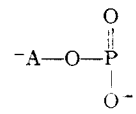
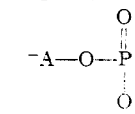
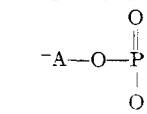
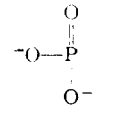
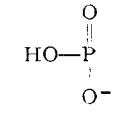
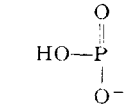
TABLE III

HEATS OF CONSTANT *p*H, UNBUFFERED HYDROLYSES OF PHOSPHATE COMPOUNDS AT 25°[a]

| Substrate | Reaction 12 x | $-\Delta H$, cal./mole | Reaction 13 y | $-\Delta H$, cal./mole | Reaction 14 z | $-\Delta H$, cal./mole |
|---|---|---|---|---|---|---|
| *p*-Nitrophenol | $O_2N\cdot C_6H_4$ | 6280 | $O_2N\cdot C_6H_4$ | 2760 | $O_2N\cdot C_6H_4$ | −1900 |
| ATP |  | 5800 |  | 5000 |  | 5000 |
| Pyrophosphate |  | 5800 |  | 5800 |  | 5800 |

[a] A represents the adenylate residue.

The primary factors in making the above comparisons so sensitive to the reaction type selected are the high heats of ionization of *p*-nitrophenol and *p*-nitrophenylphosphate. An additional factor which would have to be included in any complete discussion of the various heat values is the electrostatic interactions between the ions of various charge types. For example, reaction 13 for the ester involves no separation of charges, whereas in the case of ATP this reaction results in the separation of two doubly charged negative ions. A considerable *positive* contribution of electrostatic origin would be expected[12] in the $\Delta H$ of the ATP reaction if the effective dielectric constant for this case has a temperature coefficient similar to that of pure water.

**Acknowledgments.**—This research was supported in part by grants from the National Science Foundation and the United States Public Health Service. The author is indebted to Dr. W. Forrest and Dr. H. Gutfreund for helpful discussions.

(12) The electrostatic contributions to enthalpies of reaction, and their variation with ionic strength, will be discussed in a future communication.

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[CONTRIBUTION NO. 1243 FROM THE DEPARTMENT OF CHEMISTRY OF YALE UNIVERSITY]

# The Hydration of Desoxyribonucleic Acid

BY JUI H. WANG

RECEIVED AUGUST 6, 1954

The self-diffusion coefficient of water in aqueous sodium desoxyribonucleate solution at 25° was determined as a function of concentration The hydration of sodium desoxyribonucleate computed from the measured results is about 0.35 g. of water per g. of the dry desoxyribonucleate. The effect of added salt on the hydration and shape of the nucleic acid molecule is examined, and the broadening of the proton magnetic resonance lines in aqueous sodium desoxyribonucleate solutions is discussed.

The hydration of nucleic acids has been the subject of speculation of many investigators.[1,2] Recently Jacobson, Anderson and Arnold[3] have made some interesting measurements on the proton magnetic resonance in aqueous sodium desoxyribonucleate solutions. They concluded that desoxyribonucleic acid is much more highly hydrated than hemocyanin, egg albumin, etc., and that on adding sodium chloride to the nucleic acid solutions considerable amounts of water previously bound are released to the normal water state. Since some quantitative knowledge of this hydration is of importance both to the size and shape studies by dynamic method and to the development of the theory of line-breadth of proton magnetic resonance in aqueous solutions, the subject is further examined by means of self-diffusion measurements in the present work. The method of computing the hydration of the macromolecule from the measured self-diffusion coefficient of water in solution has been described in an earlier article.[4] The experimental procedure for self-diffusion measurements is essentially the same as that used by Wang, Anfinsen and Polestra.[5]

## Experimental

**Diffusion Measurements.**—$H_2O^{18}$ was used as tracer to determine the self-diffusion coefficients of water in sodium desoxyribonucleate solutions. This was supplied by Stuart Oxygen Company, and obtained on allocation from the Isotopes Division, U. S. Atomic Energy Commission. The experimental method has already been described.[5] The diffusion samples were analyzed directly by means of a Consolidated-401 mass-spectrometer.

