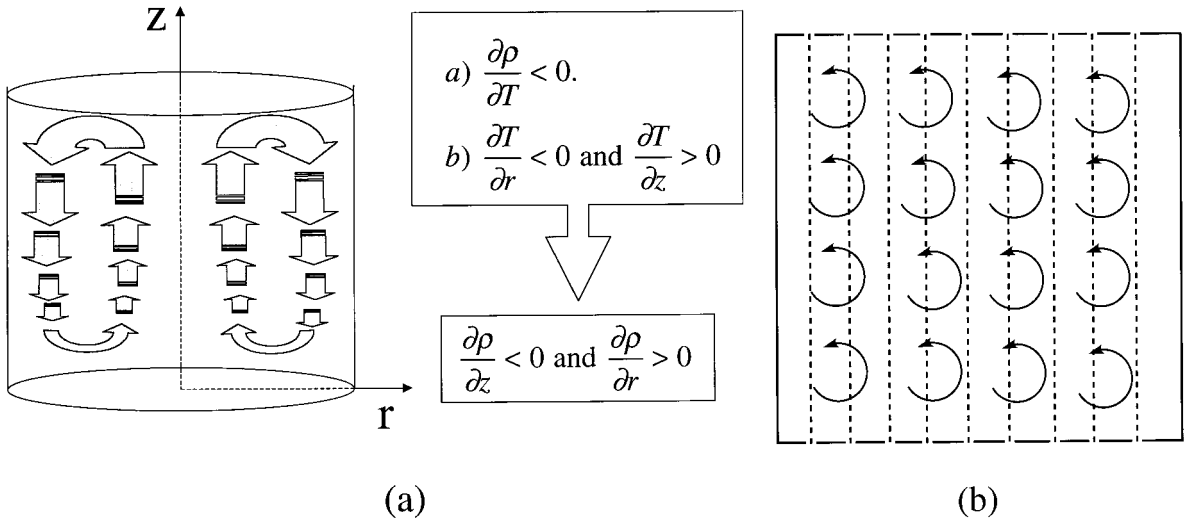

FIG. 1. (a) Schematic illustration of heat-induced convection in a cylindrical sample tube. Symbols ρ and T represent solution density and temperature, respectively. (b) The capillary walls (dashed lines) impose physical barriers to block the convection and the electrical current loops.

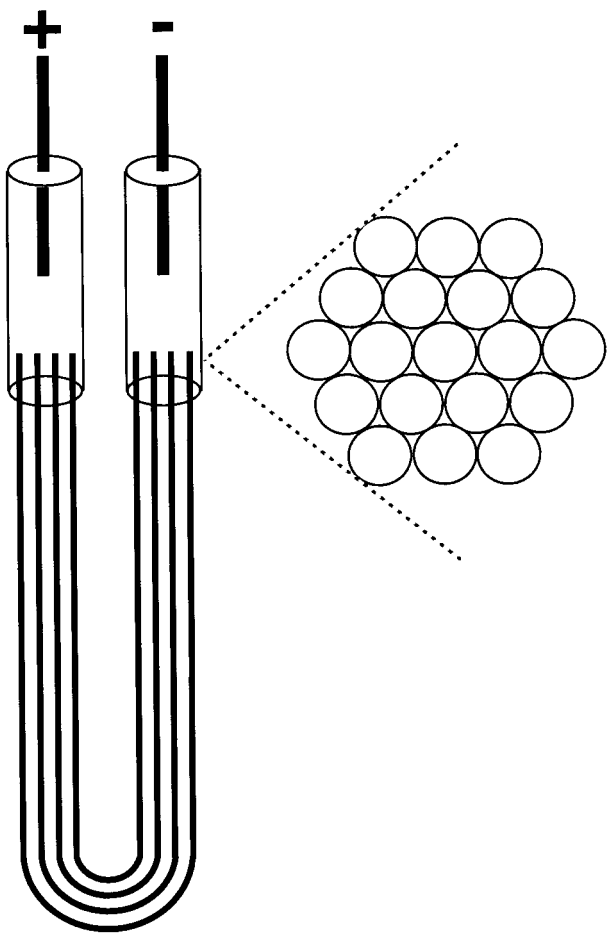

FIG. 2. Schematics of a CA-ENMR sample cell. The number of bundled capillaries varies in different experiments. All capillaries have the same length to ensure that the same resistance and electric field are produced in each tube.

