POSITRONIUM FORMATION IN MUSCLE

AN INVESTIGATION OF THE STRUCTURE OF CELL WATER

D. R. GUSTAFSON

From the Department of Physics, Wayne State University, Detroit, Michigan 48202

ABSTRACT Positronium formation in muscle at +4°C and −4°C was examined by the measurement of the angular correlation of positron annihilation radiation. Since the positronium formation rate in ice is considerably higher than it is in water, there should be a comparable increase in the positronium formation rate in muscle tissue if recent speculation that cellular water is ordered in a semicrystalline icelike state is correct. Comparison of the angular correlation from muscle at +4°C with that from water at +4°C shows no enhancement of the positronium formation rate. Frozen muscle at −4°C shows an enhancement of the positronium formation rate of approximately half that found in ice at −4°C, indicating that most cellular water undergoes a normal water-ice transition when frozen. It is concluded therefore that cell water in muscle is not ordered in a hexagonal icelike structure. While the results are consistent with the hypothesis that cell water is in the liquid state, the hypothesis that cell water is ordered in an undetermined close packed structure which transforms to the hexagonal ice structure at or near 0°C cannot be ruled out.

INTRODUCTION

Interest in the degree of structure of cell water has been renewed by the recent measurements by Cope (1969) of the nuclear magnetic resonance (NMR) spectrum of deuterated muscle and brain tissue. Cope's measurements tend to support the hypothesis that cell water is in a highly structured semicrystalline state. This hypothesis is, of course, in conflict with the classical theory of cell structure in which cell water is thought to be liquid with various ions and organic molecules in solution. Water is known to exist in a number of solid structures and, as the NMR results do not indicate any particular type of crystallinity, they all should be regarded as possible structures for cell water. However, if cell water is in a nonliquid form, the structures which exist only at extremes of pressure or temperature are certainly less likely than the forms which exist at temperatures and pressures closer to those found in living cells. Therefore, one of the more probable forms is the hexagonal structure of normal ice with, of course, no significant long-range order. A phenomenon which

is quite sensitive to the difference between ice and water, but does not require long-range order, is the positronium (Ps) formation rate (Colombino, de Benedetti, Degregori, and Trossi, 1958; de Zafra and Joyner, 1958). Ps is the hydrogen-like bound state formed by a positron and an electron. The Ps formation rate in ice is considerably greater than it is in water, primarily because the open hexagonal structure of ice has open regions large enough for the Ps (diameter $\approx$ 1 A) to form, while the more closely packed structure of liquid water does not. The Ps formation rate in muscle was measured and compared to the Ps formation rate in water. No enhancement of the Ps formation rate was observed; therefore, it is concluded that cell water is not ordered in an icelike hexagonal structure.

The positron is the antiparticle of the electron and differs from the electron by having a charge of $+e$ instead of $-e$. By itself the positron is stable; however, when the positron comes in contact with an electron, mutual annihilation occurs with the mass of the two particles being converted into electromagnetic energy. The utility of the positron as a probe of condensed material is a consequence of two things bascially: first, the positron can reach a very low energy state in the material since the limitations of the exclusion principle are negligible for the extremely low positron densities used in the study of materials; second, when the positron annihilates with an electron the resultant high energy photons (511 kev, for two photon annihilation) escape the material and can readily be analyzed for both the lifetime of the positron in the material and the momentum of the annihilation photons and consequently, the momentum of the positron-electron pair just prior to annihilation. Both the technique of lifetime analysis and measurement of the angular correlation of the annihilation radiation (which measures the momentum of the annihilation photons) have been widely employed for the study of such condensed materials as metals, liquids, and polymers. Two recent books reviewing the subject of positron annihilation, one by Stewart and Roellig (1967), the other by Green and Lee (1964), give detailed treatments of the interaction of positrons with matter.

Even though the positron usually enters a material with an energy in the kev range, the positron rapidly loses its energy through collisions with the electrons and in about 10^{-12} sec has an energy of at most a few ev. In the case of water there are then two dominant modes of annihilation. The first mode is by Ps formation where the positron captures an electron from a water molecule and binds to it to form the Ps atom. Ps is very similar to the H atom except Ps has only 10^{-3} the mass of H; the chemical behavior of Ps is therefore quite different from H. In Ps the spins of the positron and electron can be either parallel (triplet state, total spin = 1) or antiparallel (singlet state, total spin = 0). The triplet state is generally called ortho-Ps, the singlet para-Ps. Ortho-Ps decays by the emission of three annihilation photons, to conserve spin angular momentum, with a mean life of 10^{-7} sec in free space. The two photon angular correlation apparatus used in the present work was not sensitive to three photon annihilation; therefore the behavior of ortho-Ps will not be of major concern in this work. Para-Ps decays by two photon emission to conserve spin

