SPECTROSCOPIC INVESTIGATION OF CYCLODEXTRIN MONOMERS, DERIVATIVES, POLYMERS AND AZO DYES (1)

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ABSTRACT. Circular dichroism (CD) and visible spectra of inclusion compounds between Methyl Orange (MO) analogues and α-, β-, γ-cyclodextrin (cdx), 2,6-dimethyl-and 2,3,6-trimethyl-β-cdx, water soluble α-, β-, γ-cdx polymer products were investigated. In the CD-spectroscopic investigation, the complex with α-cdx epichlorohydrin condensate showed a large amplitude and splitting of the induced $\pi \rightarrow \pi^*$ band. Fractions of glyceryl ether of less than 2000 and polymer of more than 10000 dalton molecular mass were separated. Complexes of above two fractions and MO showed the same splitting spectral pattern. Job's plots from visible spectra showed the formation of the 1:1 complex and CD-data suggested the co-existence of the 2:1 MO-cdx complex. This splitting pattern showed the reversal of the signs when α-cdx-ethyleneglycol-bis(epoxypropyl) ether was used and disappeared when larger host molecules and azo dyes were used. The splitting was explained by exciton interaction.

1. INTRODUCTION

The widespread practical application of cyclodextrin (cdx) polymers is expected in the coming years. First, probably the insoluble ones will be produced and utilized on industrial scale, e.g. for chromatographic purposes [2a] or as tablet-disintegrating agents [3]. The soluble polymers also seem to be worthy of studying, partly because they can be studied in solutions, partly, because their specific

Journal of Inclusion Phenomena 2, 715–724. 0167-7861/84.15.

properties are rather promising for specific purposes. Harada et al [4] have compared the stability of the inclusion complexes of β-cdx-epichlorohydrin (Ep), β-cdx and β-cdx-acrylate and discussed the inclusion capability of cdx on a polymer chain.

Recently the molecular mass distribution of water-soluble β-and γ-cdx-Ep polymer was studied [2b]. The CD spectra of azo dyes in the presence of monomeric cdx showed to be very sensitive to conformational perturbation by complexation [5a].

Continuing these studies, the CD-spectra of Methyl Orange (MO) were recorded in presence of soluble polymers prepared from α-cdx with Ep(α-cdx-Ep)or with ethyleneglycol-bis (epoxypropyl) ether (α-cdx-DiEp) as well as in presence of 2,6-dimethyl-β-cdx and 2,3,6-trimethyl-β-cdx. An interesting splitting has been observed in the spectra in the presence of the soluble α-cdx polymers.

MO is the most appropriate azo dye for similar investigations, because it can be included into α-, β-, γ-cdx monomers [6], and form complexes with peptides [7,8], and with bilayer membranes of chiral dialkyl amphiphiles [9]. The present paper is dedicated to the possible explanation of the observed splitting in CD spectra of α-cdx-Ep and α-cdx-DiEp MO-complexes.

2. MATERIALS AND METHODS

α-, β-, and γ-cdx used are products of Chinoin Pharmaceutical and Chemical Works, Budapest. Azo dyes used are the same as described previously [5]. Other chemicals used are marketed by Reanal Fine Chemical Works, Budapest.

Cdx polymers of different molecular mass were prepared as follows; Ep or DiEp was added to the aqueous alkaline solution of cdx. The small molecules (inorganic salt, unreacted cdx and Ep) were removed from the reaction mixture by dialysis using Visking tubes (Medicell Int. LTD). The products were obtained as white powders by freeze drying. The characteristics of them are given in Table I. Cdx content was determined by iodometry after acid hydrolysis. The mass average molecular mass and the distribution of the molecular mass of the products (Fig.1) were calculated on the basis of their chromatograms on Ultrogel ACA 34 polyacrylamide gel (total volume of the column 160 ml, sample size 300 mg/5 ml water, elution rate 30 ml/hr, eluent distilled water, detection by polarimetry). 5 ml fractions were collected and united on the basis of the chromatogram to get separate fractions of different molecular mass. The chromatographic equipment used consisted of a column Pharmacia K 16/100, Vario Perpex Pump LKB 12000 and Ultra Rac Fraction Collector LKB 7000.

