

Disproportionating Transglycosylase (D-Enzyme) in Green Algae and Cyanobacteria. Partial Purification and Characterization

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D-Enzyme (4- α -glucanotransferase, EC 2.4.1.25) from cultured symbiotic *Nostoc* and *Chlorella* has been partially purified and characterized. The enzyme catalyzes the disproportionation of maltooligosaccharides and is able to form maltooligosaccharides from soluble starch and D-glucose. The properties of D-enzyme from *Nostoc* and *Chlorella* are similar with respect to substrate specificity, K_M values and pH dependence, but differ with respect to temperature optimum and molecular weight (40 °C/50 kDa and 50 °C/230 kDa for the enzyme from *Nostoc* and *Chlorella*, respectively). D-enzyme activity has been demonstrated also in free-living *Anabaena*, *Chlorella* and *Chlamydomonas*. Its physiological role in symbiotic *Nostoc* is briefly discussed.

Introduction

D-Enzyme is a transglycosylase disproportionating maltooligosaccharides in chain length, *e.g.* forming glucose and maltopentaose from two molecules of maltotriose by a maltosyltransfer (Peat *et al.*, 1956; Jones and Whelan, 1969). Its suggested physiological role is to provide maltooligosaccharides sufficiently long for phosphorolytic breakdown from those too short (degree of polymerization (DP) ≤ 4) to be a substrate for phosphorylase (Lee *et al.*, 1970; Kakefuda *et al.*, 1986). Activity of D-enzyme has been shown in several higher plants where it has been found in different organs (Peat *et al.*, 1956; Kakefuda *et al.*, 1986; Manners and Rowe, 1969; Okita *et al.*, 1979).

Since in the disproportionation of maltodextrins by D-enzyme free glucose appears as a reaction product, this reaction may be important for symbiotic phototrophs providing their host with glucose as do *e.g.* the cyanobacterial photobionts of lichens (Drew and Smith, 1967; Hill and Smith, 1972). In the present work D-enzyme from the cultivated cyanobiont, a *Nostoc* sp., of the lichen *Peltigera horizontalis* was partially purified and characterized,

and, in addition, from a *Chlorella* sp. isolated from the heliozoon *Acanthocystis turfacea*. To see whether disproportionating transglycosylase is of more general occurrence, tests were made for this enzyme also with cell-free extracts of two free-living strains of green algae and of a free-living cyanobacterium.

Materials and Methods

Plant material

Nostoc sp. isolated from the lichen *Peltigera horizontalis* (Meindl and Loos, 1990) was cultured in medium BG 11 of Stanier *et al.* (1971) in which NaNO₃ was substituted by NaCl (1 mM). Other culture conditions were as those used by Fischer *et al.* (1989). Filaments were harvested 7–13 d after inoculation in the beginning stationary phase (Bogner *et al.*, 1993).

Anabaena sp. strain PCC 7120 originated from the culture collection of Dr. P. Wolk (East Lansing, U.S.A.) and was cultured and harvested as *Nostoc*. From Sammlung für Algenkulturen Göttingen, F.R.G. were obtained *Chlamydomonas noctigama* strain 35.72 and *Chlorella* sp. strain 3.83 which is the endosymbiont of the heliozoon *Acanthocystis turfacea*. *Chlorella kessleri* was kindly supplied by Dr. N. Sauer from our institute. Liquid mineral medium according to Matzke *et al.* (1990) and Kuhl (1962) was used for growing *Chlorella* sp. 3.83 and the other strains of green algae, respectively. Other conditions were as de-

Abbreviations: DP, degree of polymerization; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate.

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scribed previously (Fischer *et al.*, 1989). Cells of *Chlorella* and *Chlamydomonas* were collected by centrifugation 4–5 d after inoculation when cell densities between 2.0 and 4.0 μl packed cells $\cdot\text{ml}^{-1}$ had been obtained.

