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Temperature Effect of the Induced Circular Dichroism in the Heparin-Acridine Orange Complex

Chong-Su Cho and Sung-Woo Kang

Chonnam National University, Kwangju 505, Korea

Summary

An optical activity was induced in the region of the absorption bands of acridine orange when the dye became bound to heparin. The magnitudes of induced circular dichroism(ICD) increased with increasing polymer to dye ratio due to the stacking of dyes. ICD of the complex was affected enormously by the temperature because of the rearrangement of aggregated dye molecules bound to heparin to the dimeric molecules. The temperature effect is reversible and the reversibility is very slow.

Introduction

Blout and Stryer(1,2) first reported that an optical activity was induced in the region of the absorption bands of acridine orange(AO) when the dye became bound to polypeptide. A similar induced optical activity was observed for AO(3) bound to DNA and for several other aminoacridines(4) bound to DNA. Inducing optical activity is called induced circular dichroism(ICD). It is the simplest technique used to understand the stereochemistry of interactions between biopolymers and low-molecular weight materials. Most ICDs have been studied on complexes of synthetic polypeptides such as poly(L-glutamic acid)(1,2),poly(L-lysine)(5,6,7) and poly(L-arginine)(8). Stone(9) studied the induced optical activities of complexes of polysaccharides,such as chondroitin sulfate,hyaluronic acid,kerato sulfate,with cationic dyes, such as AO,neutral red,methylene blue and proflavine. But very few has been known on the ICD of the complex between heparin and AO. Yamamoto(10) studied that the behavior of ICD of polymer-dye systems was dependent on pH,polymer to dye ratio(P/D),ionic strength and degree of polymerization.

In this paper,we wish to report the temperature effect on the ICD in the heparin-AO complex.

Experimental

Materials: Heparin was purchased from Diosynth,Inc., and used without further purification. The anticoagulant activity was 160 IU/mg according to the supplier. Highly pure AO was kindly provided by prof. K.Nishida of Tokyo University of Agriculture and Technology.

Preparation of heparin-AO complex: The heparin-AO complex was prepared by mixing the polymer solution and AO solution after the pH of each solution had been adjusted to pH 6.98.

CD measurement: CD spectra of the complex solution in a quartz cell with a path lengh of 10.0mm were recorded in a JASCO J-500A Recording Spectropolarimeter at room temperature. The ellipticity,(θ) values for the complex was calculated based on the mole concentration of AO.

Results and discussion

Fig. 1 shows the ICD spectra of heparin-AO complex as a function of P/D ratio. ICD spectra of heparin-AO complex

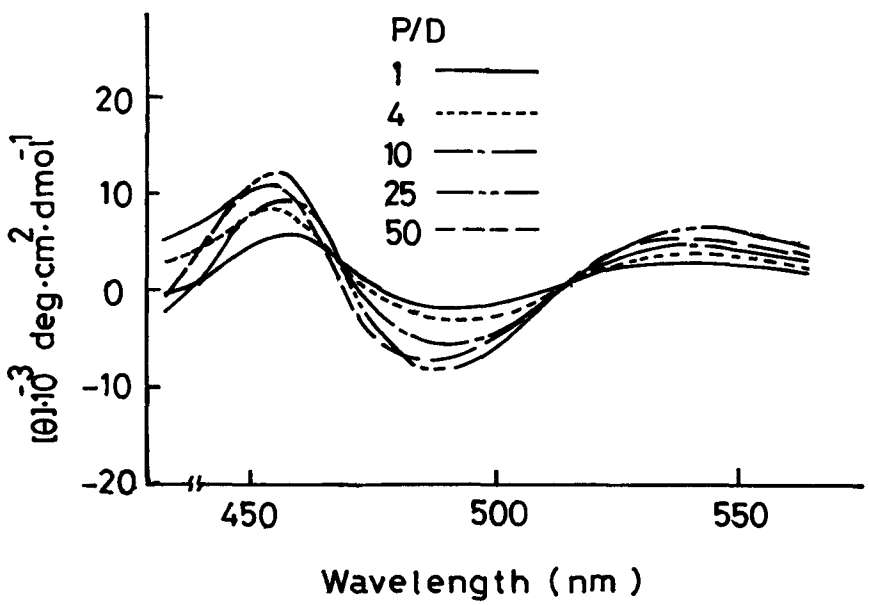

Fig. 1 ICD spectra of heparin-AO complex as a function of P/D ratio at pH=6.98. $(AO)=2\times10^{-5}M$

in P/D ratio between 1 to 50 consist of three ICD bands,i.e., two positive CD bands and one negative CD band. With increasing P/D,the magnitudes of the ICD due to the interaction between heparin and AO increased until P/D=25. This result indicates an increase of the dye-dye interaction (stacking of AO) with increasing P/D. When the P/D ratio is larger than 25,the magnitudes of the ICD decreased.

