

UC Irvine

UC Irvine Previously Published Works

Title

Correlation of IR spectroscopic, heat capacity, diamagnetic susceptibility and enzymatic measurements on lysozyme powder.

Permalink

<https://escholarship.org/uc/item/1hb0d38s>

Journal

Nature, 284(5756)

ISSN

0028-0836

Authors

Careri, G
Gratton, E
Yang, PH
[et al.](#)

Publication Date

1980-04-01

DOI

10.1038/284572a0

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

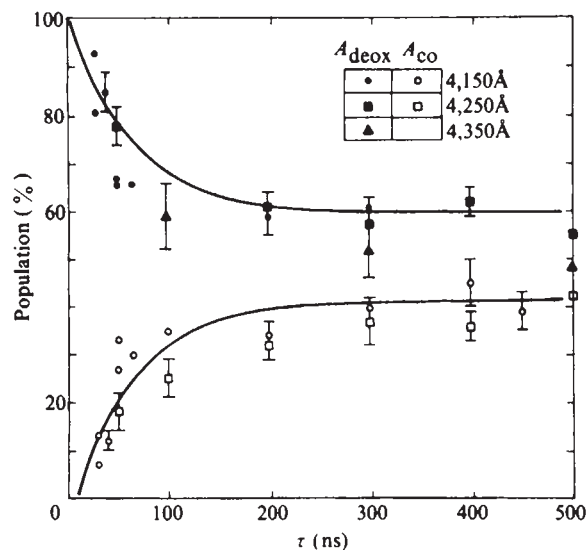


Fig. 3 Population weight factors, A_{deox} , A_{CO} , resulting from the least-squares analysis described in the text. The wavelengths in the key denote the dye laser excitation used to obtain the resonance Raman spectra.

concentration was also observed in transient absorption measurements at discrete frequencies¹⁰. In the absorption study it was also found⁹ that the amount of recombination decreased with increasing temperatures. From these dependencies we conclude that the fast recombination is, in fact, geminate in origin.

The deoxy-haem species appears in transient absorption spectra within picoseconds of photolysis⁴⁻⁶. Hence, any geminate recombination mechanism must include some means of trapping the CO molecule in the haem pocket after cleavage of the Fe-CO bond for a time τ of about 65 ns. Furthermore, this trapping, if it involves the Fe ion, must occur in a way that does not produce the HbCO absorption or Raman spectra. It seems likely, then, that the trapping is related to direct interaction of the CO molecule with the surrounding protein. This interaction probably takes the form of CO-binding sites (or potential minima) on the protein. This direct CO-protein interaction, responsible indirectly for the geminate recombination, would explain the protein and conformation dependence of the quantum yield.

The difference between the MbCO and HbCO systems could lie either in the dynamics of escape and recombination from similar sites, or in the existence of different kinds of sites. In either case, the α - and β -chains would be expected to exhibit differences in their behaviour. The conformation dependence of the quantum yield¹⁵ may also be explained on this basis. Furthermore, the proximity of the geminate recombination fraction to 50% in the *R* conformation strongly suggests that one chain predominantly undergoes recombination and the other does not. Indeed, experiments with isolated chains do indicate differences¹² at 290 K ($QY_{\beta} \approx 8\%$, $QY_{\alpha} \approx 90\%$). However, because this relationship is strongly temperature dependent, it may not hold at physiological temperatures, where the quantum yield for HbCO is much higher, and the geminate recombination is correspondingly reduced.

Thus, we have shown that transient resonant Raman spectra of photodissociated HbCO support the basic features of the suggestions made recently on the basis of transient absorption experiments^{9,10}. The better separation of the transient species in the Raman spectra has made possible a more complete determination of the populations as a function of time after photolysis. Our findings indicate that differences in the quantum yield for photodissociation of carboxyhaemoproteins can originate from differences in geminate recombination rates.

Received 22 October 1979; accepted 7 February 1980.