Purification of D-enzyme; estimation of molecular weight

To purify D-enzyme from *Nostoc* the soluble cell fraction was prepared after Bogner *et al.* (1993) except that the cells were ruptured by ultrasonic treatment (30 min, Branson sonifier B-12 set at maximal sonic output); the final dialysis was against 20 mM potassium phosphate buffer pH 6.8. Dialysate containing between 40 and 60 mg protein was applied to a hydroxyl apatite column (length 30 cm, diameter 2.2 cm) and eluted with a gradient of potassium phosphate buffer from 20 mM (pH 6.8) to 1.1 M (pH 5.8). The flow rate was 1.25 ml/min and fractions of 5 ml were collected. The active fractions were pooled, concentrated and the buffer exchanged against 20 mM imidazole pH/6.8 by ultrafiltration (Centriprep-30, Amicon). The purification proceeded with an anion exchange column (Mono Q HR 5/5 column, Pharmacia) run at 1 ml/min with a gradient from zero to 1.1 M NaCl in 20 mM imidazole pH 6.8; the fraction size was 1.0 ml. Both columns were operated with the help of a FPLC chromatographic system (Pharmacia) at room temperature whereas all other operations (except cell breakage and initial centrifugations, see Bogner *et al.*, 1993) were carried out at 0–4 °C. For purification of D-enzyme from *Chlorella* 3.83 the algal cells were washed once with 0.1 M potassium phosphate buffer (pH 6.8), suspended in fresh buffer to give a density of 150 μl packed cells $\cdot\text{ml}^{-1}$ and were broken by two passages through a French press at 140 MPa. The homogenate was centrifuged at $48,000 \times g$ for 20 min and the resulting supernatant further at $113,000 \times g$ for 1 h. The final supernatant was brought to 30% saturation of ammonium sulfate and, after removal of the greenish precipitate by centrifugation, was extensively dialysed against 20 mM imidazole/HCl pH 6.8. Further purification was achieved by column chromatography on hydroxyl apatite and anion exchange resin as described above. All procedures were carried out at 0–4 °C except for column chromatography.

The relative molecular mass of the D-enzyme from *Nostoc* and from *Chlorella* was estimated by gel filtration on a superose 12 HR 10/30 column (Pharmacia) run with 50 mM imidazole/HCl pH 6.8 at 0.4 ml/min. As molecular weight standards were used apoferritin (443 kDa), β -amylase (200 kDa), aldolase (160 kDa), bovine serum albumin (67 kDa), ovalbumin (45 kDa), cytochrome *c* (12.3 kDa) and vitamin B 12 (1.36 kDa).

Tests for enzymatic activity

Activity of D-enzyme was routinely determined by incubating enzyme preparation in a total volume of 0.35 ml at 30 °C in the presence of 20 mM imidazole/HCl pH 6.8 and 10 mM maltotriose. The reaction was stopped by heat (95 °C, 5 min) after 1 h and the control at time zero. Denaturated protein was removed by centrifugation and aliquots of the supernatant were assayed for glucose by an enzymatic test based on NADP-reduction mediated by hexokinase/glucose-6-phosphate dehydrogenase (Bergmeyer *et al.*, 1974). Checks were made to be sure of linear kinetics with respect to incubation time and enzyme concentration.

Glucosidase activity was tested by incubating enzyme preparation in the presence of 20 mM imidazole/HCl pH 6.8 with 1 mM *p*-nitrophenyl- α -D-glucoside as substrate in a total volume of 0.5 ml for 1 h at 30 °C. The reaction was terminated by addition of 1.0 ml 0.2 M sodium borate buffer pH 9.8 and the absorption of the *p*-nitrophenol formed was read at 405 nm. Activity of amylase was measured with the dinitrosalicylic acid reagent as described previously (Bogner *et al.*, 1974).

Determination of protein

Protein was determined as by Lowry *et al.* (1951) and, for samples of low (1–12 μg) content, as given by Smith *et al.* (1985).

Analysis of reaction products

After heat stop and removal of precipitated protein the reaction mixtures were desalted with ion exchange resins (Serdolit CS-2 and AS 6, Serva, Heidelberg, F.R.G.), concentrated under reduced pressure and subjected to thin-layer chromatography on silica gel sheets. Chromatograms were developed twice in acetone/*n*-butanol/water = 70/15/15 (v/v) and stained with alkaline KMnO_4 .

Radioactive spots were detected with a TLC-scanner (LB 284 from Berthold, Wildbad, F.R.G.).

