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SHORT COMMUNICATIONS

Some Factors Influencing the Magnitude of the Induced Circular Dichroism of Poly(L-lysine) Hydrobromide and Azo Dye Complexes

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The induced optical activity of dye bound to biopolymers[1] is a unique characteristic providing information on the electronic transition of the biological systems. The behavior of induced circular dichroism (ICD) of the polypeptide–dye systems reflected the mode of chiral interaction between the polypeptide and dye under a certain environment and was dependent mainly on pH (conformation) and residue/dye (R/D) ratio.[2,3] Several factors including dye structure[4] and added neutral salts[5] influenced the shape and magnitude of the ICD spectrum of the system. We have investigated the ICD properties of the poly(L-lysine) (PLL) HBr–azo dye complexes[6,7] in some detail, and wish to report the preliminary results on some additional factors which may influence the magnitude of ICD.

The PLL HBr and poly(D-lysine) (PDL) HBr samples were synthesized according to the usual *N*-carboxy anhydride method. The degree of polymerization (DP) was determined to be 50, 60, 240, 460, 980, 1520, 1720, 3260, and 4210 for PLL HBr and 1560 for PDL HBr from an empirical equation $\log DP = 1.47 \log[\eta] + 2.99$ in dichloroacetic acid at 25°C for *N*-carbobenzoxylated polylysine homologs,[8] and 20 by *N*-terminal titration in dry dioxane with 1/50 N perchloric acid in glacial acetic acid.[9] Analogous azo dyes, *p*-(4-dimethylaminophenylazo)benzenesulfonic acid sodium salt (methyl orange, MO) and *p*-(4-diethylaminophenylazo)benzenesulfonic acid sodium salt (ethyl orange, EO) were guaranteed reagents (Wako), and *p*-(4-di-*n*-propylaminophenylazo)benzenesulfonic acid sodium salt (propyl orange, PO) and *p*-(4-di-*n*-butylaminophenylazo)benzenesulfonic acid sodium salt (butyl orange, BO) were synthesized by the procedure described by Fieser.[10] The four dyes were purified according to the procedure outlined by Hickinbottom and Lambert[11] and Klotz *et al.*[12] The purity of the dyes was certified by thin layer chromatography. Sample solutions were prepared by adding the dye solutions to the PLL HBr or PDL HBr solution. pH was measured with a Toa Electronics HM-15A digital pH meter. The final dye concentrations were 2×10^{-5} M, and the ratio of R/D was 5. The absorption and CD spectra were measured with a Jasco UVIDEC-1 and a CD J-40A spectrometer, respectively. All the CD data presented below were reduced to the molar basis of the total

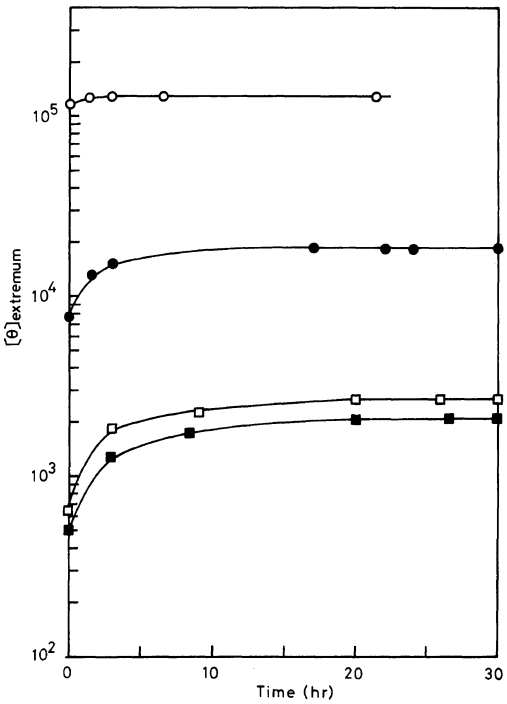

Figure 1. Time dependence of the magnitudes of ICD of the PLL HBr (DP 3260)–azo dye complexes at pH 6 and R/D = 5 in water (25°C): ○, MO complex at 359 nm; ●, EO complex at 371 nm; □, PO complex at 375 nm; ■, BO complex at 395 nm.

