radicals. Panose does not appear to be a favourable acceptor, since 6³- α -isomaltosylmaltose and related sugars are not produced. The ability to transfer to C-4 differentiates the protozoal enzyme system from various fungal maltases which can only synthesize (1 $\rightarrow$ 6)-linked oligosaccharides (e.g. Pazur & French, 1952).

The acceptor specificity of the protozoal maltase system is generally similar to that observed with extracts of *C. rupestris* (Duncan & Manners, 1958) except that the latter is unable to use monosaccharides as acceptor substrates. Since a number of pentoses and also D-galactose can accept α -glucosyl radicals with the *T. pyriformis* system, it is probable that most of the isomaltose is formed by the interaction of a glucosyl-enzyme complex with the primary alcohol group of a glucose molecule rather than by the hydrolysis of the (1 $\rightarrow$ 4)-linkage of panose. This view is supported by the fact that isomaltose synthesis and the formation of higher oligosaccharides ($R_g < 0.28$) occur simultaneously, showing that the equilibrium between hydrolysis (i.e. water as glycosyl acceptor) and trans- α -glucosylation to maltotriose favours the latter.

Isomaltose itself can function as a glucosyl donor, but the product appears to be isomaltotriose rather than panose or a maltosaccharide. One possible explanation for this is that the maltase and isomaltase activities of the *T. pyriformis* extracts are due to different enzymes. The non-identity of these activities in extracts of yeast and intestinal mucosa (for example, Larnier & Gillespie, 1956) has already been established.

SUMMARY

1. Cell-free extracts of the ciliate *Tetrahymena pyriformis* show hydrolytic activity towards

maltose, isomaltose, cellobiose, sucrose, methyl α -D-glucoside, α -D-glucosyl phosphate and starch.

2. In concentrated solution, oligosaccharides are synthesized from maltose and from isomaltose, but not from glucose.

3. Oligosaccharide synthesis from maltose results in the formation of isomaltose, maltotriose, panose, 6³- α -glucosylmaltotriose and maltotetraose.

4. The acceptor specificity for α -glucosyl radicals by the protozoal enzyme system and other maltases is discussed.

We wish to thank Professor E. L. Hirst, F.R.S., and Dr J. F. Ryley for their interest in this work, which was supported by a maintenance allowance (to A.R.A.) from the Department of Scientific and Industrial Research, and a research grant from the Rockefeller Foundation.

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Enzyme Systems in Marine Algae

3. TRANS- β -GLUCOSYLATION BY EXTRACTS OF *CLADOPHORA RUPESTRIS* AND *ULVA LACTUCA**

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(Received 30 January 1959)

During a study of the hydrolysis of cellodextrin by an extract of *Cladophora rupestris*, Duncan, Manners & Ross (1956) noted that the products of enzyme action, as examined by paper chromatography, included laminaribiose. Since the original

substrate did not contain β -1:3-glucosidic linkages, it seemed possible that this sugar had arisen from cellobiose by trans- β -glucosylation involving a double-displacement mechanism:

Cellobiose + enzyme-H $\rightarrow$ glucose + glucosyl-enzyme

Glucosyl-enzyme + H $\cdot$ OR $\rightarrow$ enzyme-H + glucosyl $\cdot$ OR

where RO $\cdot$ H is an acceptor substrate, in this case D-glucose acting at its C-3 hydroxyl group.

* Part 2. Duncan & Manners (1958).

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This possibility has now been investigated by incubating the algal extract with various concentrations of cellobiose, and with mixtures of cellobiose and other sugars. In particular, the ability of D-xylose to function as a β -glucosyl acceptor has been studied. While this work was in progress, Barker, Bourne, Hewitt & Stacey (1957) reported that cell-free extracts of *Aspergillus niger* (strain 152) could synthesize 3-O- β -D-glucopyranosyl-D-xylose from a cellobiose-xylose mixture.

The metabolism of cellobiose by an extract of *Ulva lactuca* has also been examined.

METHODS

Chromatography. (a) *Qualitative.* Descending paper chromatograms were prepared at room temperature with Whatman no. 1 or 54 paper and butanol-pyridine-water-benzene (5:3:3:1, by vol.) (A), ethyl acetate-acetic acid-formic acid-water (18:3:1:4, by vol.) (B) or ethyl acetate-pyridine-water (10:4:3, by vol.) (C) as solvent. Aniline phthalate or oxalate, *p*-anisidine hydrochloride, naphtha-resorcinol-hydrochloric acid and silver nitrate-sodium hydroxide were used as spray reagents. The rate of movement of sugars is expressed relative to D-glucose (R_g) or to D-xylose (R_x).