Preparation of [^{14}C]maltotriose

[^{14}C]maltotriose was prepared by incubating D-[^{14}C]glucose and soluble starch in the presence of D-enzyme as described in the legend to Fig. 4B followed by thin-layer-chromatographic separation (see above). The position of the label in maltotriose was determined by reduction with NaBH_4 followed by hydrolysis in trifluoroacetic acid. Thin-layer chromatography revealed glucitol as the sole radioactive reaction product indicating the label to reside in the reducing end of maltotriose.

Results

D-Enzyme from *Nostoc*

Purification of D-enzyme and isomaltase. When the soluble fraction of a *Nostoc* cell extract was incubated with maltotriose liberation of glucose was observed which, as will be shown later, was due to the action of D-enzyme. A first purification step on a hydroxylapatite column revealed a large activity peak which was used for further studies and a minor one which may be an isoform of D-enzyme (Fig. 1). Besides, at higher phosphate concentrations, a low activity hydrolyzing *p*-nitrophenyl- α -D-glucoside and isomaltase was encountered (Fig. 1). Isomaltase was enriched by hydroxylapatite chromatography (7–10-fold) with good yield, but, because of its low activity was not investigated further. From D-enzyme activity contaminating phycobiliproteids were effectively removed by anion exchange chromatography; no amylase activity could be detected in these preparations. In this stage of purification still around 20 protein bands could be discerned with the sensitive

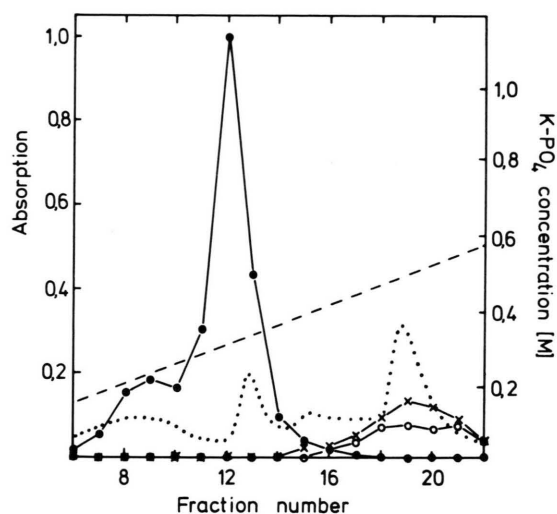


Fig. 1. Elution of D-enzyme activity (A_{340} , ●—●), isomaltase (A_{340} , ○—○), *p*-nitrophenyl- α -glucoside-cleaving activity (A_{405} , x—x) and protein (A_{280} , ·····) from a hydroxylapatite column by a potassium phosphate buffer gradient (---). The soluble fraction of a *Nostoc* homogenate originating from 11.7 g wet weight filaments was applied to the column. The maximum activities were 2.13 and 0.043 $\mu\text{mol/h}\cdot\text{ml}$ fraction for D-enzyme and isomaltase, respectively.

silver stain technique after SDS/polyacryl amide gel electrophoresis. A typical purification scheme is presented in Table I. In the following experiments D-enzyme purified by two column chromatographic steps was routinely used.

Product analyses and substrate specificity. After enzyme action on [^{14}C]maltotriose, thin-layer-chromatographic analysis revealed glucose and maltopentaose as main reaction products besides smaller amounts of higher maltooligosaccharides (Fig. 2A). Both, glucose and maltopentaose, were radioactive reaction products formed from maltotriose labeled in the reducing end; this indicates a

Table I. Enrichment of D-enzyme activity from 7.7 g wet weight *Nostoc* filaments by column chromatography.

Fraction	Protein content [mg]	Specific activity [$\mu\text{mol/h}\cdot\text{mg protein}$]	Yield [%]	Purification
Homogenate (dialyzed soluble fraction)	33.0	0.57	100	1
Hydroxylapatite chromatography, fractions 12 and 13	3.80	2.54	52	4.5
Anion exchange chromatography, fractions 11 and 12	0.247	31.2	41	54.8

