Large Hydration Structure Changes on Hydrolysing ATP

The hydrolysis of $Mg_{0.5}K_{2.5}H_{0.5}ATP$, $Mg_{0.5}K_{1.8}H_{1.2}ATP$ and $Mg_{0.5}K_{1.5}H_{1.5}ATP$ in hydrous solutions (0.3 *M*) was investigated with the aid of IR spectroscopy. The samples were located in cells described in [1] during spectra plotting. With these cells, the layer thickness of the sample was reproducible to $\pm$ 0.05 µm.

The solutions were stored at 40 °C in bottles which were completely sealed by a membrane. The spontaneous hydrolysis occurring thereby was interrupted every 24 h, a sample removed and a spectrum of this plotted at 5 °C. During the IR measurement, the hydrolysis degree of a parallel sample was determined enzymatically (test facilities supplied by Boehringer, Mannheim, Germany[2]).

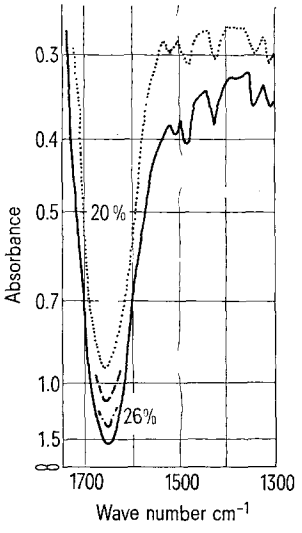

Fig. 1. H_2O scissor vibration for various degrees of hydrolysis for aqueous $Mg_{0.5}K_{2.5}H_{0.5}ATP$ solutions at 5 °C.

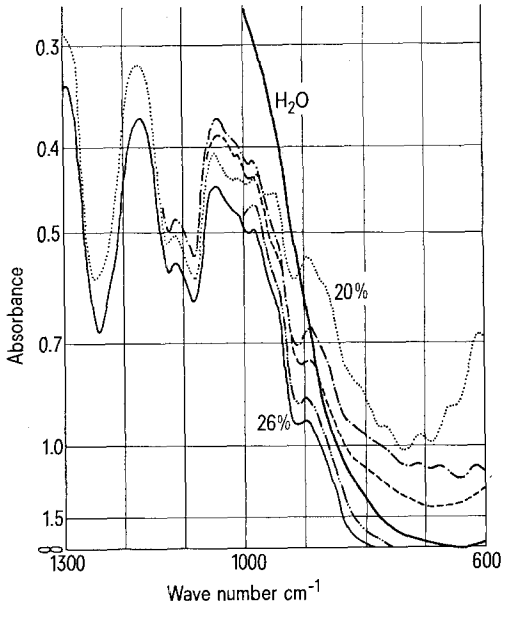

Fig. 2. H_2O torsional vibration for various degrees of hydrolysis for aqueous $Mg_{0.5}K_{2.5}H_{0.5}ATP$ solutions at 5 °C. The same with pure H_2O with equal sample thickness for purposes of comparison.

Extremely large changes in the water bands occur within small ranges as hydrolysis proceeds. Figure 1 shows the H_2O scissor vibration at about 1640 cm^{-1} and Figure 2 the broad intense band of the torsional vibration of the water molecules at about 700 cm^{-1}. This is an intermolecular vibration whereby the water molecules execute hindered librational motions relative to one another. At about 2160 cm^{-1}, a less intense combination vibration (scissor + torsional vibration)[3] occurs (for details on the vibrations of water molecules see [4] p. 28 ff.) The small bands of the ring stretching vibrations of the adenine base residues are observed in Figure 1 – besides the H_2O scissor vibration – and in Figure 2 the bands of the PO stretching vibrations of the phosphate groups.

