Carbohydrate Research, 179 (1988) 327–340
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

THE IODINE-STAINING PROPERTIES AND FINE STRUCTURE OF SOME MAMMALIAN AND INVERTEBRATE GLYCOGENS*†

STUART A. S. CRAIG, ALISON M. L. MCDONALD, DAVID J. MANNERS, AND J. ROGER STARK
Department of Brewing and Biological Sciences, Heriot-Watt University, Edinburgh EH1 1HX (Great Britain)

(Received October 24th 1987; accepted for publication, December 31st, 1987)

ABSTRACT

Glycogens from mammalian and invertebrate sources have been compared by measuring the iodine-staining spectra of the debranched polymers and the debranched beta-amylase limit dextrins. From the results, it is concluded that, whereas the interior chains of each group of glycogen are very similar, the exterior chains of the mammalian glycogens generally contain a small number of longer chains which are not found in the invertebrate glycogens.

INTRODUCTION

Although iodine staining has been widely used in studies of starch[2], its use as an analytical tool for the examination of glycogens has been less frequent. This situation may be due partly to the fact that the interaction of glycogen with iodine is weaker than with starch. However, Archibald *et al.*[3] carried out iodine-staining experiments in the presence of half-saturated ammonium sulphate which facilitated the interaction between the iodine and the (1→4)-α-D-glucan. By examining glycogens from a wide variety of biological sources, these authors derived a relationship between λ_{max} of the glycogen–iodine complex and its average chain-length (c.l.). In order to investigate further the interaction of iodine and α-D-glucan chains in the presence and absence of ammonium sulphate, Manners and Stark[4] examined the iodine-staining spectra of linear oligosaccharides of d.p. 6–22. In the presence of half-saturated ammonium sulphate, within the region d.p. 6–14, there was an approximately linear relationship with λ_{max}, whereas at higher d.p. values, the λ_{max} increased more rapidly. In the absence of ammonium sulphate, a non-linear increase was observed. More recent studies by John[5] have extended the range of oligosaccharides up to d.p. 70 although ammonium sulphate was not used. Further studies[1] of the iodine-staining characteristics of glycogens in the presence of half-saturated ammonium sulphate at different concentrations of iodine revealed

*Dedicated to Professor Bengt Lindberg.
†(1→4)-α-D-Glucans, Part XXVI. For Part XXV, see ref. 1.

0008-6215/88/$ 03.50

significant differences in the spectra of mammalian and non-mammalian glycogens. Reduction of the iodine concentration resulted in an *increase* in λ_{max} values with mammalian glycogens, but a *decrease* with non-mammalian glycogens. These results led to the conclusion that mammalian glycogens may contain some relatively long exterior chains which are not present in the other glycogens.

We have now examined the unit-chain profiles of mammalian and invertebrate glycogens and their beta-amylase limit dextrins, using a combination of gel-filtration chromatography and iodine-staining techniques.

EXPERIMENTAL

Isoamylase was obtained from Hayashibara Biochemical Laboratories, beta-amylase from Clodor (Manchester), and pullulanase from Boehringer (London). The glycogens used have been described[6–8]. A typical glycogen with an average chain-length of 12 glucose residues and a beta-amylolysis limit of 45% has average exterior and interior chain-lengths of 7–8 and 3–4 glucose residues, respectively.

General procedures. — Total carbohydrate was determined by the phenol–sulphuric acid method[9] and reducing sugars by a modification of the Nelson–Somogyi[10] technique.

Iodine-staining procedures. — The iodine-staining spectra of the glucans were examined using a Shimadzu UV240 spectrophotometer. The ratio of iodine to potassium iodide was 1:10. Test solution contained (1→4)-α-D-glucan, or the beta-amylase limit dextrin, or the oligosaccharides obtained by the debranching of these polymers (0.01%), and iodine concentrations ranging from 0.002% to 0.04% in the presence and absence of half-saturated ammonium sulphate[3]. Control experiments indicated that concentrations of up to ten times the standard quantities gave a linear relationship between the intensity of iodine colour and amount of polymer added with no difference occurring in λ_{max}. Examination of the iodine spectra of the internal chains of branched glucans was carried out using beta-amylase limit dextrins, in which the two or three glucose residues remaining on the external chains will not contribute significantly to the iodine stain.

The contribution of the external chains to the iodine spectrum was assessed by using equimolar solutions of iodine-stained internal chains, *i.e.*, from the beta-amylase limit dextrin as "blank". Digests contained glycogen (20 mg), 0.1M acetate buffer (pH 4.5, 1.0 mL), isoamylase (118 U), and pullulanase (10 U) in a total volume of 20 mL. After incubation for 24 h, each digest was heated for 5 min at 100° and then centrifuged. Portions (0.05 mL) of the supernatant solution were analysed for total carbohydrate[9]. Using the beta-amylolysis limit values, equimolar amounts of debranched glycogen and debranched beta-amylase limit dextrin were used in the iodine-staining assays. Thus, by using the iodine-stained debranched beta-amylase limit dextrin as the control and the iodine-stained debranched glycogen as the test, a scan of the exterior chains was recorded. Assays were performed on aqueous solutions and in the presence of half-saturated ammonium sulphate.