The assembled sample cells were loaded from top of a spectrometer magnet into a 5-mm RF resonator. The number of capillary array elements can be adjusted to optimize the coil filling factor. Sample temperature was regulated at 25°C by the temperature control unit of the Bruker AM 500 spectrometer, equipped with *z*-magnetic field gradient. The relatively large ratio of capillary surface area to sample volume ensures efficient heat exchange. The smaller cross-sectional area, A, of a capillary reduces the need of electric current, I_e, for generating sufficient strength of electric field, E_{dc}, and therefore, power deposition and resistive heating are decreased. Preliminary analysis indicates that temperature gradient decreases as a function of cylindrical tube diameter, and detailed thermal study results will be published. At the same time the small capillary inner space reduces resistive heating and minimizes temperature gradient and fluid density variation, and hence, convection effect. In fact, the thin capillary walls block the convective current present when using large sample tubes.

Using this CA-ENMR method, we observed a dramatic reduction of heat-induced convection in a high salt solution containing 1 mM lysozyme and 50 mM NaH_2PO_4 in D_2O (Fig. 3a). These data were acquired with a stimulated-echo ENMR pulse sequence (*1, 7*). It can be seen that a step-wise increase of DC-electric current has produced a cosinusoidal amplitude modulation of the lysozyme resonances. The magnitude of the stimulated echo signal can be expressed as

$$M(E_{dc}) = \frac{M(0)}{2} \exp\left[-DK^2\left(\tau_D - \frac{\delta_1}{3}\right) - \frac{2\tau}{T_2} - \frac{\Delta}{T_1}\right] \times \cos[(KE_{dc}\Delta)\mu], \quad [1]$$

