Conformational Changes of Acetylcholine During Spontaneous Diffusion Through a Nano/Microporous Gel

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ABSTRACT

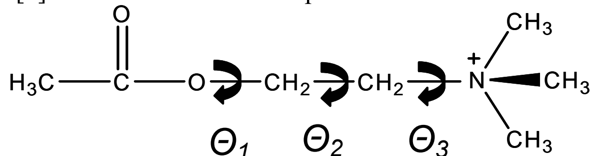
This paper reports results which aim to enhance the understanding of mechanisms and transitions taking place in biological systems [1]. In order to mimic these low energy processes, as well as to perform characterization and data acquisition on these processes, we have developed a new methodology. We study the conformational changes of acetylcholine (ACh) during propagation through a nano/microporous polymer system by measuring the fluorescence lifetime of the label molecule – fluorescein.

Keywords: acetylcholine, fluorescein, porous polymer gel, diffusion, fluorescence lifetime

1 INTRODUCTION

Acetylcholine (ACh) attracts a lot of attention due to its modulatory role in the central nervous system. However, ACh arrived within the evolutionary scheme long before the design of the nervous system and functional synapses [2].

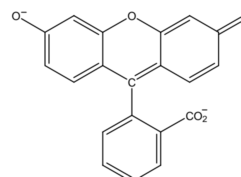
[2]. The scheme of ACh is presented below.



Torsional rotation in the ACh molecule can occur around the bonds θ_1, θ_2 and θ_3. Since the methyl groups are symmetrically disposed around θ_3 and constraints may be placed on θ_1 by the planar acetoxy group, the most important torsion angle determing ACh conformation in solution is θ_2. The *gauche* conformation is predominant in solution. However, the *trans* conformation may also be an active conformation. It was shown that the conformations of this flexible molecule differ substantially depending on the type of the adjacent reacting molecule [2]. Structural modifications change molecular activity [2].

We analyze the effect of the diffusion of ACh through the nano/microporous poly (*N*-vinylimidazole) (PVI) gel on its conformational changes. The synthesized porous chemically crosslinked polymer PVI gel has micropores with average diameter ~1.5×10^{-9} m, mesopores with diameters varying from 2×10^{-8} to 2×10^{-7} m, and interconnected macropores with an average diameter ~3×10^{-6} m [3]. Figure 1 shows a TEM image of the pores on the nanometer scale.

To investigate the conformational changes of ACh during the spontaneous diffusion through a nano/microporous gel, the fluorescence lifetime of the label molecule (fluorescein) was studied. Fluorescein is one of the best known fluorophores. It has two deprotonated forms, e.g., monoanion and dianion (scheme of the dianion is presented as),



which have lifetimes 3-4 ns and 4-5 ns [4]. The pKa of the monoanion-dianion transition is 6.3 in water. However, it substantially is increased at the lipid-water interface of micelles and bilayers [5]. Papers [4, 6] demonstrated that the monoanion and dianion behave as independent, non-interacting species [4, 6].

2 EXPERIMENTS

Fluorescence lifetime measurements were made by means of FluoTime 200 (PicoQuant GmbH) with a pulsed diode laser PDL 800-B, λ = 410 nm, pulse FWHM 54 psec, repetition frequency 40 MHz, peak power 219 mW. The Data Analysis Software FluoFit (PicoQuant GmbH) was used for lifetime measurements. For pulse control a solution of Ludox was used. The average fluorescence lifetime is evaluated as

$$\tau_{av} = \alpha_i \tau_i + \alpha_j \tau_j , \tag{1}$$

where τ_i and τ_j are the fluorescence lifetimes; α_i and α_j are the fractional coefficients describing the contribution of the mono- and di-anion, respectively.

