Exploring collagen self-assembly by NMR

N. Lisitza[1], X. Huang[2], H. Hatatu[3], and S. Patz[3]
[1]Department of Radiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States, [2]Department of Psychiatry, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States, [3]Department of Radiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States

Significance

Collagen is the principal element of a wide range of connective tissues, but surprisingly little is known about its supermolecular organization. Knowledge of collagen structure and the mechanisms of its formation at the molecular level are essential to elucidate the pathology of diseases such as fibrosis, atherosclerosis, osteogensis and rheumatoid arthritis. Collagen fibrils have been characterized by EM [1], X-ray diffraction [2] and AFM [3]. It was shown that collagen has a characteristic periodic structure with many hierarchical levels of association, but understanding the major steps of self-assembly and their mechanisms is far from complete. While traditional techniques are specialized for the study of surface properties, NMR has an advantage in that it allows noninvasive investigation of biological systems in solution, preserving their physiological conformations and functions. Recent work [4] shows that NMR can detect self-aggregation of complex molecules in solution. Here we apply NMR to study aggregation of type I collagen.

Material and Methods

Collagen solutions were prepared by diluting a standard commercial solution with either D_2O or deuterated PBS buffer; deuterated solvents and salts were used to exclude the solvent signals from NMR spectra. All solutions were equilibrated for ~ 1 hour prior to NMR measurement. The solutions were then incubated for ~ 24 hours, 2, 3, 5 and 7 days at 25°C and the NMR signal was measured at each step. All experiments were performed at 500.1 MHz on a Varian NMR Systems Inc. spectrometer, using a standard PRESAT sequence to suppress the residual H_2O signal. To increase the signal-to-noise ratio, 976 scans were averaged for each protein spectrum. Details of the experimental procedure can be found elsewhere [5].

Results and Discussion

Figure 1 shows a ^1H spectrum of collagen solution. The spectral components ranging from 0.5 to 2.5 ppm represent the collagen aliphatic protons. All spectra were integrated from 0.5 - 2.5 ppm and defined as the "collagen signal".

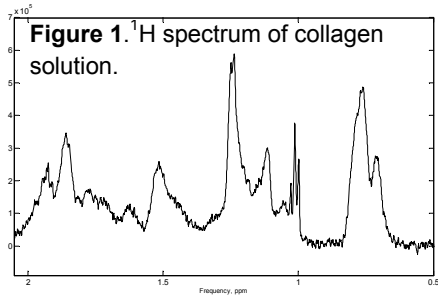

Figure 1. ^1H spectrum of collagen solution.

Figure 2A shows the dependence of the NMR collagen signal on concentration measured at different aggregation stages with pD=3.4. All curves experience a kink or deviation from linearity at ~50 μg/ml, which we interpret as due to fast T_2 relaxation for some of the species. This concentration is close to the value reported in the literature for collagen aggregation–35 μg/ml [6]. The deviation from linearity becomes even more pronounced in aged solutions. Such behavior is consistent with aggregate formation and implies signal loss occurs because of fast relaxation in the aggregates. When protein aggregates, its rotational mobility decreases and fast relaxation occurs due to less efficient motional averaging. As a consequence, the measured NMR signal becomes reduced. To determine whether similar behavior is observed for physiologically relevant conditions, collagen aggregation was also monitored in deutorated PBS solutions (pD=7.4). The initial concentration dependence at pD=7.4 (Figure 2b) is almost linear, with a kink developing after incubation. This observed behavior correlates with aggregation mechanisms proposed in the literature [7]. For acidic solutions (Fig. 2a), aggregation occurs via nucleation with a potentially complex initial structure, implying slow molecular motion and the onset of fast relaxation. The final structure of the protein aggregate becomes even more complex; consequently a more pronounced decline of the collagen NMR signal is observed. For a neutral pD, aggregation occurs via initial formation of small aggregates that combine together to produce larger species. In this regime, the initial structure is open and there is little basis for fast relaxation, which is consistent with our observed linear dependence of the NMR signal vs. concentration. The final structure becomes highly packed and motionally limited, implying accelerated spin relaxation. Therefore, a kink appears on the concentration curve after incubation.

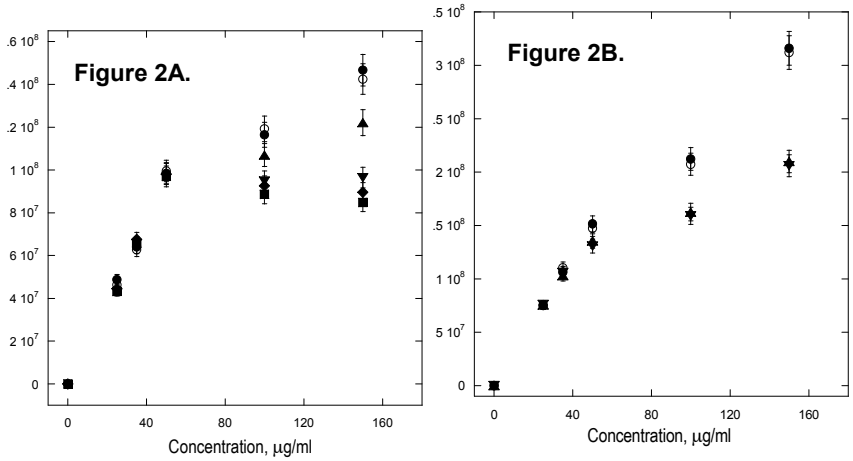

Figure 2. The collagen signal as function of protein concentration for solutions prepared with A) D_2O (pD=3.4), B) PBS buffer (pD=7.4). Measurements were made after ~ 1 hour (●), 1 day (○), 2 days (▲), 3 days (▼), 5 days (♦), 7 days (■).

Conclusion

We have demonstrated that the integrated NMR signal intensity of collagen can detect fundamental changes in structure.

Reference **[1]** R. L. Trelstad, K. Hayashi, J. Gross, *PNAS* 1976, 73, 4027, **[2]** J. P. R. O. Orgel, T. C. Irving, A. Miller, T. J. Wess, *PNAS* 2006,103, 9001, **[3]** D. A. Cisneros, C. Hung, C. M. Franz, D. J. Muller, *J. Struct. Biol*. 2006, 154, 232, **[4]** N. V. Lisitza, D. E. Freed, P. N. Sen, Y.-Q. Song. Energy & Fuels 2009, 23, 1189, **[5]** N. Lisitza, X. Huang, H. Hatabu, S. Patz. Phys. Chem. Chem. Phys. 2010, 12 (42), 14169, **[6]** A. M. Romanic, L. Spotila, E. Adachi, J. Engel, Y. Hojima, D. Prockopll, *J. Biol. Chem*. 1994, 269, 11614, **[7]** J. R. Harris, A. Reiber, *Micron* 2007, 38, 513.