Phytochemistry, 1973, Vol 12, pp 1929 to 1932 Pergamon Press Printed in England

MOLECULAR WEIGHT OF STARCH SYNTHETASE FROM *ORYZA SATIVA* LEAVES

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(*Revised Received* 21 *February* 1973 *Accepted* 9 *March* 1973)

Key Word Index—*Oryza sativa*, Gramineae, rice; starch synthesis, ADP-glucose α-1,4-glucosyltransferase

Abstract—Soluble ADP-glucose α-1,4-glucan-4-glucosyltransferase with primed activity was extracted from rice leaves and purified by $(NH_4)_2SO_4$ fractionation, gradient elution on DEAE-cellulose and finally by Sephadex G200 gel filtration or amylopectin-cellulose chromatography The purified enzyme was essentially homogeneous electrophoretically, but exhibited two peaks corresponding to MW of 22 000 and 67 000 on Sephadex G200 chromatography and five distinct bands on sodium dodecyl sulfate gel electrophoresis with MW of 11 5, 20, 35, 50 and 68 $\times 10^3$

INTRODUCTION

STARCH synthetase (ADP-glucose· α-1,4-glucan-α-4-glucosyltransferase, E.C. 2.4.1.21) is believed to play a key role in starch biosynthesis.[1-3] Recently, multiple forms of the enzyme differing in specificity for primer have been observed in the leaves of spinach,[4] corn,[5] and rice,[5] in developing grains of nonwaxy and waxy rice[5] and waxy corn[6] and in immature potato tubers.[7] One enzyme form has been found which has unprimed activity, i e it can synthesize starch in the absence of added primer [4,6,7] However, there has so far been no precise determination of the MW of starch synthetase [6,7]

The work described here deals with the determination of the MW of soluble starch synthetase, which has primed activity, from rice leaves using Sephadex G200 gel filtration and sodium dodecyl sulfate (SDS) gel electrophoresis

RESULTS AND DISCUSSION

The crude enzyme preparation obtained from the homogenate of rice leaves was purified about 10-fold by DEAE-cellulose chromatography (Table 1) Further purification was obtained either by Sephadex G200 gel filtration or by amylopectin-cellulose chromatography. These two purification steps resulted in more than a 100-fold purification of starch synthetase having primed activity from rice leaves. Analytical disc gel electrophoresis at pH 8·3 showed that P, P_s and P_a (see Table 1) were essentially homogeneous.

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SDS gel electrophoresis of starch synthetase yielded five distinct bands (Fig 1) The mobilities of the bands were the same within experimental error for enzyme fractions P, P_s

TABLE 1 PURIFICATION OF SOLUBLE STARCH SYNTHETASE FRACTIONS FROM *Oryza sativa* LEAVES

Enzyme fraction	Specific activity (units/mg protein)*	Yield† (%)
Crude extract	0 0009	
$(NH_4)_2SO_4$ cut	0 001	100
P (DEAE-cellulose chromatography of $(NH_4)_2SO_4$ cut)	0 010	81
P_s (Sephadex G200 gel filtration of P)	0 108	16
P_a (Amylopectin-cellulose chromatography of P)	0 092	13

* A unit of enzyme activity is defined as 1 μmol ^{14}C-glucose transferred per minute at 37°

† Based on the $(NH_4)_2SO_4$ cut since the crude extract and the $(NH_4)_2SO_4$ cut had similar specific activities

and P_a Parallel runs were made using reference proteins only or in combination with the enzyme, the band mobilities were the same in both cases A semi-log plot of MW against band mobility using literature values for the reference proteins[8,9] showed that the starch synthetase bands correspond to MW of 11 500 ± 2000, 20 000 ± 3000, 35 000 ± 4000, 50 000 ± 7000 and 68 000 ± 7000

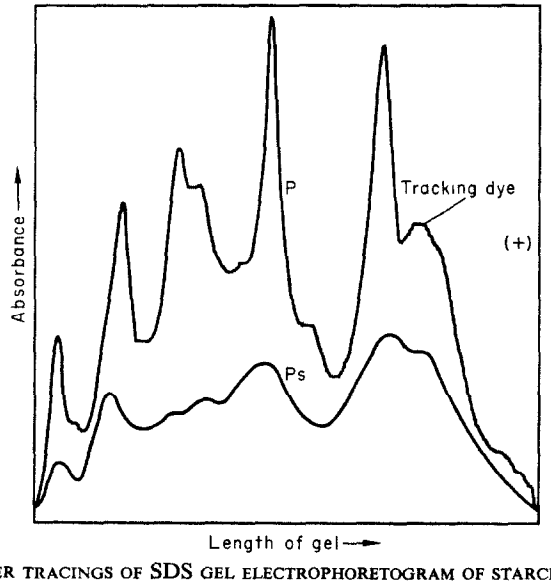

FIG 1 DENSITOMETER TRACINGS OF SDS GEL ELECTROPHORETOGRAM OF STARCH SYNTHETASE FRACTIONS HAVING PRIMED ACTIVITY

The amounts of enzymes added to the sample gels were 179 μg of P and 50 μg of P_s

