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CARBOHYDRATE METABOLISM IN *CUSCUTA*

D. V. SINGH, M. U. BEG, R. L. MATTOO, R. K. LAL, P. N. VISWANATHAN and P. S. KRISHNAN

Department of Biochemistry, Lucknow University, Lucknow, U.P., India

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Abstract—There are significant variations in the activities of enzymes of carbohydrate metabolism along the filaments of the parasite *Cuscuta reflexa* Roxb. Functionally, the filament is broadly separable with respect to the host into a proximal region and a distal region. The proximal region has an enzyme make-up preferentially directed to the elaboration of starch; the distal region is more suited for the catabolism of carbohydrate. The filament curled about the host contains a higher proportion of tissue protein in mitochondria and a higher oxidative and energy-trapping ability, in comparison with the apical region of filament.

INTRODUCTION

CONSIDERABLE differences have been found to exist in the chemical composition in different regions of the filaments of the parasite *Cuscuta reflexa*.[1-3] Differences were therefore to be expected also in the activities of enzymes along the filaments. The following is a report on the activities of enzymes of carbohydrate metabolism in various regions of the filaments of *C. reflexa*.

RESULTS

The results of determining the activity of various enzymes are recorded in Table 1. The analyses were all carried out in the proportionality region and at the respective pH optima. The pH optima for acid and alkaline phosphatase with glycerophosphate as substrate, fructose diphosphatase, phytase, phosphorylase, invertases, amylases and cytochrome *c* oxidase were experimentally determined. For assay of the other activities, the pH optima reported in literature were employed.

When the activities of two segments were found to vary by a factor of 5 or more, the assays were repeated with mixtures of the two homogenates. The observation that the mixture had an activity roughly additive, calculated from the activities of the individual homogenates (within a margin of about 15 per cent), was taken as an indication that neither activator nor inhibitor was present in either of the segments.

Enzymes of Starch Metabolism

Starch phosphorylase activity was highest in the apical portion, decreasing regularly towards the haustoria-bearing region, as observed earlier.[2] The activity in the apical region was 3-fold as high as in the haustoria-bearing region.

UDPG–starch synthetase activity was maximum in the after-haustorial region, followed by the haustoria region, while the near apical and apical regions were markedly lower in activity, with the lowest activity in the latter. The activity in the after-haustorial region was almost 4-fold that in the apical region. When the activity was calculated in terms of starch

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TABLE 1. BIOCHEMICAL HETEROGENEITY ALONG THE LENGTH OF THE VINES OF *Cuscuta reflexa*

Enzyme	Enzyme activity in regions of the filament (units/mg/protein or, in brackets, units/mg dry wt.)			
	Apical	Near apical	After-haustorial	Haustoria-bearing
Starch phosphorylase (EC 2.4.1.1, α-1,4-glucan: orthophosphate glucosyl transferase)	0·93 (0·093)	0·86 (0·059)	0·65 (0·034)	0·32 (0·013)
Starch synthetase (EC 2.4.1.21, UDP-glucose (ADP-glucose): α-1,4-glucan-4-glucosyl transferase):				
(*a*) UDP-glucose	0·094 (0·007)	0·14 (0·008)	0·35 (0·010)	0·18 (0·005)
(*b*) ADP-glucose	0·077 (0·006)	0·14 (0·008)	0·28 (0·012)	0·18 (0·005)
Amylase				
(*a*) "α"- (EC 3.2.1.1, α-1,4-glucan 4-glucanohydrolase)	5·67 (0·57)	7·14 (0·48)	4·40 (0·23)	3·10 (0·18)
(*b*) "β"- (EC 3.2.1.2, α-1,4-glucan maltohydrolase)	5·39 (0·53)	3·81 (0·25)	4·40 (0·23)	3·64 (0·14)
Maltase (EC 3.2.1.20, α-D-glucoside glucohydrolase)	1·88 (0·15)	10·50 (0·51)	14·08 (0·53)	18·88 (0·53)
UDPG/ADPG-pyrophosphorylases: (EC 2.7.7.9, UTP (ATP): α-D-glucose-1-phosphate uridylyl (adenylyl) transferase)				
(*a*) UDPG-Pyrophosphorylase	0·44 (0·044)	0·84 (0·056)	3·32 (0·17)	0·80 (0·033)
(*b*) ADPG-Pyrophosphorylase	0·44 (0·044)	1·03 (0·070)	3·13 (0·16)	2·41 (0·10)
Alkalaine phosphatase towards:				
(*a*) Fructosediphosphate (EC 3.1.3.11, D-fructose-1,6-diphosphate-1-phosphohydrolase)	2·05 (0·19)	2·72 (0·13)	1·54 (0·052)	0·86 (0·037)
(*b*) β-Glycerophosphate (EC 3.1.3.1, orthophosphoric monoester phosphohydrolase)	2·54 (0·23)	3·39 (0·14)	2·13 (0·073)	1·22 (0·053)
Sucrose phosphate synthetase (EC 2.4.1.14, UDP-glucose: D-fructose, 6-phosphate 2-glucosyl transferase)	0·82 (0·082)	0·55 (0·037)	0·46 (0·024)	0·42 (0·015)
Sucrose synthetase (EC 2.4.1.13, UDP-glucose: D-fructose, 2-glucosyl transferase)	0·29 (0·030)	0·80 (0·058)	1·34 (0·044)	1·20 (0·027)
Invertase (EC 3.2.1.26, β-fructofuranoside fructohydrolase)				
(*a*) Acidic	5·3 (0·59)	7·5 (0·37)	4·9 (0·13)	5·5 (0·16)
(*b*) Neutral	3·1 (0·36)	5·8 (0·22)	3·0 (0·087)	2·8 (0·078)
Acid phosphatase towards:				
(*a*) Phytic acid (EC 3.1.3.8, *meso*-inositol-hexaphosphate phospho-hydrolase)	0·08 (0·012)	0·17 (0·013)	0·15 (0·008)	0·15 (0·007)
(*b*) β-Glycerophosphate (EC 3.1.3.2, orthophosphoric monoester-phosphohydrolase)	0·80 (0·064)	1·65 (0·13)	1·20 (0·066)	1·40 (0·062)