Fig. 2 shows the ICD spectra of heparin-AO complex as a function of temperature at P/D=25,where the stacking of

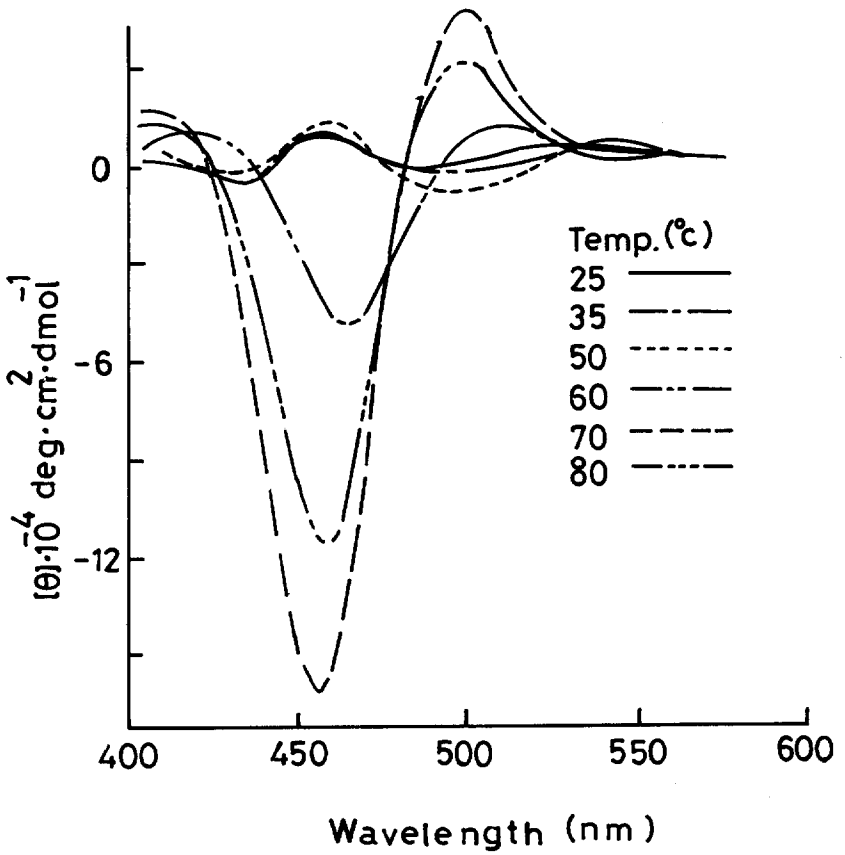

Fig. 2 ICD spectra of heparin-AO complex as a function of temperature at P/D=25,heated for 4 hrs.

AO is the largest. As the temperature increases up to 70°C, extremum in the negative band of the ICD shifted by 25 nm to shorter one and the magnitudes increased gradually. And those magnitudes decreased at 80°C. These results may be considered that aggregated dye molecules are rearranged to the the dimeric dye molecules bound to heparin with elevation of temperature below 70°C. At 80°C, the decrease of the ICD of heparin-AO complex may be regarded as the increase of the amount of unstacking dye with the breakage of ionic bonding between heparin and AO partially by elevation of temperature(10,11).

The ICD spectra of heparin-AO complex as a function of treatment time is shown in Fig.3. The magnitudes of the