Debranching of α-D-glucans for iodine-staining experiments was carried out by incubating glycogen (20 mg), isoamylase (118 U), and 0.01M acetate buffer (pH 3.8, 1.0 mL) in a total volume of 20 mL for 24 h at 37°. Digests were heated for 2 min at 98° and then centrifuged. In order to confirm that total debranching of the glycogen had occurred, a mixture consisting of an aliquot of the digest containing 2.5 mg of glycogen, 0.01M acetate buffer (pH 4.8, 0.5 mL), and beta-amylase (1 mg) in a total volume of 10 mL was incubated for 24 h at 37°. Samples were analysed for total carbohydrate[9] and maltose[10]. The conversion into maltose was always 100%, indicating that complete debranching had taken place.

Distribution of unit chains and iodine-staining properties of debranched glycogens after fractionation. — Digests containing glycogen (10 mg), isoamylase (59 U), and 0.01M acetate buffer (pH 3.8, 0.5 mL) in a total volume of 10 mL were incubated for 24 h at 37°. Each reaction was stopped by heating the digest for 2 min at 98°, centrifuging to remove any denatured protein, and determining the content of total carbohydrate[9]. A volume of solution containing exactly 5 mg of glycogen was applied to a column (2.2 × 87 cm) of Sephadex G50 and eluted with degassed distilled water. Aliquots (1 mL) of fractions (6 mL) were analysed for total carbohydrate[9]. To other aliquots (2.5 mL) were added M hydrochloric acid (0.03 mL) and 0.1 mL of an aqueous solution containing iodine (0.4%) and potassium iodide (4%). Absorbances at 450 and 500 nm were determined. For calibration purposes, rabbit muscle glycogen (100 mg) was debranched and fractionated as described above, and the fractions were analysed for total carbohydrate[9] and reducing sugars[10] in order to determine the d.p. of each fraction.

Beta-amylolysis procedure. — The beta-amylolysis limits of glycogens were determined by incubating a solution containing glycogen (2.5 mg), 0.1M acetate buffer (pH 4.8, 0.5 mL), and beta-amylase (1 mg, 100 U) in a total volume of 10 mL for 24 h at 37° under toluene. Samples (0.5 mL) were then analysed for maltose[10] and for total carbohydrate[9]. Several glycogens were tested this way and other beta-amylolysis limits were obtained from previous work[6,8].

Only typical iodine-staining spectra are shown in the Figures. Where appropriate, tables of values are included to indicate the variations in λ_{max} and E_{max} for glycogens from different sources.

RESULTS AND DISCUSSION

Debranching and gel filtration. — Glycogens were debranched and subjected to gel chromatography on Sephadex G50, and the fractions were analysed for total carbohydrate and iodine staining. The results are recorded in Table I, and typical distribution curves for a mammalian and an invertebrate glycogen are shown in Fig. 1.

The profiles produced by determining the total carbohydrate have the same general shape for both the debranched mammalian and invertebrate glycogens. However, the trend is for the maximum carbohydrate response, as determined by

TABLE I

CHROMATOGRAPHY OF DEBRANCHED GLYCOGENS ON SEPHADEX G50

Glycogen	*Maximum values*			
	Total carbohydrate		*Iodine staining*	
	$A_{490\,nm}$	*C.l.*[a]	$A_{450\,nm}$	$A_{500\,nm}$
Pig muscle	0.570	15	0.680	0.780
Rabbit muscle	0.510	15	0.555	0.620
Rabbit liver	0.622	16	0.935	1.070
Human liver	0.650	10	0.718	0.750
Mytilis edulis	0.730	10	0.700	0.670
Ascaris lumbricoides	0.750	10	0.750	0.710
Arenicola marina	0.720	11	0.540	0.445
Cardium tuberculatum	0.470	12	0.415	0.390

[a]Average chain length.

the phenol–sulphuric method[9], to be located at slightly higher values of chain length for the mammalian samples (Table I). For rabbit-muscle and *Cardium tuberculatum* glycogens, the corresponding chain lengths are 15 and 12, respectively (Fig. 1). These differences were accentuated in the iodine-staining spectra, and the use of two particular wavelengths, which gave an indication of shorter (450 nm) and longer chain-lengths (500 nm), provided a useful means of expressing these differences. The data in Table I show that the iodine-staining absorbance at 500 nm is greater than that at 450 nm in the mammalian glycogens, but the reverse is true with the invertebrate glycogens. This finding suggests a fundamental difference between the distributions of the chains in the two categories of glycogens.

Iodine staining of debranched glycogens. — Glycogens were debranched and the iodine-staining spectra of the unit chains were examined under different conditions. Fig. 2 shows the effect of changing the iodine concentration on the iodine-staining spectrum of the unit chains of a typical mammalian (Fig. 2a) and invertebrate (Fig. 2b) glycogen in aqueous solutions. The results for other parallel experiments (Table II) indicated that there was no significant difference between the curves and data for debranched mammalian and invertebrate glycogens. The values of λ_{max} were similar for all the debranched glycogens, although the values of E_{max} tended to be slightly lower for debranched invertebrate glycogens; all the values of E_{max} increased with increasing concentration of iodine.

