Phytochemistry, 1975, Vol. 14, pp. 953–956. Pergamon Press. Printed in England.

LYTIC BODIES FROM CEREALS HYDROLYSING MALTOSE AND STARCH

CLIFFORD A. ADAMS, TERENCE G. WATSON and LAWRENCE NOVELLIE

National Chemical Research Laboratory, Council for Scientific and Industrial Research, P.O. Box 395, Pretoria, South Africa

(*Revised Received* 2 *October* 1974)

Key Word Index—Gramineae; cereals; lytic bodies; maltase; carbohydrases; starch degradation.

Abstract—Protein bodies and spherosomes from sorghum contained carbohydrase activity against maltose, starch and *p*-nitrophenyl-α-D-glucoside. Maltase activities in sorghum and also in maize lytic bodies were very high; carbohydrase activities of lytic bodies from whole wheat, whole barley, sorghum aleurone, sorghum embryo and maize embryo were considerably lower. The pH response of sorghum lytic bodies was bimodal with an optimum in the range of 3·4–4·2 and a minimum or a shoulder near pH 3·8. Protein bodies from sorghum, maize, wheat and barley reduced the iodine-colouring capacity of soluble starch to give a purple colour typical of a β-limit dextrin. With spherosomes colour reduction was usually more rapid, eventually taking the breakdown of starch beyond the achroic point. The lytic bodies produce both maltose and glucose from starch, except in the case of maize when only glucose was found. The data suggest that protein bodies contain a linked β-amylase–maltase system and that spherosomes contain a linked α-amylase–maltase system.

INTRODUCTION

Maltase (α-D-glucoside glucohydrolase, E.C. 3.2.1.20) activity has been detected in a variety of ungerminated cereals; barley [1], buckwheat [2], rice [3], sorghum [4] and maize [5]. The maltase preparations from ungerminated buckwheat [6], rice [3] and from malted barley [7] were also capable of hydrolysing soluble starch, yielding glucose. Sorghum maltase is insoluble in water, but active in the insoluble state [4]. This insoluble maltase has been detected in two sub-cellular lytic bodies prepared from sorghum grain and designated protein bodies and spherosomes [8]. The present experiments have now shown that these lytic bodies contain a very active maltase and are probably the sub-cellular location of sorghum maltase. Furthermore, a starch hydrolysing activity is also associated with these sub-cellular organelles and the combined carbohydrase activities are possibly of significance in the early stages of germination.

RESULTS

Maltase activity was high in lytic bodies prepared from whole sorghum and maize (Table 1) but was much lower in those from wheat and barley. The sorghum and maize maltase was solubilized by some of the extracting media used by Watson and Novellie [4]. Lytic bodies prepared from sorghum aleurone tissue, sorghum embryos and maize embryos were much lower in maltase activity (Table 2) although still more active than the lytic bodies obtained from wheat or barley (Table 1). When soluble starch was used as a substrate, glucose was produced by sub-cellular particles from all cereals tested although sorghum was most active (Table 1).

Enzyme activity against maltose, *p*-nitrophenyl-α-D-glucoside and starch is optimal in the pH range of 3·4–4·2 for sorghum protein bodies. The hydrolysis of starch shows a distinct reproducible minimum at about pH 3·8. Maltase activity has a shoulder at pH 3·8 and activity with *p*-nitrophenyl-

Table 1. Maltase and starch hydrolysing activities of lytic bodies from various cereals

Cereal	Lytic bodies	Maltase activity (nmol Maltose/min/mg dry wt) Direct	Extracted (1)	Extracted (2)	Starch hydrolysing activity (nmol glucose/min/mg protein)
Sorghum	Protein body	19·3	33·3	2·2	12·9
	Spherosome	30·2	30·3	19·6	32·4
Maize	Protein body	30·1	39·9	1·2	1·1
	Spherosome	98·4	197·6	0·8	18·5
Wheat	Protein body	0·1	0·3	0	2·7
	Spherosome	0·2	0·2	0	17·1
Barley	Protein body	0	0·1		1·4
	Spherosome	0·3	0·1		7·2

Direct activity is that of dry bodies incubated in reaction mixture. Extracted (1) activity was solubilized by extracting dry bodies in 6 M urea and 0·1 M Na_2SO_3, pH 7 at 0° for 2 hr. Extracted (2) activity was solubilized by extracting dry bodies in M NaCl, pH 9·3 at 0° for 2 hr.