angular momentum. Para-Ps has a mean life of 10^{-9} sec in free space. Since the Ps atom will be almost at rest with at most a few times thermal energy, the two annihilation photons from para-Ps decay will be emitted almost directly opposite to each other to conserve linear momentum. Hence, the angular correlation from para-Ps decay will be a very narrow peak centered at zero deviation from exact colinearity. (Angular correlations of positron annihilation radiation are conventionally plotted versus the deviation from colinearity. In addition, since the peaks are symmetrical about zero deviation, the data are often folded about zero.) The second important mode of annihilation in water is the so-called "free" annihilation where a free positron annihilates with a bound electron of a water molecule. Annihilations with the oxygen $2s$ and $2p$ electrons will predominate because there are more of them than any other type of electron and because the positron will be attracted toward the negatively charged oxygen atom. Oxygen $1s$ electrons do not participate significantly because the electrostatic repulsion between the positron and the positively charged oxygen nucleus will prevent the positron from penetrating the oxygen atoms. In "free" annihilation the decay will nearly always be by two photon annihilation. Since the free positron will be at or very near thermal energy, it carries a negligible momentum. Thus, the total momentum carried by the annihilation photons will be essentially the electron's momentum. The momentum of the oxygen $2s$ and $2p$ electrons is such that the angular correlation will be a broad peak of nearly gaussian shape and approximately 9 mrad half-width. The exact shape can be found from an analysis of the momentum of the oxygen $2s$ and $2p$ electrons. The mean life of a positron in water annihilating by "free" annihilation is 10^{-10} sec. Since each mode of annihilation has a distinctly different angular correlation, the angular correlation provides an accurate measure of the ratio of Ps formation to "free" annihilation.

In liquid water there is little space between the closely packed molecules and the molecular rotation period is on the order of 10^{-11} sec, considerably shorter than the para-Ps lifetime. Thus, there will be relatively few open spaces of sufficient size and duration for Ps to form in and annihilate in before being drastically distorted by a neighboring water molecule. The angular correlation from liquid water therefore shows only a small Ps peak and the peak is broadened, as a result of collisions with water molecules, to the point that the Ps peak is not visually separable from the broad "free" annihilation peak. (Colombino, Fiscella, and Trossi, 1967). In ice the relatively open hexagonal structure allows a much larger fraction of the positrons to form Ps and since the period of the molecular motion in ice is about 10^{-6} sec, considerably longer than the para-Ps lifetime, the Ps can decay without being significantly distorted by collisions with the molecules. In ice, therefore, the angular correlation has an easily discernible Ps peak rising above the broad "free" annihilation peak (de Zafra and Joyner, 1958). As the thermal motion of the molecules is reduced by lowering the temperature, the Ps peak grows in height and decreases in width showing that the reduced molecular motion decreases the distortion caused by colli-

sions (Colombino et al., 1967). Since the formation and annihilation of Ps is localized to a region of a few A, the formation rate is not appreciably altered by the absence of long-range order.

If the hypothesis that cell water is ordered in an icelike structure were correct, the Ps formation rate in cell water should be much larger than it is in normal liquid water. The effect of the increased formation rate on the angular correlation would be a significant enhancement of the Ps peak in the angular correlation from cell water. If the classical theory of cell water were correct, the angular correlation from cell water should be nearly the same as that from liquid water. However, it should be noted that the classical theory is not the only possible explanation of an angular correlation from cell water which is the same as that from liquid water. There are other possible forms of structured cell water such as the high pressure cubic phase or the recently discovered and poorly understood polymeric form ("polywater") (Deryagin Talaev, and Fedyakin, 1965; Lippincott, Stromberg, Grant, and Cessac, 1969). Very little is known about the behavior of positrons in these forms; however, since they are more dense than ordinary ice it is likely that there would be no enhancement of the Ps formation rate. Hence, these forms cannot be ruled out by the absence of a Ps peak. At the present time, therefore, an examination of the angular correlation from cell water provides a test of the hypothesis that cell water has a structure similar to ice but does not provide a definite test of other possible ordered structures for cell water. In the future, it is likely that further study of the behavior of positrons in these other forms of structured water will yield information which will be useful in understanding the role of water in the living cell.