However, in the presence of half-saturated ammonium sulphate, considerable differences emerged between the two groups of glycogens. The unit chains of both sets of glycogens produced iodine spectra which showed two components (A and B in Fig. 3). The higher-wavelength component A had values of λ_{max} in the region 570–590 nm, whereas the values for component B were in the region 430–465 nm. The exact values for the debranched mammalian and debranched invertebrate

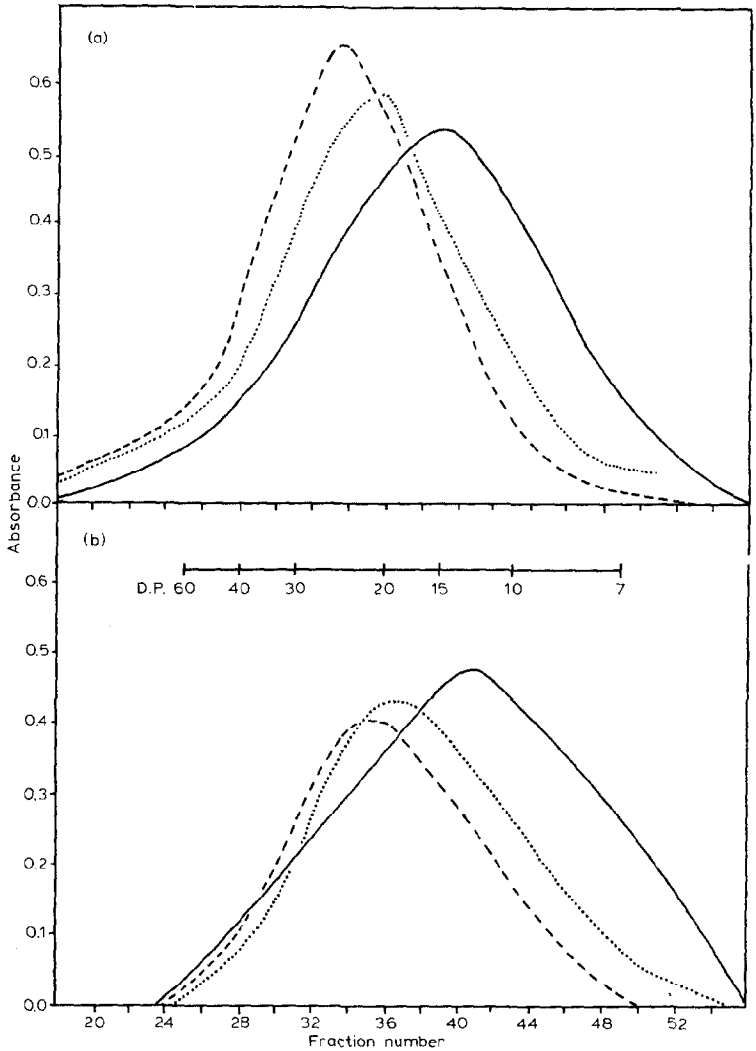

Fig. 1. Molecular weight distribution curves for debranched glycogens, (a) mammalian (rabbit muscle) and (b) invertebrate *(Cardium tuberculatum)*, by gel chromatography on Sephadex G50. Total carbohydrate (——), absorbance of iodine complex at 450 nm (·····), and absorbance of iodine complex at 500 nm (– – –).

glycogens are shown in Table III. These values of λ_{max} offer little distinction between the two groups of glycogens. However, when the values of E_{max} are compared (Figs. 3–5), it can be seen that, for the debranched mammalian glycogens, the E_{max} for component A reaches much higher values than those for component B at equivalent concentrations of iodine. With debranched invertebrate glycogens, the E_{max} of component B remains stronger than that of component A. The λ_{max} of

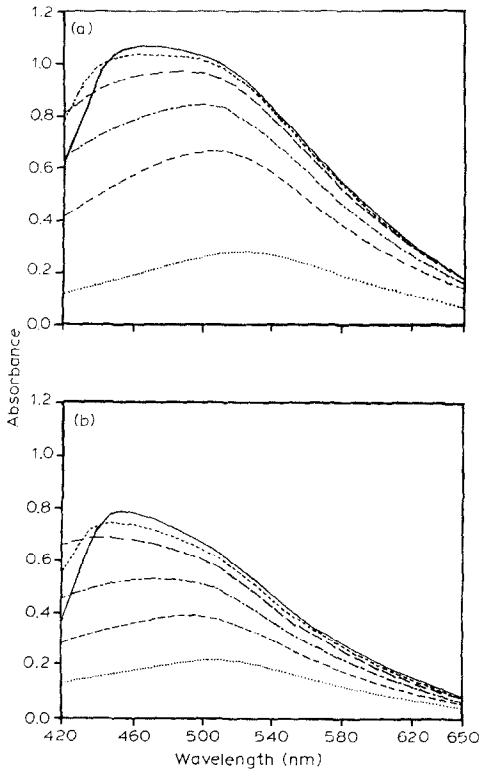

Fig. 2. Iodine-staining absorption spectra of debranched glycogens in aqueous solution: (a) mammalian (cat liver) and (b) invertebrate *(Ascaris lumbricoides)*. Iodine concentrations: 0.4% (——), 0.3% (-----), 0.2% (— — —), 0.1% (—·—·—), 0.05% (– – –), and 0.02% (·····).

