Biochimica et Biophysica Acta, 646 (1981) 356–359
Elsevier/North-Holland Biomedical Press

BBA Report

BBA 71530

DETECTION OF A PHASE TRANSITION IN RED CELL MEMBRANES USING POSITRONIUM AS A PROBE

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(Received February 3rd, 1981)
(Revised manuscript received May 26th, 1981)

Key words: Phase transition; Positronium probe; (Red cell membrane)

Positron lifetimes in human red cell ghost membranes have been measured as a function of temperature from 3°C to 25°C. A marked sudden change in the *ortho*-positronium annihilation rate was found at 16–18°C during the heating cycle and at 18–20°C in the cooling cycle. Such sudden change of microenvironment in the membranes sensed by *ortho*-positronium is attributed to the sudden change of water diffusion rate through the membranes which is a consequence of the sudden change in free volume, or fluidities in the lipid layers.

Human red blood cell membranes exhibit discontinuities of a few functional and physical parameters around 18°C [1–10]. The viscosity change of sonicated human red cell membranes with temperature showed a non-linearity at about 18–19°C [1]. This discontinuity was further observed in 90° light scattering and anilinonaphthalene-8-sulfonate fluorescence studies [2]. HCO_3^--Cl^- exchange across the red cell membrane has lower Q_{10} or Arrhenius activation energy around physiological temperature than those at lower temperatures [3]. The transition seems to be at 17°C [4], and occurs for different extracellular pH and even in the presence of anion exchange inhibitor SITS (4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid). Cl^- self-exchange shows similar temperature dependence and the transition temperature observed is about 15°C [5]. The Arrhenius plot of the water diffusion exchange is better fitted by two straight lines with junctions in the range 20°C to 30°C [6]. Glucose transport and lactate transport also manifest non-linearity in the Arrhenius plot around 18°C [7,8]. Zimmer and Schirmer [1] interpreted the discontinuity observed in viscosity studies as a phase transition maybe in the lipid bilayer lamellae of the red cell membrane, and the phase transition is the explanation for the discontinuities in the membrane transport functions which involve protein-lipid interaction [2,3,10]. Gottlieb and Eanes [11], however, detected no liquid crystalline to rigid crystalline transition in the X-ray diffraction patterns of the red cell membranes even down to −20°C. The discrepancy was attributed to the different physical form of the materials used by the two groups.

Since positron annihilation has been demonstrated to be one of the most sensitive probes in detection of micro-structural changes in molecules substance (for examples see Refs. 12 and 13), we have studies the temperature dependence of positron lifetimes in red cell membranes in hope to gain more information on the nature of the phase transition in membranes. For detailed information on positron annihilation and positronium chemistry, readers should refer to recent reviews and conference proceedings [14–17].

Human red cell ghost membranes were prepared

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Abbreviations: *o*-Ps, *ortho*-positronium; *p*-Ps, *para*-postironium.

according to Dodge et al. [18]. 5 ml of fresh human red blood cells were washed three times in isotonic saline (150 mM NaCl). The cells were then lysed in a hypotonic phosphate buffer of 20 ideal milliosmolar (imosM) and pH 7.4). The ghost membranes were washed three times under $20000 \times g$ for 40 min in the 20 imosM phosphate buffer subsequent to the hemolysis. The washed ghost button was suspended to about 6 ml in distilled H_2O. The suspension was kept overnight at 4°C before the positron lifetime measurements. The positron lifetime apparatus was a conventional fast-slow coincidence system which has a resolution of 300 ps, the FWHM of ^{60}Co prompt curve at ^{22}Na setting [19]. The positron source was a 10 μCi of ^{22}Na isotope which was carefully sealed between two thin mylar films. It was tested to be water-proof and then immersed into the sample solution in the sample cell. The temperature of the sample cell was controlled by a conventional temperature controller with a stability of ±0.1°C at each temperature setting. Lifetime spectra were measured starting from 3°C up to 25°C (the heating cycle) and then from 25°C down to 6°C (the cooling cycle). The time needed for each lifetime measurement was about 4–6 h. Occasionally, two or three spectra were measured at the same temperature, if changes in machine resolution due to electronics drift were suspected. The total time for the whole experiment was about ten days. Conlon and Outhred [6] tested the effect of storage conditions of blood samples on their NMR study of water permeability in red blood cells. No significant change was observed for blood stored at 4°C for 48 h, but a noticeable change was observed for the sample after 48 h storage at 20°C. Using this as a guideline, although ghost membranes should be more stable than red blood cells, the lifetime spectra for the heating cycle, from 3°C to 25°C, were measured within 48 h to avoid the effect of any possible deterioration of the sample.

