REFERENCES

1. Trebst, A. and Wagner, S. (1962) *Z. Naturforsch.* **17b**, 396.
2. Siegenthaler, P.-A. and Vaucher-Bonjour, P. (1971) *Planta* **100**, 106.
3. Tolbert, N. E. (1973) *Plant Physiol.* **51**, 234.
4. Sâto, M. and Hasegawa, M. (1976) *Phytochemistry* **15**, 61.
5. Hofer, A. (1964) *Planta* **62**, 137.
6. Lieberei, R. and Biehl, B. (1976) *Ber. Deutsch. Bot. Ges.* **89**, 663.
7. Lieberei, R. (1976) Thesis, T. U. Braunschweig, Bot. Inst.
8. Sâto, M. (1977) *Phytochemistry* **16**, 1523.
9. Levitt, J. (1974) cited in Lozina-Lozinskii, L. K. 766 *Cryobiology*. John Wiley, New York.
10. Katz, Y. and Mayer, A. M. (1969) *Israel J. Botany* **18**, 11.
11. Kenten, R. H. (1958) *Biochem. J.* **68**, 244.
12. Madhosingh, C. (1975) *Can. J. Microbiol.* **21**, 2108.
13. Harel, E., Mayer, A. M., Markus, Z. and Avigad, G. (1967) *Israel J. Botany* **16**, 38.
14. El-Bayoumi, M. A. and Frieden, E. (1957) *J. Am. Chem. Soc.* **79**, 4854.
15. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265.
16. McKinney, G. (1959) in *Moderne Methoden der Pflanzenanalyse* Vol. 4, p. 159. Springer–Verlag, Berlin.
17. Anderson, J. M. and Boardman, N. K. (1966) *Biochim. Biophys. Acta* **112**, 403.
18. Maurer, H. R. (1968) *Disc Electrophorese*. Walter de Gruyter, Berlin.
19. Stegemann, H. (1972) *Z. Anal. Chem.* **261**, 388.

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PROPERTIES OF POTATO α-GLUCOSIDASE

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Key Word Index—*Solanum tuberosum*; Solanaceae; potato; α-glucosidase; properties

Abstract—Potato tuber α-glucosidase has an isoelectric point of 4.7 and an apparent MW of 120000. The enzyme has a neutral pH optimum (pH 6.5–7.0) and a K_m of 0.21 mM for *p*-nitrophenol-α-D-glucoside at pH 6.8 and 30°. Maltose and higher maltosaccharides are also substrates. The enzyme exhibits transglucosidase activity.

INTRODUCTION

α-Glucosidases (α-D-glucoside glucohydrolase, EC 3.2.1.20) are exoenzymes which catalyse the hydrolysis of glucose units from the non-reducing ends of α-linked glucans. The enzymic activity has been demonstrated in a number of plant extracts [1–10] and was first detected in potato tuber extracts by Hutson and Manners [1] but the enzyme was not characterised in any detail. We report here some of the properties of a partially purified preparation of the potato tuber enzyme.

RESULTS AND DISCUSSION

The partially purified α-glucosidase preparation was found to apparently contain only one activity which hydrolysed *p*-nitrophenyl-α-D-glucoside. One activity band of low electrophoretic mobility was detected on polyacrylamide gels. On isoelectric focusing in the pH range pH 3.0–10.0 a single activity peak, having an apparent isoelectric point of 4.7, was obtained. When subjected to gel filtration on a Sephadex G-200 column, the α-glucosidase activity was eluted as a single symmetrical peak having an apparent MW of 120000.

The effect of pH on the stability and also on the catalytic activity was tested in the pH range 2.5–9·0 in McIlvane buffers. The α-glucosidase activity was stable in the pH range pH 6.0–9.0 when stored at 4° for 16 hr, and was stored in 0.1 M potassium phosphate buffer, pH 6.8, for 6 months at −20°, with no loss in activity. Maximal activity on *p*-nitrophenyl-α-D-glucoside was observed in the pH region 6.5–7.0. Little activity (0–15% of maximal activity) was observed in the pH region pH 2.5–4.5, indicating the absence of acid α-glucosidase activity such as have been observed in other plant tissues [1–10].

