Carbohydrate Research, 61 (1978) 41–52

ORDERED ARRANGEMENTS IN SOLUTIONS OF AMYLOSE–IODINE COMPLEXES DERIVED FROM FREE AND TERMINALLY FIXED AMYLOSE CHAINS*

BEATE PFANNEMÜLLER
Institute of Macromolecular Chemistry, 7800 Freiburg i.Br. (West Germany)
(Received June 27th, 1977; accepted for publication in revised form, September 2nd, 1977)

ABSTRACT

Linear, homodisperse amyloses and branched and cross-linked products are characterized by absorption and c.d. measurements of their iodine complexes in the range 400–780 nm. The long-wave Cotton effect splits into a broad, negative band and a positive band of smaller width. Dichroic absorption has maximum values at d.p. values of about 50 for iodine concentrations of 0.7, 1.0, and 1.4 I_3^- per six glucose residues. A rather stiff, helical rod is suggested for this chain length. Compared with free amyloses of equal length, amylose branches linked densely to glycogen give stronger Cotton effects, thus indicating a significantly ordered arrangement. Amylose segments in soluble, cross-linked gels, when compared with linear amyloses of equal length, show 60% of the iodine-binding capacity, but only 20–25% of the dichroic absorption. This effect is due to imperfect helix-formation. From experiments under various conditions (amylose concentration, rapid and slow addition of iodine), there is strong evidence that the time-dependent increase of c.d. results mainly from intramolecular orientation rather than from intermolecular association. The formation of larger aggregates may be ruled out, under the conditions applied.

INTRODUCTION

In previous papers[1, 2], we reported measurements of optical rotatory dispersion (o.r.d.) and circular dichroism (c.d.) for iodine complexes of enzymically synthesized amyloses having a Poisson distribution (P_w/P_n of 1.001–1.005). It was shown that the strong Cotton-effect observed in the range of the long-wave absorption maximum is most intense at degrees of polymerization (d.p.) of about 50, and decreases considerably for shorter and longer chains. It was also shown that o.r.d. and c.d. increase on aging of the solutions, whereas λ_{max} and ε_{max} of the absorption spectra remain unchanged[1–4]. Thus, the time-dependent, postordering process does not involve significant variation in the average length of the polyiodine chain.

As possible explanations, intramolecular (*1*) and intermolecular (*2*) arrange-

*Dedicated to Professor Dexter French on the occasion of his 60th birthday.

ments were considered: (*1a*) helical segments of different lengths formed initially could subsequently be converted into a thermodynamically more-stable, uniform length; (*1b*) polyiodine chains could first occupy preexisting helical segments, and unordered, coiled regions of the amylose molecule might then wind partially around the iodine chain, resulting in a more-densely packed and stiffer helix; (*2*) a further ordering may also result from some kind of intermolecular orientation through association, such as end-to-end association or a parallel aggregation of the amylose–iodine molecules.

Explanation (*2*) is suggested by electron micrographs obtained by Bittiger and Husemann[5–7] with iodine-complexed, synthetic amyloses. Depending on the concentration of the solutions, fibrils or rods of single molecules may be seen, both having diameters of about 4 nm. The lengths of the rods were directly dependent on the d.p. of the parent amyloses, suggesting that the helices may be folded parallel to the long axis.

In the meantime, some evidence has accumulated that supermolecular aggregation might play a role in the effects observed. Ultracentrifugation measurements made by Dintzis *et al.*[8] on dilute solutions of amylose–iodine complexes clearly showed an increase in sedimentation coefficients and apparent molecular weights with increasing concentration of potassium iodide and with time of aging. Cesàro and Brant[9] observed a decrease in viscosity of low-d.s. *O*-(carboxymethyl)- and *O*-(diethylaminoethyl)-amylose with increasing iodine saturation. They have tentatively attributed the fall in viscosity with the formation of aggregates. More recently, the presence of aggregates in solutions of amylose–iodine complexes was demonstrated by ultrafiltration experiments carried out by Nguyen *et al.*[10]. The aggregation model is further supported by studies of Salter *et al.*[11] on complexes of acridine orange with glycosaminoglycans. Dichroic absorption increased considerably with decreasing solubility of the dye complexes. When larger aggregates were removed by centrifugation, a fall in the c.d. was found.