TABLE II

ABSORPTION SPECTRAL DATA[a] OF DEBRANCHED GLYCOGENS AT DIFFERENT IODINE CONCENTRATIONS (AQUEOUS SOLUTIONS)

Glycogen	*Concentration of iodine added (%)*											
	0.4		*0.3*		*0.2*		*0.1*		*0.05*		*0.02*	
	λ_{max}	E_{max}	λ_{max}	E_{max}	λ_{max}	E_{max}	λ_{max}	E_{max}	λ_{max}	E_{max}	λ_{max}	E_{max}
Cat liver	460	1.07	464	1.04	480	0.97	494	0.85	503	0.67	520	0.28
Human kidney	460	0.91	457	0.88	468	0.80	495	0.69	505	0.57	520	0.33
Rat liver	475	1.02	470	0.96	478	0.90	492	0.80	502	0.65	516	0.38
Rabbit liver	480	1.10	488	1.08	495	1.02	502	0.91	507	0.75	523	0.34
Helix pomatia	456	0.70	448	0.68	454	0.62	475	0.50	490	0.37	510	0.17
Ascaris lumbricoides	456	0.78	450	0.74	462	0.69	467	0.53	488	0.39	506	0.12
Cod liver	465	0.87	460	0.85	485	0.77	495	0.67	505	0.55	520	0.30
Cardium tuberculatum	458	0.95	455	0.88	465	0.80	485	0.60	500	0.52	518	0.28

[a] λ_{max} (nm) = the wavelength of maximum absorbance; E_{max} = the absorption value at λ_{max} under standard conditions.

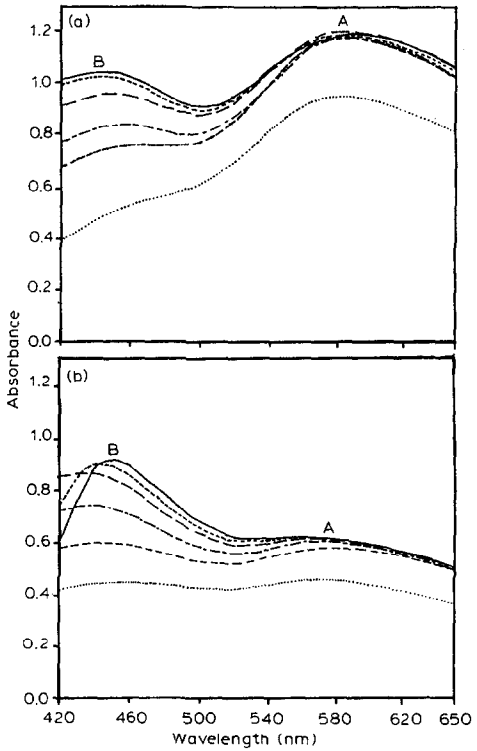

Fig. 3. Iodine-staining absorption spectra of debranched glycogens in half-saturated ammonium sulphate (a) mammalian (cat liver) and (b) invertebrate *(Ascaris lumbricoides)*. Iodine concentrations as in Fig. 2. A and B represent the component peaks at ~580 and ~460 nm, respectively.

TABLE III

WAVELENGTHS (NM) OF MAXIMUM ABSORPTION OF DEBRANCHED GLYCOGENS AT DIFFERENT IODINE CONCENTRATIONS (IN HALF-SATURATED AMMONIUM SULPHATE)

Glycogen	*Concentration of iodine added (%)*											
	0.4	*0.3*	*0.2*	*0.1*	*0.005*	*0.02*	*0.4*	*0.3*	*0.2*	*0.1*	*0.005*	*0.02*
	Peak B						*Peak A*					
Cat liver	445	448	450	458	465		585	587	589	590	590	584
Human kidney	440	445	438	449	462		585	587	588	588	588	582
Rat liver	445	443	450	455	465		585	590	590	590	587	583
Rabbit liver	445	460	460	463	482		590	590	590	588	588	581
Helix pomatia	450	442	430	438	452	465	565	570	572	575	580	575
Ascaris lumbricoides	450	444	435	434	447	459	567	569	572	578	583	572
Cod liver	450	448	442	451	460		575	577	579	583	581	575
Cardium tuberculatum	450	442	440	439	452	472	577	580	584	587	588	582

