^{1}H Spin–Lattice Relaxation Times of Imidazole and L-Histidine Treated with a Metal-chelating Resin

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Summary Paramagnetic metal ion impurities which seriously perturb the spin–lattice relaxation times of the C-2 and C-4 protons of imidazole and L-histidine were eliminated to a satisfactory degree by treating the sample solutions with a metal-chelating resin.

THE spin–lattice relaxation times (T_1) of the imidazole C-2 protons of histidine residues in some proteins were studied in order to gain an insight into the macromolecular structure and the mobility of the side chain in solution.[1] Wasylishen and Cohen pointed out that the T_1 values were seriously influenced by trace amounts of paramagnetic metal ions and, therefore, the results obtained for proteins should be interpreted with caution.[2]

It was usually believed that correct T_1 values could be obtained by the addition of ethylenediamine tetra-acetate (EDTA) which would effectively chelate paramagnetic metal ions. However, Wasylishen and Cohen showed that the effect of paramagnetic metal ions could not be completely eliminated with EDTA. An alternative method is to treat the sample solutions with Chelex-100.[3] We found that this treatment eliminated paramagnetic metal ions

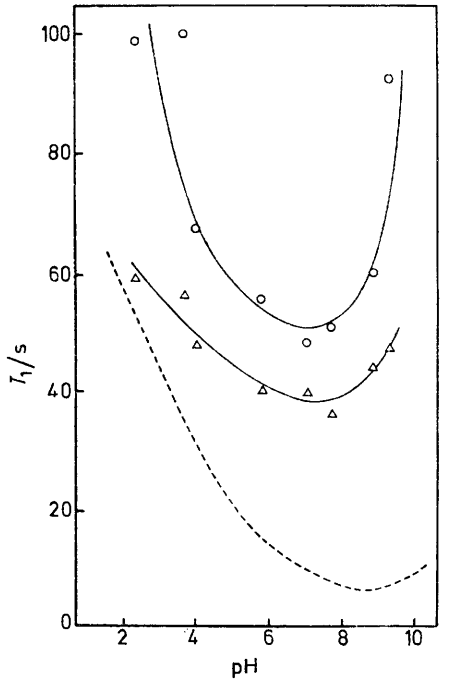

FIGURE 1. Plot of the spin–lattice relaxation time (T_1) against pH for the C-2 (○) and C-4 (△) protons of imidazole (0·1 M in 0·1 M NaCl–D_2O, 37 °C). Samples were treated with Chelex-100 and degassed. The broken line indicates the results for the C-2 proton obtained by Wasylishen and Cohen (ref. 2).

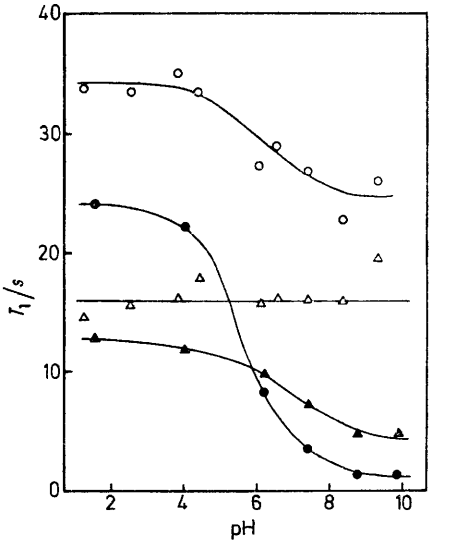

FIGURE 2. Plot of the spin–lattice relaxation time (T_1) against pH for the C-2 (○,●) and C-4 (△,▲) protons of L-histidine (0·15 M in 0·1 M NaCl–D_2O, 37 °C). Open and filled symbols indicate, respectively, the data obtained with and without the Chelex-100 treatment.

from n.m.r. sample solutions. Thus, for the C-2 and C-4 protons of imidazole and L-histidine in D_2O solutions, the observed T_1 values were much longer than the previously reported values.

All the reagents used in this experiment were of the highest quality. Glassware was cleaned thoroughly with *ca.* 50% HNO_3 before use. Each sample solution (see Figure captions) was batch-wise treated with Bio-Rad Chelex-100 and the pH was roughly adjusted with HCl and NH_4OH using pH test papers. The supernatant liquid was transferred into an n.m.r. tube, lyophilized, dissolved in D_2O, and degassed. The n.m.r. tube was then filled with N_2 and sealed. The T_1 measurements were performed with a Hitachi R-22/FT spectrometer using a standard 180°-τ-90° pulse sequence. The accurate pH (meter reading) was determined after the T_1 measurements.

In Figure 1 are shown our results for imidazole, together with the data by Wasylishen and Cohen which were obtained without the Chelex-100 treatment.[4] The T_1 values from our experiment are much longer (by 4—5 times at neutral pH) than those of Wasylishen and Cohen, indicating that paramagnetic metal ions were effectively eliminated by Chelex-100. However, the T_1 values at neutral pH are still shorter than those at acidic pH. This may be due to the remaining paramagnetic metal ions which bind to deprotonated imidazole at neutral pH. The T_1–pH profile of the L-histidine C-2 proton shows a similar trend in Figure 2, where the effect of the Chelex-100 treatment is again obvious.

If paramagnetic metal ions are responsible for the observed difference between the T_1 values at acidic and neutral pH's, the paramagnetic contribution to the relaxation rate (T_1^{-1}) may be estimated from the difference to be *ca.* 0·01 s^{-1}. This means that, even for a proton with T_1 as long as 10 s, the paramagnetic contribution to the total relaxation rate can be reduced to about 10% when the sample is carefully treated with Chelex-100. For a proton with a shorter T_1 value, therefore, the paramagnetic contribution can be made negligibly small with the same treatment.

Commercial NaOH should not be used for the pH adjustment because the paramagnetic contaminations are difficult to remove even with Chelex-100.

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