Finally, the time-dependent increase in c.d. is discussed in connection with secondary iodine binding derived from potentiometric-titration studies. This effect, first observed by Bates, French, and Rundle[12] has been ascribed to surface adsorption. For amyloses of less than d.p. 50 (ref. 13) (d.p. 100) (ref. 14), the two processes of complex formation and surface adsorption cannot be distinguished clearly from the titration curves[13–16]. The uptake of iodine was found to exceed the limiting value of amylose (~ 20% by weight)[14]. Because of the different nature of iodine bound internally or attached to the surface, and the difference in lengths of the polyiodine chains, if any longer chains can be formed at the surface, the two chromophores will absorb light at different wavelengths. Thus, by such an effect, the c.d. spectrum can be changed, but a simple increase of the c.d. absorption as observed, without any change of shape and position of bands, is not likely to occur. By resonance-enhanced Raman spectra, Heyde *et al.*[17] found differences in the vibrational frequencies between amylose–iodine and polyvinyl alcohol–iodine complexes (λ_{max} ~ 595–620 nm), which are assigned to different boundary conditions at the ends of the conjugated, iodine

segments. There remains the question as to whether adsorbed charges, probably triiodide ions, may be involved in the ordering process in a way that a closer contact between chromophore and chiral binding center is formed.

In this paper we present some data from our current studies that may shed new light on the intricate problem of the behaviour of the amylose–iodine complexes: (*a*) a more detailed analysis of the long-wave Cotton effect by use of a new light source that permits the extension of c.d. measurements from 600 to 780 nm; (*b*) the influence of the iodine concentration on the amylose chain-length dependence of the c.d.; (*c*) experiments that might prove a relation between the formation of aggregates and increase in c.d.; and (*d*) characterization of iodine-complexed molecules containing amylose chains linked as branches or fixed more rigidly in a network structure.

The results obtained will be discussed under the aspects of intramolecular orientation or intermolecular aggregation.

RESULTS

Long-wave Cotton-effect and dependence on chain length. — Absorption and c.d. spectra of free and complexed iodine are presented in Fig. 1.

According to Robin[18], the excited states of an isolated I_3^- ion, which appear as two strong bands at 287 and 353 nm in the spectrum of the iodine–potassium iodide solution, are shifted and split by an exciton interaction when *n* I_3^- ions are aligned to form a linear chain. The main bands of the complexed polyiodine chain at 353 nm and at 582 nm (Fig. 1) are assigned to $\pi \rightarrow \sigma^*$ and $\pi^* \rightarrow \sigma^*$ transitions, respectively. A weak band is located at 476 nm. All three bands are optically active.

