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Photochromic Polypeptides: Acceleration of α- Helix to Coil Transformation in Light Adapted Poly(L-Spiropyran Glutamates) by Substituent Effects

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PHOTOCHROMIC POLYPEPTIDES: ACCELERATION OF α-HELIX TO COIL TRANSFORMATION IN LIGHT ADAPTED POLY(L-SPIROPYRAN GLUTAMATES) BY SUBSTITUENT EFFECTS.

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ABSTRACT The kinetics of the coil to α-helical transformation was studied in light adapted hexafuoroisopropanol solutions of spiropyran covalently attached to poly(l-glutamates). The dark adapted polypeptide had a coil conformation and light adaptation led to ring closure of the merocyanine form with simultaneous conversion into a-helix. By suitable substitution on the pyran ring of the spiropyran, it is possible to accelerate the conformational changes on the polypeptide back bone by two orders of magnitude. Half lifes of the order of 90 seconds were obtained for reversible conformational changes. It is interesting to note that the half lifes for the peptide bound spiropyran photochromic reaction and those of helical to coil transition for different substituents, follow the relative rates of the decoloration reaction of the unbound dyes in solution.

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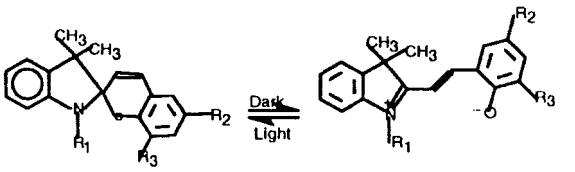
INTRODUCTION

Photochromic molecules covalently bound to polypeptides have been investigated as model systems for coupling photochemistry with conformational changes in the polypeptide backbones[1-4]. Studies have been performed with azo-modified and spiropyran-modified polypeptides[5,6]. Recently[7] we reported on the photomodulation of conformation by sunlight in spiropyran(SP) containing poly(l-glutamic acid) where the dark-adapted polymer has an disordered conformation, and the chromophore is in its merocyanine form(MC). When light adapted, reverse photochromism(MC-

SP)occurs, accompanied by a coil to a-helix transition. During dark adaptation, a slow helix to coil dark reaction (HCDR) and a SP to MC reaction(SMR) takes place(Fig.1). Detailed systematic kinetic studies of the HCDR in hexafluroisopropanol(HFIP) revealed that there were two populations of MC chromophores. The minority(populationI) were complexed to the polypeptide backbone and exhibited optical activity while the majority(population II) were hydrogen bonded to HFIP and optically inactive. The CD spectrum of MC in the dark adapted PSLG was red shifted and had a narrower bandwidth than the corresponding UV/VIS spectrum indicative of good coupling between SMR and the polypeptide.

There have been many studies reported on the kinetics of spiropyran photochromism involving the effect of substituents, which are electron withdrawing or electron releasing type, on the pyran ring or the indoline moiety, and their consequences on the rate of decoloration of the merocyanine form due to the ring closure[7]. A relationship based on the Hammett equation, which relates rates and equilibria for many reactions involving varying substituents in the phenyl ring, was attempted for spiropyrans. Among the substituents in the pyran ring, electron withdrawing groups like nitro or halogens facilitated a slower rate for ring closure compared to the electron releasing group like methoxy[8].

SP MC



PGNBIPS R_1= $OOC(CH2)_2$-Glu_n, R_2= NO_2, R_3= H
PGMNBIPS R_1= $OOC(CH2)_2$-Glu_n, R_2= OMe, R_3= NO_2
PGDBRBIPS R_1= $OOC(CH2)_2$-Glu_n, R_2= Br, R_3= Br

Figure.1 Reverse photochromism of spiropyran attached poly(L-glutamate).

In this paper, we have shown how varying the substituents in the pyran ring influences the rate of decoloration reaction and consequently

the kinetics of helical conformational changes in the polypeptide backbone. We have studied the light induced reversible conformational changes by circular dichroism spectroscopy. Our experiments show that the fast rate of zwitterion ring closure in the side chain photochromophore shortens the lifetime of the α-helical conformation of the poly(l-glutamate) by orders of magnitude. A striking observation is that the relative rates of helix to coil change for varying substituents in the pyran ring in our study parallels that of the decoloration reaction in the unbound dyes reported in the literature.