For the experiment, 0.045 g of the gel was swollen in water (the final weight is 0.548g) and placed in a solution of fluorescein for five days at the stabilized temperature of 22.0±0.1°C. To remove the fluorescein from the surface of the gel, the gel was washed at least three times in triple distilled water TDW (18 MΩ/cm). The removal of the free fluorescein was monitored by UV-VIS spectroscopy. Subsequent to washing, the porous gel, loaded with the fluorescein molecules, was cut into two pieces of equal weight. One piece was placed in a water solution of ACh and the other one in TDW. The diffusion of fluorescein out of the gel into the bath was monitored by fluorescence lifetime and spectral absorption measurements. Measurements were made in the gel and also as a function of distance from the gel surface.

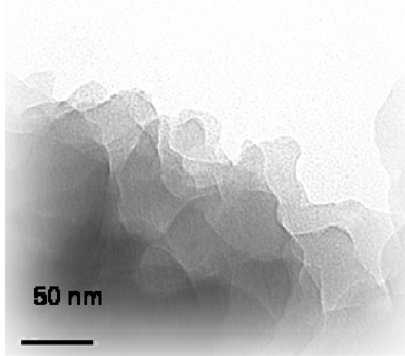


Figure 1. HR TEM image of the porous poly (*N*-vinyl imidazole) PVI gel.

3 RESULTS

There was no indication of diffusion of individual fluorescein molecules out of the porous PVI gel into the TDW bath in which it was immersed. However, the absorption spectrum of the PVI gel/ACh/TDW bath demonstrated the absorption at 488 nm. This indicates the diffusion of a fluorescein/ACh complex as reported in Figure 2.

The investigation of the fluorescence lifetime of the fluorescein provides an evidence that the fluorescence lifetime depends on:

1. Duration of the diffusion process;
2. Distance (propagation length) above the PVI gel as documented in Figure 3.

Further control experiments show that fluorescein/ACh/TDW at different ratios of fluorescein/ACh do not show any fluorescence lifetime changes during 200 hours.

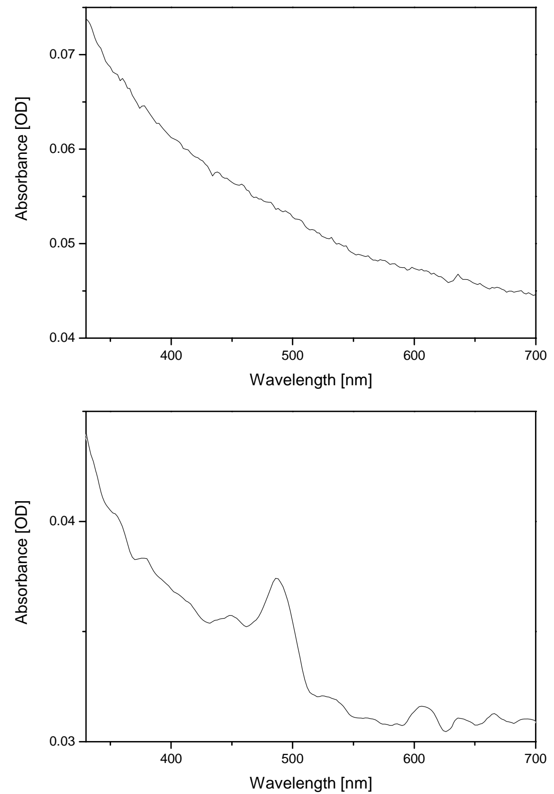


Figure 2. Absorption spectra of the bath containing both the aqueous solution of ACh and the porous gel.
(a) Immediately after immersion of the porous gel, loaded with fluorescein, in the aqueous solution;
(b) The same sample after 50 hours of storage in the dark. The number of fluorescein molecules expelled from the gel after 50 hours storage is ~2.4×10^{13}.