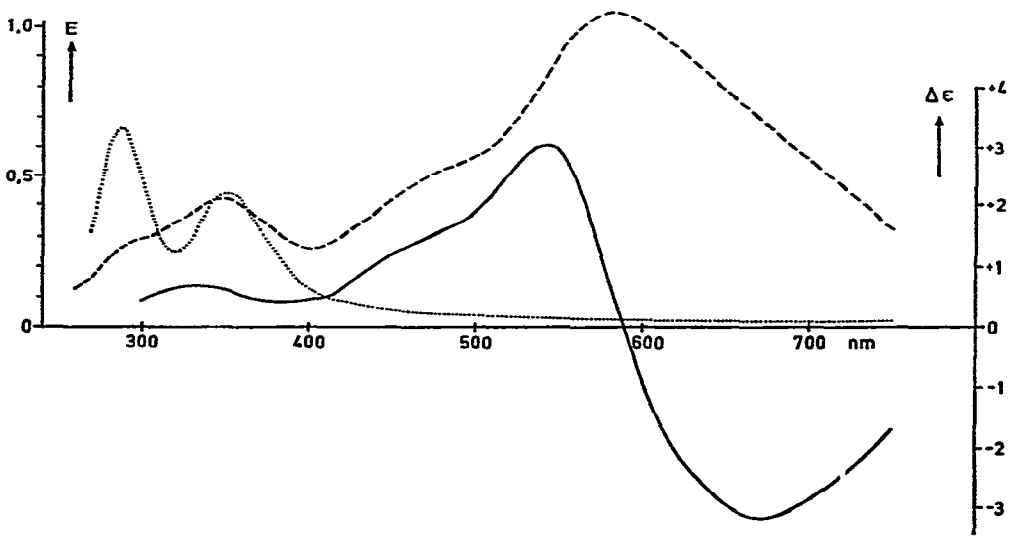

Fig. 1. Circular-dichroism (——), and absorption (– – – –) spectra of amylose–iodine complex, P_n 76; absorption of I_2–KI solution (.....).

As already suggested by the c.d. spectra[1,2] of low-molecular weight amyloses of d.p. 25 and 42, the dichroic absorption corresponding to the long-wave absorption maximum is split into two bands of opposite sign. The splitting of the c.d. caused by interaction of the chromophores with the asymmetric environment of the helical chain has been thoroughly investigated for dinucleotides[19-21]. The pair of opposite Cotton effects is centered at $\lambda_0 = 588$ nm, close to $\lambda_{max} = 582$ nm of the absorption spectrum. The two bands are not of equal shape, and this may arise from the shoulder near 480 nm.

To demonstrate the dependence on chain length, the c.d. spectra of a series of amyloses are shown in Fig. 2.

It may be seen that λ_{max} of the negative c.d. band and λ_0 are shifted continuously to higher wavelength with increasing molecular weight; however, the peak c.d. absorptions reach maximal values between d.p. 38 and 51 and decrease markedly at further increase of d.p.

Influence of iodine concentration on chain-length dependence. — At this point, the question arises as to whether the chain-length dependence observed in the c.d. spectra would be influenced by the amount of iodine added. Such dependence appeared to be conceivable, as it is known from titration experiments[2,15] that iodine binding of the short-chain amyloses occurs at higher equilibrium concentration of free iodine. To clarify this point, measurements on a series of amyloses having d.p. 25, 38, 47, 51, 76, and 92 were carried out at 1.4 M and at 0.7 M I_3^- per six glucose residues, respectively. The absorption and c.d. spectra were recorded at 10 min and 24 h after addition of iodine. The results are shown in Fig. 3 and Table I.

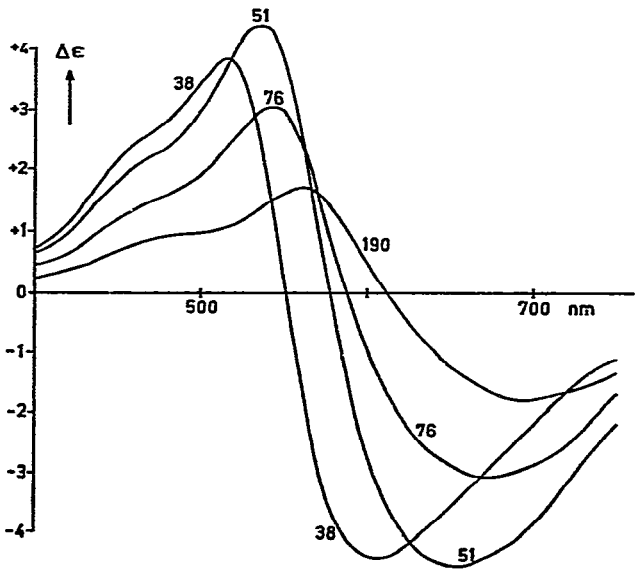

Fig. 2. Circular-dichroism spectra of amylose–iodine complexes, P_n 38, 51, 76, and 190 (amylose, *c*, 0.4 g/l; 1 I_3^- per 6 glucose residues).

