

## Photocontrol of Polypeptide Membrane Permeabilities by *cis-trans* Isomerism in Side Chain Azobenzene Groups

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The ion permeabilities of solid membranes of poly(L-glutamic acid) with azobenzene groups in the polymer side chains can be reversibly controlled by photo-irradiation.

During our studies concerning the flux regulation mechanisms of polypeptide membranes,<sup>1,2</sup> we have found that conformational changes in the polypeptides influence certain parameters, such as the charge density, partition coefficient, and free volume, of the membranes that determine their permeation properties. We now report on photocontrol of the ion permeabilities of solid membranes of poly(L-glutamic acid) (PGA) with azobenzene groups in the polymer side chains.

It has been recognized that conformational changes in biological macromolecules, induced by changes in the environment, might be the important factors in variations in natural membrane activities. Simple model systems may give

physicochemical insight into the behaviour of natural membrane systems. Photo-induced membrane permeability change has recently been observed for bilayer,<sup>3</sup> liquid membrane,<sup>4</sup> and synthetic polymer membrane<sup>5</sup> systems, with entrapped azobenzene derivatives as the ionophores. No report has appeared, however, on photo-induced permeability changes in polymer membranes containing azobenzene groups in the polymer chains.

PGA polymers with incorporated azobenzene groups (azo-modified PGA) were synthesized by the condensation reaction of PGA with *p*-phenylazoaniline in dimethylformamide at 70 °C, as reported by Ciardelli.<sup>6</sup> 12% of the L-glutamic acid residues were converted into the *p*-phenylazoanilide derivatives.† Changes in the u.v.-visible absorption spectrum of an azo-modified PGA membrane effected by u.v. irradiation [a high pressure mercury arc lamp (Rico-Kagaku Sangyo Co. Ltd., UVL 100 p 100 W) was used] are reported in Figure 1. In the dark, all the azo groups are in the *trans* form. The *trans* to *cis* photoisomerization in azo-modified PGA in chloroform has been reported,<sup>6</sup> with a photoconversion of 85–90%. Isomerization to the *cis* isomer was also induced by irradiation of the solid membrane as shown in Figure 1, although the *trans*

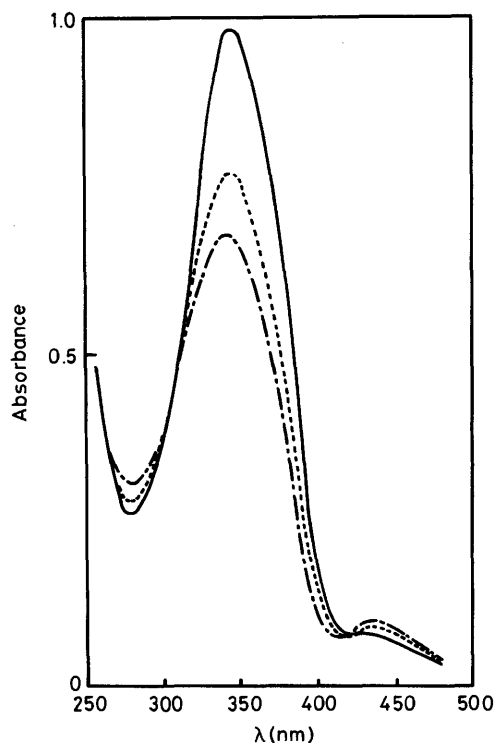


Figure 1. Absorption spectra of a solid membrane of poly(L-glutamic acid) with a 12% content of side chain azobenzene groups at 25 °C (—: in the dark, - - - - : u.v. irradiation for 30 min, — · — · — : u.v. irradiation for 2 h).

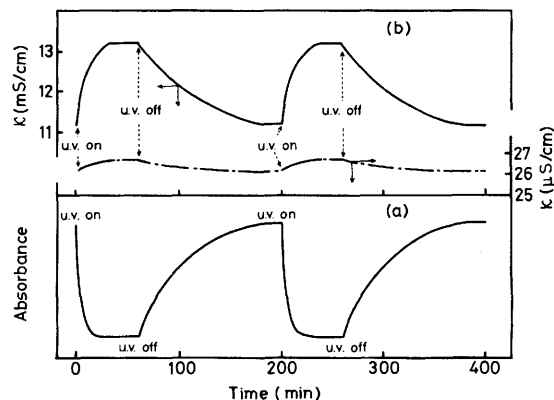


Figure 2. (a) Change in absorbance at 350 nm of the membrane at 60 °C. (b) Photo-induced cross-membrane conductance changes at 60 °C (concentration of KCl on both sides of the membrane = 1 mol dm<sup>-3</sup>; —; = 10<sup>-4</sup> mol dm<sup>-3</sup>; — · — · —).