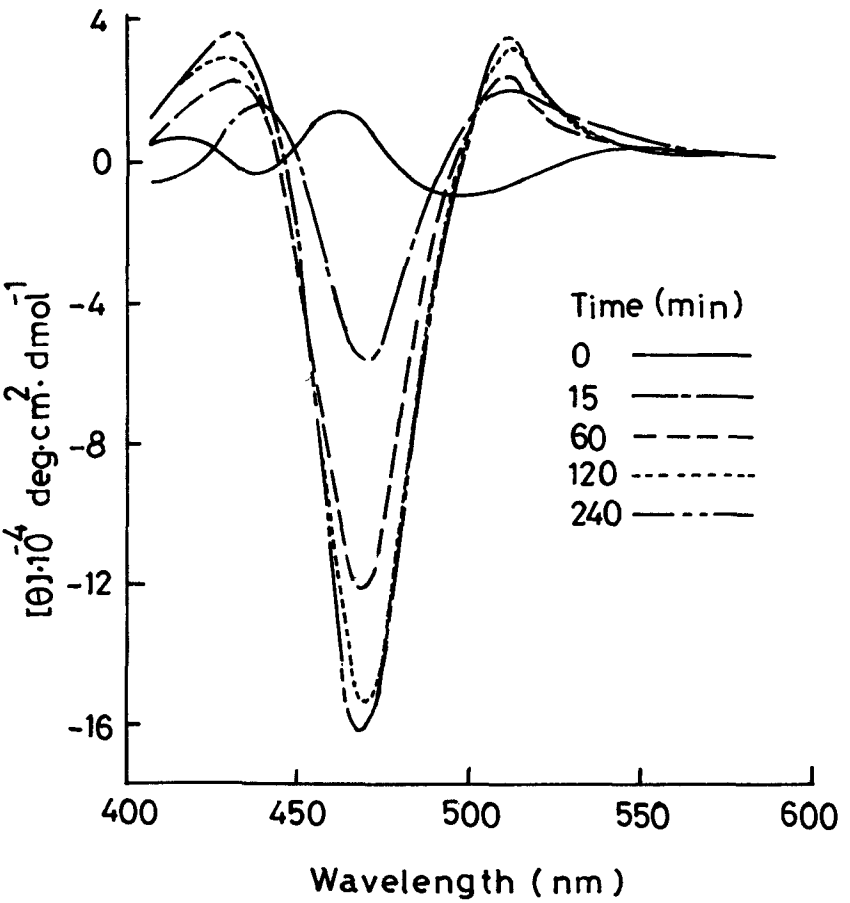

Fig. 3 ICD spectra of heparin-AO complex as a function of treatment time at 70°C, P/D=25.

ICD of heparin-AO complex increased with increasing treatment time. This result may be ascribed to increasing dimeric dye molecules with rearrangement of aggregated dye molecules by treatment time.

Fig. 4 shows the hysteretic effect of temperature on the ICD spectra of the complex after heat treatment at 70°C for 4 hrs. After heat treatment, the complex was then allowed to stand at room temperature and the spectrum was measured according to the time. The ICD of heparin-AO complex shifted to a longer wavelength gradually and decreased in magnitude with increasing the time. This result indicates that the temperature effect is reversible and the reversibility is very slow.

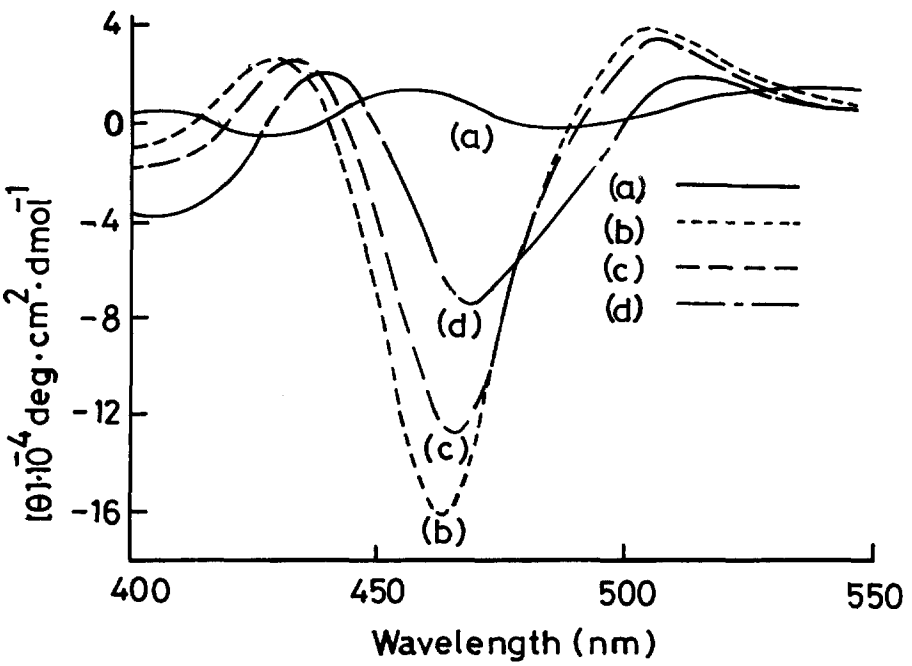

Fig. 4 ICD spectra of heparin-AO complex, P/D=25.
(a) no heat treatment
(b) measured 3 hrs after heat treatment
(c) measured 1 day after heat treatment
(d) measured 30 days after heat treatment

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