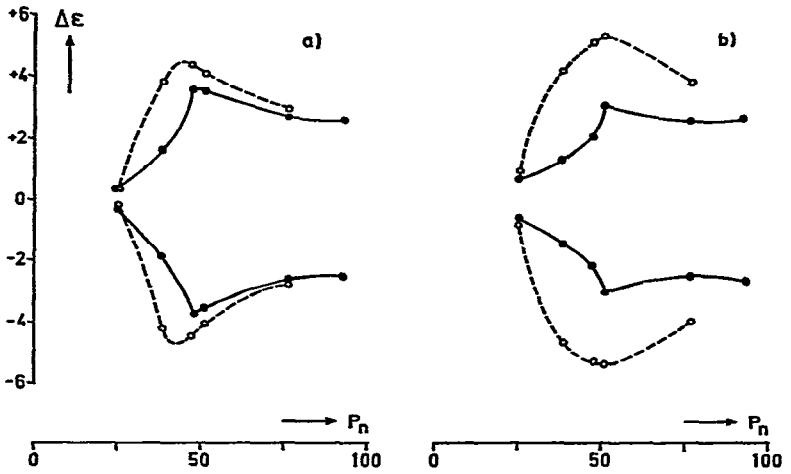

Fig. 3. Negative- and positive-peak c.d. absorptions ($\Delta\varepsilon$) and their dependence of amylose chain length (P_n), iodine concentration (●—●—●: 0.7 I_3^- per 6 glucose residues; ○—○—○: 1.4 I_3^- per 6 glucose residues), and time after addition of iodine (*a*, 10 min; *b*, 24 h).

TABLE I

ABSORPTION AND C.D. OF AMYLOSE–IODINE COMPLEXES GIVING HIGHEST COTTON EFFECTS

Amylose d.p.	*I_3^- per 6 glucose residues*	*Time of measurement*	*Absorption*		*Peak c.d. absorption*
			λ_{max} *(nm)*	ε_{max}	$\Delta\varepsilon_{max}$
47	0.7	10 min	566	3056	−3.74 +3.56
47	1.4	10 min	571	3680	−4.50 +4.30
51	0.7	24 h	568	2135	−3.07 +3.00
51	1.4	24 h	572	4165	−5.46 +5.27
			ε 1.4/ε 0.7		$\Delta\varepsilon$ 1.4/$\Delta\varepsilon$ 0.7
		10 min	1.21		1.20 (neg.) 1.21 (pos.)
		24 h	1.95		1.78 (neg.) 1.76 (pos.)

In Fig. 3a, the negative and positive peak c.d. absorptions ($\Delta\varepsilon$) are plotted against the d.p. for the freshly prepared solutions. For the two different concentrations of iodine, both curves pass through a maximum, but $\Delta\varepsilon_{max}$ is significantly larger for the higher I_3^- concentration. The increase in dichroic absorption is most pronounced at d.p. 38. This behaviour demonstrates that short chains can adopt a much better helical arrangement when sufficient iodine is present. No difference between the two iodine concentrations was observed for d.p. 25, a sample obviously having a somewhat

broader distribution of molecular weight. After 24 h (Fig. 3b), the curves for both iodine concentrations have moved apart. This separation results from an overall decrease of the c.d. at 0.7 I_3^- and a considerable increase of the c.d. at 1.4 I_3^-.

The amylose chain-length for which the maximum c.d. occurs in each curve is listed in Table I, together with the corresponding λ_{max} and ε_{max} of the absorption curve. The optimum d.p. is obviously the same for both iodine concentrations used, but it is found at d.p. 47 for the initial measurement and at d.p. 51 after 24 h. This slight shift with time may indicate a more-complete winding of d.p. 51 onto the iodine chain, as λ_{max} at 0.7 I_3^- and 1.4 I_3^-, respectively, remain unchanged after 24 h; the increase in $\Delta\varepsilon$ on aging may, on the other hand, be a sign of aggregation.