Table I. Properties of Polymer Products

Polymer products	Cyclodextrin content (%)	Mass average molecular mass
α-Cyclodextrin epichlorohydrin (1)	61	4700
α-Cyclodextrin epichlorohydrin (2)	59	1400
α-Cyclodextrin epichlorohydrin (3)	60	8200
α-Cyclodextrin ethyleneglycol-bis (epoxypropyl) ether	52	3600
β-Cyclodextrin epichlorohydrin	65	3600
γ-Cyclodextrin epichlorohydrin	59	7500

UV and visible spectra were measured with a Specord-M 40 Carl Zeiss JENA using a 0.2 cm cell. CD spectra were measured with a Roussel-Jouan Dichrographe N°III (Jobin-Yvon) using a 0.5 cm cell. ~$2x10^{-3}$M host molecule and ~$2x10^{-4}$M guest molecule were dissolved in 0,1 M phosphate buffer at pH 6.0.

3. RESULTS AND DISCUSSION

Induced CD spectra of MO in presence of different types of cdx monomers, di-,tri-methylated β-cdx and glycerylether fractions of β-and γ-cdx-Ep-polymer products show single patterns. The α-cdx-Ep-1 and α-cdx-DiEp result in the $\pm$ Cotton effects and the largest amplitudes (Fig.2). α-cdx-Ep-1 is characterized by a rather wide molecular mass distribution from 1000 to 13000 (Fig.1a). The α-cdx-Ep-2 (low molecular mass fraction = L) having a mass average molecular mass of 1400 (Fig.1b) was selectively prepared in diluted solution. The α-cdx-Ep-3 characterized by the highest (yet soluble) molecular mass was prepared by further crosslinking the α-cdx-Ep-1 and the fraction of >10000 dalton (=H) was separated (Fig.1c). The MO results in similar CD spectral patterns with both of the above mentioned fractions, nevertheless the amplitude is considerably lower with the H-fraction.

The CD data does not give exact molar ratio, the coexistence of MO: α-cdx = 1:1 and 2:1 complexes is assumed. It is noteworthy, that the low molecular fraction (L-α-cdx-Ep) results in higher Cotton-amplitudes than that of the high molecular one (H-α-cdx-Ep) (Fig.3a).

Visible spectral data (λ_{max} = 463 nm) point to the formation of 1:1 complexes [10] (Fig. 3b).

Similar CD splitting patterns were observed with some MO analogues; 4-{[4-(phenylamino)phenyl]azo}benzene-