**Preparation of Bath Solutions.**—Desoxyribonucleic acid prepared from thymus by Delta Chemical Works, New York, was used. This was dissolved in distilled water by stirring overnight at 1°. The solution was then kept in an ice-bath and slowly neutralized with dilute sodium hydroxide solution until *p*H 7.8. Some sodium chloride was now added to the solution. The mixture was then alternately dialyzed and concentrated (by evaporation through the cellulose bag at 1°) for 7 days. The resulting solution was

(1) B. Jacobson, *Nature*, **172**, 666 (1953).

(2) M. E. Reichmann, S. A. Rice, C. A. Thomas and P. Doty, THIS JOURNAL, **76**, 3047 (1954).

(3) B. Jacobson, W. A. Anderson and J. T. Arnold, *Nature*, **173**, 772 (1954).

(4) J. H. Wang, THIS JOURNAL, **76**, 4755 (1954).

(5) J. H. Wang, C. B. Anfinsen and F. M. Polestra, *ibid.*, **76**, 4763 (1954).

found to be free from chloride ion and was centrifuged to remove a minute amount of insoluble gelatinous material. The *p*H of the clear solution was still 7.8 and remained unchanged until all the diffusion measurements were completed. The concentration of sodium desoxyribonucleate[6] was determined by dry weight method. Bath solutions of different concentrations for diffusion measurements were prepared by quantitative dilution of this stock solution.

**Preparation of $H_2O^{18}$-labeled Solutions.**—Solutions of sodium desoxyribonucleate in $H_2O^{18}$-labeled water with concentration equal precisely to that of the corresponding bath solution were prepared by the following procedure. About $^1/_2$ cc. of the bath solution was put in a small glass vial and weighed on a semi-micro balance. The solution was then freeze-dried until more than $^9/_{10}$ of its water was removed. Labeled water containing about 1.5 atom per cent. of $O^{18}$ was added droplet by droplet to the residue by means of a micro-pipet until the total weight of the vial plus its content became equal to the original weight. The content of the vial was then thoroughly mixed, and the uniform solution was used to fill the diffusion capillaries.

## Results and Discussion

The results of the self-diffusion measurements in aqueous sodium desoxyribonucleate solutions with and without NaCl are summarized in Table I.

TABLE I

SELF-DIFFUSION COEFFICIENTS OF WATER IN AQUEOUS SODIUM DESOXYRIBONUCLEATE SOLUTIONS AT 25°

| % by wt. of sodium desoxyribonucleate in soln. (without added NaCl) | $D \times 10^5$ (cm.²/sec.) Soln. without NaCl | Soln. made 0.20 *F* in NaCl |
|---|---|---|
| 0.00 | 2.57 ± 0.02 | (2.55 ) |
| 3.74 | 2.40 ± .02 | 2.39 ± 0.02 |
| 6.23 | 2.28 ± .03 | 2.27 ± .03 |
| 9.26 | 2.14 ± .03 | 2.11 ± .02 |
| 12.42 | 2.02 ± .02 | 1.99 ± .02 |

The self-diffusion coefficient of water in 0.20 *F* NaCl solution without desoxyribonucleate was obtained by interpolation from previous results.[7] Each of the other values listed in Table I is the average result of at least three measurements. Values of $D$ listed in the second column of Table I are used in the computations described below.

According to the theory of the self-diffusion of water in protein solutions[4] the ratio, $D/D_0$, of the self-diffusion coefficient of water in a given protein solution to that in pure water is related to the weight-fraction, $w$, of dry protein by

$$\frac{D}{D_0} - \Delta_1 = 1 - [\bar{\alpha}(\bar{V}_p\, d_0 + H) + H]w + \Delta_2 \quad (1)$$

where

$$\Delta_1 = \frac{\bar{\alpha}\,\bar{V}_p\, d_0(\bar{V}_p\, d_0 - 1)w^2}{1 + (\bar{V}_p d_0 - 1)w}$$

$$\Delta_2 = \left[\frac{\bar{\alpha}H(\bar{V}_p d_0 - 1)}{1 + (\bar{V}_p d_0 - 1)w} - \frac{H}{1-w} + \frac{\bar{\alpha}(\bar{V}_p d_0 + H) + H}{1 + (\bar{V}_p d_0 - 1)w(1 - w)}\right] w^2$$