Influence of aggregation on c.d. — In order to obtain more information on the intricating question as to whether supermolecular orientation or aggregation are involved in the phenomena observed, a series of experiments was carried out. First of all, aged solutions of amylose–iodine complexes (0.4 g/l of amylose, 1 I_3^-/6 glucose residues) were centrifuged at 27,000 r.p.m. for 1 h. According to Salter *et al.*[11], and as indicated by the studies of Dintzis *et al.*[8], aggregated particles having apparent molecular weights in the order of 10^6 should undergo sedimentation giving rise to a decrease in $\Delta\varepsilon$ for the supernatant solution. However, after centrifugation in solutions removed from the upper layer of the supernatant, no decrease was observed in the dichroic absorption for iodine complexes of amyloses between d.p. 38 and 190. No solid material could be detected at the bottom of the tubes. Thus, precipitation of larger aggregates evidently did not take place. Secondly, upon adding 1 I_3^- per 6 glucose residues dropwise with stirring to an amylose solution (0.4 g/l) dichroic absorptions of especially high magnitude were found. Again, the effect was most pronounced with amylose of d.p. 51. By this technique, the c.d. values were found to be even higher, by a factor of 1.3–1.7, than found after rapid addition of iodine and keeping the solutions for 24 h. Thirdly, measurements were carried out in fivefold-diluted solutions (0.07 g/l amylose; 0.02 g/l iodine and potassium iodide; 1 I_3^- per 6 glucose residues) because, at lower concentrations, the tendency of the

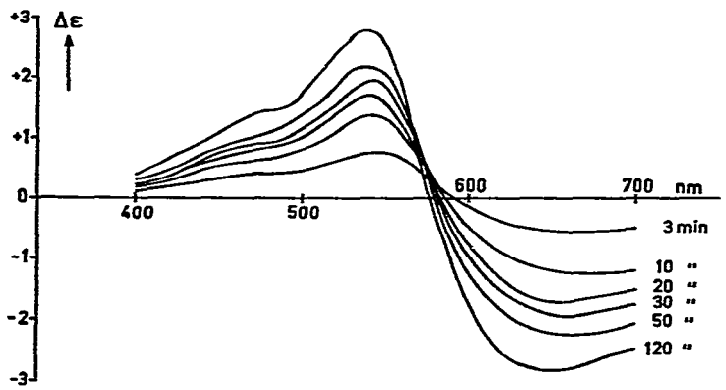

Fig. 4. Time-dependent increase of c.d. of amylose–iodine complex, P_n 47 (amylose, *c* 0.07 g/l; 1 I_3^- per 6 glucose residues).

complex to form aggregates should be decreased. Dichroic absorptions were found to be low in the freshly prepared solutions of amyloses having d.p. 38–190, but they increased considerably with time. After 24 h, the peak absorptions were equal to (d.p. 38 and 51) or even higher (d.p. 76 and 190) than those in the more-concentrated solutions. The variation of the c.d. curves during the first 2 h are presented in Fig. 4 for an amylose of d.p. 47.

Apart from the general increase of the intensity, the changes in the c.d. spectra are mainly found in the long-wave region. The values of λ_{max} and λ_0 shift continually with time to shorter wavelength. No differences could be observed in the corresponding absorption spectra during the 2-h period.

Another point of interest was the different rate of development of the negative and positive dichroic bands, respectively, in a dilute solution having d.p. 38. A similar effect was found in the c.d. spectra of high-molecular-weight amyloses of d.p. 500–5000. The positive band is seen from the very beginning after addition of iodine, whereas the negative band appears only later; simultaneously, the maximum peak of the negative band migrates to shorter wavelength and into the region of instrumental observability, (namely, $\lambda < 780$ nm). Because of the strong absorption of the high-molecular-weight samples, the spectra display noise. A detailed analysis of the spectral changes will be given elsewhere.

Circular dichroism of branched and cross-linked products. — The finding that c.d. measurements may be made for iodine complexes of high molecular-weight amyloses encouraged us to investigate several types of branched products. Table II shows the results obtained with two samples of comb-like molecules. Maltopentaose, previously linked to an amylose backbone by hydrazone bonds[22], had subsequently been elongated by phosphorolytic synthesis. The length of the branches was controlled by the concentration of primer used in the synthesis, namely, the molar ratio of maltopentaose to D-glucosyl phosphate.