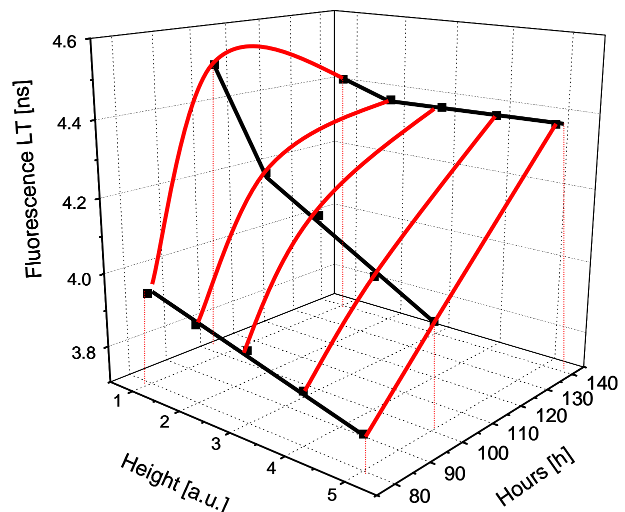


Figure 3. Fluorescein fluorescence lifetime dependence on the diffusion duration and the distance above the PVI gel. The fluorescence lifetime $\tau_{average}$, diffusion duration and distance are plotted on Z-, Y- and X-axes, respectively. The distance is plotted in the a.u, and a.u. is 0.8 cm.

The experimental data illustrates the role of diffusion of ACh into the gel, and the resulting conformational changes, on the formation of the different complexes, such as PVI/fluorescein, PVI/Ach and ACh/fluorescein.

4 DISCUSSION

The data reported in Figures 2 and 3 show that the ACh penetration in the gel induces fluorescein diffusion out of the gel in such a way that the dynamic and steady state behavior is described as

$$-\frac{dC_{ACh}}{dt} = \frac{dC_{Fl}}{dt}, \qquad (2)$$

where C_{ACh} and C_{Fl} are the acetylcholine and fluorescein molar concentrations.

In the initial phase (**I**) (0 - 80 hours) of diffusion of the fluorescein molecules out of the gel, a fluorescence lifetime of 3.6-3.8 ns was recorded around a whole volume of the sample (practically with monoexponential decay). That means that the fluorescein molecules are present as the monoanion throughout an entire PVI/Fl/ACh/water solution. The fluorescein molecules exhibit the same fluorescence lifetime (3.6-3.7 ns) in aqueous solution with the concentration 1×10^{-5} M^{-1}.

During the second phase (**II**) of the diffusion (80-100 hours) in the area located near the gel, as shown in Figure 3, fluorescein's fluorescence lifetime is significantly increased to ~4.5 ns (the long fluorescence lifetime component constitutes ~65%) in addition to the prolongation of the diffusion duration, and an increase in the concentration of the fluorescein molecules in solution. The absorption spectra are not shown in Figure 3. The value of the fluorescence lifetime is evidence for the presence of the dianion near the surface of the gel. With increasing propagation length, the fluorescence lifetime is reduced to 3.7 ns which is typical for the monoanion.

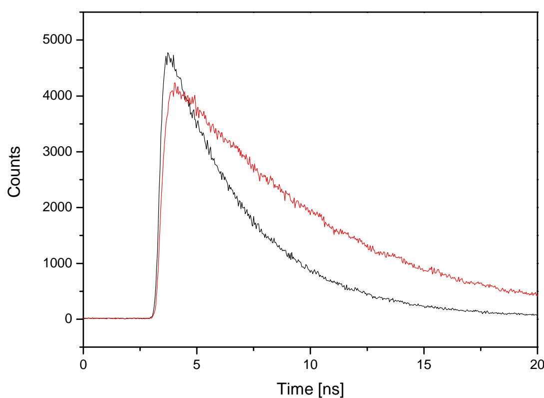


Figure 4. Fluorescence intensity decay of the fluorescein inside the gel (red curve) and in the solution (black curve) after solution storage during 80 hours.

The third phase (**III**) (100-140 hours) is a saturation (steady state) behavior of the concentration of the fluorescein molecules. The average fluorescence lifetime of the fluorescein was 4.35 nsec, which corresponds to a double exponential decay. This value was consistently measured over the whole volume of the sample.