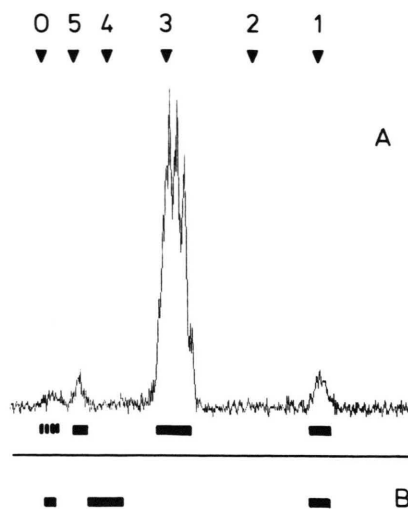
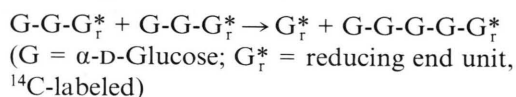


Fig. 2. Thin-layer-chromatographic analysis of a digest of (A) [^{14}C]maltotriose and (B) maltotetraose by D-enzyme from *Nostoc*. The original incubation mixture contained 10 mM oligosaccharide substrate, 20 mM imidazole/HCl pH 6.8 and D-enzyme preparation (43 and 7.4 μg protein/ml in (A) and (B), respectively). Specific radioactivity of maltotriose was $5.3 \cdot 10^5$ Bq/ μmol . The incubation time was 1 h (30 °C); for further details see Materials and Methods. At the top are given the positions of the origin (0) and of reference substances (1 = glucose, 2 = maltose, 3 = maltotriose etc.). Stained regions are indicated by bars, weak staining by a broken bar.

disproportionation took place by transfer of a maltosyl group containing the nonreducing end of the donor maltotriose to another maltotriose molecule according to the following scheme:



Maltotetraose yielded glucose and oligosaccharides with a DP > 6 as reaction products (Fig. 2B); in no case was observed the production of maltose. These patterns of action are characteristic of a disproportionating transglycosylase for which cleavage of the bond at the nonreducing end and of the bond penultimate to the reducing end are "forbidden" [cf. Peat *et al.*, 1956; Jones and Whelan, 1969].

When the *Nostoc* enzyme was incubated with soluble starch and D- ^{14}C]glucose, radioactive maltooligosaccharides with a DP \geq 3 and only traces of maltose were formed (data not shown;

similar to Fig. 4B). This shows glucose to be an acceptor for maltosyl- and oligosaccharyl residues derived from starch through the action of D-enzyme, similar as with the D-enzyme from other sources (Walker and Whelan, 1957; Lin and Preiss, 1988).

The highest rates of glucose formation were observed with maltotriose as substrate, followed by higher maltooligosaccharides. Attack of cellotriase, disaccharides, glycogen and starch was not detectable except for a very slow cleavage of maltose (Table II). A similar pattern of specificity was observed in the raw soluble fraction of *Nostoc*, which contained neither an appreciable maltase nor invertase activity. A relatively low maltase activity was observed also in *Anabaena* (compare Table IV).

Properties of D-enzyme. D-enzyme activity had a broad pH optimum around pH 7.4 (half maximal values at pH 5.7 and pH 8.8) with maltotriose as substrate and a similar one with maltoheptaose (data not shown). Differences with these substrates, however, were seen in the temperature dependence; maximal rates were attained around 40 °C and 45 °C with maltotriose and maltoheptaose, respectively (data not shown). A K_M value of 5.5 mM was found for maltotriose and a somewhat lower value (2.6 mM) for the analogous heptaose (Fig. 3A). No significant effect on enzyme

Table II. Substrate specificity of the D-enzyme from *Nostoc* and from *Chlorella* 3.83 in catalyzing glucose release. Concentration of oligo- and disaccharides was 10 mM and that of starch (soluble Zulkowsky starch, Merck) and of glycogen (from oyster, type II, Sigma) was 1% (w/v). Other conditions are given under Materials and Methods. A glucose release of 100% corresponded to 11.9 and 200.0 $\mu\text{mol/h} \cdot \text{mg}$ protein for the enzyme from *Nostoc* and *Chlorella*, respectively.

Substrate	<i>Nostoc</i> Glucose release [%]	<i>Chlorella</i> 3.83 Glucose release [%]
Maltose	1.5	n.d.
Maltotriose	100	100
Maltotetraose	74	78
Maltoheptaose	27	45
Isomaltose	n.d.	n.d.
Sucrose	n.d.	n.d.
Cellotriase	n.d.	n.d.
Starch	n.d.	n.d.
Glycogen	n.d.	n.d.

n.d. = not detected.