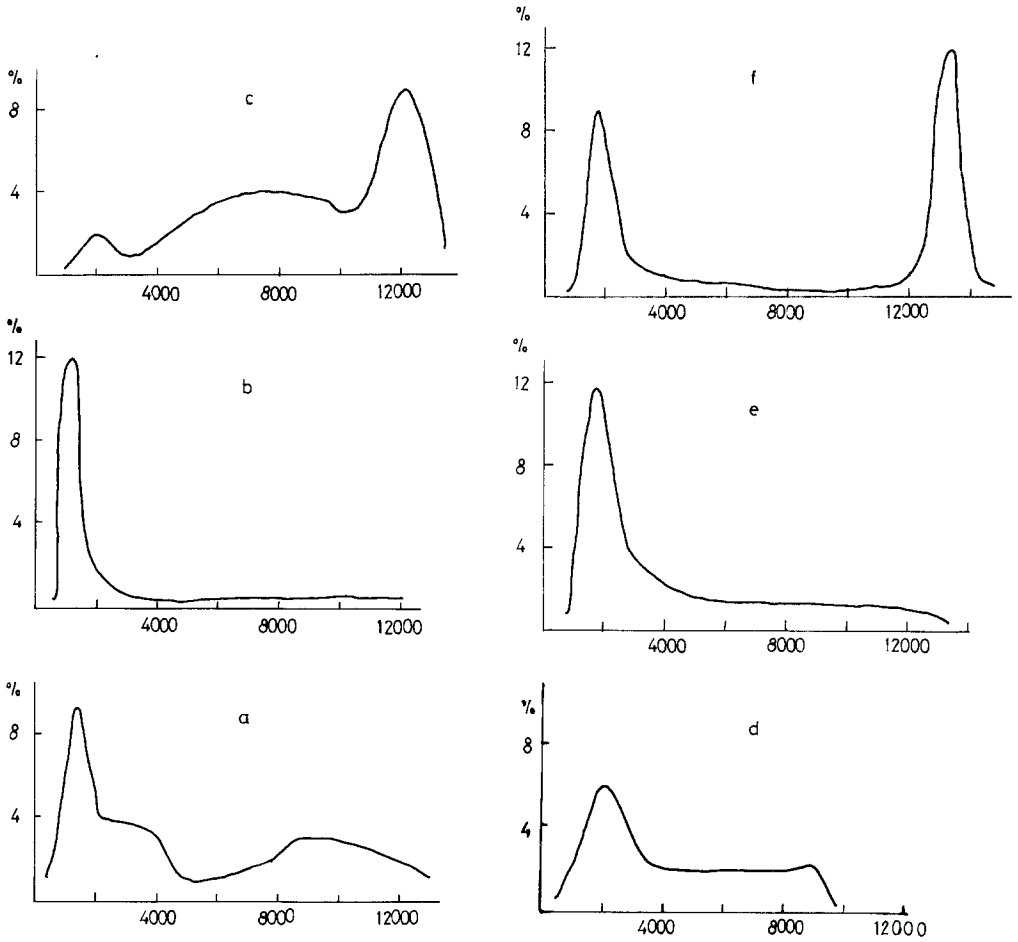


Fig.1. Cyclodextrin Polymer Percentage as a Function of Molecular Mass

a : α-Cyclodextrin epichlorohydrin 1
b : α-Cyclodextrin epichlorohydrin 2
c : α-Cyclodextrin epichlorohydrin 3
d : α-Cyclodextrin ethyleneglycol bis (epoxypropyl-) ether
e : β-Cyclodextrin epichlorohydrin
f : γ-Cyclodextrin epichlorohydrin

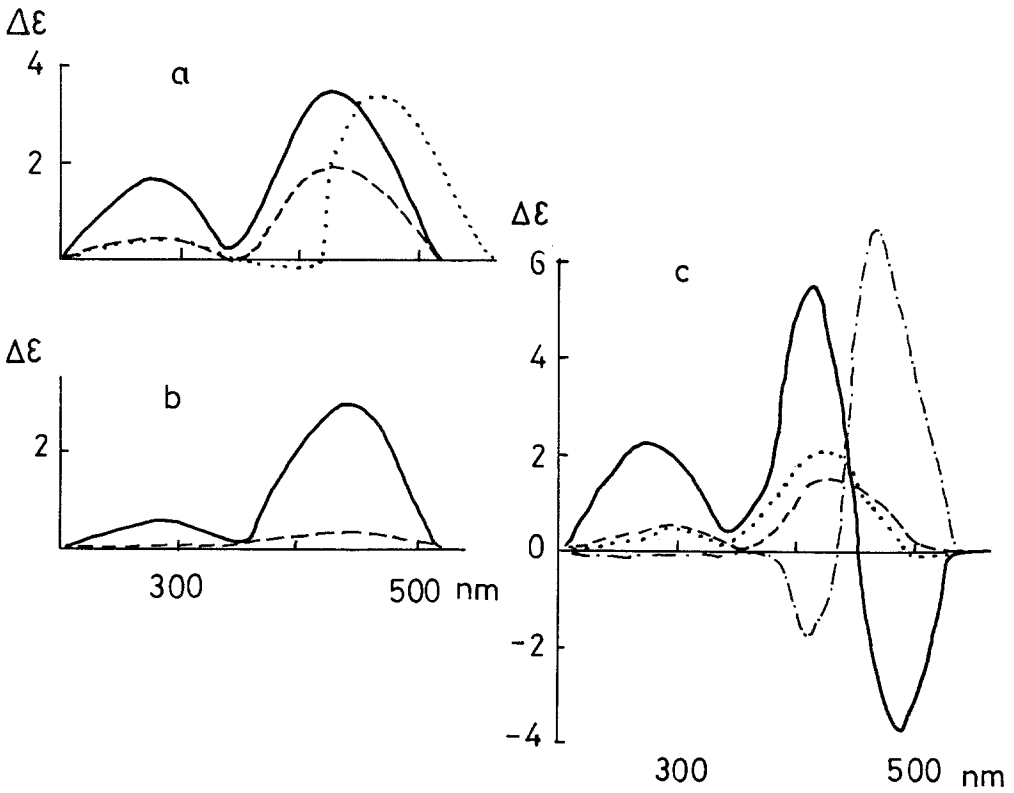


Fig.2. CD Spectra of Methyl Orange Complexes

Cyclodextrin
a: α(——), β (— —), γ (····) Monomers
b: 2,6-Dimethyl-β(——) , 2,3,6-trimethyl-β(— —) monomers
c: Products with the linking agents α-epichlorohydrin(——) , β-epichlorohydrin (— —), γ-epichlorohydrin (.....), α-ethylene-glycol bis (epoxypropyl) ether(— . —)

