Nuclear magnetic resonance spin-spin relaxation time in hydrated protein powders. A two site dynamic exchange model

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investigations with other combinations of semiconductors and sensitizing dyes.

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References and Notes

(1) H. Tributsch and H. Gerischer, *Ber. Bunsenges. Phys. Chem.*, **73**, 251, 850 (1969); **72**, 437 (1968).
(2) B. Pettinger, H. R. Schöppel, and H. Gerischer, *Ber. Bunsenges. Phys. Chem.*, **77**, 960 (1973).
(3) E. Daltrozzo and H. Tributsch, *Photogr. Sci. Eng.*, **19**, 308 (1975).
(4) H. Gerischer, *Faraday Discuss., Chem. Soc.*, **58**, 219 (1974); H. Gerischer and M. Lübke, *Z. Phys. Chem. (Frankfurt am Main)*, **98**, 317 (1975).
(5) K. Hauffe, H. Pusch, and J. Range, *Z. Phys. Chem. (Frankfurt am Main)*, **64**, 122 (1969); U. Bode, K. Hauffe, Y. Ishikawa, and H. Pusch, *ibid.*, **85**, 144 (1973); K. Hauffe and O. Haeggqwist, *ibid.*, **85**, 191 (1973).
(6) H. J. Danzmann and K. Hauffe, *Ber. Bunsenges. Phys. Chem.*, **79**, 438 (1975).
(7) K. Hauffe and U. Bode, *Faraday Discuss., Chem. Soc.*, **58**, 281 (1974); K. Hauffe, *Photogr. Sci. Eng.*, **20**, 124 (1976).
(8) W. P. Gomes and F. Cardon, *Ber. Bunsenges. Phys. Chem.*, **75**, 914 (1971).
(9) M. Matsumura, Y. Nomura, and H. Tsubomura, *Bull. Chem. Soc. Jpn.*, **49**, 1409 (1976).
(10) A. Fujishima, T. Iwase, T. Watanabe, and K. Honda, *J. Am. Chem. Soc.*, **97**, 4134 (1975); T. Watanabe, A. Fujishima, and K. Honda, *Ber. Bunsenges. Phys. Chem.*, **79**, 1213 (1975).
(11) T. Watanabe, A. Fujishima, O. Tatsuoki, and K. Honda, *Bull. Chem. Soc. Jpn.*, **49**, 8 (1976).
(12) M. T. Spitler and M. Calvin, *J. Chem. Phys.*, **66**, 4294 (1977).
(13) W. D. K. Clark and N. Sutin, *J. Am. Chem. Soc.*, **99**, 4676 (1977).
(14) T. Iwasaki, S. Sumi, A. Fujishima, and K. Honda, *Photogr. Sci. Eng.*, **23**, 1 (1979); T. Abe, S. Ohkouchi, M. Sukigara, and K. Honda, *Nippon Kagaku Kaishi*, 569 (1976).
(15) H. Gerischer and H. Selzle, *Electrochim. Acta*, **18**, 799 (1973).
(16) R. Memming and H. Tributsch, *J. Phys. Chem.*, **75**, 562 (1971); R. Memming, *Photochem. Photobiol.*, **16**, 325 (1972).
(17) R. Memming, *Faraday Discuss., Chem. Soc.*, **58**, 261 (1974); M. Gleria and R. Memming, *Z. Phys. Chem. (Frankfurt am Main)*, **98**, 303 (1975).
(18) H. Kim and H. A. Laitinen, *J. Electrochem. Soc.*, **122**, 53 (1975).
(19) T. Osa and M. Fujihira, *Nature (London)*, **264**, 349 (1976).
(20) T. Miyasaka, T. Watanabe, A. Fujishima, and K. Honda, *J. Am. Chem. Soc.*, **100**, 6657 (1978).
(21) H. Gerischer and F. Willig, *Top. Curr. Chem.*, **61**, 31 (1976).
(22) W. N. Hansen, T. Kuwana, and R. A. Osteryoung, *Anal. Chem.*, **38**, 1810 (1966); N. Winograd, H. N. Blount, and T. Kuwana, *J. Phys. Chem.*, **73**, 3456 (1969).
(23) T. Sawada and H. Kamada, *Jpn. Anal.*, **22**, 882 (1973).
(24) S. Oda, T. Sawada, and H. Kamada, *Anal. Chem.*, **50**, 865 (1978); *Proc. Jpn. Acad.*, **54**, B 189 (1978).
(25) H. Lerner, *Photogr. Sci. Eng.*, **13**, 103 (1969); Z. M. Jarzebski and J. P. Marton, *J. Electrochem. Soc.*, **123**, 333C (1976).
(26) W. West and P. B. Gilman, "The Theory of the Photographic Process", 4th ed, T. H. James, Ed., Macmillan, New York, 1977, pp 251–290.
(27) V. A. Myamlin and Y. V. Pleskov, "Electrochemistry of Semiconductors", Plenum Press, New York, 1967.
(28) F. Möllers and R. Memming, *Ber. Bunsenges. Phys. Chem.*, **76**, 469, 475 (1972).