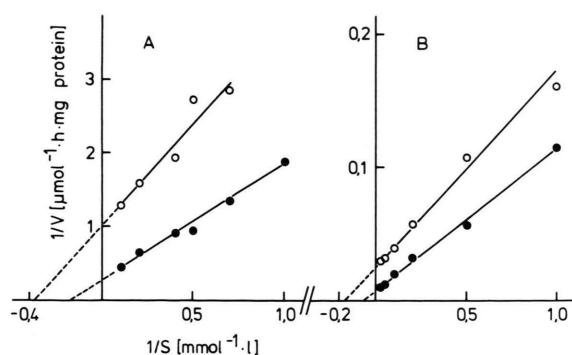


Fig. 3. Lineweaver-Burk plot of substrate dependence of D-enzyme activity from *Nostoc* (A) and from *Chlorella* 3.83 (B). Substrates were maltotriose (●—●) and maltoheptaose (○—○).

activity had the presence of either of the following: MgCl_2 , CaCl_2 , EDTA (1 mM each). Gel filtration indicated an apparent molecular weight of 50 kDa for the D-enzyme.

D-Enzyme from *Chlorella* 3.83

Purification. With ammonium sulfate (30% saturation) practically all of the green material of the soluble cell fraction could be precipitated giving a slight enrichment on a protein basis (Table III). Precipitation at higher ammonium sulfate concentration caused large losses of activity and was not employed therefore. In the following chromatography on hydroxylapatite principally two elution patterns of D-enzyme activity could be discerned: either all of the activity eluted at low phosphate concentration (0.20 M) or the activity split in two peaks, one eluting at the low phosphate concentration and the second one at higher concentration

(0.30 M). This is considered to be due to subtle alterations in the state of the enzyme (for still unknown reasons) since *e.g.* in rechromatography the second peak eluted at the position of the first one and since in one preparation both types of activity profiles were registered in two adjacent runs. For further purification all of the active fractions were pooled and subjected to anion exchange chromatography. After this step an enrichment between 25- and 70-fold was achieved in five different preparations with yields ranging from 27 to 67% (see Table III). In anion exchange chromatography as well as in a following gel filtration run D-enzyme activity always eluted in one single peak (data not shown). After the gel filtration step hardly measurable amounts of protein were contained in the active fraction in which, after SDS/polyacryl amide gel electrophoresis and silver staining, still 12 protein bands were detectable. For the following analyses enzyme preparations purified according to the scheme in Table III were used throughout.

Product analyses and substrate specificity. When maltotriose was offered as substrate, only glucose and maltopentaose were detected as early reaction products and, after longer incubation, homologous maltooligosaccharides and in no case maltose. As with the corresponding enzyme of *Nostoc* maltopentaose formation was achieved by transfer of a maltosyl group from the nonreducing side of the maltotriose molecule [*cf.* Fig. 2]. From maltoheptaose appeared as first reaction products glucose and maltooligosaccharides with a DP > 6 and, after prolonged incubation, also shorter homologues whereas maltose was undetectable (data not shown).

Table III. Purification of D-enzyme from 5.2 ml packed cells of *Chlorella* 3.83. Fraction 12 of anion exchange chromatography eluted at 0.48 M NaCl.

Fraction	Protein content [mg]	Specific activity [$\mu\text{mol/h} \cdot \text{mg protein}$]	Yield [%]	Purification
Homogenate (dialyzed soluble fraction)	144	1.50	100	1
Ammonium sulfate fractionation, supernatant after 30% saturation	95.9	2.03	90	1.36
Hydroxylapatite chromatography, pooled active fractions	26.8	6.17	76	4.11
Anion exchange chromatography, fraction 12	0.492	72.1	33	48.1

Besides maltotriose (see above) glucose served as an acceptor in transglycosylation, too. This was seen when a mixture of maltotriose and labeled glucose was incubated with the enzyme: the predominant labeled reaction product was maltotriose (Fig. 4A). In an analogous experiment soluble starch instead of maltotriose was successfully used as donor substrate, resulting in a higher percentage of larger maltooligosaccharides ($DP \geq 4$) in the reaction products (Fig. 4B) which probably is due to considerable maltooligosaccharyl transfer directly from starch to glucose. All of these characteristics are in agreement with those reported for D-enzyme from *Nostoc* (see above) and from higher plants (Peat *et al.*, 1956; Jones and Whelan, 1969; Manners and Rowe, 1969; Walker and Whelan, 1957).