In equation 1, $H$ is the hydration of the protein expressed in g. of bound water per g. of dry protein, $\bar{V}_p$ is the apparent specific volume of dry protein in solution, $d_0$ is the density of pure water and $\bar{\alpha}$ is a dimensionless numerical factor. $\Delta_2$ is usually negligible and may often be omitted in numerical computations.

Equation 1 was derived for compact macromolecules of definite shape. As long as the mobility of the hydrated macromolecules is negligibly small as compared to that of the "free" water molecules, the hydration, $H$, can be evaluated from self-diffusion and density data by means of equation 1 without knowing the actual molecular weight of the macromolecule. Since desoxyribonucleic acid and its salts are highly ionized polyelectrolytes, we can no longer consider the nucleic acid or its salt as a single kinetic unit in self-diffusion measurements because most of the hydrogen or sodium ions can obviously self-diffuse much faster than the giant macromolecular poly-ion. Likewise there is ambiguity in the meaning of the term hydration which was previously defined[4] as the statistically average number of g. of water carried by 1 g. of the dry macromolecule when the latter migrates through solution. However, we may generalize the above definition to include the present case by considering this hydration to include both the water bound directly to the desoxyribonucleate poly-ions and the water bound to the sodium ions which are in turn bound, as the case may be, to the desoxyribonucleate poly-ions.

Values of the apparent specific volume, $V_p$, of dry sodium desoxyribonucleate in solution at 25° were determined from the density measurements. The results are listed in Table II together with the values of $D/D_0$, $\Delta_1$ and $(D/D_0) - \Delta_1$ in equation 1.

TABLE II

DATA FOR COMPUTING THE HYDRATION OF SODIUM DESOXYRIBONUCLEATE AT 25°

| $w$, wt. fraction of sodium desoxyribonucleate in soln. | $\bar{V}_p$, cc./g. | $D/D_0$ | $\Delta_1$ | $(D/D_0) - \Delta_1$ |
|---|---|---|---|---|
| 0.0000 | | 1.000 | 0.0000 | 1.000 |
| .0374 | 0.525 | 0.933 | .0006 | 0.932 |
| .0623 | .526 | .887 | .0016 | .885 |
| .0926 | .527 | .832 | .0037 | .828 |
| .1242 | .529 | .785 | .0068 | .778 |
| .176 | .544 | | | |

We may, with sufficient accuracy, use 0.53 cc./g. as the average value of $\bar{V}_p$ for solutions of concentration between $w = 0.000$ and 0.125 in which the diffusion measurements were made. The value of $\bar{\alpha}$ for prolate ellipsoids varies from 1.500 for spherical molecules to 1.667 for infinite thin rods.[4] Although we do not yet know the exact shape of the desoxyribonucleate poly-ion, considerable evidence has been accumulated in the last few years[8,9,2] to indicate that the general shape of these macromolecular ions can be approximated by long chains with considerable degree of stiffness. Inasmuch as the value of $\bar{\alpha}$ is not shape-sensitive,[4] we may use $\bar{\alpha} = 1.667$ in computations for the present system.

The plot of $(D/D_0) - \Delta_1$ listed in Table II *vs.* $w$ is linear within experimental uncertainties as predicted by equation 1. The slope of this experimental line is −1.800 which should be equal to $-[\bar{\alpha}(\bar{V}_p d_0 + H) + H]$. Using $\bar{\alpha} = 1.667$, $\bar{V}_p =$

(6) J. M. Gulland, D. O. Jordan and C. J. Threlfall, *J. Chem. Soc.*, 1129 (1947).

(7) J. H. Wang, *J. Phys. Chem.*, **58**, 686 (1954).

(8) D. O. Jordan, *Ann. Rev. Biochem.*, **21**, 209 (1952).