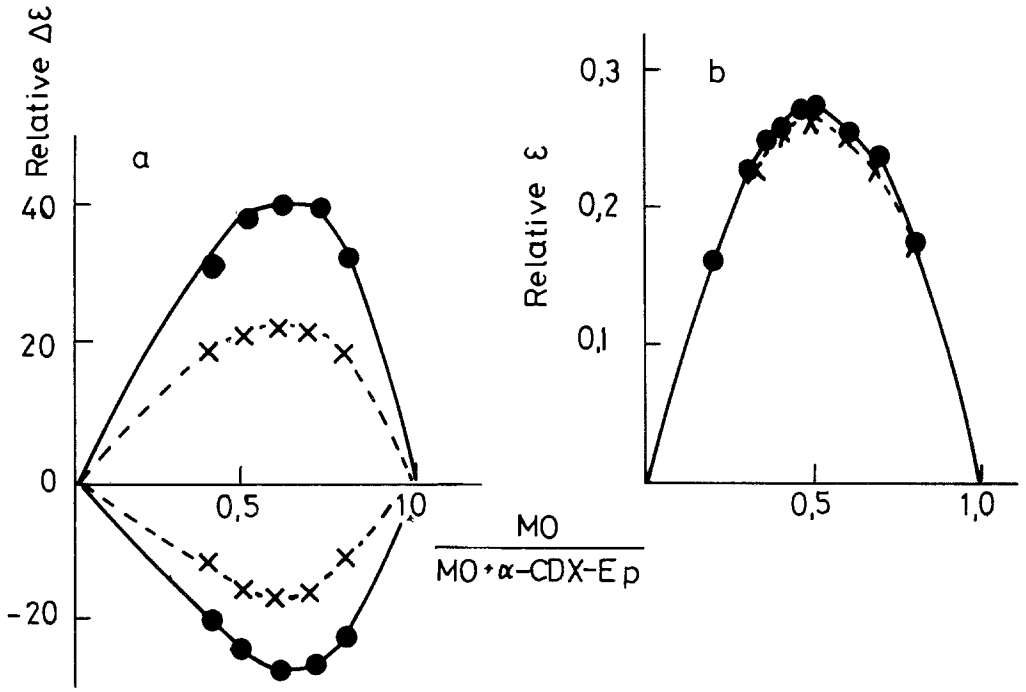


Fig.3.a. Continuous Variation Plots of the Molar Circular Dicroism at 413 and 485 nm of α-Cyclodextrin epichlorohydrin Polymer Products and Methyl Orange
—●— The fraction in which the molecular weight is less than 2000.
--X-- The fraction in which the molecular weight is more than 10000
Total concentration: 4×10^{-4} M
Cell length 0,5 cm.

Fig.3.b. Continuous Variation Plots of the Absorption Coefficient at 463 nm of α-Cyclodextrin epichlorohydrin Polymer Products and Methyl Orange
Cell length 0,2 cm.

sulfonic acid monosodium salt (I) and dimethylamino-azobenzene (II) but not with bulky dyes; 2{[4- (dimethylamino) phenyl] azo} benzoic acid (III), Orange I, Orange II, Yellow V, Fast Red E and Red II in which one or both sides of the N=N bridge bear a naphthalene nucleus.

The shape and the sign of the spectral pattern is quite different when the crosslinking agent is DiEp. Azobenzene does not show the splitting pattern in $\pi \rightarrow \pi^*$ region (330 nm) (11) in presence of α-cdx-Ep and α-cdx-DiEp. Apparent amplitudes indicate that the shorter crosslinkages containing α-cdx-Ep fits better for shorter azo dye molecules and the larger cross-linkages containing α-cdx-DiEp for the longer ones (Table II).

The splitting pattern in the CD spectra of α-cdx-Ep and α-cdx-DiEp complexes may arise from exciton interaction of two MO molecules, because absorption maximum of the visible spectra and the cross-over point of the CD couplet practically coincide. The amplitudes of the Cotton effects are always different. This fact suggests the co-existence of two components; the predominant one is the 1:1 complex, which results in a single peak at ~420 nm but there is another one which results in the exciton splitting. There are two possibilities concerning this second component.

1. Intermolecular association of the 1:1 complex. Goto et al (12) found that several dyes associate in solution and show strong Cotton effects. In the present case, this splitting pattern can be observed until the MO concentration is 4×10^{-7} M at which the signal almost disappears.

