New Approaches to Research on Cereal Carbohydrates

Edited by

Robert D. Hill
Lars Munck

ELSEVIER

Progress in Biotechnology

VOLUME 1

Progress in Biotechnology 1

New Approaches to Research on Cereal Carbohydrates

Proceedings of the International Conference on New Approaches to Research on Cereal Carbohydrates, Copenhagen, Denmark, June 24–29, 1984

Edited by

Robert D. Hill

The University of Manitoba,
Department of Plant Science,
Winnipeg, Manitoba, Canada

Lars Munck

Carlsberg Research Laboratory,
Department of Biotechnology,
Copenhagen, Denmark

ELSEVIER
Amsterdam – Oxford – New York – Tokyo 1985

ELSEVIER SCIENCE PUBLISHERS B.V.
Molenwerf 1
P.O. Box 211, 1000 AE Amsterdam, The Netherlands

Distributors for the United States and Canada:

ELSEVIER SCIENCE PUBLISHING COMPANY INC.
52, Vanderbilt Avenue
New York, NY 10017, U.S.A.

ISBN 0-444-42434-2 (Vol. 1)
ISBN 0-444-42435-0 (Series)

Printed in The Netherlands

New Approaches to Research on Cereal Carbohydrates

Progress in Biotechnology

Volume 1 New Approaches to Research on Cereal Carbohydrates (Hill and Munck, Editors)

PREFACE

The Conference of which these proceedings are the record was suggested to us by Professor Roy L. Whistler; it is the third meeting of its type held in conjunction with the International Carbohydrate Symposia. In arranging this meeting, held at the Carlsberg Research Center in June, 1984, we had the pleasure of working jointly with the International Association of Cereal Science and Technology (ICC) and the Danish Cereal Society.

The properties and uses of cereal carbohydrates are of interest to a wide and diverse group ranging from the basic research scientist to the industrial technologist. The presentations within this volume are witness to that diversity. The topics vary from a discussion of the number of binding sites on an enzyme, and the use of starch polymers or granules in the production of biodegradable films, to the structure and utilization of straw cellulose fibres for feed and paper.

We are at a critical point in our understanding of the properties of cereal carbohydrates. There has been a hiatus in development of further knowledge on the synthesis and structure of the starch granule. New ideas and directions are required to stimulate further research. In the past several years, great progress has been made in field of structural cereal carbohydrates but we have still only scratched the surface of this tremendously complex subject.

Cereal carbohydrate utilization has, in the past, varied little from traditional food uses. Within the next decade, we are likely to see a major diversion from traditional utilization as current research knowledge is implemented to substitute cereal carbohydrates as raw material for industrial manufacture.

We hope that these proceedings will give the reader an impression of the optimism and enthusiasm felt by this Conference audience with regard to the possibilities of development in the science and technology of cereal carbohydrates.

There are a number of individuals who have spent long hours in organizing the Conference and in assisting with the editing of this volume. Their acknowledgment here is only a small measure of our appreciation for their efforts. We are especially grateful to the Conference Chairman, Professor Roy L. Whistler, for initiating this meeting, to our co-members in the Programme Committee, Dr. Bent S. Enevoldsen and Dr. Finn Rexen, to Mrs. Britha Jensen for the conference arrangements and to Mrs. Kirsten Kirkegaard for helping us edit the proceedings.

September 1984

Robert D. Hill Lars Munck

Proceedings of the International Conference
on New Approaches to Research on Cereal Carbohydrates
held at Carlsberg Research Center, Copenhagen, Denmark
June 24-29, 1984.

Conference Chairman:	R.L. Whistler Purdue University Department of Biochemistry West Lafayette Indiana, U.S.A.
Editorial Committee:	B.S. Enevoldsen, Department of Brewing Chemistry, Carlsberg Research Laboratory R.D. Hill L. Munck F. Rexen, Department of Biotechnology Carlsberg Research Laboratory
Organized by:	L. Munck, Chairman of Group 38 (Scientific Communication and Education) International Association of Cereal Science and Technology (ICC)

CONTENTS

STARCH CHEMISTRY

STARCH TECHNOLOGY

NON-STARCH POLYSACCHARIDE CHEMISTRY

NON-STARCH POLYSACCHARIDE TECHNOLOGY

NUTRITION

GENERAL CONSIDERATIONS

New Approaches to Research on Cereal Carbohydrates, edited by R.D. Hill and L. Munck
Elsevier Science Publishers B.V., Amsterdam, 1985 — Printed in The Netherlands

VARIOUS ASPECTS IN THE REGULATION OF STARCH BIOSYNTHESIS

JACK PREISS, FRASER D. MACDONALD, BIJAY K. SINGH, NINA ROBINSON, and KATHLEEN McNAMARA

Biochemistry and Biophysics Dept., University of California, Davis, CA 95616

INTRODUCTION

Starch is the most abundant storage reserve polymer present in plants and the three main reactions in nature responsible for its synthesis are catalyzed by ADPglucose synthetase (E.C. 2.7.7.27; reaction 1), starch synthase (E.C. 2.4.1.21; reaction 2) and branching enzyme (E.C. 2.4.1.24; reaction 3), respectively.