Nuclear Magnetic Resonance Spin–Spin Relaxation Time in Hydrated Protein Powders. A Two Site Dynamic Exchange Model

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The NMR spin–spin relaxation time of water in hydrated protein powder is analyzed by using a two-site dynamic exchange model. An expression is derived for the population fraction of stronger bound water molecules by using Guggenheim adsorption isotherm. The model is applied to the published experimental results from the NMR of lysozyme. A linear relationship is found between the spin–spin relaxation time of water fixed to primary binding sites and water content.

Introduction

NMR spectra of water in hydrated protein powder show a single spin–spin relaxation time (T_2). Zimmerman and Brittin[1] have shown that two or more exchangeable spin populations with different dynamic states can give a single observable transverse relaxation time if a fast exchange condition is satisfied. The formulation of a detailed model for the dynamical behavior of water in hydrated protein presents serious difficulties. Even the simplest exchange model (two sites) that one can propose needs independent determination of, or, what is more serious, severe assumptions, on different parameters.

The purpose of this work is to show how a two-state dynamical model can adequately describe the NMR transverse relaxation behavior of water in hydrated protein powder. The main point is the use of the population fraction of primary binding sites derived from the adsorption isotherm. The model is worked out by using published experimental data.

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The Exchange Model

The inverse of the spin–spin relaxation time can be expressed, assuming chemical fast exchange conditions, as

$$1/T_2^{\text{obsd}} = \sum_i p_i/T_{2i} \quad (1)$$

where T_{2i} is the spin–spin relaxation time corresponding to the ith state and p_i the probability of finding a molecule in such a state (i.e., the population fraction). For the case of a two state model, eq 1 is reduced to

$$1/T_2^{\text{obsd}} = f/T_2^{\text{b}} + (1-f)/T_2^{\text{m}} \quad (2)$$

where T_2^{b} is the relaxation time of the strongly bound molecules, with population fraction f, and T_2^{m} is the time corresponding to the more freely adsorbed water ("multilayer" water). If T_2^{b} remains constant with changes in water content we have a static model, otherwise we obtain a dynamic model.

From the point of view of NMR experiments eq 2 has three unknowns: T_2^{b}, T_2^{m}, and f. We will consider first

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how f can be obtained by means of an independent experiment.

The Population Fraction of Primary Binding Sites

If we consider that water molecules with spin–spin relaxation time T_2^b can be identified with water fixed at primary binding sites detected in adsorption experiments, while the molecules with characteristic T_2^m correspond to "multilayer" water, the adsorption isotherm can be of great help in the evaluation of f.

Let us consider one particular formulation of the adsorption isotherm. We will use the formalism due to Guggenheim[2] although a similar final result can be obtained with others. The Guggenheim approach is based on the use of grand partition functions. For details and proofs regarding the isotherm the reader is referred to the original work and ref 3.

It is useful to consider the absolute activity, $\lambda = \exp(\mu/RT)$, with μ being the thermodynamic potential. The standard thermodynamic potential for the ith site is defined as

$$\mu_i = -RT \ln q_i \tag{3}$$

where q_i is the ordinary partition function and the term "standard" refers to a full occupation of sites. It is considered that for the primary binding sites the partition function is q_1 while for the secondary ones we have $q_2 = q_3 = \ldots = q$. Both partition functions are proportional, so that we can write $q_1 = cq$. The number of molecules bound for a certain amount of adsorbent can be written

$$n = Nc(p/p^*)/\{[1 + (c-1)p/p^*](1 - p/p^*)\} \tag{4}$$

where N is the number of primary binding sites, p is the vapor pressure, and p^* is a parameter that stems from the relation $\lambda q = p/p^*$. This isotherm is reduced to that of BET[4] for $p^* = p_0$, being p_0 the saturated vapor pressure.