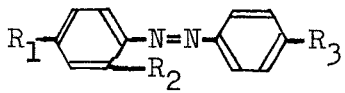
2. Interaction of 2MO within the 2:1 complex. Job's plot from CD data suggests the existence of MO: α-cdx = 2:1 complex. The longitudinal molecular axis of MO is longer than the depth of the α-cdx cavity. If the protruding fragment of the complexed MO molecule interacts with another MO molecule entangled between the side chains, such splitting can be expected. Generally, dimeric MO results in blue shift in the visible spectra. Hatano et al (7) observed a blue shift (360 nm) in the visible spectrum of MO and two Cotton peaks of opposite signs in the CD spectrum in presence of poly-L-lysine. The likely explanation for this observation was the formation of dimeric MO molecules bound to poly-L-lysine.

According to Clark et al (13) the γ-cdx forms MO: γ-cdx = 2:1 and 2:2 complexes and a parallel arrangement of the included two MO moieties to the annular axis of γ-cdx was assumed. The λ_{max} of this complex is 435 nm and does not show splitting in the CD spectrum (Fig.2a).

In our case however the λ_{max} of the α-cdx-Ep-MO complex is 463 nm which coincides with that of the MO itself. The same phenomenon was observed with other azo dyes and glyceryl ether fraction of the γ-cdx-Ep, in which the Job's plot from split CD spectra indicated obviously the formation of azo dyes: L- γ-cdx-Ep = 2:1 complex.

Table II. Absorption Maxima and Induced CD Values of Complexes between Azo Dyes and Glycerylether Fractions of Cyclodextrin Polymer Products

Azo Dyes



	R_1	R_2	R_3	Linking Agents: Epichlorohydrin λ_{max}	CD $_{max}$	$\Delta\varepsilon$	Ethyleneglycol bis (epoxypropyl) ether λ_{max}	CD $_{max}$	$\Delta\varepsilon$
MO*	$NaSO_3$	H	NMe_2	463	415 482	+ 5,6 - 4,0	460	410 472	- 2,1 + 6,3
I	$NaSO_3$	H	NH-⬡	444	413 480	+ 3,0 - 1,8	450	410 470	- 1,3 + 5,8
II	H	H	NMe_2 sat.**	426	400 467	(+3,8)*** (-2,8)	421	400 460	(-0,2) (+2,2)
III	H	COOH	NMe_2 sat.	429			429		
IV	H	H	H sat.	324, 430	325 440	(+14,0) (-6,5)****	323, 430	330 440	(+2,9) (+1,1)****

* MO= methyl orange
** sat.= saturated solution
*** ($\Delta\varepsilon$) = apparent $\Delta\varepsilon$
**** $n \rightarrow \pi^*$

H and L-α-cdx-Ep complexes show the same splitting spectral pattern and the amplitude of the latter is larger than that of the former.

Harada et al [4] reported that 4-dimethylaminoazobenzene forms much more stable inclusion complexes with β-cdx-Ep than with β-cdx and this is due to the co-operation in binding of adjacent two cdx units on a polymer chain. In the present case, the small amplitude of the H-α-cdx-Ep complex stops such complex formation. The more crowded surrounding around the entrance of the cavity of H-α-cdx-Ep as compared to the L-one may reduces the ability of complex formation.

Table II shows that such splitting does not depend on the electrostatic interaction, the determining factor is the length of azo dye molecule; presence of $-NMe_2$ and $-SO_3$ groups is not important. The length of the linking agent may determine the angle between the longitudinal axis of the interacting two MO molecules and the inverted signs observed at different kinds of polymers may be due to the difference of the chirality originating in the relative orientations of the dye molecules.

Lack of the splitting pattern in the $\pi \rightarrow \pi^*$ band of azo benzene and α-cdx-Ep (or DiEp) complexes may be that the bare portion of azo benzene is too short to interact with others.

Glycerylether fractions of β-and γ-cdx Ep polymer products show only single pattern in induced CD spectra of MO complexes, but L-γ-cdx-Ep shows the splitting CD pattern and an increase in the amplitudes with other azo dyes.

While the helical amylose forms rather stable complexes with some azo dyes, disordered amylose or branched amylopectin are not complex forming agents [14]. The observed CD spectra can be explained probably by assuming a more or less ordered array of the azo dye molecules complexed by the cdx-polymers.

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