TABLE 1—*continued*

Enzyme	Enzyme activity in regions of the filament (units/mg/protein or, in brackets, units/mg dry wt.)			
	Apical	Near apical	After-haustorial	Haustoria-bearing
Hexokinase, towards:				
(*a*) Glucose (EC 2.7.1.2, ATP:D-glucose-6-phosphotransferase)	1·17 (0·093)	1·88 (0·091)	4·39 (0·17)	9·90 (0·28)
(*b*) Fructose (EC 2.7.1.4, ATP:D-fructose-6-phophotransferase)	3·51 (0·28)	4·67 (0·22)	10·70 (0·40)	19·68 (0·55)
Phosphohexoisomerase (EC 5.3.1.9, D-glucose 6-phosphate Ketol-isomerase)	2·74 (0.22)	3·06 (0·14)	3·71 (0·14)	2·71 (0·076)
Phophoglucomutase (EC 2.7.5.1, α-D-glucose-1-6-diphosphate α-D-glucose-1-phosphate phosphotransferase)	18·65 (1·52)	15·51 (0·73)	12·81 (0·47)	3·60 (0·10)
Mitochondrial activity				
Yield of mitochondrial protein (%)	5·0	6·3	6·5	8·0
Cytochrome oxidase (EC 1.9.3.1, cytochrome c:O_2 oxidoreductase) (μmole/min)	0·017 (0·0017)	0·021 (0·0014)	0·024 (0·0013)	0·033 (0·0013)
Oxidative phosphorylation, succinate as substrate (μatom/hr)				
Orthophosphate uptake	0·054 (0·0054)	0·048 (0·0033)	0·066 (0·0035)	0·121 (0·0050)
Oxygen uptake	0·036 (0·0036)	0·034 (0·0023)	0·050 (0·0026)	0·080 (0·0033)
P:O ratio	1·50	1·40	1·40	1·50

The part of the parasite curling round the host constituted the haustoria-bearing region. A 10-cm terminal length of the filament constituted the apical region. The intervening portion, measuring about 40 cm, was cut into two equal lengths, the sample adjacent to the curling portion designated as after-haustorial and the other as the near-apical region.

Enzyme assays in cloth-strained homogenates were as reported in text. The activities in the isolated starch granules and mitochondria were related to the protein in the particular region of the filament. The yield of mitochondrial protein was calculated as % of the protein in the tissue sample.

The various assays were carried out with different samples of filaments during a period of 2 months. There were changes in the protein content, so that the relation between enzymic activity in terms of protein and % dry solids of tissue was not a constant in the different estimations.

or protein in the preparation of the isolated granules, the activity was again the highest in the after-haustorial region. ADPG–starch synthetase activity followed the same pattern, the activity being almost the same as with UDPG.

The amylase activity in the apical region was found to have pH optimum at 5·5 (acetate buffer) and 7·0 (phosphate buffer). On the basis of the influence of 1×10^{-4} M $HgCl_2$, stability at acid pH, heat treatment in the presence of Ca^{2+} and rate of lowering of blue value on incubation with starch, it was concluded that the filament possessed both α- and β-amylase activity,[4a–f] the former with optimum at pH 7·0 and the latter at pH 5·5. The two activities were of

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almost equal magnitude in the various segments except in the near-apical region in which α-amylase was twice as active as β-amylase. There was a tendency for maximum amylase activity in or near the apical region, the haustoria-bearing region having about half the activity of the apical region.