EXPERIMENTAL PROCEDURE

The angular correlations were measured using a standard long slit apparatus shown schematically in Fig. 1. Slits 1 and 2 were fixed as were the 60 mCi Cobalt-58 positron source and the sample under study. Slits 3 and 4 were mounted on a movable arm which pivoted about a horizontal axis through the sample. Slits 1 and 4 were set to define an angular resolution of ½ mrad while slits 2 and 3 were set at a wider opening and served only to shield the detectors from the direct radiation from the positron source. The annihilation photons were detected by NaI(Tl) scintillation detectors.[1] The signals from the detectors were analyzed with a standard pulse-analysis system. The apparatus was programmed to measure the coincidence rate as a function of θ, the angular deviation from exact colinearity. In order to minimize the effects of drift in the pulse-analysis circuitry, the coincidence rate as a function of θ was measured several times and the results of the individual sweeps were summed. The sample under study and the positron source were both enclosed in a small refrigerated chamber which was sealed to prevent undue evaporation of water during the course of the experiment. The chamber was refrigerated by circulating cold nitrogen gas through coils soldered to the chamber. The cold gas was obtained by the controlled evaporation of liquid nitrogen. The controller maintained a stability of ±0.1°C. Each run took about 50 hr.

[1] The scintillation detectors were 2 × 16 inch NaI(Tl) cylinders manufactured by the Harshaw Chemical Co., Cleveland, Ohio. They were coupled to RCA 8575 photomultiplier tubes.

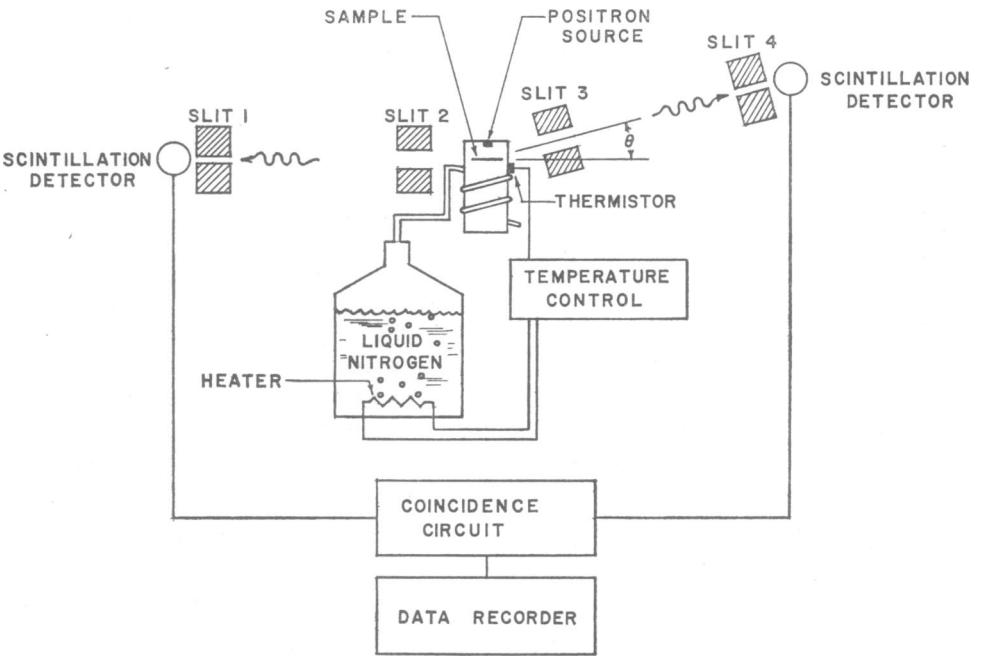

FIGURE 1 Schematic diagram of the apparatus.

The muscle sample was a section of the abdominal muscle from an adult male white rat killed with chloroform. The sample was removed and cooled to +4°C within 2 hr after death. +4°C was chosen to minimize deterioration during the necessarily long run and also to minimize the thermal motion of the molecules and therefore increase the amount of molecular order. At the end of this run, the muscle was rapidly frozen by circulating liquid nitrogen through the coils surrounding the chamber. The muscle sample was thermally coupled to the coils through a large piece of copper to insure rapid freezing ($\approx$ 1 min) and thus minimize resultant cell damage. The sample was then warmed to −4°C and the angular correlation from frozen muscle was measured. For comparison, the angular correlations were measured in distilled water at +4°C and in ice at −4°C. The ice was frozen rapidly by circulation of liquid nitrogen through the coils.

