THE BIOCHEMISTRY OF GREEN PLANTS

THE BIOCHEMISTRY OF GREEN PLANTS

DAVID W. KROGMANN

Professor of Biochemistry
Purdue University

Prentice-Hall, Inc., Englewood Cliffs, New Jersey

Library of Congress Cataloging in Publication Data

KROGMANN, DAVID W.
The biochemistry of green plants.

(Foundations of modern biochemistry series)
Bibliography: p.
1. Botanical chemistry. I. Title.
QK861.K7 581.1'92 73-7637
ISBN 0-13-076463-9
ISBN 0-13-076455-8 (pbk.)

Printed in the United States of America.

10 9 8 7 6 5 4 3 2 1

PRENTICE-HALL INTERNATIONAL, INC., *London*
PRENTICE-HALL OF AUSTRALIA, PTY. LTD., *Sydney*
PRENTICE-HALL OF CANADA, LTD., *Toronto*
PRENTICE-HALL OF INDIA PRIVATE LIMITED, *New Delhi*
PRENTICE-HALL OF JAPAN, INC., *Tokyo*

PREFACE

This text is directed to the needs of students who are interested in the biochemistry of green plants – not only those students specializing in biochemistry but also those in botany, plant physiology, agronomy, plant pathology, horticulture, forestry, and ecology. The contents can be covered in a one-semester course and are by no means comprehensive. Since a course in general biochemistry is a *sine qua non* for any advanced study in the life sciences, such an instruction is taken for granted as a prerequisite to the more specialized areas covered in this text. Since botanical terms, plant structures, and even the generic names for plant species are often of use but rarely remembered, a glossary has been appended. At the end of most chapters a few general references are given to lead the reader to more detailed summaries; footnotes are cited in the text by number and collected as references at the back of the book to provide leads into the research literature.

There is little point in cataloging all of the biochemical processes common to all living things and noting their occurrence in plants. Usually these general processes were first worked out with mammalian or bacterial preparations which are inherently easier to do biochemistry with. The subsequent discovery of these

CONTENTS

1 INTRODUCTION

At the outset of a study of plant biochemistry, one must ask if there is any unique province for the biochemistry of plant cells. Much of the metabolism of plant tissue is identical to that found in animal or bacterial cells. The biochemical activities of plants are clearly unique with respect to

pigments
photosynthesis
cell walls
chloroplasts
photoregulation
metabolism of certain inorganic elements
phytohormones
secondary metabolites

The tendency in the discussions that follow is to concentrate on biochemical processes which are general to most plant tissue and to avoid that which is peculiarly characteristic of a single species or group.

The architecture of the plant cell includes the

nucleus
mitochondria
endoplasmic reticulum bearing ribosomes
Golgi apparatus
microbodies
plasma membrane
fat droplets
carbohydrate granules
protein bodies

which are generally similar to structures found in animal cells. In addition, the plant cell has

a rigid cell wall
a vacuole
chloroplasts
glyoxysomes

1.1 EXPERIMENTAL CONSIDERATIONS UNIQUE TO PLANTS

The unique difficulties in doing biochemical experiments with plants are formidable and arise from the special character of plant cell structure.

1. Much of the bulk of fresh plant material is water – 90% of a spinach leaf is water, so one is working with material that is inherently dilute.

2. The cell wall makes up one-fourth to one-half of the dry weight of plant material. The wall is practically inert in a biochemical sense and is frequently so tough in a physical sense that breaking the wall often involves breaking the structured contents of the cell. Many marine algae are essentially unbreakable. A variety of mechanical devices are available for homogenizing plant tissue so that one can usually find circumstances that allow isolation of a particular organelle. Enzymatic digestion of the plant cell wall can be used to produce protoplasts which can then be broken under very gentle conditions.

3. In addition to wall material, some plants may accumulate various substances which, when the cell is broken, form a gel that enmeshes all the cell contents in an unfractionatable mess. Parsley and red algae show this property. In principle, one should be able to find an enzyme to hydrolyse this polysaccharide but in practice such enzymes are often hard to obtain.

4. Most plants contain phenolic compounds that oxidize and polymerize when the cell is broken open. The polymers are frequently acidic, frequently bind irreversibly to proteins, and are always colored – yellow

to dark brown – which makes spectrophotometric assays difficult. The oxidation of these compounds can be minimized by grinding the plant tissue with a reducing agent like ascorbic acid or glutathione and by using a metal chelator to suppress some of the metal or metalloenzyme catalysed oxidations. Some of the oxidized polymeric phenols can be absorbed on an added synthetic polymer like polyvinyl pyrolidone, nylon powder, or even ion exchange resins.