Using *p*-nitrophenyl-α-D-glucoside at concentrations varying from 0.25–2.0 mM and standard assay conditions (pH 6.8, 30°), a Lineweaver–Burk plot was obtained. The value of K_m, found from the plot, was 0.21 mM and this value is similar to that reported for the yeast enzyme [11]. At 5.0 mM and higher concentrations of *p*-nitrophenol-α-D-glucoside substrate inhibition was observed. EDTA (10 mM) and 2-mercaptoethanol (10 mM) had no effect on the enzymic activity. The α-glucosidase activity was inhibited (100%) by Tris (0.1 M) at pH 6.8. Inhibition of other α-glucosidase preparations by Tris has been extensively documented [9, 12–16].

The anomeric form of the D-glucose released by enzymic action on *p*-nitrophenyl-α-D-glucoside was determined as by Semenza *et al.* [17]. This procedure is based on the known specificity of fungal glucose oxidase for β-glucose. It was found that the rate of oxidation by the glucose oxidase of the D-glucose released from the *p*-nitrophenyl-α-D-glucoside by the α-glucosidase was increased after mutarotation by heating [17]. Thus the α-glucosidase released α-D-glucose, i.e. hydrolysis took place with retention of the configuration of the anomeric carbon atom of the released glucose molecule. This result strongly indicated that the activity is a true α-glucosidase (i.e. maltase) rather than an exo-α-glucosidase which would be expected to release the glucose as β-D-glucose [18].

The enzyme preparation also hydrolysed the maltosaccharides, maltose, maltotriose, maltotetraose, maltopentaose and maltohexaose, when each was incubated with enzyme preparation. The products were qualitatively analysed by paper chromatography and glucose was the predominant product in each case. On heavy loading of the chromatograms traces of the intermediate products were also detected tentatively indicating a multichain action pattern. In no case were higher maltosaccharides than the substrates detected showing that the enzyme preparation was free of D-enzyme activity. D-Enzyme is a transglucosidase, first detected in potato [19], that disproportionates maltotriose and higher maltosaccharides [20]. Panose and isomaltose were also hydrolysed by the α-glucosidase preparation but it was not determined if this α-1,6-hydrolytic activity was due to the same enzyme or a separate α-1,6-hydrolytic activity in the preparation.

When the α-glucosidase was incubated with high concentrations of maltose (0.5 M, pH 6.8, 30°) transglucosidase activity was observed. Paper chromatography of the reaction mixture resolved unhydrolysed maltose, glucose, panose and an unidentified slow migrating spot. This activity is reminiscent of potato T-enzyme activity [21].

The physiological significance of the α-glucosidase has not been clearly elucidated. It functions presumably to hydrolyse maltose and other maltosaccharides, produced by amylase action on starch, to glucose [22].

EXPERIMENTAL

Enzyme Assays. α-Glucosidase activity was assayed with *p*-nitrophenyl-α-D-glucoside as substrate (at pH 6.8, 30°) as by Halvorsen and Ellias [11]. Incubation of the enzyme preparation with maltose and other maltosaccharides was carried out in 1 ml incubation mixtures containing the maltosaccharide (final concentration, 2 mM), 0.1 M K–Pi buffer, pH 6.8, and the enzyme preparation. The incubations were terminated by heating (100°, 3 min) and then deionised by shaking with carbonated mixed ion exchange resin AG 501-x8 (Bio-Rad Laboratories) prior to PC [23] on Whatman No. 1 paper in EtOAc–Py–H_2O (4:2:1). The sugars were detected on the chromatograms with $AgNO_3$/NaOH [24].

Enzyme preparation. Potato tubers (2 kg, cv Kerry Pink, stored at 4°) were washed, peeled and juiced. The juice was made 0.01 M with respect to Na_2SO_3 to prevent darkening of the juice and subjected to $(NH_4)_2SO_4$ fractionation. The protein precipitating between 0.20 and 0.45 saturation was dialysed for 16 hr at 4° against 6 l. of distilled H_2O, pH 8.5 (adjusted with dilute NH_4OH). The resulting suspension was centrifuged (25000 g, 30 min) and to the supernatant were added 2 vol. of distilled H_2O, pH 8.5. After standing at 4° for 30 min a further precipitate was obtained and removed by centrifugation as before. The supernatant was again subjected to $(NH_4)_2SO_4$ fractionation and the protein precipitating between 0.25 and 0.50 saturation was collected by centrifugation (25000 g, 30 min). This precipitate was redissolved in a minimal volume of 0.04 M buffer, pH 7.5 containing 0.01 M EDTA and 0.25 M NaCl and incubated at 30° for 30 min. Inert protein was removed by centrifugation as above. The supernatant (50 ml) was then subjected (in 10 ml aliquots) to gel filtration on a Sephadex G-200 column (2.5 × 65 cm) equilibrated with 0.04 M K–Pi buffer, pH 7.5 containing 0.01 M EDTA and 0.25 M NaCl. The active fractions were pooled and concentrated by the addition of $(NH_4)_2SO_4$ to 0.5 saturation. The precipitate was dialysed against 0.1 M K–Pi buffer, pH 6.8 and stored at −20°. Using this procedure the α-glucosidase was purified 10-fold with a 50% recovery of activity.

