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POSITRON LIFETIMES IN PHOSPHOLIPID DISPERSIONS

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Summary

Positron lifetimes have been determined in phospholipid dispersions. In fluid phosphatidylcholines, a lifetime of 3.3 ns is found, and a lifetime of 2.8 ns is found for frozen phosphatidylcholines. In dispersions where fluid and frozen phases coexist due to lateral phase separation, an intermediate lifetime is found.

Introduction

It is generally assumed that the "fluidity" of the phospholipid components of biological membranes is essential in their biological functions. While the concept of fluidity in single phospholipids is well defined, in heterogeneous mixtures of phospholipids, such as are found in natural membranes, it is not clear-cut. This is because the different spectroscopic techniques (e.g. magnetic resonance [1], fluorescence [2]) used to measure the motional properties of lipids tend to detect preferentially the more mobile components in mixtures of phospholipids [1,2].

We present here a new method, "borrowed" from the physicists, that can be used to detect both the gel and liquid crystalline components in phospholipid dispersions. The measurements involve the introduction of positrons, positively charged anti-electrons, into the lipid matrix and the determination of their decay times. This can in principle provide information on the structural organisation of molecular substances.

Method

The properties of positrons are currently investigated in experimental physics and theoretical chemistry [3—5] and they have been recently

reviewed [6]. Since, however, the concepts and measurements are not familiar to most biologists we shall briefly summarise them here.

Positrons are positively charged particles with the mass of an electron. There are several sources of such particles, but the most conveniently and commonly used is the isotope ^{22}Na which decays to an isotope of neon by positron emission. This emission is accompanied by a gamma photon from the excited state of neon, and for most purposes the gamma emission and the positron emission can be regarded as simultaneous. Once formed, the positron must dissipate most of its energy before annihilation with an electron is possible. The positron may then either remain free, in which case it has a natural lifetime of the order of tens of picoseconds before annihilation, or else it may bind an electron without being immediately annihilated. The bound state is known as "positronium" and may be formed in either the singlet or triplet state, with a yield weighted by the multiplicity. The singlet state, *p*-positronium, has a lifetime of the order of 125 ps (picoseconds) in free space before self-annihilation with the emission of two simultaneous 0.511 MeV gamma photons at 180°. The triplet, *o*-positronium, is much longer lived with a free space lifetime of about 140 ns whereafter it decays by the emission of three simultaneous gamma photons at 120° to conserve momentum. Since the triplet state is comparatively long-lived, the species is susceptible to various "quenching" processes which shorten the measured decay time by providing alternative routes for annihilation. One such process is inter-system crossing with subsequent fast annihilation of the singlet positronium, while another is of chemical interest, the oxidation leading to

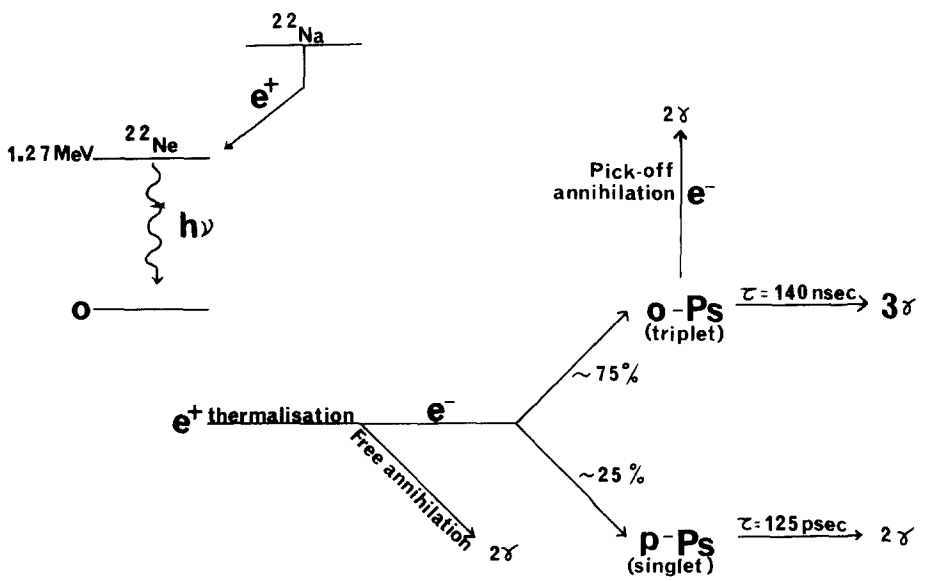

Fig. 1. Production and Fate of Positrons. *o*-Ps ortho-positronium; *p*-Ps, para-positronium. Excited state of neon, generated by positron emission from ^{22}Na, simultaneously (< 10 ps) emitts 1.27 MeV photon, used to start electronic timing. Photons emitted by two-photon events (*p*-Ps decay and *o*-Ps "pick-off") are 0.511 MeV, those in three-photon events (*o*-Ps) 0.334 MeV. Electronic discrimination using single channel analysers only allows two-photon events to be recorded. For instrumental details, see refs. 3—6.