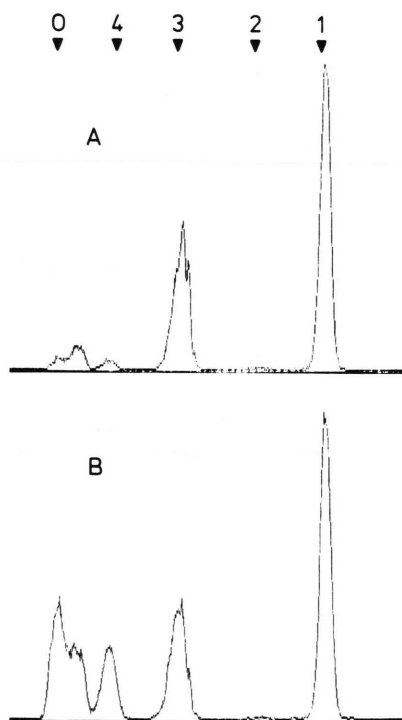


Fig. 4. Thin-layer-chromatographic analysis of the products formed by transglycosylase from *Chlorella* 3.83 with D-[^{14}C]glucose as acceptor and (A) maltotriose or (B) starch as donor. The incubation mixture contained 10 mM D-[^{14}C]glucose ($2.94 \cdot 10^8$ Bq/ μmol), 14 mM imidazole/HCl pH 6.8, D-enzyme preparation (0.7 mg protein/ml) and either 10 mM maltotriose (A) or 82.5 mg/ml soluble starch (B). The incubation time was 1 h (30°C). For further details see Materials and Methods. Positions of reference substances are indicated as in Fig. 2.

When D-enzyme acted on maltodextrins the rate of released glucose fell with the chain length of the substrate and was not detectable for disaccharides, cellotriose, starch and glycogen (Table II). This is essentially the same substrate dependence as found for the D-enzyme from *Nostoc* (Table II) and from *Arabidopsis* (Lin and Preiss, 1988).

Properties of D-enzyme. The enzyme showed maximal action around pH 7.2 and more than 80% of this activity between pH 6.0 and pH 8.4. At pH 4.8 the activity had dropped to 6% and at pH 9.6, depending on the buffer used, to 71% (glycine/NaOH) or 11% (1,3-bis[tris(hydroxymethyl)methylamino]propane). The temperature optimum of D-enzyme activity was located at 49.5°C with a steep decline towards higher temperatures (30% activity at 57°C) and was similar for maltotriose and maltoheptaose (data not shown). For the latter substrate a K_M of 5.9 mM was determined and for maltotriose a somewhat higher value (14.2 mM; Fig. 3 B). No significant effect on enzyme activity was noticed by the addition of each of the following: MgCl_2 , CaCl_2 and EDTA (1.0 mM final concentration). An apparent molecular weight of 230 kDa was estimated for D-enzyme by gel filtration which is an almost five-fold higher value than found for the respective enzyme from *Nostoc*.

Occurrence of D-enzyme in free-living cyanobacteria and green algae

Anabaena PCC 7120, *Chlorella kessleri* and *Chlamydomonas noctigama* were tested for the presence of D-enzyme. With all three organisms the soluble fraction of a cell homogenate readily catalyzed liberation of glucose when maltotriose or maltotetraose were offered as a substrate (Table IV). This should not be due to the action of α -amylase since maltotriose is no (Whelan *et al.*, 1953) or only a poor (Meyer and Gonon, 1951) substrate for this enzyme; besides, EDTA was hardly inhibitory (Table IV) dismissing the involvement of Ca-requiring α -amylases. A β -amylase obviously does not participate either since maltotriose is an extremely poor substrate for it and since it should not produce glucose from maltotetraose (Whelan *et al.*, 1953). Maltose was cleaved very slowly suggesting only a limited role of α -glucosidase. Thin-layer chromatography of the digest of maltotriose revealed for all species glu-

Table IV. Glucose release by the soluble cell fraction of *Anabaena* and two species of green algae with the substrates maltose, maltotriose and maltotetraose. Cell breakage and centrifugations were as described for the purification of D-enzyme from *Chlorella* 3.83; activity tests were as the one routinely used for D-enzyme (see Materials and Methods) but with substrates as listed below. A relative rate of 100% corresponded to 2.09, 2.41 and 4.89 $\mu\text{mol/h} \cdot \text{mg}$ protein in *Anabaena*, *Chlorella* and *Chlamydomonas*, respectively.