α-D-glucoside also shows a slight but reproducible decline at pH 3·8. Sorghum spherosomes have a somewhat similar pH response for hydrolysis of starch and maltose, showing a minimum close to pH 3·8. However, activity with *p*-nitrophenyl-α-D-glucoside was quite different and had a pH optimum of 5.

The starch hydrolysing activity of the sorghum lytic bodies was further substantiated by following the reduction of the iodine-colouring capacity of a solution of soluble starch. Protein bodies catalysed an initial rapid decline (from $A565 = 0·87$) in A for about 1 hr, followed over the next 4 hr by a more gradual loss (to $A565 = 0·59$). The control samples also showed a small initial decrease in A. This was probably caused by some residual activity in the particles. The protein bodies are highly insoluble organelles [8], possibly somewhat resistant to heat denaturation. Similar results were obtained for the protein bodies of wheat, barley and maize. Doubling the concentration of the protein particles and extending the reaction time, brought the starch breakdown to a point where iodine gave a purple colour typical of a β-limit dextrin. Even a reaction time of 24 hr did not produce any further breakdown.

Table 2. Maltase activity of lytic bodies from aleurone and embryo tissue

Lytic bodies	Source of lytic bodies: Sorghum aleurone	Sorghum embryo	Maize embryo
Protein body	4·3	3·3	7·7
Spherosomes	1·2	2·0	6·7

Activity is that of dry bodies incubated in reaction mixture and is expressed as: nmol maltose utilized/min/mg dry wt.

With sorghum spherosomes, the initial fall in A was usually more rapid than with the protein bodies and progressed further in 5 hr from $A565 = 1$ to $A565 = 0·28$. Lengthening the reaction time took the breakdown beyond the achroic point. In contrast to the protein bodies, spherosome control samples showed no starch degrading activity. The spherosomes have more soluble protein than the protein bodies [8] and are probably more readily denatured by heat.

PC revealed that protein bodies and spherosomes produced both maltose and glucose from soluble starch except in the case of those from maize where only glucose could be found. The maltase activities of the maize particles are the highest which have so far been observed.

DISCUSSION

The maltase activity per weight of dry lytic bodies is from 3–6 × greater than that reported in whole grain by Watson and Novellie [4]. Therefore, a considerable enrichment in maltase activity was obtained by preparing these sub-cellular particles. This would be expected if the maltase of whole grain was located in these particles. Lytic bodies from sorghum embryos and aleurone tissue

were relatively poor in maltase (Table 2) suggesting that most of the maltase-containing particles are located in the endosperm. Maize has a similar distribution and rice endosperm is known to contain maltase [9]. Wheat and barley, however, appear significantly different in that no enrichment of maltase activity was achieved by preparing the lytic bodies. Also Jørgensen [1] has shown by histochemical techniques that barley maltase is located in the embryo and aleurone tissue, rather than in the endosperm. Furthermore, barley maltase could not be extracted in the same media as sorghum maltase [4], again suggesting a fundamental difference between sorghum and barley.

Spherosomes from whole grain were more active than protein bodies in their carbohydrase activities (Table 1). This was also the case for several other enzymes previously studied in sorghum lytic bodies [8]. Both protein bodies and spherosomes have their pH optima for maltose and starch hydrolysis in a range similar to that reported for rice [3] and maize [5]. Furthermore, the pH activity curves resemble those reported for α-glucosidase activity in extracts of mature maize [5]. In both cases a bimodal pH curve with a minimum at about pH 3·8 was observed. This indicates a close similarity in the α-glucosidase activities of sorghum and maize and also suggests that more than one enzyme is involved. Further work is needed to explain this unusual pH effect. The hydrolysis of *p*-nitrophenyl-α-D-glucoside by spherosomes is possibly catalysed by other glycosidases as well as by maltase which would account for the observed pH response.

The pattern of starch breakdown shown by protein bodies is consistent with the action of β-amylase producing a β-limit dextrin. Spherosomes hydrolyse starch in a manner consistent with the action of α-amylase reducing the starch finally beyond the achroic point. The occurrence of maltose and glucose in the reaction mixture after starch hydrolysis suggests that a linked amylase–maltase system is operative. In the case of maize, the extremely high maltase activity is probably responsible for the failure to detect maltose in the digests.