1) ATP + αglucose 1-P $\rightleftharpoons$ ADPglucose + PP_i

2) ADPglucose + $(G)_n \longrightarrow (G)_{n+1}$ + ADP

3) Linear α1,4 glucan $\rightarrow$ branched glucan; 4-7% α1,6 linkages

Much information has been reported and reviewed on the above three enzymes with respect to their properties (1-5) and their localization in the plant cell (6,7). However the detailed steps on the mechanism of amylose and amylopectin formation and how they are combined to form the native starch granule remains obscure. Moreover it has been shown that there are multiple forms of starch synthase and branching enzyme (1-5). Their exact roles in the formation of the starch granule are still not clear.

This report will present some of the recent data we have obtained bearing on the regulation of starch synthesis and further information on the relationship between the multi-forms of starch synthase and branching enzyme.

REGULATION OF STARCH SYNTHESIS

ADPglucose Synthetase Regulation

ADPglucose synthetase was shown in 1966 (8,9) to be activated by 3-P-glycerate (3PGA) the primary CO_2 fixation product in photosynthesis and inhibited by inorganic phosphate (P_i). The ADPglucose synthetase may be isolated from leaves of either C_3 or C_4 plants (5). The enzyme from at least 13 different leaf sources has been shown to be activated by 3PGA. Recently the enzyme from leaves of 2 plants that assimilate carbon during photosynthesis via crassulacean acid metabolism (CAM) has also been shown to be inhibited by P_i and activated by 3PGA (10).

As seen in Fig. 1 the leaf ADPglucose synthetase of the CAM plant, Hoya carnosa is activated 14-fold by 3PGA. The concentration of 3PGA required for half maximal stimulation ($A_{0.5}$) is 0.25 mM. 3PGA also increases the apparent affinity of the substrates, glucose-1-P and ATP, about 14- and 5-fold respectively (10). Also seen in Fig. 1 is the inhibition caused by P_i. The $A_{0.5}$ value

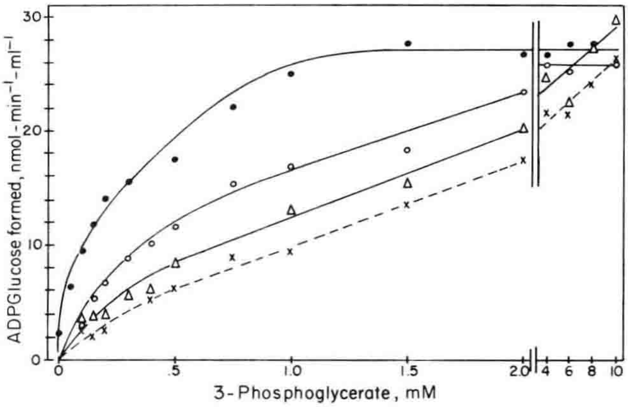

Fig. 1. Activation of ADPglucose synthesis catalyzed by Hoya carnosa ADPglucose synthetase by 3PGA and inhibition of activation by P_i. ADPGlucose synthesis was measured as previously described (10). P_i concentrations are none -●-; 0.1 mM -O-; 0.2 mM -Δ-; 0.3 mM *.

of 3PGA is increased in the presence of P_i and is 6.4-fold higher, 1.6 mM, in the presence of 0.3 mM P_i. The P_i concentration required for 50% inhibition in the absence of 3PGA is 0.22 mM. Thus the regulatory properties observed for the CAM plant leaf ADPglucose synthetase is very similar to that observed for the C_3 and C_4 plant leaf enzymes. Namely that the ratio of 3PGA/P_i modulates ADPglucose synthesizing activity.

It was thus postulated that starch synthesis during photosynthesis was stimulated by activation of ADPglucose synthesis from glucose-1-P via increases of 3PGA concentration in the chloroplast and a decrease of P_i due to photophosphorylation also occurring during photosynthesis (1-5). At night, due to the absence of photosynthesis, the concentration of P_i may increase. Indeed, it has been shown by Kobayashi et al. (11) and by Kaiser and Bassham (12) that energy charge values in the chloroplast drastically decrease in the dark and the ATP values of 1.0 mM in the light decrease to about 0.2 mM in the dark. These conditions would thus inhibit starch and ADPglucose synthesis and starch degradation would ensue because of the higher P_i concentration stimulating phosphorylase action.

Studies of a number of intact leaf systems have shown that changes in 3PGA and P_i concentrations can be correlated with the observed changes in the rates of starch synthesis (13-17).