RESULTS AND DISCUSSION

The measured angular correlations are shown in Fig. 2. All four angular correlations were normalized to the same height at 4 mrad, well outside the Ps peak. The counting rate is in arbitrary units. The angular correlations from water and ice are the same as found by previous investigations (Colombino et al., 1967). The angular correlations from water and muscle at +4°C are nearly identical; the small difference between the two is probably due to "free" annihilations with the various ions and proteins of the muscle. The data from the first sweep in muscle at +4°C, which was completed 8 hr after death, were identical, within statistical accuracy, to the final results. Clearly, the Ps formation rate in muscle is no greater than it is in liquid water. The angular cor-

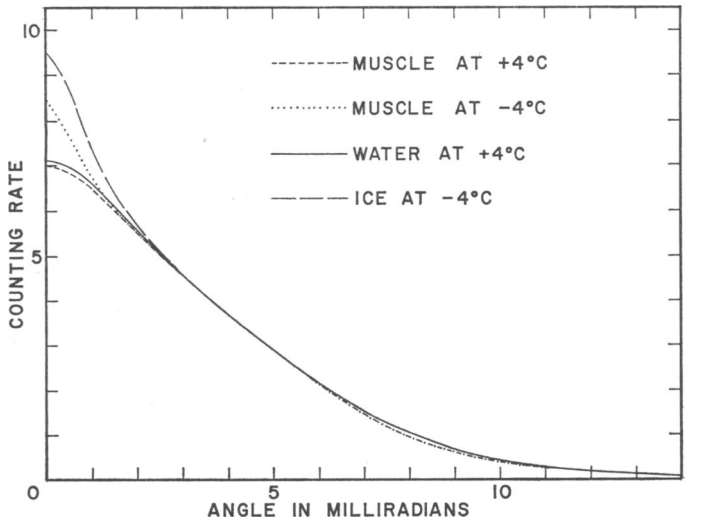

FIGURE 2 Angular correlations from muscle at +4°C, frozen muscle at −4°C, water at +4°C, and ice at −4°C. For clarity, each curve shown is a smooth curve drawn through 66 individual data points. The statistical error of all of the individual points was less than ±0.5% of the peak height.

relation from frozen muscle at −4°C, has a Ps peak approximately half the height of the peak in ice at −4°C showing that there is a large enhancement of the Ps formation rate when cell water is frozen.

The positrons are emitted with energies which are more than sufficient to penetrate into all parts of the interiors of the muscle cells. While the charges of the proteins and the various ions might cause the positron to favor some particular location over others, there is no reason to believe that this would cause anything but minor fluctuations in the positron density over the cell. Since the cells are about 80% water, it is assumed that roughly 80% of the positrons annihilate in the cell water. This assumption is consistent with the observations; the angular correlation from muscle at +4°C is nearly the same as that from liquid water and the angular correlation from muscle shows the same type of dependence on phase as water does.

The fact that the Ps formation rate in muscle is no greater than it is in liquid water indicates that cell water is in a form that does not contain open regions sufficiently large for Ps to form in. Thus, it is extremely unlikely that cell water is ordered in an icelike hexagonal structure.

The large increase in the Ps formation rate observed when the muscle was frozen indicates that the major portion of the cell water undergoes a phase transition to the ice structure at or very near to the normal freezing point of water. The reduction in the Ps formation rate in frozen muscle, as compared to ice, was no doubt caused by the presence of ions, proteins, and other organic material which reduced the number of open spaces in the frozen muscle. The fact that cell water undergoes a phase tran-

sition at the normal freezing point of water makes it unlikely that polywater is a major constituent of cell water. This is because polywater does not have a liquid-solid phase transition but instead exhibits a gradual increase in viscosity as the temperature is lowered, as most polymers do. However, since so little is known about polywater and practically nothing is known about the behavior of the positron in polywater, further study is required before polywater can be definitely ruled out as a primary constituent of cell water.

It has been suggested that cell water may be structured in an amorphous state similar to that of liquid water, but with essentially no molecular motion. This structure would be expected to show a small, but easily measurable, Ps peak in the angular correlation. This is because, as Colombino et al., (1967) point out, there is a certain amount of Ps formed in liquid water; the reason that it is not apparent in the angular correlation is that the molecular motion broadens the peak beyond visual recognition. If the molecular motion were halted, the Ps peak should become readily observable. As there was no sign whatever of an increase in the Ps peak in muscle, it does not seem likely that cell water exists in such a structure.

In conclusion, the simplest explanation of the present results is that cell water is primarily in the liquid state. However, the hypothesis that cell water is ordered in an undetermined close packed structure which transforms to the hexagonal ice structure at or near the normal freezing point of water cannot be ruled out.

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