a "bare" positron. One other process leading to premature annihilation of the triplet state positronium is "pick-off" annihilation. In this process, the *o*-positronium, after reaching thermal equilibrium interacts with one of the electrons from an outer orbital of a surrounding atom. The *o*-positronium is thus annihilated in a two-photon event. In molecular materials, such as those studied in the present work, this "pick-off" annihilation is the predominant route for loss of *o*-positronium (Fig. 1). Thus annihilation occurs almost exclusively by two-photon events. Events which correspond to *p*-positronium annihilation or intersystem crossing occur with half-times of the order of hundreds of picoseconds, and thus only contribute to the early part of the decays.

The pick-off annihilation rate is sensitive to the physical state of the material, and consequently it is possible to distinguish different phases of a given substance by measurement of this rate. Several investigators have studied phase equilibria in this way [5]. Our object was to determine whether the approach could be extended to the study of phospholipid dispersions, and ultimately to biological membranes, since phase equilibria and lateral phase separation are thought to be of importance in the modulation of biological membrane activity. One difficulty is that the large amount of water in biological materials results in a significant contribution to the pick-off annihilation. Previously an external source of ^{22}Na sandwiched within two thin mica films and placed between the scintillation detectors of the measuring apparatus was used. We have taken advantage of the presence of water in biological membranes by incorporating ^{22}Na directly into the solution. In this approach a relatively small amount of isotope is needed and the effect of pick-off annihilation within the walls of the containing vessel is minimised.

Materials and Apparatus

Positron decay times were measured using fast-slow coincidence circuitry [8]. The operational time resolution was 0.7 ns or better using ^{60}Co as calibration source. The prompt 1.27 MeV gamma photon emission coincident with positron emmission from ^{22}Na is used to provide a "start" signal to the time-to-pulse height converter after discrimination and subsequent 0.511 MeV radiation signals the annihilation event which stops the converter. The side channel discriminates for the energy range of annihilation radiation and provides a signal which gates the output of the converter into a multichannel analyser used in its pulse height analysis mode. The side channel ensures that only events which correspond to annihilation are recorded in the analyser: events which correspond to cosmic radiation or 1.27 MeV photons are not recorded, and the converter resets after a fixed period determined by its internal time base. After many events have been recorded the data in the analyser give a histogram of the number of annihilations at a given time after positron emission as a function of that time. This represents the decay time curve convoluted with an instrument response function. The instrument response function is measured using the prompt coincident gamma emission from ^{60}Co as source with given instrumental settings. This

way the data can be deconvoluted. Deconvolution is important if short lifetime components in the decay are to be studied, but with the relatively long decay times characteristic of pick-off annihilation in lipids it was found to be unnecessary. Approximate decay times were measured from the slope of the later portion of the decay.

Results

Typical decay time spectra obtained for aqueous dispersions of well-characterised phospholipids [1] are shown in Fig. 2. The "tail" of the

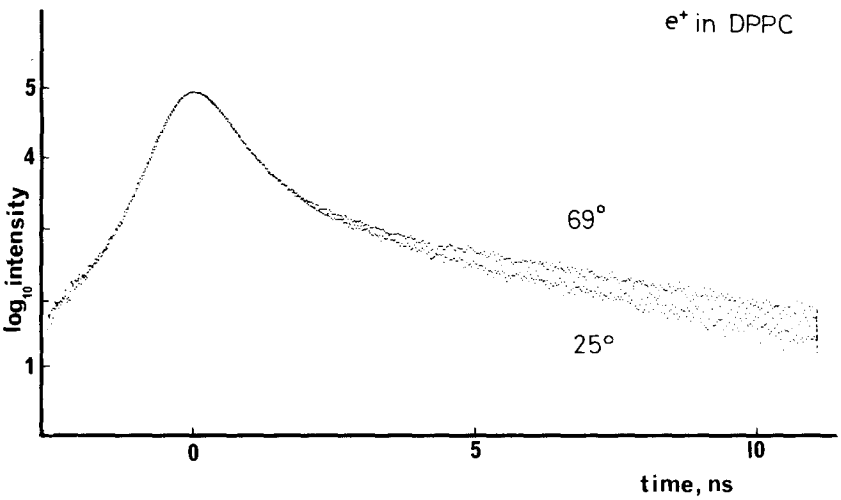

Fig. 2. Decay time spectra for positrons in dipalmitoyl phosphatidylcholine (DPPC) at 69 and 25° C. Log (intensity) is plotted against time, and least squares analysis of the decay "tails" gives lifetimes of 2.8 and 3.3 ns for 25 and 69° C, respectively. Gel-liquid crystalline transition temperature for this lipid is 41.5° C. For conditions, see Table I. Instrument prompt response function, evaluated using ^{60}Co as source, has half-height width of 700 ps or less, and thus does not significantly affect data at longer times.