where E_{dc} and Δ are the amplitude and the duration of the

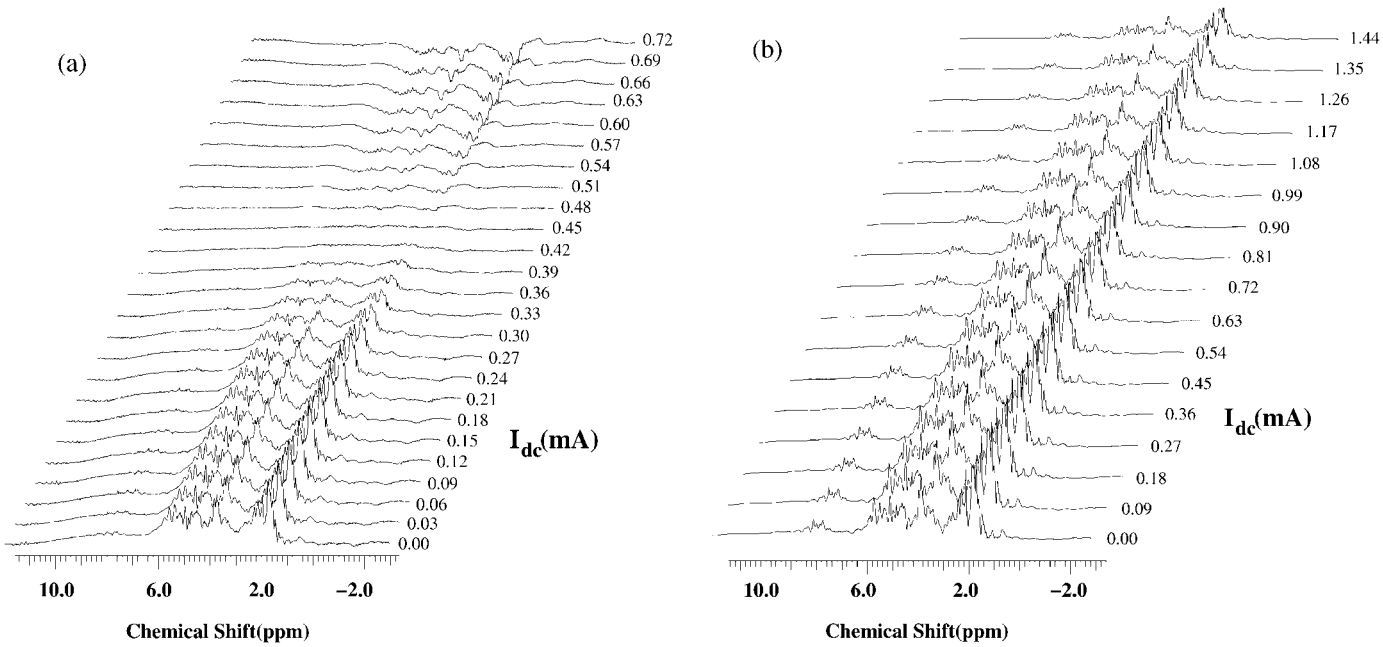

FIG. 3. Bruker AM 500 MHz electrophoretic NMR interferograms of 1 mM lysozyme in a D_2O solution of 50 mM NaH_2PO_4. Data were obtained at 25°C with (a) a 12-bundle capillary array U-tube (i.d. = 250 μm) and (b) a conventional glass U-tube. Parameters are: (a) pH 5.89, κ = 4.21 mS cm^{-1}; Δ = 0.6097 s, τ = 2.575 ms, τ_D = 0.6136 s, NS = 512, T_R = 4 s; $g_1 = g_2$ = 426.3 mT m^{-1}, δ_1 = 1 ms, δ_2 = 6 ms; E_{dc} = 0 to 29.03 V cm^{-1} and $\mu = 1.3 \times 10^{-4}$ cm^{2}V^{-1}s^{-1}. (b) E_{dc} = 0 to 9.10 V cm^{-1}, A = 3.758 mm^2, and NS = 128. Other parameters are the same as in (a). The spectral linewidth in CA-ENMR was typically 4 Hz after shimming. The pulse sequence and symbols are consistent with our earlier studies (*1*).

electric field, μ is the electrophoretic mobility of the protein, τ_D is the self-diffusion time, D is the molecular diffusion coefficient, $M(0)$ is the initial magnetization, and T_1 and T_2 are the spin–lattice and spin–spin relaxation times, respectively. Parameter $K = \gamma g_1 \delta_1$ is determined by γ, the gyromagnetic ratio of the nucleus, g_1 and δ_1, the amplitude and the duration of the labeling and refocusing gradient pulses, respectively. The sample conductivity was 4.21 mS cm^{-1}, and the solution pH was 5.89. The maximum total DC electric current was 0.72 mA (60 μA per capillary), producing an electric field of 29.03 V cm^{-1}. By contrast, a control experiment performed using a regular glass U-tube of larger diameter (A = 3.758 mm^2) yielded a maximum electric field of only 9.10 V cm^{-1}, although this required twice the maximum electric current (1.44 mA).

TABLE 1
NMR Probe Q Factors of High Salt Samples[a]

	Saline (0.9% or 154 mM NaCl)	1 mM lysozyme 50 mM KH_2PO_4 in D_2O	Deionized water (κ = 0.43 ± 0.02 mS cm^{-1})
5 mm NMR tube	131.8 ± 1.7	186.2 ± 1.5	222.8 ± 1.1
Large U-tube (diameter = 2.55 mm)	186.4 ± 3.0	208.6 ± 3.1	227.8 ± 2.2
Capillary array ENMR sample cell (12-bundle)	192.5 ± 1.5	218.1 ± 2.9	230.3 ± 1.9

[a] The sampling size is 5.

