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NMR Relaxation Study of the Thermal Denaturation of Lysozyme

D. Pumpernik, B. Fajt, S. Lapanje, and A. Ažman

Chemical Institute Boris Kidrič and Department of Chemistry, University of Ljubljana, Ljubljana

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Relaxation, Lysozyme, Thermal Denaturation

Lysozyme relaxation times T_1 and T_2 have been measured. Methyl groups T_1 are sensitive to thermal denaturation of the lysozyme. The exchange of the water molecules between bounded and free is slow.

The thermal denaturation of lysozyme was very extensively studied [1] by various methods including high resolution NMR spectroscopy [2]. The purpose of this contribution is to report the results of NMR measurements of the proton spin-lattice (T_1) and spin-spin (T_2) relaxation times. The measurements have been made on lysozyme dissolved in D_2O and H_2O. Salt-free egg-white lysozyme (6 x cryst., Lot 7102) was supplied by Miles Laboratories, Inc. The concentration of lysozyme was in both cases 10% and pD 5.6. The relaxation times were measured on a conventional Bruker pulse spectrometer B-KR 322 s.

Lysozyme in D_2O

A. The measured spin-lattice relaxation time can be accurately separated into three contributions. In Figs 1 and 2, two of them are depicted. The third is temperature independent with the value 1.6 sec. The dominant relaxation mechanism for the protons is the dipole-dipole one. From the minimum of T_1^1 one can calculate the reorientational correlation time $\tau = 9.8 \times 10^{-10}$ sec and the proton-proton distance $r = 2$ Å. Near the minimum of T_1^1 the activation energy has the value 2.24 kcal/mol. All these three numbers suggest that the main contributors to the T_1^1 minimum are the methyl groups.

The contributions of other groups to T_1^1 are not negligible. The shape of T_1^1 is not symmetrical around the minimum, and the activation energy far from it has a value of approximately 12 kcal/mol which strongly indicates that larger groups than the methyl ones influence the relaxation time. The

Requests for reprints should be sent to Dr. A. Azman, Department of Chemistry, University of Ljubljana, Murnikova 6 — P.O.B. 537, *61001 Ljubljana*, Yugoslavia.

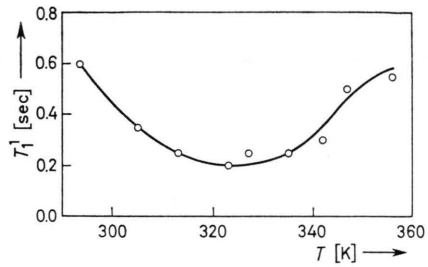

Fig. 1. Spin-lattice relaxation time T_1^1 of lysozyme in D_2O.

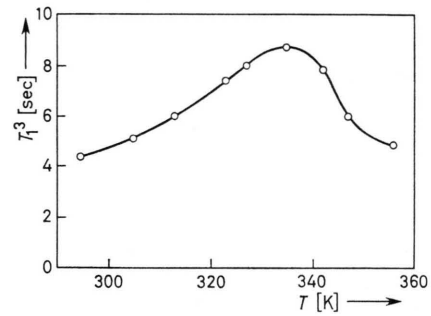

Fig. 2. Spin-lattice relaxation time T_1^3 of lysozyme in D_2O.

methyl groups as monitored by T_1^1 are a sensitive indicator of pretransition changes occurring in the thermal transition [3]. T_1^3 results from the constituent motions that are not activated in the thermal transition.

B. The spin-spin relaxation time T_2 can be separated into two contributions, Figs 3 and 4. From the temperature dependence of T_2^1 the conclusion can be drawn that the main contributions stem from larger groups whose ordering in lysozyme is constant between $T = 320$ and $T = 340$ K. At higher temperatures motions of these groups are uncooperative like in simple liquids with T_2 approaching T_1. The maximum of T_2^2 is at the same temperature as the minimum of T_1^1. From the known

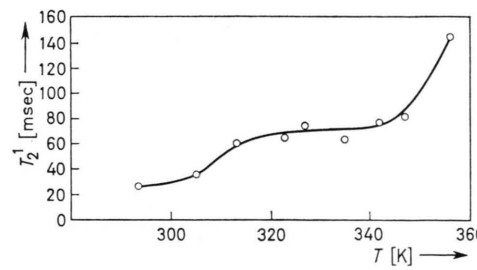

Fig. 3. Spin-spin relaxation time T_2^1 of lysozyme in D_2O.