- Haldane, J. & Smith, J. L. *J. Physiol., Lond.* **20**, 497-520 (1896).
- Gibson, Q. H. *Biochem. J.* **71**, 293-298 (1959).
- Lyons, K. B., Friedman, J. M. & Fleury, P. A. *Nature* **275**, 565-566 (1978).
- Shank, C. V., Ippen, E. P. & Bersohn, R. *Science* **193**, 50-51 (1976).
- Noe, L. J., Eisert, W. G. & Rentzepis, P. M. *Proc. natn. Acad. Sci. U.S.A.* **75**, 573-577 (1978).
- Greene, B. I., Hochstrasser, R. M., Weisman, R. B. & Eaton, W. A. *Proc. natn. Acad. Sci. U.S.A.* **75**, 5255-5259 (1978).
- Albert, B., Banerjee, R. & Lindqvist, L. *Biochem. biophys. Res. Commun.* **46**, 913-919 (1972).
- Albert, B., Banerjee, R. & Lindqvist, L. *Proc. natn. Acad. Sci. U.S.A.* **71**, 558-562 (1974).
- Duddell, D. A., Morris, R. J. & Richards, J. T. *JCS chem. Commun.* 75-76 (1979).
- Albert, B., El Mohani, S., Lindqvist, L. & Tfibel, F. *Chem. Phys. Lett.* **64**, 11-16 (1979).
- Austin, R. H., Beeson, K. W., Eisenstein, L., Frauenfelder, H. & Gunsalus, I. C. *Biochemistry* **14**, 5355-5360 (1975).
- Alberding, N. *et al. Biochemistry* **17**, 43-49 (1978).
- Sawicki, C. A. & Gibson, Q. H. *J. biol. Chem.* **251**, 1533-1542 (1975).
- Austin, R. H. *et al. Science* **181**, 541-542 (1973).
- Sawicki, C. A. & Gibson, Q. H. *J. biol. Chem.* **254**, 4058-4062 (1979).
- Friedman, J. M. & Lyons, K. B. *Proc. 2nd USA-USSR Light Scattering Symp.* (ed. Cummins, H.) (Plenum, New York, in the press).
- Srivastawa, R. B., Schuyler, M. W., Dosser, L. R., Purcell, F. J. & Atkinson, G. H. *Chem. Phys. Lett.* **56**, 595-598 (1978).
- Woodruff, W. H. & Farquharson, S. *Science* **201**, 831-832 (1978).
- Dallinger, R. F., Nestor, J. R. & Spiro, T. G. *J. Am. chem. Soc.* **100**, 6251-6252 (1978).
- Shelnutt, J. A., Rousseau, D. L., Friedman, J. M. & Simon, S. R. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4409-4413 (1979).
- Saffran, W. A. & Gibson, Q. H. *J. biol. Chem.* **22**, 7955-7958 (1977).
- Lyons, K. B. & Friedman, J. M., in preparation.

Correlation of IR spectroscopic, heat capacity, diamagnetic susceptibility and enzymatic measurements on lysozyme powder

G. Careri*, E. Gratton†, P.-H. Yang‡ & J. A. Rupley‡§

* Department of Physics, Gruppo GNSM-CNR, University of Rome, Rome, Italy

† Department of Physics, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

‡ Department of Biochemistry, University of Arizona, Tucson, Arizona 85721

The interaction between protein and water is of fundamental importance for processes ranging from protein folding and enzymatic activity¹ to anhydrobiosis². In this letter we bring together results from diverse types of measurements to give a unified picture of the hydration process for lysozyme. The data come principally from experiments with protein films and powders. The principal aim is to examine the relationship between the sites of water interaction, the extent of coverage, and the enzymatic activity, thus providing a better understanding of the relationship between water and enzyme dynamics³.

Figure 1 describes the results of measurements on the heat capacity⁴, enzymatic activity⁵, IR spectroscopic properties⁶, and diamagnetic susceptibility⁷ of lysozyme powders as a function of hydration level (h , g of water per g of protein).