A well defined c.d. curve, displaying sharp negative and positive bands, is found for the sample CMA-32. The position of λ_0 is close to λ_{max} of the absorption curve. By comparison with linear amyloses, a branch length of about d.p. 300 may be estimated. As seen from λ_{max} of the absorption, longer branches are present in

TABLE II

ABSORPTION AND C.D. MEASUREMENTS ON IODINE COMPLEXES OF COMB-LIKE BRANCHED AMYLOSES[a]

Product	*Absorption*		*Dichroic absorption*				
	λ_{max} *(nm)*	ε_{max}	λ_{max} *(nm)*	$\Delta\varepsilon_{max}$	λ_0 *(nm)*	λ_{max} *(nm)*	$\Delta\varepsilon_{max}$
CMA-16.3	632	6236	730	−0.110	654	570	+0.660
CMA-32.0	620	5737	725	−0.632	630	560	+0.824

[a]Amylose, *c* 0.4 g/l; $1I_3^-$ per 6 glucose residues.

TABLE III

ABSORPTION AND C.D. MEASUREMENTS ON IODINE COMPLEXES OF GLYCOGEN-AMYLOSE STAR MOLECULES[a]

Product	*Absorption*		*Dichroic absorption*				
	λ_{max} *(nm)*	ε_{max}	λ_{max} *(nm)*	$\Delta\varepsilon_{max}$	λ_0 *(nm)*	λ_{max} *(nm)*	$\Delta\varepsilon_{max}$
GM-6	632	4558	720	−0.151	655	567	+0.508
GM-2	625	4988	740	−0.275	643	560	+0.646
GM-24	618	5124	735	−0.728	634	562	+1.003
GM-48	612	4376	720	−1.333	620	560	+1.374
GM-80	607	4330	720	−2.006	610	558	+1.828
GM-120	600	3855	706	−2.748	601	550	+2.350
GM-160	595	3400	695	−3.010	592	546	+2.570
GM-200	587	3445	677	−3.834	585	542	+3.160
Amylose d.p. 76	585	4435	680	−2.110	592	543	+2.125

[a]See footnote, Table II.

TABLE IV

ABSORPTION AND C.D. MEASUREMENTS ON IODINE COMPLEXES OF CROSS-LINKED AMYLOSES[a]

Product	*Absorption*		*Dichroic absorption*				
	λ_{max} *(nm)*	ε_{max}	λ_{max} *(nm)*	$\Delta\varepsilon_{max}$	λ_0 *(nm)*	λ_{max} *(nm)*	$\Delta\varepsilon_{max}$
AC-60	578	2980	655	−0.980	575	528	+0.975
Amylose d.p. 51	577	4775	658	−3.754	582	540	+3.767
AC-90	568	2777	648	−0.714	576	528	+0.764
Amylose d.p. 47	570	3680	640	−3.800	568	535	+3.560

[a]See footnote, Table II.

CMA-16. This is also indicated by a typical asymmetric c.d. spectrum: a low dichroic-absorption of the negative band and a rather high peak value of the positive band are observed. This effect, as yet unexplained, is most probably connected with the longer time-period needed for long chains to pass from a coiled to a more-helical state.

Measurements on a series of star molecules, prepared by elongation of the short outer branches of glycogen with muscle phosphorylase[23], are listed in Table III.

With increasing concentration of primer (6–200 mg of glycogen per 2 g D-glucosyl phosphate), and consequent decreasing length of the amylose branches, the λ_{max} of the absorption curve is shifted to shorter wavelength. Characteristic changes in the peak dichroic-absorption are found. As compared with the positive band, the negative band again gives lower dichroic absorptions for longer branches and higher

ones for shorter branches. With decreasing branch length, the λ_0 values of the c.d. curves are shifted closer to the absorption maximum (λ_{max}). It is interesting to compare the sample GM-200 with a linear amylose of equal λ_{max}, even though the molecular-weight distribution of the newly synthesized branches in GM-200 may be assumed to be broader[23]. As seen from ε_{max} in Table III, the iodine-binding capacity of GM-200 is lower than that of free amylose of d.p. 76, because the molecules contain a 25% glycogen nucleus that is not stained by iodine. Nevertheless, the $\Delta\varepsilon$ values are markedly higher than those of the free amylose. Similar results are obtained for GM-120 and GM-160. Aside from any influence of differences in molecular-size distribution, it appears reasonable to state that, in these densely branched molecules, amylose chains complexed with iodine are especially well oriented. This may result from the branches being fixed at one end. It may also indicate a strong interaction between neighbouring branches. This would favour the idea that increased c.d. values might result to some extent from intermolecular association. In aqueous solution, the uncomplexed star molecules have a tenfold higher rate of retrogradation than the free amyloses of equal chain-length.