5. The plant cell vacuole often serves as a cess pool in this creature which has no excretory system. The frequently noxious material accumulated in the vacuole is mixed with the protoplasm when the cell is broken open and may denature or inhibit the enzymatic machinery. In the case of acid accumulating Crassulacean plants, this problem has been overcome by infiltrating the tissue with alkaline buffer before homogenizing.

6. The metabolic rates in many plant tissues are relatively low compared to bacterial and animal cells (the problem of dilution with water and cell wall plus a slower rate of cell replication). Thus, most plant metabolism studies begin with isotope measurements.

7. The problem of bacterial contamination of many tissues is quite a danger when studying low-rate phenomena in tissue homogenates – many experiments on amino acid incorporation into chloroplasts and plant mitochondria went down the drain when bacterial contamination was properly indentified. The use of axenic cultures of algae or of higher plant cells in tissue culture is one obvious way around this problem. Otherwise, careful controls must be used to subtract or correct for the misleading effects of bacterial contamination.

To illustrate the dilution problem, consider that of the one-half of the dry weight (i.e., 5% of the fresh weight) which is not cell wall, roughly half is soluble cytoplasmic constituents and half is particulate material. For either fraction, roughly half is protein. To obtain one gram of unfractionated leaf cytoplasmic protein, something over 80 grams of fresh leaves are required.

The size and number of cellular constituents are indicated in Table 1.1. In 1947, one guessed that a cell might contain 1,000 different kinds of enzymes.

TABLE 1.1 SIZES AND NUMBERS OF PLANT CELL STRUCTURES

	Diameter in Angstroms	*Number per Cell*
Nucleus	$5\text{–}20 \times 10^3$	1
Chloroplasts	$4\text{–}10 \times 10^3$	50–200
Mitochondria	$1\text{–}5 \times 10^3$	500–2,000
Microbodies	$2\text{–}15 \times 10^3$	300–3,000
Ribosomes	250	$5\text{–}50 \times 10^5$
Enzymes	20–100	$5\text{–}50 \times 10^8$

Bonner[1] has revised the estimate by one order of magnitude to 10,000 kinds of enzymes and adds another 100 kinds of non-enzymic proteins per cell. The non-enzymic proteins – structure proteins and nitrogenous reserves – are frequently much more abundant than individual enzymes, so that in enzyme isolation one usually needs very large quantities of plant material to obtain a reasonable amount of pure enzyme.

1.2 THE MAGNITUDE OF PLANT PROCESSES

Before plunging into the detailed substance of plant biochemistry, consider briefly the magnitude of the total process of plant metabolism. M. Kamen is fond of decrying the twin vices of "mammalian chauvinism and temporal solipsism" which restrict enthusiasm for plants and their works.[2,3] Some gee-whiz numbers might stimulate a look beyond the more conventional horizons. Approximately 100 billion (10^{11}) tons of carbon dioxide are consumed by photosynthesis per year.[4,5] This activity may be compared to the 4 billion (4×10^9) tons of fossil fuel consumed or the 400 million (4×10^8) tons of steel produced per year in the last decade.[6] In terms of magnitude of per annum activity, green plants handle about 250 times as much material as produced by the steel industry. The total net deposit of photosynthesis in terms of fossil fuel reserve on this planet is estimated at 10^{13} tons.

The temporal magnitude of photosynthesis is only vaguely reflected in the fossil fuel figures, and recent geochemical studies indicate that photosynthesis has been going on for a long time. Barghoorn has made interesting attempts at establishing chemical evidence for the antiquity of life beyond the age of obvious fossil deposition. The age of the earth is currently set at 4.9×10^9 years by radioactive uranium-thorium dating. Barghoorn took carbonaceous deposits that could be accurately dated by radiochemical measurements and he observed what appear to be fossil algae in electron micrographs of these samples.[7] In order to obtain chemical evidence, these rocks were carefully surface cleaned to remove contemporary contaminants, pulverized, extracted, and the extract hydrolyzed in hydrochloric acid to release amino acids from possible peptides.[8] The extract was then run through an amino acid analyzer, with the results as shown in Table 1.2 and Figure 1.1. Even without peptide hydrolysis, the samples

TABLE 1.2 FREE AMINO ACID CONTENT OF CHERT

Specimen	*Age* $\times 10^9$ *Years*	*Glycine nmoles/10 gm Sample*
Bitter Springs	1.0	18.1
Gunflint	1.9	9.8
Fig Tree	3.1	5.9