Because of insufficient strength of the electric field in the regular large diameter U-tube, no phase inversion of lysozyme resonances was observed in the cosinusoidal ENMR interferogram shown in Fig. 3b. The substantially weaker electric field in high salt conditions rendered the electrophoretic mobility measurements impossible using such large U-tubes. The reduced cross-sectional area of the capillary tubes in CA-ENMR permits a significant increase of the strength of the electric field, leading to improved signal resolution in the new dimension of motion. This is the major advantage of CA-ENMR over conventional ENMR. The trade-off for accomplishing this higher motional resolution is reduced coil filling factor. In the CA-ENMR experiment reported here, the sample volume was only 16% of that of the large U-tube. The resulting reduction of signal sensitivity, however, was offset by the fact that higher probe Q-factor are maintained in CA-ENMR (Table 1).

In both experiments, electroosmotic flow was reduced by methylcellulose coating of the sample tubes. This was accomplished by injecting the standard coating solutions (*18*) into the capillary array sample tubes. However, the coating did not alter the effective capillary diameter. The average diameters (±s.d.d.) of the capillary tube were respectively 256 μm (±5; n = 6) before coating, and 254 μm (±11; n = 12) after coating. Figure 3 shows frequency-domain spectra obtained after Fourier Transformation of the first chemical shift dimension.[4] Spectral white noise was subsequently removed by a

[4] Using Felix, an NMR software by Biosym and Molecular Simulations (San Diego).

wavelet shrinkage denoising program (*19–21*). The signal attenuation due to high electric current, which comes from residual convection in the capillaries, can be further removed by spectroscopic compensation of the convective flow. We have evaluated this effect, and detailed results are being prepared for publication.

Capillary walls also block electrical eddy current, which often reduces NMR signal sensitivity of high salt samples. To verify this, we measured the Q factors (*22*) of the NMR probe loaded with different samples (Table 1), using an HP 8753A Network Analyzer (300kHz–3.0GHz). The probe Q factor decreased as the solution ionic strength increased; however, a smaller change of the probe Q factor was observed as the area of the sample tube was decreased. Thus, the probe Q factor of a saline solution in a 5-mm NMR tube was 41% lower than that of water, whereas the probe Q factor was only 16% less than that of water when the capillary array sample cells were used. Note that this simple sensitivity comparision is solely based on Q-factor measurements and does not take into account other determining factors such as sample volume and RF coil filling factors. However, the results with CA-ENMR suggest that the higher Q factor that is achieved with the capillary array tube in high salt solutions compensates for the decrease in signal sensitivity due to the smaller sample volume and coil filling factor.

We routinely achieved excellent magnetic field homogeneity with this system since capillary array chambers introduce no magnetic susceptibility effect (*23, 24*). Each capillary can be viewed as an infinitely long circular cylinder with uniform magnetization. No external magnetic field is produced by each capillary since the cylinder behaves like a solenoid with nonzero, uniform surface current (*25*).

In summary, the significant increase of the effective electric field and the dramatic reduction of the heat-induced convection effect that CA-ENMR achieves enable electrophoretic NMR studies of protein samples in high salt biological buffer solutions. Furthermore, CA-ENMR maintains high probe Q factors and minimizes spectral lineshape distortions, which are important in obtaining high resolution spectra of proteins. Our results have demonstrated the potential of investigating multiple protein conformations and protein interactions in biological buffer solutions of high ionic strength. Lithographic technology (*26, 27*), microcoil NMR detector, and ultra-high electric field devices may be adapted to further improve signal sensitivity and resolution (*24, 28–30*). CA-ENMR does not require physical separation of proteins and can be implemented on commercially available NMR spectrometers equipped with magnetic field gradients.

ACKNOWLEDGMENTS

We thank Dr. Douglas L. Rothman for the use of Bruker AM 500 spectrometer in the Magnetic Resonance Center of Yale University School of Medicine, and Terry Nixon at Yale University for technical assistance. We are grateful to Hui He (Duke University), Zhaohui Wei, Drs. Douglas L. Rothman, Richard P. Kennon (Yale University), Ian Greenshields, T. C. Ting, and Dikoma C. Shungu (Columbia University) for discussions. The work was supported by NSF (Grant MCB-9707550), NIH (Grant RR12774-01), ACS-PRF (Grant PRF 32308-G4), University of Connecticut Research Foundation (Grant 441833), and the new faculty startup equipment funds.

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