Maltase activity was 10-fold as high in the haustoria-bearing as in the apical region. UDPG- and ADPG-pyrophosphorylases were maximum in the after-haustorial region with just 1/7 of the activity in the apical region. ADPG-pyrophosphorylase differed from UDPG-pyrophosphorylase in being highly active also in the haustoria-bearing region. Alkaline phosphatase, tested with both fructose-1,6-diphosphate and β-glycerophosphate, was about 3-fold as active in the near-apical as in haustoria-bearing region.

Enzymes of Sucrose Metabolism

Sucrose–phosphate synthetase was maximum in the apical region, with about one-half the activity in the haustoria-bearing region. Sucrose synthetase, on the other hand, was present in the highest concentration in the after-haustorial region, where its activity was over 5-fold that in the apical region.

Acid phosphatase activity (assayed against either phytic acid or β-glycerophosphate) was nearly doubled when passing from the apical to the adjoining region of filament and thereafter the enzymic activity was more or less unaltered.

Invertase activity had two pH optima in every segment of the vine, one "acidic" (pH 5·0–5·5) and the other "neutral" (pH 6·5–7·0). The activity at the neutral optimum was slightly higher than that at the acidic optimum, except in the after-haustorial region, in which the latter activity was higher than the former. Both types of invertases were maximally active in the near-apical region. The acidic activity in the haustoria-bearing region was about 3/4 and the neutral activity 1/2 of that in the near-apical region.

Enzymes of Hexose Metabolism

Glucokinase and fructokinase were maximally active in the haustoria-bearing region, with respectively 1/8 and 1/5 of the activity in the apical region. Phosphoglucomutase activity was 5-fold as high in the apical as in the haustoria-bearing region. Phosphohexo-isomerase was most active in the after-haustorial region, but the lowering observed in the other regions was not marked.

Mitochondrial Activity

A higher proportion of tissue protein was present in the mitochondria in the haustoria-bearing than in the apical region. In the experiment reported, the increase was 60 per cent, but the range (five experiments) was 50–150 per cent.

Cytochrome *c* oxidase was 2-fold as active in the haustoria-bearing as in the apical region, calculated in terms of the tissue protein in the respective regions of the parasite filament.

The rate of phosphate esterification and oxygen uptake when the mitochondria oxidized succinate was 2-fold as high in the preparation from the haustoria-bearing as in that from the apical region, when the calculations were in terms of tissue protein. The P:O ratio was essentially a constant.

DISCUSSION

Metabolism of Starch

Phosphorylase may play an essential role in the synthesis of starch *ab initio* in the proximal region of filament. The lowering in orthophosphate concentration, necessary for the syn-

thesis of starch from glucose-1-phosphate,[5a] will be mediated through the increased activity of mitochondria in this region and the increased formation of phytic acid (unpublished data). The high phosphatase activity in this region may, however, operate by hydrolysing esters and increasing the concentration of orthophosphate. The concentration of orthophosphate is not known with certainty in the different segments of the vine. The malto-oligosaccharides needed as primer may be formed by reversal of maltase activity.[5b] The after-haustorial region had the highest UDPG/ADPG pyrophosphorylase activity and sucrose synthetase activity—two pathways whereby glycosyl donors for starch synthesis are elaborated. Sucrose could enter the reaction pathway also after action of invertase followed by hexokinase, although additional expenditure of energy would be involved in the process.

The after-haustorial region was thus ideally suited for the synthesis of starch from translocated sucrose by the pathway of de Fekete and Cardini.[6] The content of starch relative to protein, as determined in cloth-strained homogenates, was nearly the same in the haustoria-bearing and the after-haustorial regions and higher than the rest of the vine (0·76, 0·72, 0·65 and 0·51, as mg/mg tissue protein, respectively in the haustoria-bearing, after-haustorial, near-apical and apical regions; the corresponding values in mg/g dry solids were 33, 40, 49 and 75). The synthesis of the polysaccharide from hexoses was more favoured in the after-haustorial region and the haustoria-bearing region than in the other two regions also because of the favourable ratio existing between phosphoglucomutase and pyrophosphorylase activities, two enzymes competing for glucose-1-phosphate.[7] The occurrence of fructose diphosphatase was suggestive of the active operation of the pentose phosphate reductive pathway[8] and of gluconeogenesis.[9]

A high phosphorylase activity in the terminal portion of the vine would, by phosphorolytic action, channel the amyloplast starch and any chloroplast starch to the metabolizable form of sugar phosphate. The "endoenzymic" activity of α-amylase, together with the "exoenzymic" activity of β-amylase, could effect the hydrolytic decomposition of starch to maltose units.