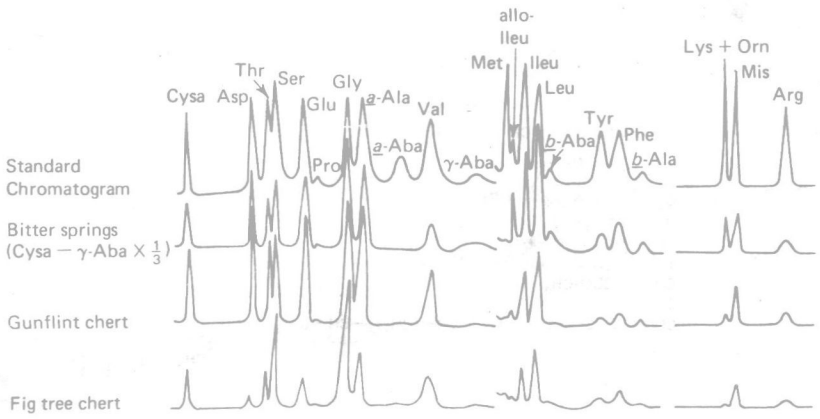


Figure 1.1 Amino acid analyzer chromatograms of the acid hydrolysates of three Precambrian cherts. [From Schopf, J. W., K. A. Kenvolden, and E. S. Barghoorn, *Proc. Natl. Acad. Sci. USA*, 59, 639 (1968).]

are seen to contain free amino acids whose concentration is related to the antiquity of the sample. The acid hydrolysates from the rock extract showed amino acid analyzer patterns of decreasing complexity with increasing age, presumably because of the relative instability of different amino acids. Identity of eleven amino acids was checked by derivitization and vapor-phase chromatography. Although this kind of data does not rule out an abiogenic origin of the compounds, it is an interesting probe at the biochemical information available in fossil material.

Another more frequently cited evidence of ancient plant life is the appearance of porphyrins and certain hydrocarbons in oil shale. The hydrocarbons pristane and phytane are particularly interesting since they can be detected with great sensitivity and unambiguous accuracy in the mass spectrometer. These compounds are presumed to arise from the degradation of chlorophyll via the reactions in Figure 1.2. Pristane and phytane have been identified in the Gunflint chert and in the Fig Tree material dating chlorophyll back to more than 3 billion years.

The Gunflint deposits show structures that may be fossil blue-green algae and other evidence suggests that this material was deposited at the beginning of atmospheric oxygen production. On taxonomic grounds the blue-green algae are the most primitive of oxygen producing plants. Contemporary blue-green algae show little respiratory oxygen consumption and no activity in the mixed function oxidase reactions where oxygen is used for biosynthetic purposes. It is reasonable that organisms responsible for the appearance of oxygen in the atmosphere would not need oxygen for growth.

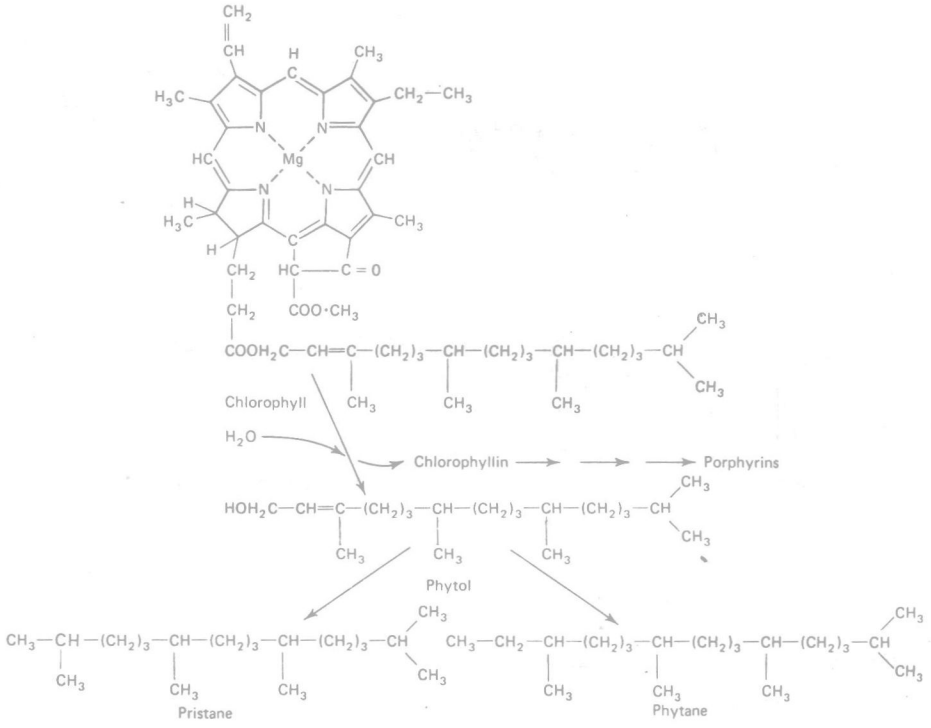


Figure 1.2 The degradation of chlorophyll to stable hydrocarbons of characteristic structure.