The appearance of a positive peak at $1,580 \text{ cm}^{-1}$ in the IR difference spectrum (Fig. 1a) indicates the formation of carboxylate species⁸. Apparently, drying the protein below $0.05h$ has produced inversion of the pK order for carboxylic and basic groups, resulting in charge neutralisation by proton transfer. This implies that interaction with water is associated with proton redistribution—that is, normalisation of the pK order with deprotonation of carboxylic acid groups and protonation of basic groups, presumably amino groups, to give the ionisation state found for the protein in solution. A proton redistribution process is expected to produce a rise and fall in the heat capacity, as is observed at $0.05h$ (Fig. 1d).

§ To whom correspondence should be addressed.

The discontinuity in several properties at 0.07h must represent at least changes in water-water and water-protein interactions, because there are effects on the IR spectroscopic properties of both water (Fig. 1c) and protein (Fig. 1a, b). The change in slope of the heat capacity function at 0.07h suggests that the water-protein arrangements have greater freedom above the discontinuity. In this regard, the partial specific heat capacity of the water bound below 0.07h is similar to that of ice or water vapour, while that above 0.07h is greater than that of liquid water, which has twice the specific heat capacity of ice. It is important that the fine structure of the amide I' band is unchanged above 0.07h, and thus significant contributions to the heat capacity from changes in protein conformation can be ruled out⁶.

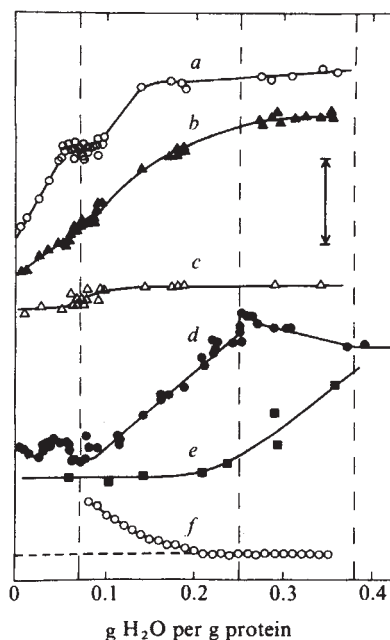


Fig. 1 Properties of the lysozyme-water system as a function of water content. The correspondence between the vertical bar in the figure and the units of measurement is given separately for each curve. From top to bottom the curves are: *a*, Absorbance at the carboxylate band maximum ($1,580\text{ cm}^{-1}$), measured at 38°C ; units, 0.35 A . *b*, Change in the amide I' band, measured at 38°C as the difference between the negative ($1,690\text{ cm}^{-1}$) and positive ($1,645\text{ cm}^{-1}$) extrema about the isosbestic point of the differential spectrum; units, 0.17 A . *c*, Frequency of the highest intensity maximum of the OD stretching band of adsorbed D_2O , measured at 38°C . The total shift is from $2,550\text{ cm}^{-1}$ to $2,580\text{ cm}^{-1}$. *d*, Apparent specific heat capacity of lysozyme, for 25°C ; units, $0.2\text{ J K}^{-1}\text{ g}^{-1}$. This function is a measure of the excess heat capacity of the system per g protein. *e*, Enzymatic activity, measured at 25°C ; units, $5 \times 10^{-6}\text{ s}^{-1}$. *f*, Diamagnetic susceptibility, measured at 25°C and expressed as differential susceptibility per g of water adsorbed; units, $0.55 \times 10^{-6}\text{ e.m.u. g}^{-1}$. The high hydration limit is equal to the value for liquid water ($0.721 \times 10^{-6}\text{ e.m.u. g}^{-1}$).

The continuous changes in heat capacity and IR spectroscopic properties from 0.1 to 0.2h represent binding of water at amide and carboxylate sites and presumably also other charged or polar sites. Saturation of these sites, if it is complete before significant covering of nonpolar elements, is expected to require^{8,9} about half the water needed for monolayer coverage, as is observed (0.2 versus 0.38h). The polar atoms are largely in the backbone and side-chain amide groups. The change in the amide I' band is a local effect produced by association of the amide group with water and not the result of changes in protein conformation¹⁰.