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Gel filtration through Sephadex G200 of enzyme fractions P, P_s and P_a resulted in two peaks having primed activity These peaks correspond to MW of 22 000 ± 5000 and 67 000 ± 8000 These values correspond to two of the bands obtained from SDS gel electrophoresis and are a factor of 3 relative to each other

The multiple bands obtained after SDS electrophoresis of the enzyme may be explained by aggregation of the separated subunits during electrophoresis The MW corresponding to these bands were related to one another by whole numbers, within experimental error, and indicate aggregation of the polypeptide chains into oligomers There is, therefore, a strong probability that the enzyme consists of more than one polypeptide chain and it exists in at least two active oligomeric forms of MW 22 000 and 67 000 under the experimental conditions used

An alternative explanation of the two enzyme peaks produced by gel filtration is that they correspond to different enzymes This is unlikely because rechromatography of either one of the two peaks on Sephadex G200 yielded both peaks Furthermore, the SDS electrophoretic patterns for the enzymes corresponding to the two peaks were similar Preliminary experiments indicate that the unprimed starch synthetase U or U_s (U purified by Sephadex G200 chromatography) probably exhibits a similar aggregation phenomenon but has a higher MW than the primed enzyme

EXPERIMENTAL

Purification Soluble starch synthetase was isolated from 2-week-old seedlings (variety IR8) and purified at 2–4° The leaves were frozen in dry ice and ground in a Waring blendor Crude enzyme from the leaf powder was extracted with pH 7 5 buffer (0 05 M Tris-HCl –0 002 M EDTA–0 01 M dithiothreitol (DTT)–0 005 M potassium phosphate) [10] The suspension was filtered through 8 layers of cheesecloth, and the filtrate was centrifuged at 15 000 *g* for 15 min Starch synthetase was obtained from the fraction of the filtrate precipitating between 30 and 60% saturation with $(NH_4)_2SO_4$ The resulting precipitate was dissolved in a minimum volume of pH 7 5 buffer (0 005 M sodium phosphate –0 001 M EDTA–0 001 M DTT), then dialyzed against the same buffer overnight

The enzyme solution was chromatographed through a DEAE-cellulose column (3 5 × 35 cm) with NaCl gradient elution according to the procedure of Doi *et al* [11] Two distinct enzyme fractions having mainly unprimed (*U*) or primed (*P*) activity were eluted one after the other, pooled and concentrated through a Diaflo PM-10 or UM-10 filter The enzyme solutions were further purified separately by passing them through a Sephadex G200 column (3 5 × 42 cm) and eluting them with pH 7 5 buffer (0 05 M Tris-HCl–0 1 M KCl) The active fractions were pooled and concentrated by ultrafiltration In a separate experiment *P* was chromatographed on an amylopectin–cellulose (1 corn amylopectin 10 Whatman standard grade cellulose, w/w) column (1 5 × 17 cm) [12] The active fractions were pooled and concentrated as before The fractions gradually lost enzyme activity on standing in solution at 0–4° but were more stable when stored as $(NH_4)_2SO_4$ precipitates Analytical disc electrophoresis of the primed enzyme was done at pH 8 3 according to the method of Davis [13]

Enzyme assay Starch synthetase activity was measured with and without primer The procedure followed was essentially that of Sanwal *et al* [10] In the enzyme assay without primer, the latter was omitted but the concentrations of the other reagents in the reaction mixture were maintained ADP-glucose and UDP-glucose were found efficient glucose donors for the primed and unprimed enzymes, respectively, each glucose donor was therefore used in the corresponding enzyme assay Because oyster glycogen was a better primer than maltose, potato amylose or corn amylopectin, it was used in the assay for primed activity Protein level was determined by the method of Lowry *et al* [14] using bovine serum albumin as standard

MW determination The procedure of Andrews[15] for gel filtration on Sephadex G200 at pH 7 5 was used The column employed was the same as that used in the purification of enzyme *P*, this procedure served as a

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check of the MW and homogeneity of the enzyme For SDS gel electrophoresis, the method of Weber and Osborn[8] was followed Both gel filtration and SDS electrophoresis were performed at 2–4° The reference proteins used were β-galactosidase (*E coli*), α-chymotrypsinogen-*A* (bovine pancreas), and ribonuclease (bovine pancreas) from Worthington, fumarase (pig heart) and aldolase (rabbit muscle) from Boehringer Mannheim, glyceraldehyde phosphate dehydrogenase (rabbit muscle), cytochrome-*c* (horse heart) and γ-globulin (bovine) from Sigma, mitochondrial malate dehydrogenase (pig heart) from Mann, trypsin (beef pancreas) from Nutritional Biochemicals, and serum albumin (bovine) from Armour