EXPERIMENTAL

The sodium salt of poly(L-glutamic acid PGA), MW 74,000 was purchased from Sigma Chemical Co. The photochromic dyes, namely, 1'-β-hydroxyethyl-6-methoxy-8-nitro-3',3'-dimethylspiro-[2H-1-benzopyran-2,2'-indoline], abbreviated as MNBIPS, 1'-b-hydroxyethyl-6,8-dibromo-3',3'-dimethylspiro-[2H-1-benzopyran-2,2'-indoline], abbreviated as DBRBIPS, and 1'-b-hydroxyethyl-6-nitro-3',3'-dimethylspiro-[2H-1-benzopyran-2,2'-indoline], abbreviated as NBIPS, were purchased from Chroma Chemicals, Dayton, Ohio. Dicyclohexyl dicarbodiimide(DCCI), dimethyamino pyridine DMAP, dimethyl formamide(DMF), hexafluro-2-propanol(HFIP) were purchased from Aldrich Chemical Co,. All chemicals were used without purification.

Synthetic procedure for modifying poly(l-glutamate) with spiropyran was given in detail in our earlier publication[7]. The polypeptide-bound dyes were synthesised via DCCI coupling reaction[7]. Prior to coupling, the Na-PGA was protonated with 1 M HCL, centrifuged, and freeze-dried. A mixture of 0.0012 M of PGA, 0.0024 M of the dye, 0.0013 M DCCI and 0.0006 M DMAP were dissolved in DMF and the reaction was slightly heated for 48 hours, the precipitated dicyclohexyl urea was filtered. The polypeptides attached to the photochromic dyes, namely, PGNBIPS, PGMNBIPS, PGDBRBIPS, were precipitated by the addition of ethanol and the process of precipitation and dissolving in DMF was repeated atleast three times to ensure the purity of the products. The purity was checked by thin layer chromatogram and also FT-IR. FT-IR spectrum of a KBr pellet of the purified products showed an ester absorption band at 1739 cm-1. Gel permeation chromatography analysis of

the photochromic polypeptides using microstyrogel coloumn(10^4 A., Waters Instrument)) in DMF showed that PGMNBIPS had a broad distribution with molecular weights ranging from 250,000 to 100,000, where as the PGDBRBIPS had a relatively narrower distribution with molecular weights ranging from 150,000 to 100,000.

A Jasco model 720 spectropolarimeter was used to measure circular dichroism CD. The instrument was calibrated using an aqueous solution of ammonium-d-camphor-10-sulfonate supplied by Jasco. UV/VIS spectra were measured with a Perkin-Elmer Lamda 9 spectrophotometer. Polypeptide solution was illuminated with broad band white light source and upon bleaching placed into spectropolarimeter where ellipticity was monitored over time. Similar experiment was done in the spectrophotometer to follow changes in the spiropyran photochromism.

RESULTS AND DISCUSSION

The spiropyran-attached PGA's were studied in HFIP solutions. In HFIP, the polypeptides were highly colored(pinkish or orange), demonstrating the stabilization of the colored MC form. We measured the CD of the polypeptides in HFIP down to 190 nm. The HFIP solutions when dark adapted, exhibited random coil conformation as seen by the CD. The CD of all the three PGA's showed maxima at 194 and 223 nm characteristic of the random coil conformation. When light adapted, the decolored solutions exhibited maxima at 190, 208 and 222 nm characteristic of the α-helix. Fig. 2 shows the CD spectrum of dark adapted PGDBRBIPS.

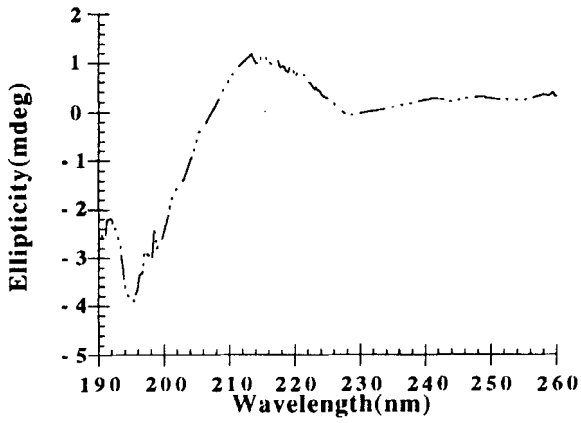


Figure.2 Dark adapted CD spectrum of PGDBRBIPS in HFIP.