(b) *Preparative.* Charcoal-Celite columns were prepared as previously described (Duncan & Manners, 1958).

Oxidation by periodate. The reduction of periodate was determined spectrophotometrically (Aspinall & Ferrier, 1957). The remaining methods were those described by Duncan & Manners (1958) and Anderson & Manners (1959).

Enzyme preparations. For the preliminary experiments, unfractionated extracts of *C. rupestris* and *U. lactuca* were used (Duncan *et al.* 1956). In the large-scale digest, an extract of *C. rupestris* was fractionated with ammonium sulphate and the protein precipitated between 0.18 and 0.36 saturation was collected, dissolved in water, dialysed against running tap water for 24 hr. at room temperature and freeze-dried. This fraction had the highest β -glucosidase activity. Toluene was used to maintain aseptic conditions in the enzymic reactions.

EXPERIMENTAL AND RESULTS

C. rupestris extract

Action on cellobiose. Digests containing cellobiose (1, 5, 10, 20%, w/v) and *C. rupestris* enzyme preparation (1%, w/v) were incubated at 35°. In all digests, glucose was detected after 3.5 hr. The other results were as follows: (a) 1% cellobiose, hydrolysis complete within 96 hr.; (b) 5% cellobiose, oligosaccharides detected after 96 hr.; (c) 10 and 20% cellobiose, oligosaccharides produced within 24 hr. These were identified, by paper chromatography in three solvents, as laminaribiose, gentiobiose and cellotriose. The cellotriose was formed first, and in greatest amount; we are indebted to Dr W. J. Whelan for an authentic sample for comparison.

In a further experiment, cellobiose (6%, w/v) and extract were incubated for 14 days. In addition to the above oligosaccharides, three sugars with R_g values of 0.12, 0.08 and 0.06 were also produced (solvent A).

Since these results were generally similar to those reported with β -glucosidase preparations from various moulds and plants (Crook & Stone, 1957) and, in particular, from barley in which the identity of the laminaribiose and gentiobiose was confirmed by chemical methods (Anderson & Manners, 1959), it was decided to examine the acceptor specificity for β -glucosyl residues in greater detail.

Action on cellobiose-sugar mixtures. Digests were prepared containing cellobiose (15%, w/v) with D-xylose, L-xylose, L-sorbose or N-acetyl-D-glucosamine (15%, w/v) and enzyme extract (1%, w/v) and were incubated at pH 5.6 and 35° for 10–15 days. The last two digests contained the same series of sugars as with cellobiose alone. The pentose-containing digests contained additional sugars which stained pink with aniline oxalate. In solvent B, those from the D-xylose digest had R_g values 0.70 and 0.40; in the L-xylose digest, R_g 0.72 and 0.42. The sugars with R_g 0.70 and 0.72 were eluted from paper chromatograms with cold water, hydrolysed with sulphuric acid, neutralized and rechromatographed. Each hydrolysate contained glucose and pentose in apparently equivalent quantities, and no other sugar. The sugars with R_g 0.40 and 0.42, which were present only in small amounts, were similarly examined; both yielded glucose and pentose. It is possible that these sugars are diglucosylxylose trisaccharides.

The presence of more than one new component in the pentose digests was confirmed by chromatography on small charcoal-Celite columns. Elution with water gave a mixture of monosaccharides, with 5% aqueous ethanol yielded cellobiose, and with 10% ethanol the following: with D-xylose as acceptor, R_g 0.74, 0.60 (trace) and 0.42 (solvent B); L-xylose as acceptor, R_g 0.73 and 0.45 (solvent B), all of which gave pink stains with aniline oxalate.

A comparison of the suitability of other pentose sugars to act as glycosyl acceptors was then carried out by incubating D-lyxose, D-ribose, or L-arabinose (7%, w/v) with cellobiose (7%, w/v) and algal extract (0.7%, w/v). A control cellobiose-D-xylose mixture was also prepared. In all cases, new sugars were formed which appeared to be glucosylpentose disaccharides. The R_x values of the products were: from D-lyxose 0.38 (solvent B), from D-ribose 0.44, from L-arabinose 0.28, and from xylose 0.43 and 0.27 (trace). With ribose and xylose, the new sugar could be detected within 24 hr.; with lyxose and arabinose, 8 and 15 days' incubation respectively were required.