In contrast to the normal mesophyll leaf cell the guard cells degrade starch during the day during stomatal control (18). At night starch reaccumulates in the guard cell. Thus the pattern of starch synthesis and its utilization in the guard cell is opposite to what is seen in the mesophyll leaf cell. This raised the question if the mode of starch regulation in the guard cell was different. However studies with guard cells from Commelina communis (19) from Argenteum a mutant of Pisum sativum (Table 1) and from Vicia fabia (20) have shown that the guard cell ADPglucose synthetase has the same regulatory properties as the mesophyll leaf cell enzyme; 3PGA activates ADPglucose synthesis and P_i inhibits it. Fig. 2 shows that higher concentrations of 3PGA desensitizes the Commelina communis enzyme to inhibition by P_i. This phenomenon had been seen before with many mesophyll cell ADPglucose synthetases (8-10,21).

Since the guard cell does not contain ribulose bisphosphate carboxylase (Rubisco) activity (22) and therefore does not make 3PGA during photosynthesis or at night from CO_2 it is of interest to know what the source of 3PGA is for activation of the guard cell ADPglucose synthetase. Starch as well as the starch biosynthetic enzymes are localized in the mesophyll leaf cell in the chloroplasts (6). Most probably this is also true for the guard cell and since it is devoid of Rubisco the source of 3PGA and triose phosphates of the guard cell chloroplast is most likely from the cytosol and obtained from other cell types. Moreover the only glycolytic metabolites appreciably permeable to the mesophyll chloroplast (and most probably the guard cell chloroplast) are the triose phosphates and 3PGA (22). This occurs via an exchange of the triose phosphates with inorganic phosphate (23). It is quite possible that a carbon source such as sucrose from the mesophyll cell is transported to the guard cell and then is metabolized to the triose phosphate/3PGA level and then translocated into the chloroplast. The ATP required at night for starch synthesis most probably is formed via oxidative phosphorylation that would occur from

TABLE 1

Effect of 3PGA and P_i on ADPglucose synthetase activity of Argenteum mutant of Pisum sativum leaf mesophyll and guard cells

Activity/Conditions	Guard Cells		Mesophyll Cells
		$\text{nmol-min}^{-1}\text{-mg}^{-1}$	
ADPG Synthetase	11.7		68
- 3PGA, 1 mM	< 0.24		--
+ P_i, 1 mM	0.24		--
		$\mu\text{mol-min}^{-1}\text{-mg}^{-1}$	
Rubisco	0.012		0.34

[a] Rubisco is the abbreviation for ribulose bisphosphate carboxylase.

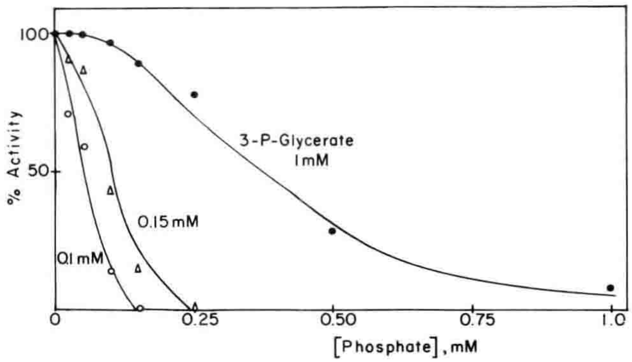

Fig. 2. Inhibition of Commelina communis guard cell ADPglucose synthetase activity by P_i. The assay procedure was previously described.

oxidation of the malate that accumulated during the day. Guard cells have been shown to have a greater ratio of mitochondria to chloroplasts than the Vicia fabia mesophyll cells (24). Thus the questions to be answered in the future about guard cell biochemistry is what is the source of carbon for starch synthesis? What is the enzyme composition of the guard cell chloroplast as well as the cytosol with respect to starch and sucrose metabolism? What are the energy charge levels in guard cells in light and dark conditions as compared to the mesophyll cell?

It is also becoming apparent that many of the regulatory patterns of starch synthesis seen in the leaf cell are also observed for the non-photosynthetic plant cells such as in maize endosperm (25) and potato tubers (26). The endosperm and tuber ADPglucose synthetases are activated by 3PGA and inhibited by P_i. In addition the starch as well as the starch biosynthetic enzymes are localized in a subcellular organelle, the amyloplast. MacDonald and ap Rees have succeeded in isolating intact amyloplasts from protoplasts of soybean cell cultures and have shown that the ADPglucose synthetase and starch synthase activity are localized in the cell in the amyloplast fraction (7). It is quite possible therefore that in the non-photosynthetic plant cell conversion of sucrose to starch requires that the sucrose be metabolized to the triose phosphate level before it can enter the amyloplast; i.e. the amyloplast may be similar to the chloroplast. If so, the only glycolytic intermediates able to