Other methods. Polyacrylamide disc gel electrophoresis was carried out in 6% gels as by Ornstein [25] except that K–Pi buffer was used. The gel and reservoir buffers were 0.02 M and 0.01 M K–Pi buffer, pH 7.5, respectively. Following electrophoresis the α-glucosidase activity was detected as a yellow band (due to liberated *p*-nitrophenol) by incubating the extruded gels in 3 ml incubation mixtures containing 0.1 M K–Pi buffer, pH 6.8 and 1 mM *p*-nitrophenyl-α-D-glucoside at 30°. Isoelectric focusing was carried out at 4° in a vertical glass column in a sucrose density gradient containing synthetic ampholytes (LKB-Ampholine) in the pH range pH 3.0–10.0 as by Vesterberg and Svensson [26]. The apparent MW of the α-glucosidase was determined according to Andrews [27] by gel filtration on a Sephadex G-200 column (2.5 × 65 cm) equilibrated with 0.04 M K–Pi buffer, pH 7.5 containing 0.01 M EDTA and 0.25 M NaCl. The protein standards used to calibrate the column were R-phycoerythrin, R-phycocyanin, ovalbumin and myoglubin [27]. Maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, panose and isomaltose were obtained from Koch-Light and were purified by preparative PC on Whatman No. 3 paper in EtOAc–Py–H_2O (4:2:1) [24]. *p*-Nitrophenol-α-D-glucoside was obtained from the Sigma Chemical Co. R-Phycoerythrin and R-phycocyanin were isolated from the red algae *Rhodymenia palmata* and *Porphyra laciniata* respectively as by O'Carra [28].

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REFERENCES

1. Hutson, D. H. and Manners, D. J. (1965) *Biochem. J.* **94**, 783.
2. Manners, D. J. and Rowe, K. L. (1969) *Carbohydrate Res.* **9**, 441.
3. Jorgensen, B. B. and Jorgensen, O. B. (1963) *Acta Chem. Scand.* **17**, 1765.
4. Nakayama, S., Amagase, S., Tahara, M. and Okomura, K. (1969) *J. Biochem. (Tokyo)* **65**, 751.
5. Swain, R. R. and Decker, E. E. (1966). *Biochim. Biophys. Acta* **122**, 87.
6. Nomura, T., Kono, Y. and Akazawa, T. (1969) *Plant Physiol.* **44**, 765.
7. Takahashi, M. and Shimomura, T. (1968) *Agric. Biol. Chem* **32**, 929.
8. Takahashi, N., Shimomura, T and Chiba, S. (1971) *Agric. Biol. Chem.* **35**, 2015.
9. Kanaya, K., Chiba, S., Shimomura, T. and Nishi, K. (1976) *Agric. Biol. Chem.* **40**, 1929.
10. Marshall, J. J. and Taylor, P. M. (1971) *Biochem. Biophys. Res Commun.* **42**, 173.
11. Halvorsen, H. and Ellias, L. (1958) *Biochim Biophys. Acta* **30**, 28.
12. Dahlqvist, A. (1961) *Biochem. J.* **80**, 547.