decay is a result of pick-off annihilation in the lipid matrix: the component due to water contributes to the earlier part of the decay (Table I). It is not possible to estimate accurately the contribution from the water component, due to the well-known difficulty of separating several exponentials of similar lifetimes. However, the lifetime of the water component is known to be independent of temperature over the temperature range used here, so that the changes observed in the long-lifetime component observed can be ascribed to the phospholipid dispersion. The lifetime of the component resulting from pick-off annihilation in the lipid remains constant within the accuracy of the analysis at temperatures below the gel-liquid crystalline transition temperature. Above this temperature, the decay time becomes longer, and again remains constant at higher temperatures. Decay times of 2.8 and 3.3 ns are found for the frozen and fluid phases, respectively. For dioleoyl and dilauroyl phosphatidylcholine, which are fluid at room temperature, pick-off decay times of 3.3 ns are found.

It is known that mixtures of dioleoyl and dipalmitoyl phosphatidyl-

TABLE I

PICK-OFF DECAY TIMES FOR *o*-POSITRONIUM IN PHOSPHOLIPIDS

The phospholipids used were highly purified synthetic phosphatidylcholines, whose preparation and purity have been described elsewhere [1]. Egg phosphatidylcholine was Grade I, obtained from Lipid Products, Redhill, Surrey, U.K. Dispersions were made up in 0.5 ml buffer (10 mM trishydroxymethylaminoethane, pH 8.5, to which 25—50 μCi of $^{22}Na^{+}$ (as NaCl, from the Radiochemical Centre, Amersham, U.K.) was added. Samples typically contained 20 mg lipid, and were allowed to hydrate over saturated NaCl solution overnight. Lifetimes were obtained by fitting tail of decay to a single exponential, when decay processes due to *p*-positronium and intersystem crossing are effectively non-contributory. The reported lifetimes for lipids are likely to be shorter than the actual lifetimes because of the contribution from water (but see text).

System	Temperature (°C)	Lifetime (ns)*	State of lipid
Water	18	1.9	—
Dioleoyl phosphatidylcholine	18	3.3	liquid crystal
Dilauroyl phosphatidylcholine	18	3.3	liquid crystal
Egg phosphatidylcholine	18	3.25	liquid crystal
Dipalmitoyl phosphatidylcholine	18	2.8	gel
Dipalmitoyl phosphatidylcholine	65	3.3	liquid crystal
Dipalmitoyl phosphatidylcholine/dioleoyl phosphatidylcholine (1:1 molar ratio)	18	3.05	gel and liquid crystal coexisting

* The standard deviations for the observed lifetimes are never more than 0.03 ns.

choline exhibit phase separation in the plane of the bilayer over a limited temperature range [1]. Here gel and liquid crystalline phases coexist at room temperature. The positron pick-off annihilation rate here would be expected to resolve into components characteristic of annihilation in fluid and frozen phases. Since these rates are similar (decay times of 3.3 and 2.8 ns, respectively), with equal weighting of components the resultant decay would be difficult to analyse but would visually resemble a single component of intermediate lifetime. To check this conclusion, ignoring the short lifetime components present in a real decay, data were simulated by convoluting single exponentials of 2.8, 3.3 ns and an equally weighted sum of these with an instrument response function measured using a ^{60}Co source. The three decays were visually compared, and the decay calculated using the weighted sum of exponentials was as predicted. The measured positron pick-off decay time for the mixed lipid system was 3.05 ns (Table I).

In conclusion, we have shown that decay times of positrons are sensitive to the fluidity of phosphatidylcholine-water systems. Positron lifetimes characteristic of fluid and frozen lipids have been established. In a system where fluid and frozen phases coexist at room temperature on account of lateral phase separation an intermediate apparent lifetime is found. Careful analysis of decay times is thus likely to provide a method for estimating the extent of phase separation in biological membranes. A further application of this method related to the mechanism of lateral diffusion in biological membranes [7]. Positrons trapped in defects have annihilation rates different from those of the crystal bulk [3], the lifetime being greater for the trapped positrons. If diffusion in biological membranes involves vacancies, we consider that such vancancies might be detected by the use of more intense positron sources ($\approx$ 1 Ci). The intense source is required because the longer lifetime is obscured by the noise, and thus impossible to characterise.

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