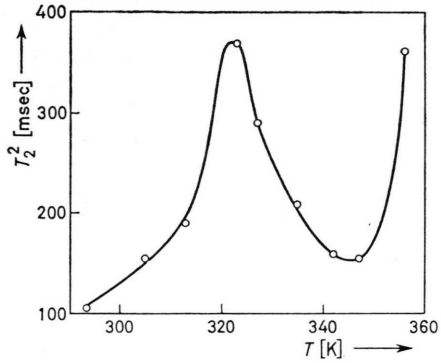

Fig. 4. Spin-spin relaxation time T_2^2 of lysozyme in D_2O.

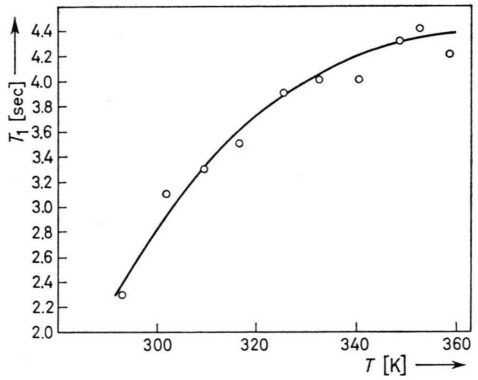

Fig. 5. Spin-lattice relaxation time T_1 of lysozyme in H_2O.

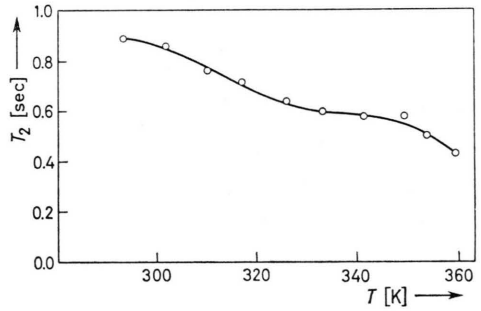

Fig. 6. Spin-spin relaxation time T_2 of lysozyme in H_2O.

expression [4] for T_2 and from the data calculated from T_1[1], the T_2^2 can be reproduced in the temperature interval $T = 315 - 335$ K. Thus the reorientational motions of methyl groups contribute to T_2^2 in this temperature interval.

Lysozyme in H_2O

Measurements of T_1 (T_2) of lysozyme in H_2O have given only one value for T_1 (T_2), Figs 5 and 6. The relaxations times T_1 and T_2 are due to the proton motion of water. From the difference between T_1 of lysozyme in H_2O and pure water the fraction of the "immobilized" water at 25 °C is estimated to be about 14%. This is the upper limit if the assumption is made that T_1 of the bound water is much smaller than that of the free one. It is interesting that this is not supported by measurements of the diffusion coefficients. Within the experimental error, the diffusion coefficients of pure water and the water in lysozyme-water solution are the same. The sensitivity of T_1 and the diffusion coefficients to the amount of the bound water is apparently not the same.

The spin-spin relaxation time T_2 decreases with temperature. This behaviour suggests that the exchange of the "free" and "bound" water molecules is slow [5]. The difference $(1/T_2 - 1/T_1)$ increases logarithmically with temperature, the exchange energy being 3.9 kcal/mol.

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[1] T. Imoto, L. N. Johnson, A. C. T. North, D. C. Phillips, and J. A. Rupley, The Enzymes (P. B. Boyer, ed.), **Vol. VII,** Chapter 21, Academic Press, New York and London 1973.

[2] C. C. McDonald, W. D. Phillips, and D. D. Glickson, J. Amer. Chem. Soc. **93,** 235 [1971].

[3] N. N. Khechinashvili, P. L. Privalov, and E. I. Tiktopulo, FEBS Lett. **30,** 57 [1973].

[4] A. Abragam, The Principles of Nuclear Magnetism, Oxford University Press, London 1961.

[5] B. D. Sykes, P. G. Schmidt, and G. R. Stark, J. Biol. Chem. **245,** 1180 [1970].