Depending on the degree of hydration, the following changes in the bands of the water molecules are observed: 1. The integral absorbance of the torsional vibration of the water (at 700 cm^{-1}) changes, for instance with $Mg_{0.5}K_{2.5}H_{0.5}ATP$ in the range of 20–26% degree of hydrolysis, by about 2 orders of magnitude (see Figure 2, logarithmic scale). 2. In the case of slight absorbance, the band possesses a structure and its maximum lies at 710 cm^{-1}; with strong absorbance it lies at 650 cm^{-1}; both values for solutions at 5 °C. 3. A similar but less pronounced intensity change is observed with the scissor vibration at 1640 cm^{-1} (see Figure 1). 4. The H_2O combination vibration shifts parallel to the torsional vibration from 2160 cm^{-1} about 12 cm^{-1} toward larger wave numbers. 5. The changes in the background, which are observed with the spectra in Figure 1 in the region 1600–1300 cm^{-1}, are caused by the formation of easily polarizable H-bonds[4–8]. These bonds are formed between the base residues as well as between hydrogen phosphate ions. 6. The change in the integral absorbance of the H_2O scissor vibration follows that of the torsional vibration, except in the hydrolysis ranges in which the formation of polarizable hydrogen bonds is indicated by the rise of the background, i.e., by the occurrence of the continuous absorption.

In the latter case the absorbance change is explained by a coupling of the torsional vibration with transitions in hydrogen bonds in which the proton fluctuates and which are thus easily polarizable[4–8].

The band shift of the torsional vibration, and that of the combination vibration toward larger wave numbers, signifies the formation of a hydrate structure with stronger hydrogen bonds. Fox and MARTIN[9] studied this with pure water at the band at 2160 cm^{-1}, investigating its temperature sensitivity. The magnitude of the change of the transition moment of the torsional vibration in the ATP solutions shows that large ranges of the hydration structure are affected by slight alterations of

[1] K. P. HOFMANN and G. ZUNDEL, Z. Naturforsch. Ser. C, in press.
[2] *Testfibel* (Boehringer, Mannheim 1971).
[3] G. E. WALRAFEN, J. chem. Phys. *40*, 3249 (1964); J. chem. Phys. *44*, 1546 (1966).
[4] G. ZUNDEL, *Hydration and Intermolecular Interaction* (Academic Press, New York 1969 and Mir, Moscow 1972).
[5] E. G. WEIDEMANN and G. ZUNDEL, Z. Naturforschung *25a*, 627 (1970).
[6] R. JANOSCHEK, E. G. WEIDEMANN, H. PFEIFFER and G. ZUNDEL, J. Am. chem. Soc. *94*, 2387 (1972).
[7] R. JANOSCHEK, E. G. WEIDEMANN and G. ZUNDEL, J.C.S. Faraday II *69*, 505 (1973).
[8] W. SESSLER and G. ZUNDEL, Chem. Phys. Letters *14*, 356 (1972).
[9] J. J. Fox and A. E. MARTIN, Proc. R. Soc. London Ser. A *174*, 234 (1940).

the degree of hydrolysis. This supports the supposition of GEORGE et al.[10] that changes in the solvation enthalpy are primarily of significance as regards the change in the free enthalpy on ATP hydrolysis. These changes in the hydrate structure must be connected with a correspondingly large change in the dielectric properties of the systems. The hydrolysis of the ATP hence controls the intermolecular interactions in the system investigated. Since hydrolysing ATP is present in most biological systems, it is clear that the effect observed is of biological significance.

Zusammenfassung. Es wurde die Hydrolyse von ATP in wässrigen Lösungen IR-spektroskopisch untersucht. Innerhalb kleiner Hydrolysegrad-Bereiche treten enorme Veränderungen an den Banden der Wassermoleküle, insbesondere an der Torsionsschwingung auf. Die Bedeutung dieses Effekts wird diskutiert.

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6 August 1973.

[10] P. GEORGE, R. J. WITONSKY, M. TRACHTMAN, C. WU, W. DORWART, L. RICHMAN, W. RICHMAN, F. SHURAYH and B. LENZ, Biochim. biophys. Acta *223*, 1 (1970).

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Comparison of Preparations of Erythrocyte Membranes and Membrane Proteins by SDS-Gel Electrophoresis

In the past decade a large number of procedures for preparation of red cell membranes and red cell membrane protein fractions have appeared. The resulting array of protein fractions and properties has recently been described as 'bewildering'[1]. If attention is confined only to protein preparations made by perturbation of ionic strength, there are rarely two preparations of water-soluble protein that are made in exactly the same way whether the starting ghosts are the same or not. These variations make it extremely difficult to compare the results of different laboratories, or even of the same laboratory at different times. Yet it seems probable that many methods should yield preparations with similar properties, since the preparation methods are similar in principle if not identical in detail.