Control digests showed that oligosaccharide

synthesis did not occur from glucose or xylose alone.

The greatest trans- β -glucosylation occurred with xylose, and this reaction was therefore examined on a larger scale.

Enzymic synthesis of glucosylxylose. Cellobiose (10.0 g.), D-xylose (10.0 g.) and *C. rupestris* fraction (0.3 g.) in water (40 ml.) were incubated at 35° for 14 days. Paper-chromatographic analysis (solvent B) showed the presence of a satisfactory amount of the component with R_x 0.43. The digest was heated to inactivate the enzyme, concentrated under reduced pressure, and placed on a charcoal-Celite column (86 $\times$ 6 cm.). The glucose and xylose were eluted with water, and the disaccharides (cellobiose and a sugar shown below to be glucosylxylose) with increasing concentrations of aqueous ethanol (0 $\rightarrow$ 10%). Approx. 770 mg. of the glucosylxylose was collected; this was freed from traces of glucose and cellobiose by chromatography on Whatman 3MM paper with solvent B, and crystallized from ethanol.

Characterization of glucosylxylose. The sugar was homogeneous by paper chromatography, with R_g 1.02 and R_x 0.64 (solvent A), and R_x 0.43 (solvent B), and gave a pink stain with aniline phthalate and *p*-anisidine; it had m.p. 120°, $[\alpha]_D^{20}$ -6.4° in water (*c*, 2.8) and the reducing power of a disaccharide (xylose standard).

Hydrolysis with 0.67N-sulphuric acid for 6.5 hr. at 100° gave, by Pridham's (1956) method, equimolar proportions of glucose and xylose. After oxidation of the disaccharide with hypiodite, an acid hydrolysate contained glucose but not xylose. Incubation with emulsin at pH 5.0 and 35° gave glucose and xylose.

In view of the mode of synthesis of the sugar, and of the fact that D-xylose exists in solution in the pyranose form, the above preliminary experiments indicate that it is a β -D-glucopyranosyl-D-xylopyranose. The following evidence shows the presence of a 1:3-linkage:

(1) On heating with sodium acetate-phenylhydrazine hydrochloride, a crystalline osazone was formed; after two recrystallizations from ethyl acetate, the bright-yellow crystals had m.p. 205–206° (cf. m.p. 213–215° reported by Barker *et al.* 1957). An acid hydrolysate of the osazone contained glucose and not xylose. These findings eliminate the possibility of a 1:2-linkage.

(2) On oxidation with sodium metaperiodate in the dark at room temperature, a rapid initial reduction of 3.02 mol.prop. was observed. After 22 hr. this had increased to only 3.07 mol.prop. The theoretical values for a 1:3- and 1:4-linked disaccharide are 3.00 and 4.00 mol.prop., respectively. Under alkaline conditions, the sugar was over-oxidized by periodate, since on prolonged

oxidation more than 7 mol.prop. of periodate was reduced. This behaviour is typical of oligosaccharides which give rise to intermediates containing malonaldehyde groups (cf. Hough & Perry, 1956).

(3) Confirmation of a 1:3-linkage was provided by a comparison of R_x values (solvents B and C) with those of an authentic sample of 3-O- β -D-glucopyranosyl-D-xylopyranose kindly given by Dr S. A. Barker (cf. Barker *et al.* 1957).

Ulva lactuca extract

A digest containing cellobiose (16%, w/v) and algal extract (1%, w/v) was incubated at pH 5.0. The presence of laminaribiose, gentiobiose and cellotriose was detected by paper chromatography within 24 hr. Similar results were obtained after 3, 5 and 7 days' incubation. In contrast, a 1% solution of cellobiose was completely hydrolysed. During the hydrolysis of cellodextrin (1.7%, w/v), traces of laminaribiose were detected during the first 3 days; after this time, only cellosaccharides were present.

Attempts to demonstrate trans- β -glucosylation from cellobiose to D-galactose, D-mannose, phenol or methanol were unsuccessful. Digests with cellobiose-acceptor mixtures did not contain new sugars (other than those obtained with cellobiose alone) or glucosides after incubation for periods up to 16 days; the phenol and methanol partly inhibited the enzymic reaction.