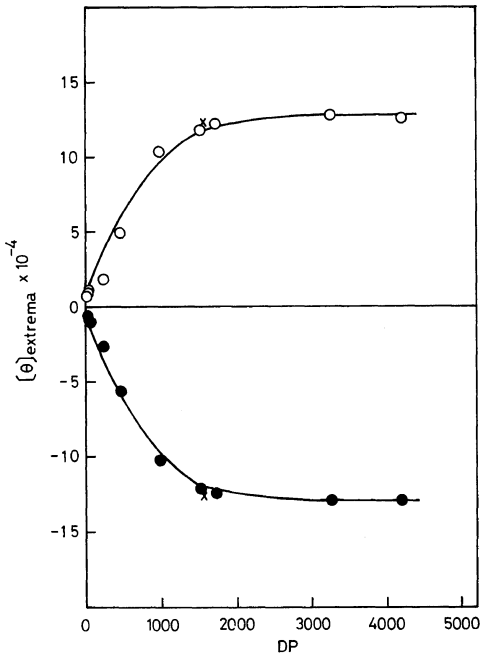

Figure 2. Chain length dependence of the magnitudes of ICD of the PLL HBr–MO complex at pH 6 and R/D = 5 in water (25°C): ○, at 359 nm; ●, at 371 nm. Symbol × denotes the magnitude of the PDL HBr (DP 1560)–MO complex under the same experimental conditions.

dye concentration, and expressed by the ellipticity, $[\theta]$ (degree cm^2 d mol^{-1}). The pH and R/D dependences of ICD of the PLL–azo dye complexes have been reported previously.[6,7] Briefly, the random coiled complexes at pH between 4 and 8 induced CD for the dyes and the R/D ratio of 5 showed the largest ICD values.

The following findings are described in this paper. The ICD spectra of the PLL HBr–dye complexes in water were found to be time dependent on standing without stirring at pH 6 and R/D = 5. For example, immediately after preparing the mixed solution, the ICD values of the PLL HBr–EO complex were −4600 at 387 nm and 7600 at 371 nm. After 17 h at 25°C, the values increased to −15200 and 18200, respectively. Figure 1 summarizes the time dependence of the ICD values of the PLL HBr–azo dye complexes (DP 3260 sample). Final ICD values were −128000 (371 nm) and 129000 (359 nm) for the MO complex, −15200 (387 nm) and 18200 (371 nm) for the EO complex, −2000 (430 nm) and 2700 (375 nm) for the PO complex, and −1800 (465 nm) and 2100 (395 nm) for the BO complex. Next, the MO complex showed the plateau value after 2 h, the EO complex after 17 h, and the PO and BO complexes after 24 h. These suggest that the bulkiness of the *N*-dialkyl moieties in the azo dyes plays an important role in aligning the dye molecules in a nearly parallel orientation along the peptide chain.[6,13] In this connection, the absorption spectra of the MO and EO complexes showed the blue-shifted peaks at 366 nm (MO) and 392 nm (EO), while the PO and BO complexes only the shoulders at around 407 nm (PO) and 410 nm (BO).

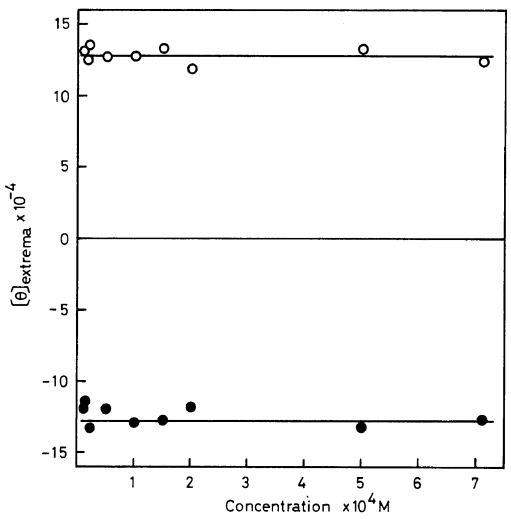

Figure 3. Concentration dependence of the magnitudes of ICD of the PLL HBr (DP 3260)–MO complex at pH 6 and R/D=5 (25°C): ○, at 359 nm; ●, at 371 nm. Concentration is based on lysyl residues.

Since the blue-shifted absorption bands result from the interaction between the delocalized π–π^* transition dipoles, the bulkier *N*-dialkyl moieties may disturb the interaction between the transition dipoles and take longer times for the stable complex formation.

Figure 2 shows the chain length dependence of the ICD magnitude of the PLL HBr–MO complex in water at pH 6 and R/D=5. The critical chain length for the ICD magnitude was found to be about DP 1500. The PDL HBr (DP 1560)–MO complex showed ICD values of 120000 at 371 nm and −124000 at 359 nm, and was of just the opposite sign to the PLL HBr (DP 1520)–MO complex.

Figure 3 shows the concentration dependence of the extreme values of ICD of the PLL HBr (DP 3260)–MO complex at pH 6 and R/D=5. In the concentration range from 10^{-5} to 7×10^{-4} M (based on polypeptide residues), the ICD values were not dependent on concentration. This means that no aggregation of the complex occurs. Some additional factors influencing the magnitude of ICD will be reported elsewhere.

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