1.3 THE EVOLUTION OF PLANT CONSTITUENTS

With the consolidation of descriptive knowledge of biochemistry, it has become possible to try to distinguish ancient from more modern biochemical processes or to seek this history in the structure of macromolecules. A few examples are of interest at this point, but the theme of biochemical evolution will turn up again and again.

The ability to synthesize certain complex metabolites is an indication of the position of the plant tissue on the evolutionary scale. Sterols and certain unsaturated fatty acids are found in red algae and higher plants but not in bacteria and only occasionally in blue-green algae.[9] This is consistent with the theory that blue-green algae are responsible for the introduction of oxygen in the earth's atmosphere since these compounds are formed through oxygenase type reactions. These processes could only evolve after atmospheric oxygen became available.

The structure of complex macromolecules contains detailed information about the evolutionary position of the organism and amino acid sequencing has opened a whole new world of taxonomic and evolutionary comparisons. The ferredoxin molecule is an interesting case in point. This is a low molecular weight protein found in certain anaerobic heterotrophic bacteria and in photosynthetic autotrophs. Since ferredoxin is a small protein and is readily purified, it is admirably suited for sequence work. A ferredoxin sequence is shown in Figure 1.3. A comparison of the two halves of the ferredoxin isolated from the heterotrophic bacterium *Clostridium pasteurianum* indicates that this molecule arose by the doubling of a relatively short gene.[10]

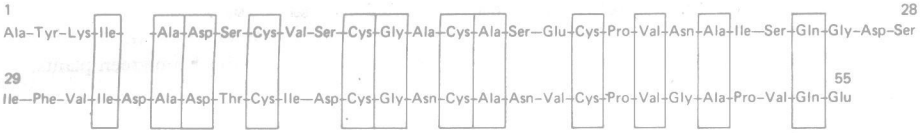


Figure 1.3 Amino acid sequence of ferredoxin from *Clostridium pasteurianum*. Twelve of the first twenty-eight residues are found in corresponding positions in the latter half of the sequence from residue 29 to 55. A detailed comparison of the nucleic acid codons responsible for positioning each of these amino acids suggests that most of the variations between the two halves resulted from a change of only one nucleotide per codon.

The ferredoxin from the photosynthetic bacterium *Chlorobium* has been isolated and appears similar to Clostridial ferredoxin in size and amino acid composition. However, the ferredoxin from the photosynthetic bacterium *Chromatium* is larger – 81 residues instead of the 53 to 57 residue lengths found for other bacterial ferredoxins. Ferredoxins from blue-green algae, green algae, and from a number of higher plants show a characteristic size of between 97 to 105 residues. The clear indication of gene doubling in the short bacterial ferredoxins prompts the hypothesis that the gene tripled in *Chromatium* and quadrupled in algae and higher plants.[11] More sequences of bacterial and algal ferredoxins are needed to provide convergent evidence for this suggestion.

Amino acid sequences for higher plant ferredoxin are rapidly becoming available and four sequences are shown in Figure 1.4. The sequences are for ferredoxins from *Leucaena glauca* – a small, leguminous tree, spinach, alfalfa, and *Scenedesmus* – a green alga. In all but the first line, only the positions of variance are shown so that even a cursory glance indicates that the molecules are very similar.

In the *L. glauca* sequence, there are four positions where two amino acid residues are indicated for the same location (6, 12, 33, and 96).[12] Positions 6 and 12 occur within the same tryptic peptide fragment, and this peptide is found in two forms (6-Leu, 12-Pro and 6-Val, 12-Ala) indicating microheterogeneity

						Leu			10			Pro							20				
L. glauca	Ala	Phe	Lys	Val	Lys	Val	Leu	Thr	Pro	Asp	Gly	Ala	Lys	Glu	Phe	Glu	Cys	Pro	Asp	Asp	Val	Tyr	Ile
Spinach	Ala	Tyr			Thr	Leu	Val			Thr		Asn	Val			Gln							
Alfalfa	Ala	Tyr			Lys	Leu	Val			Glu		Thr	Gln			Glu							
Scenedesmus	Ala	Tyr			Thr	Leu	Lys			Ser		Asp	Gln	Thr	Ile	Glu					Thr		