DISCUSSION

Recent investigations have shown that cellobiase preparations from various higher plants (e.g. sweet almonds, gentian roots, barley) and moulds (e.g. *Myrothecium verrucaria*, *Aspergillus niger*, *A. aureus*, *A. flavus*) catalyse the formation of oligosaccharides from concentrated solutions of cellobiose (Crook & Stone, 1957; Anderson & Manners, 1959). The present work shows that marine algae contain similar enzyme systems. The tentative identification of laminaribiose, gentiobiose and cellotriose suggests that, with cellobiose as β -glucosyl donor, both glucose (OH at C-3 and C-6) and cellobiose may function as acceptor substrates. By contrast, D-galactose, D-mannose, L-sorbose, *N*-acetyl-D-glucosamine, phenol and methanol do not appear to satisfy the specificity requirements. In a similar experiment with *A. oryzae* β -glucosidases, Jermyn & Thomas (1953) were able to demonstrate the transfer to methanol of β -glucosyl residues from *p*-nitrophenyl β -D-glucoside, but not from cellobiose.

The synthesis of 3-O- β -D-glucopyranosyl-D-xylopyranose from a cellobiose-xylose mixture is of interest since D-xylopyranose has, with the

exception of C-5, the same configuration as D-glucopyranose. The formation of this disaccharide thus parallels that of laminaribiose. This behaviour is similar to that reported by Barker *et al.* (1957) for *A. niger* (152) and differs from that of another strain of this mould (E19) which was unable to effect appreciable trans- β -glucosylation to D-xylose (Crook & Stone, 1957). It is presumed that the hydroxyl groups at C-2 and C-4 of D-xylose, and at C-2, C-3 and C-4 in the other pentoses are less favourable acceptor sites for the algal cellobiase.

D-Xylose is not an acceptor substrate for trans- α -glucosylation by extracts of *C. rupestris* (Duncan & Manners, 1958).

SUMMARY

1. Extracts of *Cladophora rupestris* and *Ulva lactuca* catalyse the synthesis of oligosaccharides from concentrated solutions of cellobiose. Laminaribiose, gentiobiose and cellobiose have been identified by paper chromatography.

2. On incubation of *C. rupestris* extract with cellobiose-pentose mixtures, glucosylpentose disaccharides are produced. 3-O- β -D-Glucopyranosyl-D-xylopyranose has been isolated from a cellobiose-xylose mixture and characterized.

3. The acceptor specificity of cellobiases from various biological sources is discussed.

We wish to thank Professor E. L. Hirst, F.R.S. for his interest in this work, which formed part of a research programme sponsored by the Institute of Seaweed Research and supported by a maintenance grant (to W.A.M.D.). We are also indebted to Dr A. G. Ross and Mr W. L. Cunningham for experimental assistance, and the Rockefeller Foundation for a research grant.

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Metal Ions as Precipitants for Nucleic Acids and their Use in the Isolation of Polynucleotides from Leaves

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(Received 20 January 1959)

Many genetical, virological and metabolic studies will require better methods for isolating nucleic acids than are yet available. It is necessary to aim at complete extraction of the polynucleotides from the tissues, since it cannot be assumed that even a large fraction is representative of the whole; and the product should be obtained as nearly as possible in its native state, uncontaminated by other cell polymers. In the procedure to be described an attempt is made to fulfil these requirements. It is assumed that the principal variables affecting the solubility of polynucleotides are polynucleotide concentration, temperature, hydrogen-ion concentration, ionic composition, ionic concentration and dielectric constant of the medium.

It has been found that a high proportion of the nucleic acid extracted from leaves in 1.7 M-sodium chloride at 100° is precipitated when the solution is

kept at 0°. Williams (1945) found that comparable extracts from plants made with 2.1 M-sodium chloride precipitated the extracted nucleic acid at pH values from 0.4 to 3.4 when kept at 2–5°, maximum precipitation occurring at pH 0.4–1.6. Holden & Pirie (1955a) obtained fractions of nucleic acids from leaves and yeast by precipitation from extracts by sodium chloride at pH 3.0. Crestfield, Smith & Allen (1955) used this salt at pH 7.0 to precipitate nucleic acid from extracts of yeast in an attempt to prepare undegraded yeast ribonucleic acid. No detailed study of this type of method has been published, nor has there been a systematic study of the interaction of multivalent cations with nucleic acids, although La³⁺, Cu²⁺ and some other multivalent metal ions are known to be good polynucleotide precipitants. A study of certain details of these reactions has been made, primarily as a guide to the