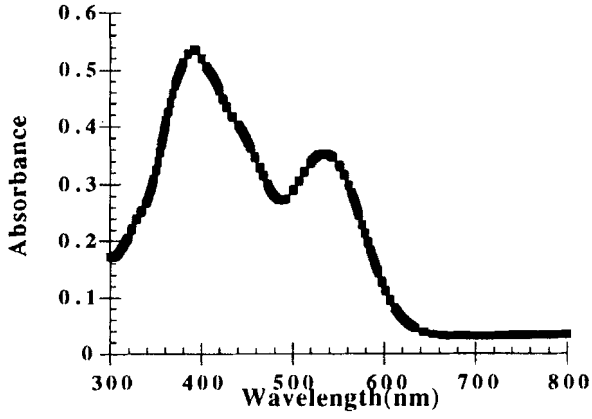


Figure.3 The absorption spectrum of PGDBRBIPS in HFIP-dark adapted.

Fig.3 shows the absorption spectrum of the dark adapted PGDBRBIPS suggesting a coil conformation. We studied the MC to SP reaction by following the absorption change at 535 nm. The kinetics of α- helix to random coil change was followed by the ellipticity changes at 222 and 195 nm extrapolated to time=o, a positive ellipticity at 195 nm and a negative

ellipticity at 222 nm gives evidence for light-induced helix formation. Fig. 4 illustrates the results of the kinetic study.

The rate of decay of the α-helical conformation is seen at 195 nm and 222 nm. Both the decay of the helix and the growth of the coil follow first order kinetics with half life of approximately 90 seconds. It is also interesting to note the absorbance change at 535 nm which corresponds to the MC to SP reaction. The half life was close to 90 seconds thus illustrating that the ring opening dictates the transformation of the helix conformation of the poly(l-glutamate) backbone to coil in the same time scale. The synchronization of SP to MC and helix to coil reaction in a macromolecule is very intriguing.

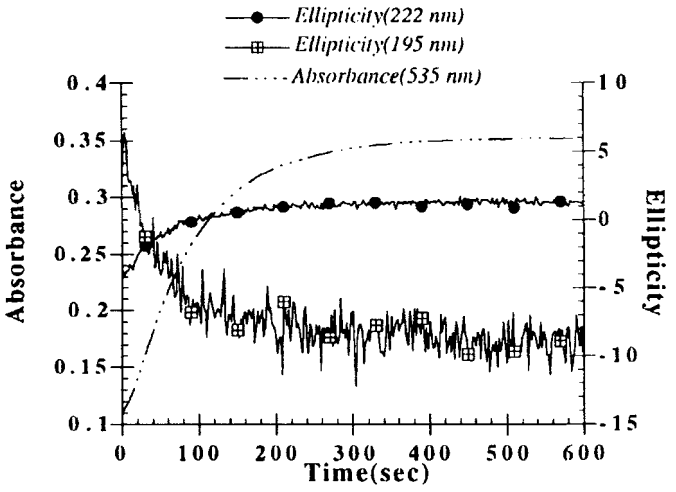


Figure.4 CD spectroscopic kinetic study of reverse conformational changes in polyglutamate. Also shown are the photochromic changes.

The half life of the helix to coil reaction of PGDBRBIPS was about two orders of magnitude faster than the corresponding PGNBIPS($t_{0.5}$= 190 minutes). This correlates well with the half lifes of the decoloration reaction of the unbound dyes NBIPS and DBRBIPS, 269 and 2.9 minutes respectively, measured in ethanol[8,9]. It was also reported that the half life of the MNBIPS is about 0.8 minutes. We were unable to observe the helix to coil change in PGMNBIPS. Based on the relation between MC to SP reaction in the free dye

and the peptide bound, we would expect a half life of 30 seconds for the helix to coil change, a change too fast to observe in our experimental set up. Transient CD spectroscopy measurements will be very valuable to study these fast conformational changes. Our studies, thus far, have demonstrated that it is possible to speed the rate of backbone conformational changes by orders of magnitude through substitution in the spiropyran ring which may have some practical implications for making dynamic filters for lasers. Further studies on photochromic polypeptides containing methoxy substituted spiropyrans are in progress.

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