13. Carter, J. and Smith, E. E. (1973) *Arch. Biochem. Biophys.* **155**, 82.
14. Takahashi, N. and Shimomura, T. (1973) *Agric. Biol. Chem.* **37**,67.
15. Jorgensen, B. B. and Jorgensen, O. B. (1967) *Biochim. Biophys. Acta* **146**, 167.
16. Galand, G. and Forstner, G. G. (1974) *Biochem. J.* **144**, 281.
17. Semenza, G., Curtius, H.-Ch., Raundhardt, O., Hore, P. and Muller, M. (1969) *Carbohydrate Res.* **10**, 417.
18. Reese, E. T., Maguire, A. H. and Parrish, F. W. (1968) *Can. J. Biochem.* **46**, 25.
19. Abdullah, M., Fischer, E. H., Querishi, M. Y., Slessor, K. N. and Whelan, W. J. (1965) *Biochem. J.* **97**, 9P.
20. Jones, G. and Whelan, W. J. (1969) *Carbohydrate Res.* **9**, 483.
21. Abdullah, M. and Whelan, W. J. (1960) *Biochem. J.* **75**, 12P.
22. Manners, D. J. (1974) in *Plant Carbohydrate Biochemistry* (Pridham, J. B., ed.) p. 109. Academic Press. London.
23. Aspinall, G. O. and Ferrier, R. J. (1957) *J. Chem. Soc.* 4191.
24. Trevelyan, W. G., Procter, D. P. and Harrison, J. S. (1950) *Nature* **166**, 444.
25. Ornstein, L. (1964) *Ann. N.Y. Acad. Sci.* **121**, 321.
26. Vesterberg, O. and Svensson, H. (1966) *Acta Chem. Scand.* **20**, 820
27. Andrews, P. (1964) *Biochem. J.* **91**, 222.
28. O'Carra, P. (1965) *Biochem. J.* **94**, 171.

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HYDROCARBONS OF *MACROCYSTIS PYRIFERA* BLADES

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Key Word Index—*Macrocystis pyrifera*; Lessoniaceae; giant kelp; hydrocarbons.

INTRODUCTION

Among lipids in benthic marine algae, hydrocarbons are least understood and they are known only from analyses of plants common to the Atlantic seaboard [1]. Hydrocarbons in macroalgae of Pacific Ocean waters are even less well characterized. We wish to report the characterization of the major saturated and unsaturated hydrocarbons in the giant kelp (*Macrocystis pyrifera*), a prominent brown alga of west coastal waters.

RESULTS AND DISCUSSION

Hydrocarbon constituents of *Macrocystic pyrifera* blades are given in Table 1. Dry material comprised 7 ± 1.8% (s.d.) of the fr. wt, and 4.4 ± 1.3 mg (0.1 ± 0.03% dry wt) non-saponifiable lipid material was extracted. Total hydrocarbons accounted for between 0.4 and 0.6% of these lipids. Saturated hydrocarbons made up from 43 to 48% of the total and *n*-alkanes were the dominant saturated species (62.4% of total saturates). Branched alkanes were generally absent from giant kelp blades.

Together, the alkenes 3,6,9,12,15,18-heneicosahexaene (HEH) and 3,6,9,12,15-heneicosapentaene (HEP) accounted for 51.5–81% of the total unsaturates present. It is presumed that HEH and HEP contain all-*cis* saturated bonds, as observed in blades of the closely related alga, *Laminaria saccharina* [1]. Squalene (2,6,10,-15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene), which has not been reported in earlier work with marine algae [1], was an important constituent in giant kelp blades, comprising 5.1–19.2% of the total hydrocarbon.

The amounts of *n*-alkanes in *M. pyrifera* blades are presented in Table 2. The dominant alkane was *n*-pentadecane, which comprised from 67.9 to 92.2% of the total *n*-alkanes, accounting for roughly 15% of the total hydrocarbons in kelp blades. Lesser, yet significant, concentrations of *n*-heptadecane were observed. Alkanes within the range C_{18}–C_{22} were generally absent. Saturated molecules with chain lengths greater than C-32 were also poorly represented. Odd-number carbon chain length compounds occurred in greater abundance than did even-number alkanes, resulting in high carbon preference indices. All the above characteristics are common to other brown algae species [2, 3]. Samples collected from two locations, San Miguel Island and Coal Oil Point, exhibited *n*-alkane profiles which deviated from the above, and suggest the presence of petroleum contamination. Total hydrocarbon levels in these samples were also elevated when compared to others. Several characteristic components of petroleum were also present in suspect samples, and absent from others viz. (a) a mixture of unresolved complex (branched and cyclic) hydrocarbons (U.C.M.), particularly pronounced in saturate fractions; (b) significant concentrations of *n*-alkanes in the range C_{18}–C_{22}; simultaneous occurrence of pristane and phytane (trace quantities detected only in suspect kelp samples); (c) reduced predominance of odd-number chain length *n*-alkanes C_{23}–C_{30} [4]. Retention indices of polynuclear aromatic hydrocarbon standards did not correlate with those of any compounds presently noted in *M. pyrifera* blades. Aromatic hydrocarbons have been detected in severely polluted marine Chlorophyceae, but were absent from algae sampled from pristine locations [4]. One must stress