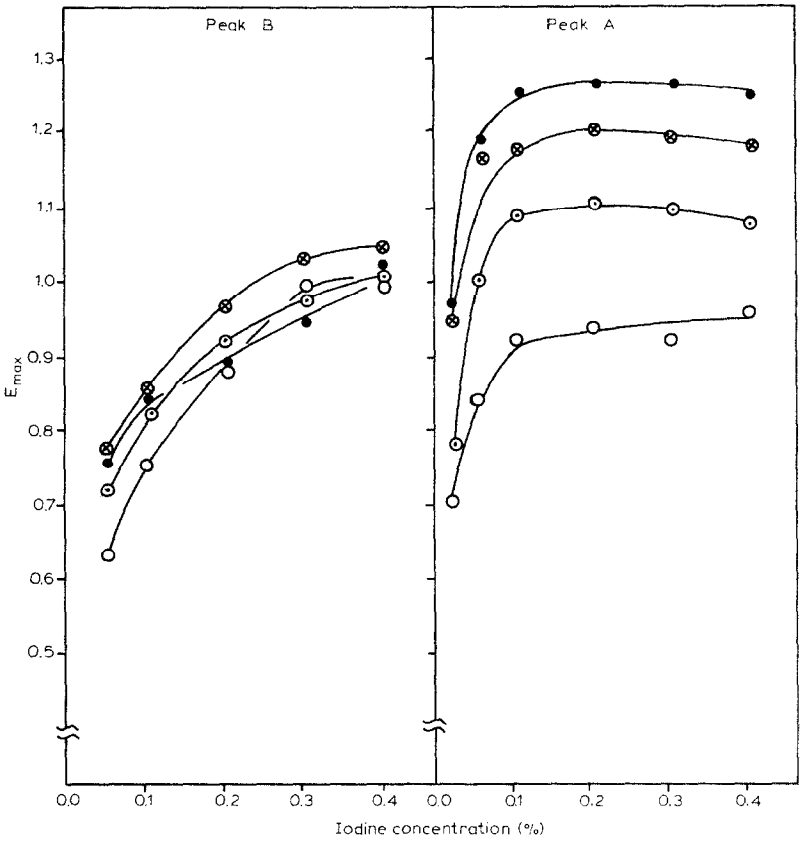

Fig. 4. The effect of iodine concentration on the E_{max} values of the component peaks A and B, for debranched *mammalian* glycogens in the presence of half-saturated ammonium sulphate: cat liver (⊗——⊗), human kidney (○——○), rat liver (⊙——⊙), and rabbit liver (●——●).

component B increases gradually over the range of iodine concentrations used, whereas that of component A increases rapidly as the iodine concentration is increased up to 0.1% but thereafter the values level out (Figs. 4 and 5). This finding clearly indicates some fundamental difference in the mode of iodine binding between components A and B.

Iodine-staining properties of debranched glycogen beta-amylase limit dextrins. — In order to investigate further the two-component system produced on iodine staining of debranched glycogens, an assessment of the contributions from the exterior and interior portions of the glycogen molecule was made. As a good approximation, the iodine-staining spectrum of beta-amylase limit dextrins of glycogens will give the contribution of the interior chains of the glycogen molecules. Therefore, similar spectra were obtained for debranched mammalian and invertebrate glycogen beta-amylase limit dextrins in aqueous solution and in the presence of half-saturated ammonium sulphate. Typical curves are shown in Fig. 6.

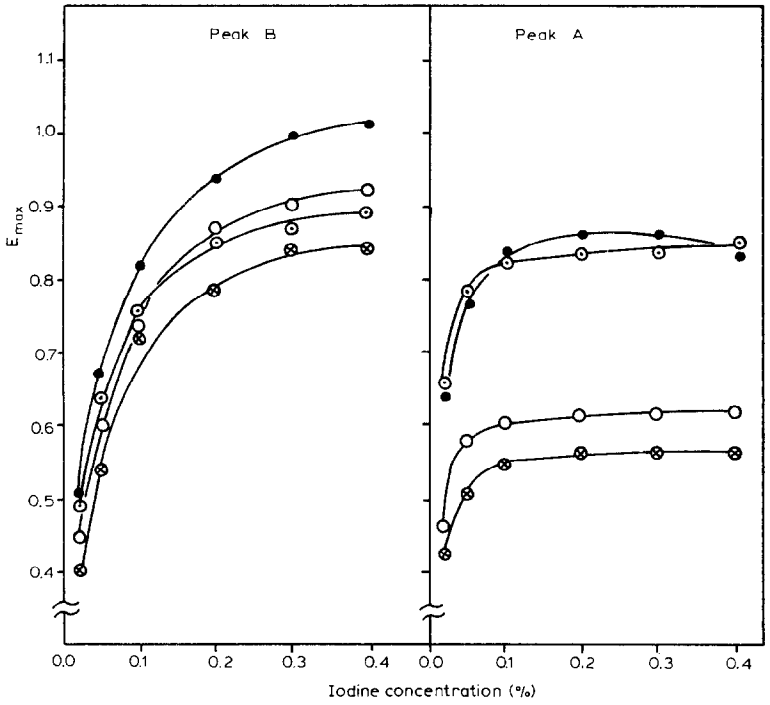

Fig. 5. The effect of iodine concentration on the E_{max} values of the component peaks A and B for debranched *non-mammalian* glycogens in the presence of half-saturated ammonium sulphate: *Helix pomatia* (⊗——⊗), *Ascaris lumbricoides* (○——○), cod liver (⊙——⊙), and *Cardium tuberculatum* (●——●).