(9) M. Goldstein and M. E. Reichmann, THIS JOURNAL, **76**, 3337 (1954).

0.53 cc./g. and $d_0 = 1$, we obtain $H = 0.35$ g. of water per g. of dry sodium desoxyribonucleate. This result shows that the hydration of sodium desoxyribonucleate is not very much higher than that of proteins in general.

There are two uncertainties in the above calculation. First, since sodium desoxyribonucleate is highly ionized, a substantial fraction of the sodium ions may self-diffuse independently instead of moving together with the giant poly-ions. This means that we should have used the weight-fraction of the desoxyribonucleate ions plus part of the sodium ions that are bound to the giant poly-ions as $w$ in the above plot. The use of the weight-fraction of total sodium desoxyribonucleate as $w$ would make the computed value of $H$ too small. Secondly, we should have used the apparent specific volume of the giant poly-ions plus the sodium ions that are bound to them as $\bar{V}_p$. Since sodium ions are known to have a pronounced electrostrictive effect, the use of the apparent specific volume of total sodium desoxyribonucleate would make $\bar{V}_p$ too small and hence the computed value of $H$ too large. Since the total volume of the hydrated "free" sodium ions is small as compared to that of the giant desoxyribonucleate ions, neither of the two errors mentioned above can be very large. Furthermore, since these two errors are in opposite directions, it is very unlikely that the above computed value of $H$ can be in serious error.

A related problem of great interest is the effect of added electrolyte on the hydration and shape of the desoxyribonucleate ions. Jacobson, Anderson and Arnold[3] observed that the broad proton magnetic resonance lines of aqueous sodium desoxyribonucleate solution are sharpened considerably by the addition of 0.04 formula wt. of NaCl per l. of solution, and concluded that this added electrolyte causes the macromolecular ions to contract so that considerable amounts of previously bound water are released to the normal water state. If this conclusion is true, we would expect the addition of NaCl to increase the self-diffusion coefficient of water in desoxyribonucleate solutions. Values listed in the third column of Table I show that the addition of 0.20 formula wt. of NaCl per l. of desoxyribonucleate solution does not cause pronounced increase in the measured values of $D$. The measured self-diffusion coefficients of water in these solutions are actually slightly lower than the corresponding values in the absence of NaCl. This is probably due to the obstruction and direct hydration effects of the added $Na^+$ and $Cl^-$ ions on the self-diffusion of water molecules which are not bound to the macromolecular ions. Indeed had we used the values listed in the third column of Table I together with $2.55 \times 10^{-5}$ cm.$^2$/sec. as $D_0$ in the above computations, we would have obtained the same numerical value for the hydration. This shows that the addition of NaCl has no appreciable effect on the hydration of these macromolecular ions, and consequently the observed sharpening of the proton magnetic resonance lines after the addition of salt must be due entirely to the change in shape of the desoxyribonucleate ions.

In view of the low hydration value obtained in the present work, it is clear that the remarkable line-broadening observed by Jacobson, Anderson and Arnold cannot be attributed completely to a simple hydration effect. A possible explanation suggested by Professor L. Onsager is that although at any given instant the number of water molecules subjected to the direct orienting influence of the desoxyribonucleate ions is small, rapid exchange of water molecules between "free" and "bound" water can make the orienting influence of the macromolecular ions unusually pronounced if both the molecular relaxation times of the macromolecular ions and the nuclear relaxation times of protons are very long. On the addition of simple electrolytes, the spacial extension and relaxation times of the desoxyribonucleate ions decrease because of the change in molecular shape, and so does the over-all orienting influence of these macromolecular ions. Consequently the observed line-breadth decreases in spite of the fact that the hydration remains unchanged. The measured results of Jacobson and co-workers show that the observed total line-broadening due to both the direct hydration effect and the effect of water exchange is not proportional to the concentration of the desoxyribonucleate ions.

**Acknowledgment.**—The author wants to thank Professor Lars Onsager, who suggested the present work, for his encouragement and advice. The measurements were supported in part by Contract (30-1)-1375 between the U. S. Atomic Energy Commission and Yale University.

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