Studies with soluble, cross-linked amyloses are shown in Table IV. The substances were prepared by treatment of amylose with epichlorohydrin in aqueous, M sodium hydroxide[24]. The reaction was stopped at intervals before gelation occurred.

The two samples, AC-60 and AC-90, are compared with synthetic amyloses having about the same λ_{max}, irrespective of a fairly broad distribution of molecular weight of the amylose segments in the cross-linked products. Whereas the extinction coefficients for both cross-linked samples are 60% of the corresponding linear amyloses, the c.d. absorptions are only 25% (AC-60) and 20% (AC-90) of those of the free amyloses. Thus, iodine binding is somewhat decreased, and c.d. indicates a much less-ordered helix.

The presence of dangling amylose branches, which would give rise to a certain iodine binding, could be ruled out as no detectable degradation by beta amylase was found. It is remarkable, therefore, that amylose segments enclosed between cross-links are flexible enough to adopt a helical conformation. A colouring with iodine was also observed with solid gels of amylose[25]. Depending upon the degree of cross-linking, the colour changed from blue-violet to orange.

DISCUSSION

The results of the new experiments may be summarized as follows:

1. By extension of the region of measurements to longer wavelengths (750–780 nm), the c.d. spectra are recorded now almost completely, even for amyloses of high molecular-weight. A splitting of the long-wave Cotton effect into a broader negative band and a positive band of smaller width, as well as typical changes in the development and the magnitude of both bands, is quite clearly noticeable. The spectra are well reproducible and of low noise, unless samples exhibit low c.d. and very high

absorption. The good quality of the spectra affords more-detailed information on the properties of the iodine complexes than known before.

2. The experiments at iodine concentrations of 0.7 I_3^- and at 1.4 I_3^- per six glucose residues on a series of amyloses of d.p. 25–97 confirm the results of earlier measurements at 1 I_3^- per 6 glucose residues. The iodine complexes of amyloses in the vicinity of d.p. 50 give the strongest Cotton effects. With the exception of d.p. 25, the $\Delta\varepsilon$ values increase when iodine concentration is changed from 0.7 to 1.4 I_3^-. The increase is most pronounced at d.p. 38 and becomes gradually smaller with growing d.p. The concomitant increase in the extinction coefficients is a sign that a higher percentage of sugar residues are interacting with iodine.

A time-dependent increase in c.d. is found only at higher iodine concentrations (1 I_3^- and 1.4 I_3^- per 6 glucose residues), but not at 0.7 I_3^- per 6 glucose residues*. Simultaneously, the extinction coefficients and λ_{max} of absorption remain almost unchanged.

The simplest interpretation of this behaviour would be the formation of helices with time. However, as ε was found not to increase within the same period of time, new helices are evidently not formed. All effects may be explained by assuming a growing ordering of the helical loops to a more-perfect helix of higher rigidity. It is well known from theoretical considerations by Brahms[19] and by Tinoco and Cantor[20] that c.d. is increased by colinear orientation of the transition moments, which are supposed to be mainly in the direction of the iodine chain[18]. A gradual change from a flexible iodine-chain to a rigid one, induced by intramolecular interaction between the helix turns, would fit the observations. A similar increase in c.d. could, on the other hand, be caused by head-to-tail association of helically wound amylose–iodine molecules. This type of association was recently proved to occur for some polypeptides[26,27]. A parallel aggregation may certainly be ruled out, because in such arrangements the transition moments would be aligned parallel to each other, and this would cause a decrease in c.d. The same arguments apply for a folding of the amylose–iodine chains.