The variations in E_{max} and λ_{max} with concentration of iodine did not reveal any significant differences between the two types of glycogen. The results of these experiments suggest that the exterior chains were responsible for the differences observed in the spectra of the debranched glycogens (Figs. 3–5).

Analysis of exterior chains by iodine staining. — For a study of the iodine-staining absorption spectra of the exterior chains of glycogen, the spectra of the interior chains and debranched glycogens were examined simultaneously. In aqueous solution, all three spectral profiles (for interior, exterior, and all chains) were similar for mammalian and invertebrate glycogens, *i.e.*, only one peak was observed at concentrations of iodine of 0.2 and 0.02%. However, E_{max} and λ_{max} were greater for the mammalian glycogens (Table IV), again implying the presence of some longer chains in the mammalian glycogens. Furthermore, the values of E_{max} and λ_{max} were greater for the exterior chains than for the interior chains. This finding is consistent with the greater chain length of the exterior chains, usually 7–8 glucose residues, compared to an interior chain length of 3–4 glucose residues.

In the presence of half-saturated ammonium sulphate, the spectra of the exterior chains of mammalian and invertebrate glycogens were very different, par-

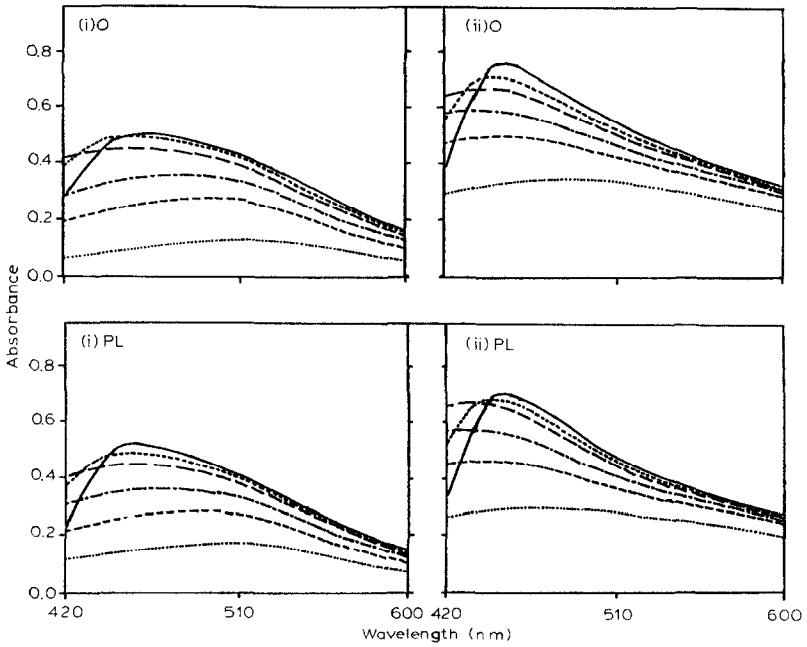

Fig. 6. Iodine-staining absorption spectra of debranched beta-amylase limit dextrins in (i) aqueous solution and (ii) half-saturated ammonium sulphate. The iodine concentrations are as in Fig. 2. The beta-limit dextrins are derived from oyster (O) and pig-liver (PL) glycogens.

ticularly at higher concentrations (0.2%) of iodine (Fig. 7). Component B (the lower-wavelength component) corresponded to the spectrum of the interior chains and also to component B of the debranched glycogens (Figs. 8 and 9). This finding indicates that component B in the iodine-staining spectrum of the debranched glycogens represents the complete contribution from the interior chains and a partial contribution from the exterior chains (*i.e.*, some of the exterior chains may

TABLE IV

IODINE STAINING OF UNIT CHAINS OF GLYCOGEN IN AQUEOUS SOLUTIONS

Glycogen source	*0.2% of iodine*						*0.02% of iodine*					
	Exterior chains		*Interior chains*		*Exterior and interior chains*		*Exterior chains*		*Interior chains*		*Exterior and interior chains*	
	λ_{max}[a]	E_{max}	λ_{max}	E_{max}	λ_{max}	E_{max}	λ_{max}	E_{max}	λ_{max}	E_{max}	λ_{max}	E_{max}
Ascaris lumbricoides	467	0.490	459	0.195	463	0.685	517	0.150	509	0.090	513	0.205
Rabbit liver	505	0.790	483	0.265	500	1.06	528	0.330	517	0.140	528	0.395

[a] λ_{max}, nm.