We will now look at the occupation of primary binding sites. It can be ssen that the relative probability of finding an empty site is 1, whereas λcq, $\lambda^2 cq^2$, $\lambda^3 cq^3$, ..., are proportional to the probability of finding a site filled with one, two, three, ..., molecules. If S_0 is the number of empty sites, we have for $S_0 \propto 1$

$$N \propto 1 + \lambda cq + \lambda^2 cq^2 + \ldots \tag{5}$$

and, consequently, we can write

$$S_0/N = 1/(1 + \lambda cq + \lambda^2 cq^2 + \ldots) \tag{6}$$

The ratio of occupied sites to the total is

$$(N - S_0)/N = 1 - S_0/N \tag{7}$$

and the fraction of the total number (n) of molecules present that are fixed to S primary binding sites becomes

$$f = (S/n)(1 - S_0/N) \tag{8}$$

Using eq 5 (in terms of p/p^*) and 3, we found

$$f = (S/N)(1 - p/p^*) \tag{9}$$

Finally, if we consider all the primary binding sites (i.e., $S = N$), we have

$$f = 1 - p/p^* \tag{10}$$

Expression 9 has been used by Grigera and Berendsen[5] for the analysis of NMR spectra of collagen.

We will now look at the existing difference between the present approach and the most usual assumption that all the primary binding sites are filled *before* any molecule goes to a secondary site. This assumption implies that f' $= N/n$ for $n > N$ and 1 otherwise. We denote as f' the fraction obtained in this way. Figure 1 shows the ratio f/f' for collagen and lysozyme. It can be seen that the maximum difference between the values derived by both approaches is found for $n = N$.

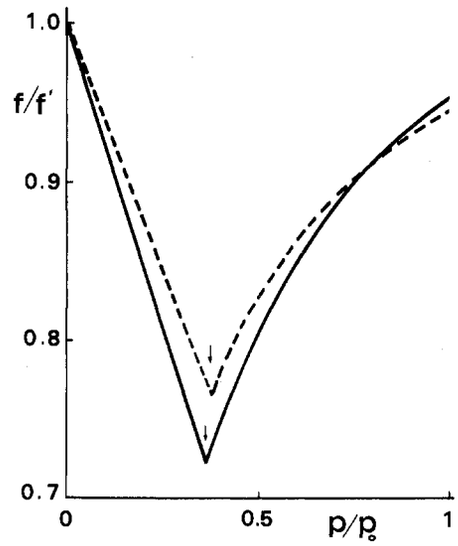

Figure 1. Ratio between population fractions computed from the adsorption isotherm (f) and assuming total occupation of primary binding sites before "multilayer" is formed (f') against relative partial vapor pressure for collagen (----) and lysozyme (—). The arrows point the p/p_0 value for which $n = N$ in each case.

The Spin–Spin Relaxation Time of Water Bound to Secondary Sites

In hydrated protein powders T_2^m can be evaluated by using the information available from dielectric relaxation experiments. These experiments are not suitable to detect water fixed to primary sites. This is due to the existing dielectric inhomogeneities that produce interfacial effects. Experiments carried out for collagen over a wide frequency range[6] have shown that water with dielectric relaxation time longer than 10^{-10} s cannot be detected. Reported results for lysozyme[7] can be interpreted in the same way and it is probable that this interpretation can be applied to any protein. Since the dielectric relaxation time, τ_D, is of the same order as the NMR correlation time, τ_c, it is possible to estimate T_2^m by using well-known relations.[8] With τ of the order of 10^{-10}, a spin–spin relaxation time of about 300 ms is obtained.

For a typical observed T_2 (<6 ms) eq 2 can be replaced by

$$1/T_2^{obsd} \simeq f/T_2^b \tag{11}$$

provided that p/p^* is not close to one.

Application to Experimental Data

To compare the above presented approach with experimental results one needs to know the spin–spin relaxation time at different water content and the corresponding adsorption isotherm. The results reported by Hilton, Hsi, and Bryant[9] satisfied this requirement. We will discuss the model by using these data.