We summarize the diffusion phases as:

(**I**) (0-80 hours) This stage demonstrates a homogeneous distribution of the fluorescein monoanions over almost the whole volume of the sample, and the lifetime is 3.7 ns, e.g., mainly the monoexponential decay;

(**II**) (80-100 hours) This phase demonstrates that dianion fluorescein molecules are expelled near the gel surface, and the lifetime becomes 4.5 ns, corresponding to the monoexponential decay;

(**III**) In the saturated steady state, the distribution of lifetimes is almost constant throughout the sample volume with an average value of ~4.35 ns. We calculated the differential absorption spectra of the solutions as the difference of the absorption of the solution during [80 100] hours and [100 140] hours diffusion time. The results are documented in Figure 5. The measurements were made at the distance from 0.8 to 2.4 cm above the gel.

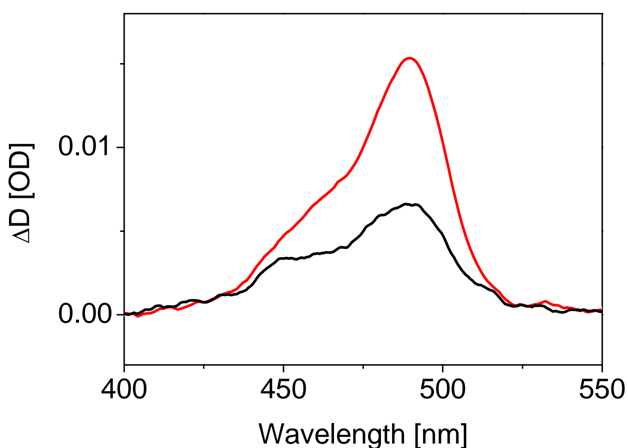


Figure 5. Differential absorption spectra of the solution (distance is from 0.8 to 2.4 cm above the gel) during the period [80 100) hours diffusion time (red curve) and during [100 140] hours diffusion time (black curve)

In agreement with the fluorescence lifetime measurements, these spectra show a decrease in the rate of the diffusion of fluorescein molecules in solution as the sample approaches saturation due to a redistribution of the mono- and dianions during the spontaneous diffusion. The maximum absorption of the fluorescein monoanion is at

455 nm, while the maximum absorption dianion is at 488 nm [4].

It seems that during the initial phase, ACh molecules expel the free fluorescein molecules located in the micropores. The expelled molecules in the monoanion form diffuse into solution through the nanopores. The value of the fluorescence lifetime shows that the monoanion form of the fluorescein is present throughout practically a whole volume of the sample. This means that the ACh molecules in the solution exist in the non-reactive, "closed" *gauche* form, which does not form complexes with fluorescein.

During the second phase, ACh molecules interact with fluorescein molecules which are bound to PVI at the pore surface. In this case, the fluorescein exhibits a fluorescence lifetime ~4.5 ns as reported in Figures 3 and 4. The fluorescence lifetime, which is ~ 4.5 ns, is similar to that of the free fluorescein dianion [3].

During the equilibrium phase (saturation), the collective effect of the combination of the two different forms of the fluorescein, results in an average fluorescence lifetime from 4.3 to 4.4 ns.

5 CONCLUSIONS

We conclude that:

1. ACh molecules expel fluorescein molecules from the micro/nanoporous PVI gel.
2. Intermolecular interactions of ACh with fluorescein molecules which are bound to PVI result in different forms of the fluorescein molecules being expelled from the gel.
3. Our data illustrate the role of diffusion of ACh into the gel and the resulting conformational changes, i.e. from the non-reactive, *gauche* closed form in solution to the active *trans* form inside the gel pores. These changes are in turn due to competition among the three different molecular complexes- PVI/fluorescein, PVI/Ach and Ach/fluorescein. Our experiments confirm that the flexible Ach can play the role of a regulator in the process of molecular transport.

6 ACKNOWLEDGEMENT

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