	53							60									70							
L. glauca	Glu	Gly	Asp	Leu	Asp	Gln	Ser	Asp	Gln	Ser	Phe	Leu	Asp	Asp	Glu	Gln	Ile	Glu	Glu	Gly	Trp	Val	Leu	Thr	Cys
Spinach	Thr		Ser		Asn		Asp								Asp			Asp						
Alfalfa	Ala		Glu	Val	Asp		Ser		Gly						Asp			Glu						
Scenedesmus	Ala		Thr	Val	Asp		Ser		Gln						Ser		Met	Asp	Gly		Phe			

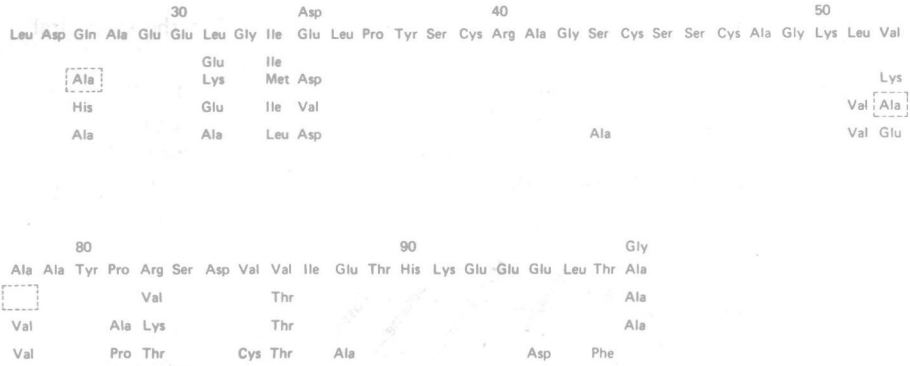
Figure 1.4 A comparison of amino-acid sequences of ferredoxins from green plants.

within this protein. Since this heterogeneity in sequence is found in a protein sample isolated from a single tree, it is not because of subspecies variation among plants.[13] These sequence ambiguities might result from polyploidy – a duplicate set of chromosomes or genes are present in the cell but are not exact duplicates as a result of independent mutations, so they code for slightly different proteins.

One can quantitate the comparison of these ferredoxins by noting that the higher plant proteins differ among themselves in from 18 to 21 positions while the algal ferredoxin differs in 28 or 29 positions from the higher plant ferredoxins. Since an amino acid substitution might be the result of 1, 2, or 3 nucleotide changes, the degree of difference between proteins can be refined by considering the nucleotide triplet that constitutes the codon for each residue. The degree of difference in nucleotide sequence can be expressed as the minimum base difference per codon for the proteins to be compared. Thus, the following comparisons;

L. glauca/spinach	MBDC = 0.30	(This agrees with the classical taxonomic positions of these
L. glauca/alfalfa	MBDC = 0.26	plants in which *L. glauca* is more closely related to alfalfa
spinach/alfalfa	MBDC = 0.28	than to spinach.)
Scenedesmus/spinach	MBDC = 0.41	(Obviously, algae are more remotely related to higher plants
Scenedesmus/alfalfa	MBDC = 0.42	than higher plants are related to one another.)

Comparison of the amino acid sequences and attendant codon sequences of the ferredoxins from *Scenedesmus* and spinach suggests a mechanism for the evolution at residues 51 to 53. A frame shift mutation as illustrated in Figure 1.5 could have occurred by dropping a deoxyguanilic from the Val codon at position 51 and by adding a deoxyadenylic at the Ala codon at position 53.



The evidence for gene duplication or quadruplication in the higher plant ferredoxins is largely obliterated from the sequences since a lot of mutations may have occurred within the subsections. It is interesting to note that the four pairs of Ala-Ala in the alfalfa protein start segments of 26, 25, 26, and 20 residues in length with most of the variable positions clustered near the amino terminal end of these segments.

Figure 1.5 A possible frame shift mutation in the evolution of ferredoxin. Deletion of a nucleotide in codon 51 and insertion of a nucleotide in codon 53 allows the preservation of the amino acid sequence beyond position 53.

The prime example of the contribution of amino acid sequence to evolutionary and taxonomic comparisons has come from comparing sequences of the protein cytochrome c. Although most of the sequences have been done for mammalian cytochromes, the few sequences for plant cytochromes permit interesting inferences. Kamen has obtained a sequence for cytochrome c_2 of the photosynthetic bacterium *Rhodospirillum rubrum*.[14] First, there is only slight homology to mammalian cytochrome c so this is a sequence that is largely altered over the early stage of evolution. In addition, the sequence of *R. rubrum* cytochrome c of 112 amino acids shows a 13 to 20 amino acid repeating