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The solubilization site of 5,10,15,20-tetrakis-(2,6-dichlorophenyl)-porphyrin-Mn(III) in DPPC vesicles: A spectrophotometric and tensiometric study

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Abstract

The solubilization site of 5,10,15,20-tetrakis-(2,6-dichlorophenyl)-porphyrin-Mn(III) (MnTDPPC) in L-α-dipalmitoylphosphatidyl choline (DPPC) vesicles was determined in a systematic UV–vis spectrophotometric study. Measurements of the MnTDPPC absorbance have been conducted at constant temperatures (in the interval $30\,^{\circ}C \leq T \leq 48\,^{\circ}C$) and varying concentrations of phospholipid. In the entire temperature range, the absorption due to MnTDCCP was found to increase with increasing DPPC concentration, until it reaches a plateau value. The data have been analyzed using a pseudo-phase model yielding the binding constant of MnTDCPP to vesicular aggregates. No significant temperature dependence of the partition coefficient of MnTDCPP between the aqueous phase and the lipophilic domain of DPPC vesicles could be detected in the studied temperature interval (which also includes the main transition temperature of DPPC double layers, $T_m = 41.3\,^{\circ}C$), unambiguously indicating that MnTDCPP is confined in the palisade layer of DPPC bilayers. The nature of the interactions between the different reaction components have been investigated by tensiometric measurements, which provide further support for hydrophobic interaction between MnTDCPP and the DPPC vesicles. This solubilization locus makes this porphyrin – which is used as an important compound in biomimetic models for the cytochrome P450 model systems – accessible for electron donation by suitable, water soluble electron donors.

Keywords: Vesicle; Model system; Cytocrome P450; Binding constant

1. Introduction

In living organisms, the vast majority of enzymes are membrane-bound. However, in order to investigate the properties of enzymes under well controlled conditions, most studies of enzyme reactivity have been performed using purified enzymes in homogeneous reaction media. Although the data acquired in such studies are very useful for elucidating many enzymic reaction mechanisms, the properties of enzymes – and specially their reactivity – in homogeneous medium often differs from those of membrane-bound enzymes. A frequently used approach to mimic membrane environment is to incorporate the respective components (enzymes) into phospholipid bilayers, and to study these "reconstituted" systems [1,2]. This approach yields insights on the reactivity of the system in question which resemble much closer to the real biological conditions than those using purified enzymes in homogeneous medium.

Phospholipid vesicles possess numerous structural characteristics of membranes, in addition, they offer the advantage of being thermodynamically or kinetically stable and easily reproducible. Therefore, they are employed to study a great variety of problems related to biological systems, even because they provide easier access for exploiting various techniques in solutions and suspensions than at biological interfaces. The peculiar structure of vesicles, which consists of an inner water pool surrounded by a surfactant double layer, deeply resembles that of biological membranes. Thus, they may faithfully reproduce the conditions encountered in membranes. Depending on the nature of the constituents, vesicles can be classified as natural (or liposome) or synthetic. The former are based on phospholipidic monomers

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while in the latter the surfactant can be anionic or cationic. Generally, they are prepared from thin films [3], or by sonication [4], injection or dilution with a surfactant [5]. Depending on the preparation procedure, the characteristics of the vesicle may differ.

An important property that distinguishes the vesicles from other aggregated systems is the tuneable and temperature-dependent fluidity of the double layer [6,7]. These double layers undergo very well-defined structural changes at certain temperatures, the most prominent being the main phase transition temperature (T_m). At this temperature, the molecular arrangement of the phospholipids in the vesicle changes from a highly ordered, rigid state in which the alkyl chains have an all-trans conformation, to the fluid one, where the alkyl chains assume the gauche conformation [8]. The rigid state, below T_m, is commonly called "gel-like" while the fluid one corresponds to the "liquid-crystal like phase", since the alkyl chains move in a cooperative way forming small transitory domains. The fluidity of the double layer can also be modified by the presence of species interacting with it [9,10]; for instance, addition of cholesterol to phospholipid double layers is known to decrease the fluidity of the vesicle [11].

In biological systems, enzymes may be involved in a series of at times rather complex reactions which may induce complex dynamical behavior, such as periodic or aperiodic oscillations (or rhythms). For such complex dynamics to occur, reactions that follow non-linear kinetic rate laws must be involved in the reaction system. In physiological context, these types of complex non-linear behavior are frequently associated with biological function, for instance in signal and information transduction [12,13] or as protection of the enzyme against deactivation [14].