Substrate [10 mM]	Maltose	Maltotriose	Maltotriose + 5 mM EDTA	Maltotetraose
Rate of glucose release [%]				
Organism				
<i>Anabaena</i> PCC 7120	4.3	100	101	73
<i>Chlorella kessleri</i>	9.5	100	90	83
<i>Chlamydomonas noctigama</i>	9.6	100	93	36

cose, maltopentaose and some higher maltodextrins as reaction products, but never maltose (data not shown). This all is taken as evidence for the occurrence of D-enzyme in cyanobacteria and green algae.

Discussion

The pattern of reaction products formed from maltooligosaccharides by D-enzyme from *Nostoc* and *Chlorella* agrees well with that reported for D-enzyme from different higher plants (Peat *et al.*, 1956; Jones and Whelan, 1969; Manners and Rowe, 1969; Lin and Preiss, 1988). An exception to the present work and other results (Peat *et al.*, 1956; Jones and Whelan, 1969; Manners and Rowe, 1969) is the early formation of maltotriose and glucose from maltotetraose by the enzyme from *Arabidopsis* (Lin and Preiss, 1988) instead of the commonly observed products maltoheptaose and glucose. The ability of disproportionating transglycosylase to transfer maltosyl- and maltodextrinyl residues to glucose as acceptor (see Fig. 4) confirms previous data on this reaction (Manners and Rowe, 1969; Walker and Whelan, 1957; Lin and Preiss, 1988). Obviously, the reactions catalyzed by D-enzyme from as different groups as cyanobacteria, green algae and higher plants are in general quite uniform. Considerable differences relate, however, to other characteristics of D-enzyme from the various sources; *e.g.* the molecular weight was 50 kDa (*Nostoc*) or 230 kDa (*Chlorella*), temperature optima were

found at 50 °C (*Chlorella*) or 37 °C (Rowe and Manners, 1969) and pH optima were encountered at pH 7.4 (*Nostoc*), pH 6.7 (Peat *et al.*, 1956) or pH 5.4 (Rowe and Manners, 1969). Isoforms of the enzyme have been found (Lin and Preiss, 1988) and are also indicated for *Nostoc* (*cf.* Fig. 1).

Disproportionating transglycosylase occurs in cyanobacteria and green phototrophs most of which synthesize α -glucans as reserve carbohydrate. Thus, the suggested function of D-enzyme in assisting phosphorolytic breakdown of starch (Lee *et al.*, 1970) seems reasonable for these organisms. In the symbiotic *Nostoc*, however, another role of D-enzyme may be to provide from oligosaccharides free glucose which in the intact lichen is known to move to the fungal partner (Drew and Smith, 1967; Hill and Smith, 1972). This pathway appears unsatisfactory at first sight since with each glucose liberated an elongated maltodextrin is generated, which, when grown too long for D-enzyme attack, had to be cut down again by amylolysis or phosphorolysis. Alternative ways of glucose formation, however, seem problematic too; for glucose phosphate as precursor a specific phosphatase had to be assumed and cleavage of maltose and sucrose is negligible in *Nostoc* extracts. A glucosidase hydrolyzing *p*-nitrophenyl α -D-glucoside has been reported from *Nostoc* (Bogner *et al.*, 1993); this enzyme probably is an isomaltase as suggests the similar elution behaviour in hydroxylapatite chromatography (Fig. 1). Because of its low activity and a relatively low substrate level *in vivo* it should liberate only limited amounts of glucose. To clarify the actual way of glucose for-

mation experiments should be undertaken with the freshly isolated cyanobiont which in many respects differs from the cultivated form (Green and Smith, 1974).

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Note added in proof

A related report on D-enzyme has appeared recently: Takaha T., Yanase M., Okada S. and Smith S. M. (1993), Disproportionating enzyme (4- α -glucanotransferase; EC 2.4.1.25) of potato. Purification, molecular cloning, and potential role in starch metabolism. *J. Biol. Chem.* **268**, 1391–1396.