3. No decrease in c.d. was found in the supernatant solutions after high-speed centrifugation (amylose, $c = 0.4$ g/l). The formation of larger aggregates cannot, therefore, be responsible for increased c.d. values. Especially strong Cotton effects were measured when iodine was added slowly with stirring. Presumably, amylose molecules are more regularly filled and oriented to helices when iodine is added slowly. The formation of smaller, soluble aggregates, possibly promoted by the shear stress of stirring, cannot be excluded. However, because of the increase in c.d., these aggregates must be oriented head-to-tail as outlined before. In fivefold diluted solutions (amylose, $c = 0.07$ g/l), where aggregation is less probable, the increase in c.d. on keeping the solutions is even more pronounced. This again is in favour of intramolecular ordering-

*At the lower concentration of iodine c.d. values decrease with time for d.p. 38–51. This decrease may readily be explained by the concomitant decrease in ε_{max} and a shift of λ_{max} to shorter wavelengths, indicating a loss of iodine bound and decreased length of the polyiodine chain.

processes and supports the interpretation of a slow rearrangement of the initially imperfect and flexible helices.

4. The dependence of the magnitude of the Cotton effects on d.p. was confirmed for amylose chains linked as branches or fixed more rigidly in a network. Amylose chains linked densely as branches to the spherical glycogen seem to be especially well oriented as compared with free amyloses. The smaller Cotton-effects obtained for amylose segments enclosed between cross-links may be due to the constraint that the chains are fixed at both ends, which obstructs formation of a perfect helix.

The results are not necessarily in disagreement with the findings of Dintzis *et al.*[8], who determined, in dilute solutions of amylose–iodine complexes (amylose, $c = 0.03$ g/l), apparent molecular-weights corresponding to clusters of 4–11 monomers. It should be emphasized that the aggregates were found at a much higher concentration of potassium iodide (3.6×10^{-3} M) than used in the present experiments (0.63×10^{-3} M; amylose, $c = 0.4$ g/l). It seems conceivable that the formation of aggregates is promoted by high concentration of salts, as known for other polyelectrolytes.

EXPERIMENTAL

Stock solutions of iodine contained 0.5225 g of iodine and 0.5225 g of potassium iodide in 1000 ml of redistilled water.

Amyloses were synthesized from D-glucosyl phosphate with potato phosphorylase[28,29]. The amylose sample of d.p. 25 was obtained from Nakarai Chemicals Ltd. The preparation of the branched and cross-linked amyloses is described elsewhere[22–24]. Solutions of amylose–iodine complexes were generally prepared by dissolving 5.0 mg of amylose in 0.5 ml of dimethyl sulphoxide. To the diluted solutions, 2.6 ml of I_2–KI solution was then added, and the final volume was brought to 12.5 ml. The solutions contained: amylose, 0.4 g/l (2.47×10^{-3} M glucose residues); I_2, 0.1045 g/l (0.412×10^{-3} M), and potassium iodide, 0.1045 g/l (0.63×10^{-3} M). The ratio of iodine to glucose residues was 1 I_2 per 6 glucose residues. The concentration of iodine was varied by adding 1.82 ml and 3.64 ml of I_2–KI solution, which corresponds to a ratio of 0.7 I_2 per 6 glucose residues and 1.4 I_2 per 6 glucose residues, respectively. For measurements in more-dilute solution, the final volume was brought to 70 ml. The solutions contained: amylose, 0.07 g/l (0.441×10^{-3} M glucose residues); I_2, 0.0185 g/l (0.0735×10^{-3} M); and potassium iodide, 0,0185 g/l, (0.1125×10^{-3} M).

Centrifugation of aged solutions was carried our in a Spinco Model L apparatus for 60 min at 27,000 r.p.m. and 20°.

Measurements of absorption and c.d. were performed simultaneously and at the times indicated. The solutions were kept at 20°.

Absorption spectra were recorded with a Perkin–Elmer spectrophotometer, Hitachi 200, by using 0.1-cm, fused-silica cuvets. C.d. spectra were determined in a Cary 61 c.d. spectropolarimeter with fused-silica cuvets having path-lengths of 0.2, 0.5, and 1 cm.

ACKNOWLEDGMENTS

This work was supported financially by the Deutsche Forschungsgemeinschaft. I thank Professor W. Burchard for his interest and advice. I am also grateful to G. Ziegast and C. Hölzner for their assistance in recording the spectra.

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