At 0.2–0.25h the carboxylate and carbonyl sites are saturated (Fig. 1a, b). The full coverage of the hydrogen-bonding sites is reflected in the differential diamagnetic susceptibility, which at this hydration level reaches the value for liquid water. At 0.25h the heat capacity rises (Fig. 1d) and then falls to the dilute solution value at 0.38h. This behaviour must be associated with coverage of the nonpolar elements of the protein surface, in view of the IR spectroscopic results that show saturation of hydrogen-bonding sites at lower hydration. The rise and fall in the heat capacity can be understood as a transition associated with covering of the least-strongly interacting regions of the surface. Statistical mechanical arguments indicate that a transition is expected at high coverage for adsorption of water on a heterogeneous surface¹¹. The magnitude of the 0.25h heat effect indicates that as many as 100 water molecules could be involved⁴.

The appearance of enzymatic activity at 0.2–0.25h (Fig. 1e) coincides with the saturation of the hydrogen-bonding sites and with the beginning of the coverage of the nonpolar regions of the surface. The catalytic activity is not simply reflecting water as a substrate in the hydrolytic reaction, because the dependence of the reaction velocity on water activity is tenth order. The new water arrangements on the protein surface that obtain above 0.25h may be required for catalysis. Spin-label studies⁵ show that these have greater motional freedom.

The apparent specific heat capacity shows no change between 0.38h and dilute solution. Because the heat capacity reflects all equilibria of non-zero enthalpy as well as the heat capacities of the components of the system, we regard this as sufficient evidence for the statement that the changes in thermodynamic properties associated with protein hydration are complete both for the protein and the solvent at 0.38h. This amount of water is barely sufficient to constitute monolayer coverage⁴ and corresponds to 300 molecules of water per protein molecule. Higher hydration is required to establish fully the kinetic properties for the dilute solution state.

We infer that between 0.1h and dilute solution, there can be no substantial change in protein structure. This is concluded from the monotonic behaviour of the IR spectroscopic properties of the amide and carboxylate groups between 0.07 and 0.25h, from the monotonic rise of the enzymatic activity observed above 0.2h, and from the constant apparent specific heat capacity observed from 0.38h to higher levels of hydration.

The following picture of the hydration process emerges from the findings and comments given above. The first water bound interacts with ionisable groups to produce proton redistribution. There is a clear transition in the hydrogen bonding between water and protein and water and water at 0.07h. This probably represents a change in the water molecule distribution about the protein surface, followed by an increase in freedom of the water-protein system. At 0.2–0.25h there is a major event in the hydration process, which is seen most clearly in the heat capacity and which coincides with the onset of enzymatic activity and the reaching of the final value of the response of the system to a magnetic field. Completion of monolayer coverage is at 0.38h, a value substantially greater than that for first observation of enzymatic activity. The protein with monolayer hydration shell must mesh simply with the bulk solvent.

This work was supported in part by NATO (Research Grant 1336) and the NIH.

Received 29 June 1979, accepted 13 February 1980.

- Kuntz, I. D. & Kauzmann, W. *Adv. Protein Chem.* **28**, 239–345 (1974).
- Clegg, J. S. in *Cell-Associated Water* (eds Drost-Hansen, W. & Clegg, J. S.) 363–413 (Academic, New York, 1979).
- Careri, G., Fasella, P. & Gratton, E. *A. Rev. Biophys. Bioengng.* **8**, 69–97 (1979).
- Yang, P.-H. & Rupley, J. A. *Biochemistry* **18**, 2654–2661 (1979).
- Rupley, J. A., Yang, P.-H. & Tollin, G. in *Water in Polymers* (ed. Rowland, S. P.) (American Chemical Society, in the press).
- Careri, G., Giansanti, A. & Gratton, E. *Biopolymers* **18**, 1187–1203 (1979).
- Careri, G., DeAngelis, L., Gratton, E. & Messana, C. *Phys. Lett.* **60A**, 490–491 (1977).
- Lee, B. & Richards, F. M. *J. molec. Biol.* **55**, 379–400 (1971).
- Shrake, A. & Rupley, J. A. *J. molec. Biol.* **79**, 351–371 (1973).
- Doyle, B. B., Bendit, E. G. & Blout, E. R. *Biopolymers* **14**, 937–957 (1975).
- Hill, T. L. *J. chem. Phys.* **17**, 762–771 (1949).