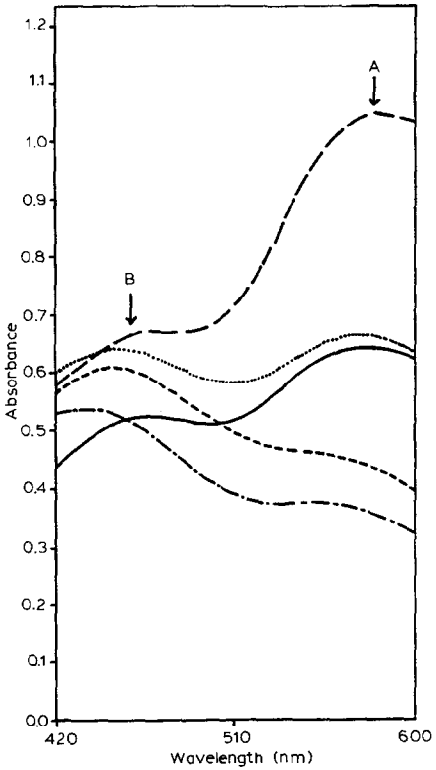

Fig. 7. Iodine-staining absorption spectra of the exterior chains of glycogens using 0.2% of iodine and in the presence of half-saturated ammonium sulphate. Invertebrate glycogens: *Ascaris lumbricoides* (– – –), *Mytilis edulis* (—·—·—); mammalian glycogens: pig liver (——), human liver (·····), and rabbit liver (— — —).

have lengths similar to those of the interior chains). Component A, with the greater λ_{max} therefore is the result of iodine staining of longer exterior chains, which occurs to a greater extent in mammalian glycogens than in invertebrate glycogens.

At the higher concentration of iodine, the iodine-staining spectra of the exterior chains of invertebrate glycogens showed the E_{max} of component A to be less than that of component B. In contrast to this finding, the exterior chains of the mammalian glycogens have a value of E_{max} of component A in excess of that of component B, implying that some of the exterior chains are much longer than the average value of 7–8 glucose residues and produce component A, possibly by helical entrapment of the iodine.

The λ_{max} for component A in the spectra of the external chains in mammalian glycogens (583–585 nm) is greater than that in the invertebrate glycogens (545–550 nm), as shown in Table V. This finding indicates that not only are there more longer exterior chains in mammalian glycogens but also that the length of these chains exceeds that of the longest exterior chains in invertebrate glycogens. Linear

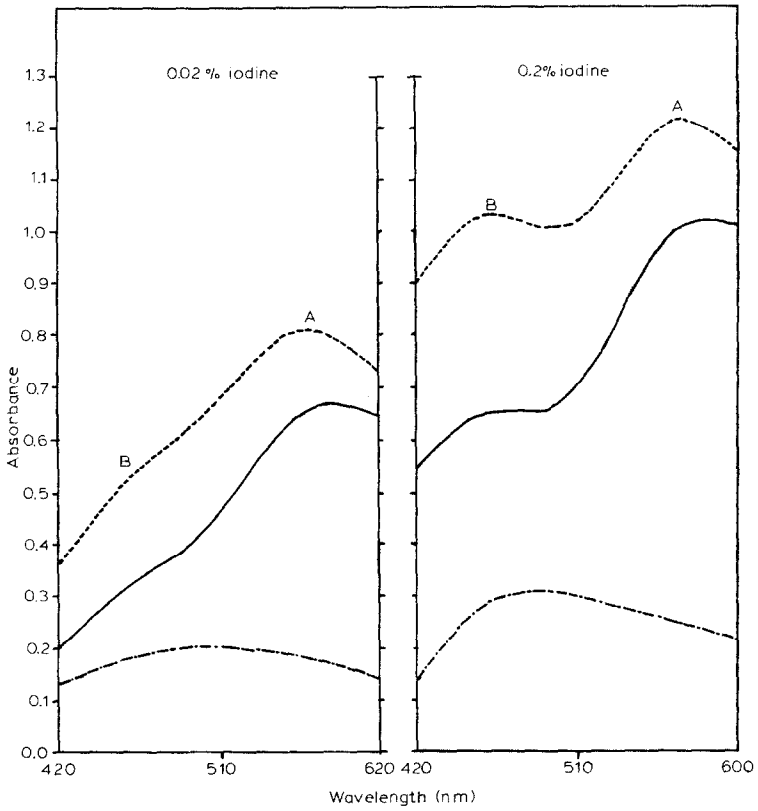

Fig. 8. Iodine-staining absorption spectra of the chains of rabbit-liver glycogen. Exterior and interior chains (------), exterior chains only (——), and interior chains only (-·-·-).

malto-oligosaccharides of d.p. 20–22 give an iodine spectrum in half-saturated ammonium sulphate with λ_{max} 460–473 nm[4]. The observed λ_{max} for component A suggests that the longer exterior chains are well in excess of 20–22 glucose residues.

The concept that glycogens may contain a wide range of lengths of unit chains has been recognised in other ways. Heller and Schramm[11] showed that there are regions within glycogen molecules which contain very short interior chains and very tight packing, such that these regions are not susceptible to the action of alpha-amylase. These regions are released as macrodextrins during alpha-amylolysis. Therefore, it follows that, in order to maintain the average chain-length, there must be a small proportion of chains which are correspondingly long. In fact, this feature is evident in the chain profile of rabbit muscle glycogen obtained by debranching with isoamylase (Fig. 1a), which indicates a small proportion of chains with a chain length of >40 glucose residues. In the present study, it has been shown that a distinguishing feature of mammalian glycogens is the presence of a small number of exceptionally long chains which are absent from invertebrate glycogens (see also Fig. 1b). This finding may reflect differences in the rates of metabolism of