The positron lifetime spectra were decomposed into two lifetime components, a short-lived component, which is the result of *para*-positronium (*p*-PS) and free positron annihilation, and the long-lived component from the annihilation of *ortho*-positronium (*o*-Ps). The short-lived component, due to its complicated origins and the limited timing resolution, gives less direct information on the properties of the sample. We will focus our attention on the decay rate λ_2 of the long-lived component because it depends on the interaction of *o*-Ps with the surrounding molecules. In our sample, the observed *o*-Ps decay rate is actually the weighted average of the decay rates for *o*-Ps annihilation in water and in membranes. The annihilation rate of *o*-Ps in pure water is about 0.57 ns^{-1} [20]. The annihilation rate of *o*-Ps in membranes not only depends on the composition of the membranes but also depends on the micro-voids inside the membranes. The long lived *o*-Ps atom inside the membranes is most likely to spend most of its lifetime in the free space between lipid layers of the membranes. Therefore, the sudden change in slope of λ_2-T curve as shown in Fig. 1, indicates that the environment in the membranes sensed by *o*-Ps undergoes a sudden change at 18 ± 2°C.

Normally, when a sample undergoes a phase transition, λ_2 has a lower value at the high temperature phase than at the low temperature phase due to the increase of free volume with increasing temperature. However, our result shows that λ_2 has a higher value of $T > 20$°C than at $T < 16$°C. The sudden increase of λ_2 can not be interpreted simply as due to the free volume change in the lipid layers because there is no reason to believe that the free volume in the lipid layers decreases with increasing temperature. It is most likely that the free volume does increase when temperature is increased to above 18°C which allows water molecules or ions to diffuse through the lipid layers. In such cases, *o*-Ps, which is in the space

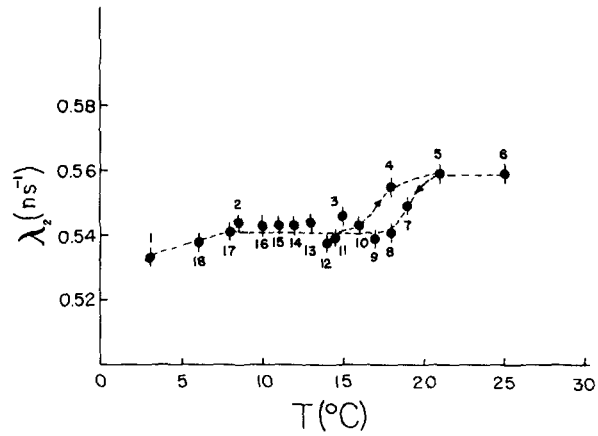

Fig. 1. Pickoff quenching rate of *o*-Ps in red cell membranes as a function of temperature. The numbers indicate the sequence of the lifetime measurement.

between lipid layers, will sense more water-like environment instead of lipid-like environment, and hence λ_2 increases to a higher value and reaches to about 0.56 ns^{-1} at $T = 25°C$. This value is indeed very close to the *o*-Ps annihilation rate in pure water, 0.57 ns^{-1}. Therefore, the sudden change of λ_2 at $T = 18 \pm 2°C$ is attributed to the sudden change of water diffusion permeability of the membranes which is consequence of the sudden change of free volume, or fluidity, of lipid layers. A similar result and interpretation have been reported by Kano et al. [21] for their positron study of DODAC vesicles in water. Positron lifetimes have been measured in phospholipid dispersions in both frozen and liquid crystal phases by McGrath et al. [22]. It was found that the *o*-Ps lifetime, or $1/\lambda_2$, increases about 18% from the rigid gel to liquid crystalline phase in phospholipid dispersions. If we can apply their finding to the case of red cell membranes, the sudden change in λ_2 in our experiment cannot be attributed to the rigid-liquid crystalline structural change in the membrane which agrees with the previous X-ray study [11]. Therefore, the sudden change of the membrane function detected by positronium is attributed to the microscopic structural change, which occurs usually at the less dense area, instead of the macroscopic morphological change, such as the rigid-liquid crystalline phase change, in the membrane structure.

It is interesting to compare our result with the NMR study on water diffusion permeability of *p*-chloromercuribenzoate treated red cell membranes by Conlon and Outhred [6]. It was proposed that *p*-chloromercuribenzoate blocks the protein pathway for water flow, leaving a lipid pathway only [23]. If this is true, the temperature dependence of water diffusion permeability of *p*-chloromercuribenzoate-treated cell should show a similar behavior as that observed in our λ_2-T curve. The result of Colon and Outhred [6] showed that *p*-chloromercuribenzoate-treated cell has a small and almost constant water diffusion rate at $T < 10°C$ and has a large increase in the slope of the Arrhenius plot of the rate at $T > 15°C$. This agrees well with our result. However, our λ_2-T curve shows a saturation for $T > 20°C$. This indicates that positronium decay rate is highly sensitive to the microenvironment where positronium is confined. The hysteresis of the λ_2-T curve at the transition is similar to that observed in the micelles [21] and also in liquid crystals [20]. This may be a characteristic property of the membranes, or it may be due to the slight deterioration of the membranes after the heating cycle. More experiments are needed to clarify this point.

In conclusion, from the present experiment, it is clear that the human red blood cell membranes undergo a microstructural change at $18 \pm 2°C$, and that it is possible to use positronium as a means for investigating microscopic properties of biomolecular systems.

One of the authors (S.Y.C.) thanks Marjorie Lou for her valuable discussions. This work was partially supported by the National Science Council of the Republic of China.

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