† The product was repeatedly precipitated from methyl alcohol and chloroform alternately, to remove unreacted azobenzene monomers.

to *cis* conversion of azobenzene groups in the solid membrane was lower than that in chloroform. On the basis of the changes in the absorbance at 350 nm, it was estimated that 40% of the *trans* forms were converted into the *cis* isomers. The half-life of the metastable *cis* isomers in the solid membrane was *ca.* 60 h at 25 °C.

The u.v. irradiations did not affect the main chain  $\alpha$ -helix structure of the azo-modified PGA membrane, as shown by c.d. spectra. Repeated reversible *trans-cis* isomerization was achieved at 60 °C by switching the u.v. lamp on and off, with 45% of the polymer side chains in the *cis* form after u.v. irradiation, Figure 2(a). The thermal (*cis*→*trans* isomerisation in the dark) and photochemical reactions were completely reversible, with a half-life for the *cis* isomers of 60 min, and the photochromic cycles were repeated several times with no apparent fatigue in the polymer at 60 °C.

A membrane of *ca.* 1  $\mu\text{m}$  thickness was prepared for permeation measurements by coating a 2% chloroform solution of the azo-modified PGA onto a porous filter and allowing the solvent to evaporate. The dependence of the cross-membrane conductance using KCl solution,  $\kappa_{\text{KCl}}$ , on u.v. irradiation of the membrane was observed at 60 °C. The results are illustrated in Figure 2(b). When the concentration of KCl on both sides of the membrane was 1 mol dm<sup>-3</sup>, a positive shift of  $\kappa_{\text{KCl}}$  was induced by u.v. irradiation, the increase in  $\kappa_{\text{KCl}}$  being 20% after the u.v. irradiation. Conversely, the enhancement of  $\kappa_{\text{KCl}}$  was only 2% when the ionic concentration was 10<sup>-4</sup> mol dm<sup>-3</sup>. Changes in other membrane parameters were also observed on u.v. irradiation, *i.e.*, enhancement in water uptake by the membrane and increase in membrane potentials. The increase in the water content of the azo-modified PGA membrane from 0.165 (wt% of water in the membrane) in the dark to 0.2 wt% after u.v. irradiation can be associated with the decrease in hydrophobicity of the side chain azobenzene groups (with increase in the content of the *cis* isomer).

The increase in membrane potential which was observed with a KCl concentration ratio across the membrane of  $c_1/c_2 = 4$ , indicates an increase in the degree of dissociation of the L-glutamic acid CO<sub>2</sub>H side chains, with increase in water content of the membrane on u.v. irradiation. That is, the enhancement of water uptake by the membrane due to u.v. irradiation has two effects: one is to increase the free space in the membrane and the other is to accelerate acid side chain

dissociation. The former increases the diffusion of ions through the membrane and the latter may decrease the solubility of ions in the membrane in accordance with the Donnan exclusion effect.<sup>7</sup> At high ionic concentrations, where the Donnan effect is negligibly small, the increase in diffusion of ions through the membrane with u.v. irradiation may result in the relatively large positive shift of  $\kappa_{\text{KCl}}$  at 1 mol dm<sup>-3</sup> of KCl [Figure 2(b)]. At low concentrations, (*ca.* 10<sup>-4</sup> mol dm<sup>-3</sup>), the Donnan exclusion effect, dominates and the solubility of ions in the membrane decreases so the increase in  $\kappa_{\text{KCl}}$  due to u.v. irradiation is only slight.

Thus, when photo-stimulation was applied to the azo-modified membrane, different conductances were observed depending on the concentration of the surrounding KCl solution. This phenomenon can be explained in terms of a co-operative effect between the photo-reaction of the azobenzene side chain and the dissociation of the L-glutamic acid CO<sub>2</sub>H side chains in the membrane. One additional important aspect should also be noted, *i.e.*, that the photo-induced  $\kappa_{\text{KCl}}$  changes were entirely consistent with the absorption changes at 350 nm of the azo-modified PGA membrane.

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