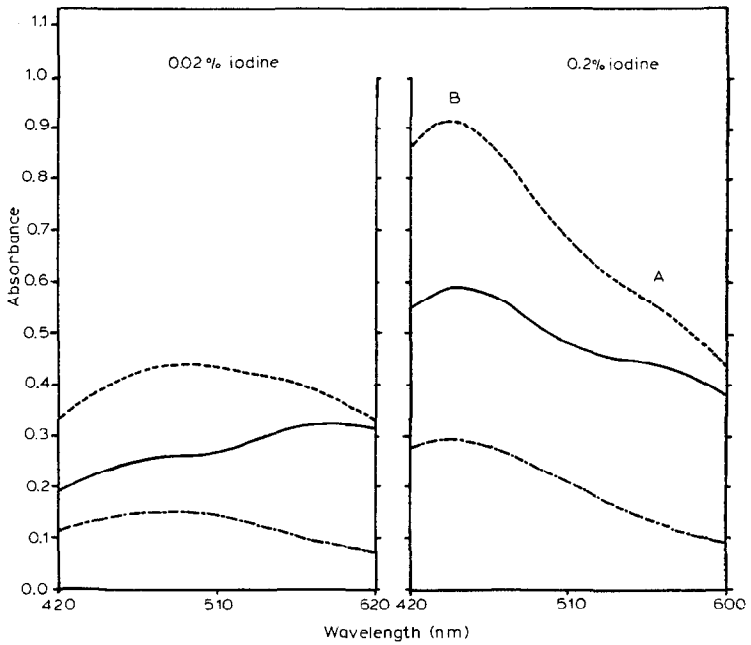

Fig. 9. Iodine-staining absorption spectra of the chains of *Ascaris lumbricoides* glycogen. Exterior and interior chains (------), exterior chains only (——), and interior chains only (–·–·–).

TABLE V

VALUES OF λ_{max} (nm) FOR COMPONENTS A AND B OF IODINE-STAINED GLYCOGEN CHAINS IN THE PRESENCE OF HALF-SATURATED AMMONIUM SULPHATE

Glycogen source	*Exterior chains*		*Interior chains*		*Exterior and interior chains*	
	Peak B	*Peak A*	*Peak B*	*Peak A*	*Peak B*	*Peak A*
With 0.02% of iodine						
Ascaris lumbricoides	490	582	485	—	495	Shoulder
Mytilis edulis	505	559	487	—	488	Shoulder
Rabbit liver	Shoulder	583	504	—	Shoulder	560
Pig liver	Shoulder	568	495	—	Shoulder	564
Human liver	Shoulder	567	489	—	Shoulder	555
With 0.2% of iodine						
Ascaris lumbricoides	449	545	442	—	442	Shoulder
Mytilis edulis	437	550	446	—	442	Shoulder
Rabbit liver	475	585	484	—	463	570
Pig liver	567	585	446	—	454	552
Human liver	451	583	441	—	453	555

the two categories of animals, with the much higher activity of the mammalian species resulting in a more vigorous synthesis of α-(1→4)-glucosidic linkages following feeding. This view is in accord with the earlier observations of Chapman *et al.*[12] on the iodine absorption spectra of newly formed rat-liver glycogen. By contrast, marine invertebrates or parasitic worms exist in a passive metabolic environment.

ACKNOWLEDGMENT

The Trustees of the Eda, Lady Jardine Charitable Trust are thanked for the award of a research studentship to (S.A.S.C.).

REFERENCES

1 D. J. MANNERS, J. R. STARK, AND V. THAMBYRAJAH, *J. Jpn. Soc. Starch Sci.*, 30 (1983) 13–18.
2 J. HOLLO AND J. SZEITLI, in J. A. RADLEY (Ed.), *Starch and its Derivatives*, 4th edn., Chapman and Hall, London, 1968, pp. 203–246.
3 A. R. ARCHIBALD, I. D. FLEMING, A. M. LIDDLE, D. J. MANNERS, G. A. MERCER, AND A. WRIGHT, *J. Chem. Soc.*, (1961) 1183–1190.
4 D. J. MANNERS AND J. R. STARK, *Staerke*, 26 (1974) 78–81.
5 M. JOHN, J. SCHMIDT, AND H. KNEIFEL, *Carbohydr. Res.*, 119 (1983) 254–257.
6 D. J. MANNERS AND A. WRIGHT, *J. Chem. Soc.*, (1962) 1597–1602.
7 O. KJOLBERG AND D. J. MANNERS, *J. Chem. Soc.*, (1962) 4596–4600.
8 O. KJOLBERG, D. J. MANNERS, AND A. WRIGHT, *Comp. Biochem. Physiol.*, 8 (1963) 353–365.
9 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350–356.
10 J. F. ROBYT AND W. J. WHELAN, in J. A. RADLEY (Ed), *Starch and its Derivatives*, 4th edn., Chapman and Hall, London, 1968, pp. 432–433.
11 J. HELLER AND M. SCHRAMM, *Biochim. Biophys. Acta*, 81 (1964) 96–100.
12 D. D. CHAPMAN, J. M. FELTS, AND I. L. CHAIKOFF, *Experientia*, 11 (1955) 283–285.