It is clear that lytic bodies prepared from ungerminated seeds of sorghum and other cereals contain the necessary enzymes to produce glucose from soluble starch. It is known that *in vivo* undamaged granular starch is attacked by α-amylase only and not by β-amylase. Sandstedt's work [10] has clearly shown the granules to be etched by the α-amylase. The material removed by this action apparently diffuses away. A study of sorghum starch granules during germination (Novellie and Malherbe unpublished results) reveals etching followed by granule disintegration. β-Amylase is known to attack fragmented granules [10]; it will certainly attack solubilized degradation products [11]. The maltose so produced will be attacked by the α-glucosidase also present in the lytic bodies yielding glucose. It has already been shown that the organelles from sorghum have the ability to produce, autolytically, amino acids and inorganic phosphate [8]. These lytic bodies containing preformed enzymes, therefore, are capable of generating the basic metabolites necessary for germination and growth, namely amino acids, inorganic phosphate and glucose. Consequently, they are likely to be of significance at the onset of germination.

EXPERIMENTAL

Material. Barnard's Red sorghum (*Sorghum bicolor* (L.) Moench); white maize (*Zea mays* L.); barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.). Aleurone tissue and embryos were prepared as previously described [12].

Preparation of lytic bodies. The procedure described in [12] was followed. The process essentially involves firstly, homogenization of an Me_2CO powder in 1% $Na_2S_2O_5$ soln. The protein bodies are then separated by differential centrifugation and the spherosomes precipitated from the final supernatant by addition of Me_2CO. Samples for assays based on dry wt were dehydrated in Me_2CO and air-dried before use. Samples for assays based on wt of protein, were transferred without drying to 0·1 M citrate buffer pH 6·2 and used as a suspension.

Enzyme assays. Maltase was assayed with maltose as a substrate according to [4]. The reaction mixture for starch hydrolysis contained: 5 ml of 2% soluble starch in 0·1 M citrate buffer pH 3·75 and 1 ml of a suspension of the lytic bodies. The mixture was incubated at 40° for 45 min and then 1 ml aliquots removed and added to 1 ml of 0·2 M NaOH. Denatured protein was sedimented by centrifugation and the glucose content of the supernatant determined as in the maltase assay. The starch hydrolysis reaction mixture was also used to measure the decrease of the starch–I_2 complex [13]. In this case incubation was for 5 hr at 40°, and 0·5 ml aliquots were removed at 30 min intervals and added to 0·5 ml of 0·2 M NaOH. Denatured protein was sedimented by centrifugation and 0·1 ml of the supernatant mixed with 10 ml of I_2 reagent. The *A* at 565 nm was then determined.

PC. The vol. of starch hydrolysis mixture was increased × 5, and incubated for 45 min at 40°. The reaction was terminated by adding 120 ml of EtOH. After centrifugation, the supernatant mixtures were evaporated to dryness and dissolved in 5 ml of EtOH–H_2O (4:1). This soln was examined by descending PC

using EtOAc–C_5H_5N–H_2O (10:4:3). The separated sugars were visualized by spraying with *p*-anisidine solution.

Protein determination. All suspensions were assayed using the method of McGrath [14].

Acknowledgement—The authors are grateful to Miss Gerlinde Klamp for technical assistance.

REFERENCES

1. Jørgensen, O. B. (1965) *Acta Chem. Scand.* **19,** 1014.
2. Takahashi, M. and Shimomura, T. (1968) *Agr. Biol. Chem.* **32,** 923.
3. Takahashi, N., Shimomura, T. and Chiba, S. (1971) *Agr. Biol. Chem.* **35,** 2015.
4. Watson, T. G. and Novellie, L. (1974) *Phytochemistry* **13,** 1037.
5. Marshall, J. J. and Taylor, P. M. (1971) *Biochem. Biophys. Res. Commun.* **42,** 173.
6. Takahashi, M. and Shimomura, T. (1968) *Agr. Biol. Chem.* **32,** 929.
7. Jørgensen, O. B. (1964) *Acta Chem. Scand.* **18,** 1975.
8. Adams, C. A. and Novellie, L. (1975) *Plant Physiol.* **55,** 1.
9. Nomura, T., Kono, Y. and Akazawa, T. (1969) *Plant Physiol.* **44,** 765.
10. Sanstedt, R. M. (1955) Supplement to *Cereal Chem.* **32,** "Presentation of the Thomas Burr Osborne Medal to R. M. Sandstedt".
11. Novellie, L. and Schütte, R. J. (1961) *J. Sci. Food Agr.* **12,** 552.
12. Adams, C. A. and Novellie, L. (1974) *Plant Physiol.* **55,** 7.
13. Briggs, D. E. (1967) *J. Inst. Brew.* **73,** 361.
14. McGrath, R. (1972) *Anal. Biochem.* **49,** 95.