In the course of studies of the principal water-extractable proteins we have compared the protein composition of a number of preparations of ghosts and of water-soluble ghost proteins by gel electrophoresis, to facilitate comparison of our results with the literature.

Materials and methods. Blood was obtained as fresh units unsuitable for transfusion and used within a week. Cells washed 3 times with 0.16 *M* NaCl were used for most ghost preparations; for preparations requiring low ionic strength media, NaCl-washed cells were washed twice in large volumes of 0.32 *M* glucose, which produced clumping.

All chemicals were obtained from commercial sources and used without further purification. Distilled water was deionized and redistilled from glass.

Aliquots of ghost preparations and of preparations of water-soluble protein were analyzed by sodium dodecyl sulfate gel electrophoresis according to the method of FAIRBANKS et al.[2]. The monomer concentration was reduced to 4%, at constant acrylamide: *bis*acrylamide ratio. After staining for protein with Coomassie blue[2], gels were photographed. Protein bands are numbered according to CARRAWAY and SHIN[3].

Results. Ten preparations of ghosts were made, according to each author's directions. SDS-gel electrophoretic patterns of these preparations are shown in Figure 1. The preparations made by low ionic strength hemolysis at neutral to alkaline pH (A–G) are quite similar in composition, with the principal variations being in the low molecular weight bands 8–10 and hemoglobin. Band 10 is not retained by ghosts below 20 imos*M* (note that the preparations in F and G are made at about 10 imos*M*) while band 8 appears to be minimally retained at 20 imos*M*. The presence of EDTA in the lysis mixture does not affect these results, since A and F (prepared with EDTA) are respectively equivalent to C and G (prepared without). The depletion of band 10 below 20 imos*M* correlates with loss of the permeability barrier to ATP[5] and with destabilization of membrane protein conformation[19]. Ghosts prepared at pH 4.6 (H) gave blurred patterns, and show depletion in band 8 and apparent enrichment in bands 1, 9, and 10. The preparations made with Triton X-100 at zero ionic strength (I) are also comparable to the other ghost preparations, with loss of band 10 and possible loss of bands 5 and 6. Preparations in Triton X-100 at non-zero ionic strength are depleted in bands 4, 5, 6, 8, and 10. It should be noted that the apparent variations in bands 1–4 in the gels shown in Figure 1 are primarily due to overloading, as can be seen by comparison of gels with less protein (not shown). Overloading was deliberate, to increase the intensity of the minor bands.

Nine preparations of water soluble proteins from ghosts made by extraction at low ionic strength are shown in Figure 2. These were also made according to the

[1] A. H. MADDY, Sub-Cell Biochem. *1*, 293 (1972).

[2] G. FAIRBANKS, T. L. STECK and D. F. H. WALLACH, Biochemistry *10*, 2606 (1971).

[3] K. L. CARRAWAY and B. C. SHIN, J. biol. Chem. *247*, 2101 (1972).

[4] J. T. HOOGEVEEN, R. JULIANO, J. COLEMAN and A. ROTHSTEIN, J. Membrane Biol. *3*, 156 (1970).

[5] T. A. BRAMLEY, R. COLEMAN and J. B. FINEAN, Biochim. biophys. Acta *241*, 752 (1971).

[6] J. T. DODGE, C. MITCHELL and D. J. HANAHAN, Arch. Biochem. Biophys. *100*, 119 (1963).

[7] S. L. MARCHESI, E. STEERS, V. T. MARCHESI and T. W. TILLACK, Biochemistry *9*, 50 (1969).

[8] R. BALLANTINE, J. comp. Cell. Physiol. *23*, 21 (1944).

[9] D. MAZIA and A. RUBY, Proc. natn. Acad. Sci., USA *61*, 1005 (1968).

[10] H. FURTHMAYR and R. TIMPL, Eur. J. Biochem. *15*, 301 (1970).