Metabolism of Sucrose

The concentration of sucrose phosphate synthetase in the apical region and of sucrose synthetase in the after-haustorial region would be understandable if the former enzyme were to be actively involved in the photosynthetic formation of sucrose in the apical region and the latter enzyme involved in UDPG generation in the after-haustorial region. *Cuscuta* filaments contain chlorophyll and are capable of carrying out photosynthesis, though at a much slower rate than the host.[10a]

Acid phosphatase might be effective in the hydrolysis of sucrose phosphate. It may be involved also in the release of orthophosphate from phytic acid; a role in the hydrolysis of ATP is also likely. Invertase activity, by conversion of sucrose to hexoses, would permit utilization for catabolic purposes. The enzyme may play an active role also in the transport of sugar.[10b]

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Metabolism of Hexoses

The maximally occurring hexokinase activity in the haustoria-bearing region and the higher activity in the after-haustorial region in comparison with the rest of the vine suggested that priming reaction proceeded at a fast rate in these regions.

The lowered level of hexokinase in the apical region may mean that the phosphorolytic degradation of starch played an important role, although the activity of amylase was higher than that of phosphorylase. The maximally occurring activity of phosphoglucomutase emphasized the transformation of hexose along the catabolic pathway in the apical region of filament.

Mitochondrial Activity

The increased content of mitochondria in the haustoria-bearing region, in relation to the other regions, of the parasite filaments suggested that in spite of its physiological age the proximal part of the filament possessed the maximum oxidative and energy trapping ability. The ATP generated in the process will enable a number of biosynthetic reactions to be initiated in the filaments, inclusive of those relating to the elaboration of starch and of phytic acid. The activity of mitochondria may also enable the uptake of cations and metabolites from the tissues of host. The energy of ATP, may, by analogy with the reactions in pea tendril,[11] be expended in the coiling by the filaments associated with the protrustion of haustoria.

Conclusion

The observed profile of enzymic and mitochondrial activity suggested that different phases of carbohydrate metabolism tended to be accentuated in different regions of the vine. The proximal part of vine with reference to the endophytic system had a dominant role in the elaboration of starch while the distal, unattached, part was equipped for the utilization of carbohydrate. The filament curled round the host played an active role also in the process of parasitization.

EXPERIMENTAL

Plant Material

Cuscuta reflexa Roxb. was harvested from *Lantana camara* Linn. The arbitrary demarcation of the filaments into four regions was after Singh *et al.*[1,2]

Preparation of Homogenates

Ten per cent (w/v) cloth-strained homogenates in neutralized 0·017 M cysteine were used in the assays of all enzymes, except those associated with starch granules and mitochondria. Tissue dispersion was effected with a Waring blender.

Isolation of Starch Granules

Granules were isolated from homogenates prepared in mannitol, (0·4 M) citrate, (0·05 M) cysteine, (0·0167 M) medium, with the aid of a Waring blender, according to Pottinger and Oliver[12] and used in the determination of starch synthetase activity.

Isolation of Mitochondria

Mitochondria were isolated in the form of particles sedimenting between 1600 *g* and 15,000 *g* from homogenates prepared in 0·25 M sucrose containing 0·37 M mannitol, 0·05 M phosphate buffer, pH 7·5, 0·005 M EDTA, 0·006 M cysteine, 0·1% bovine serum albumin and 1% polyvinylpyrrolidone of average mol. wt. 40,000, employing a mortar and pestle for cell dispersion.

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Enzyme Assays

The enzymes assayed were starch phosphorylase,[13] ADPG/UDPG starch synthetase,[14] amylase,[15] maltase,[16] ADPG- and UDPG-pyrophosphorylase,[17] fructosediophosphatase,[18] sucrose synthetase and sucrose phosphate synthetase,[19] acid phosphatase,[20] invertase,[10] phosphokinase activity towards glucose and fructose,[21] phosphohexoisomerase and phosphoglucomutase.[22] Cytochrome *c* oxidase activity of mitochondria was assayed according to Cooperstein and Lazarow,[23] and oxidative phosphorylation according to Pierpoint[24] using succinate as substrate.

Unit of Enzyme Activity and Specific Activity

The unit of enzymic activity, except in the case of cytochrome *c* oxidase, was the conversion of a μmole of substrate, or its equivalent, in 30 min at 30° under the assay conditions. Cytochrome *c* oxidase activity was calculated according to Yonetani.[25] The enzymic activities of starch granules and mitochondria were related to mg protein in homogenate.

Protein determination using the phenol reagent was according to Khanna *et al.*[26] and Mattoo.[27]

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