In order to better understand the origins of complex dynamics – or in a more general sense, the behavior of certain functionalities of a complex system – it is often convenient to reduce the system's complexity, so that the desired functionalities are retained while the "superfluous" are omitted. Such a reduction may be achieved by studying so-called biomimetic systems. They consist of tailor-made synthetic components that reproduce the main characteristic structural and functional features of their natural counterparts; however, the complexity of the reaction system is decreased since only the key features are mimicked. In this sense, biomimetic systems can be considered as reduced (if not minimal) model systems for their naturally occurring counterparts. Studies of biomimetic models may shed light into the fundamental mechanisms and properties of living systems, thus allowing for a straightforward determination of the origin of some phenomena otherwise inaccessible to the investigator.

The biomimetic approach has often been used to obtain some additional insights into the processes taking place in the cytochrome P450 reaction system [15–17]. To this purpose, a series of biomimetic models have been developed for this system, each of them aimed at studying a given problem, e.g. to reproduce the reactivity of the enzyme system, or to gain more insight into the mechanism of electron transport from the aqueous phase to the lipid domain of a membrane [17–19]. Among these models, one has a particularly interesting feature, namely the ability to show periodic oscillations in the oxidation state of the enzyme model compound [20]. In this reaction system, the synthetic enzyme model compound is hydrophobic and it is incorporated into a phospholipid bilayer. It consists of a manganese porphyrin complex, reflecting the nature of the active center of the cytochrome P450 (where it is a heme) and accounting for the electrochemical potential of the enzyme [18]. The role of the NADPH-cytochrome P450-reductase is played by a rhodium complex which is anchored to the phospholipid bilayer by two alkyl chains. The substrates are confined in the aqueous phase. This model system reproduces the ability of the natural system to epoxidize unreactive double-bonds of lipophilic compounds [18]. This reaction system has been reported to display chemical oscillations when the oxygen concentration in solution is low [20]; however, the mechanism leading to oscillatory dynamical behavior remains unrevealed. In order to shed some light into this question, the present paper reports on investigations of the partition of manganese(III) tetrakis(2,6-dichlorophenyl)porphyrin between the aqueous and the vesicle phase. In fact, we present evidence for the location of the manganese porphyrin in the palisade layer of the phospholipid bilayer and discuss its availability for hydrophilic substrates or reaction partners. This information will be a prerequisite for further studies aimed at unveiling the source of rhythmic dynamics.

2. Experimental

The porphyrins 5,10,15,20-tetrakis-(2,6-dichlorophenyl)-porphyrin-Mn(III) chloride (MnTDCPP) and 5,10,15,20-tetrakis-(2,6-dichlorophenyl)-21,23H-porphyrin (H_2TDCPP) were purchased from Porphyrin Systems and used without purification. L-α-Dipalmitoylphosphatidyl choline (DPPC), ethanol, and standard HCl solutions were obtained from Fluka and used as received. *N*-ethylmorpholine (NEM) (Aldrich) was distilled before use. Deionized water from reverse osmosis (Elga, model Option 3), having a resistivity higher than 1 MΩ cm, was used to prepare all solutions.

The experimental conditions have been chosen to match that used for the biomimetic system presented in [20]. Standard 0.05 mol dm^{-3} aqueous solution of NEM at pH 7.0 have been prepared by weighting the proper amount of NEM and adjusting the pH by addition of HCl. Stock aqueous vesicle dispersions have been prepared by sonication of an appropriate amount of the lipid in 0.05 mol dm^{-3} NEM buffer at pH 7.0 and 55 °C. Sonications have been carried out for 30 min with a Pabisch-High Intensity Ultrasonic Processor set at 60 W.

Ethanolic stock solutions of MnTDCPP and H_2TDCPP were prepared by weight before use.

The aqueous vesicle dispersions containing either 2.4×10^{-6} mol dm^{-3} MnTDCPP or H_2TDCPP have been prepared by adding a proper aliquot of the ethanolic porphyrin solution to the aqueous vesicle dispersion at the desired concentration. These dispersions have been gently stirred at constant temperature for 20 min. Then, in order to verify the stability of the porphyrins in vesicles, we have monitored the UV–vis spectra as a function of both the surfactant concentration and time. The spectra were registered for 2 h at intervals of 5 min. Moreover, at each surfactant concentration, the UV–vis