Although adsorption results have considerable scattering it has been possible, using a least-square procedure, to fit them reasonably well with the Guggenheim formula (eq 4) with $N = 14.82$ g of H_2O/100 g of dry weight; $c = 6.76$ and $p^* = 1.309p_0$. Computing f and T_2^b with eq 10 and 11, using the experimental T_2^{obsd}, it is possible to plot T_2^b against water content. This is done in Figure 2, where a lineal relationship can be observed. The least-square fit

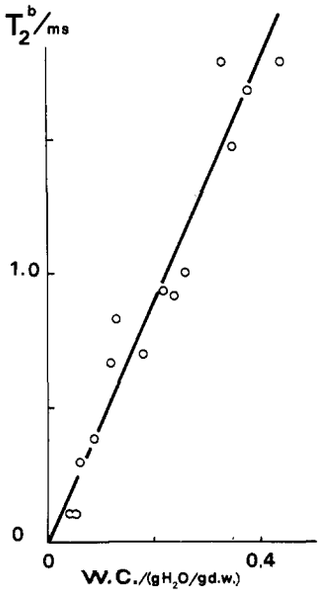

Figure 2. Spin–spin relaxation time for the stronger bound water against water content for lysozyme.

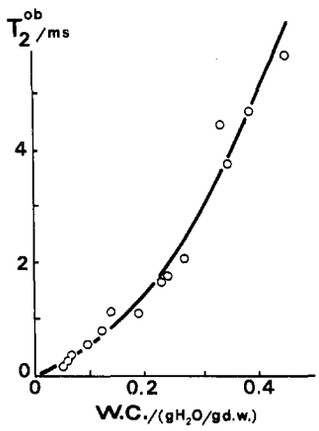

Figure 3. Observed spin–spin relaxation time against water content for lysozyme. Circles correspond to experimental points from ref 9 and the line to computed values with the present model.

of points corresponds to the straight line drawn in the figure, obeying the relation $T_2^b = aw$; where w is the water content in g of H_2O/g of dry weight and $a = 4.5$ ms/(g of H_2O/g of dry weight).

The first conclusion we can draw is that the static model (T_2^b = constant) cannot be supported. Instead we must assume that spin–spin relaxation time changes with water content.

Using the linear relation of T_2^b with water content we can see what will be the variation of T_2^{obsd} with moisture changes. Figure 3 shows the experimental T_2^{obsd} against water content as was obtained by Hilton et al.,[9] together with the curve obtained with the aid of eq 11 and the previous results. The fit seems to be quite acceptable.

Conclusions

The fact that T_2^b is not a constant introduces some doubts on the assumptions made in the derivation of the isotherm and therefore in the values of the population fraction of primary sites. The conclusion that the assumption about the equality of partition functions is not strictly valid can also be reached from calorimetric studies[3] and have been considered as an approximation from the very beginning in the derivation of the isotherm.[2] However, the successful fit of experimental data by this isotherm for the several cases tested[10] suggests that it describes reasonably well the adsorption phenomenon.

Although many different models can be built in order to explain the experimental results, the one presented here is probably the simplest one that can account adequately for the results of the experiments. It can be argued that the assumption of only one T_2^b is an oversimplification, and it is probably true. However, the experimental situation still does not allow us to discriminate between the probably existing "subset" of relaxation times.

The existence of cross relaxation invalidates any attempt to directly generalize this treatment to the spin–lattice relaxation time.

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References and Notes

(1) Zimmerman, J. R.; Brittin, W. E. *J. Phys. Chem.* **1957**, *61*, 1328.
(2) Guggenheim, E. A. "Application of Statistical Mechanics"; Clarendon Press: Oxford, 1966.
(3) Berendsen, H. J. C. In "Water. A Comprehensive Treatise", Franks, F., Ed.; Vol. 5, Plenum Press: New York, 1975.
(4) Brunauer, S.; Emmet, P. H.; Teller, E. *J. Am. Chem. Soc.* **1938**, *60*, 309.
(5) Grigera, J. R.; Berendsen, H. J. C. *Biopolymers* **1979**, *18*, 47.
(6) Grigera, J. R.; Vericat, F.; Hallenga, K.; Berendsen, H. J. C. *Biopolymers* **1979**, *18*, 35.
(7) Harvey, S. C.; Hoeckstra, P. *J. Phys. Chem.* **1972**, *76*, 9987.
(8) Carrington, A.; Mc Laughlan, A. "Introduction to Magnetic Resonance"; Harper & Row: New York, 1967.
(9) Hilton, B. D.; Hsi, E.; Bryant, R. G. *J. Am. Chem. Soc.* **1977**, *99*, 8487.
(10) Grigera, J. R.; Cabada, M. O. *Acta Physiol. Lationam.* **1975**, *25*, 446; Mogilner, I.; Grigera, J. R. unpublished. See also ref 2 and 5.