

Enzymatic Reaction Mechanisms Regarding Animal Waste Natural Organic Compounds Plant Absorption Conceptual Study

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Abstract

In this conceptual study, the plant absorption pathway of extracellular organic compounds has been explained by applying fundamental notions of organic chemistry. Indeed, as soon as animal waste nutrients, particularly carbohydrates, proteins, or lipids, are combined with plant cellular membrane constituents—essentially glycoproteins, glycolipids, or phospholipids—they bind together or interact due to their functional groups at the level of which organic reactions occur. As a result, carbohydrates, proteins, or lipids will be hydrolyzed to produce glucose, amino acids, or fatty acids, which will react with glycoproteins, glycolipids, or phospholipids to generate their analogs or the corresponding products, which will then be able to diffuse smoothly across the plant cellular membrane and therefore reach the cytoplasm, where they will be hydrolyzed to release glucose, amino acids, or fatty acids for plant development or growth.

Keywords

Carbohydrates, Proteins, Lipids, Enzymes, Cellular Components

1. Introduction

1.1. Terminology

1) Enzymatic reaction mechanisms involve the delocalization of electrons from one atom to another within chemically reactive entities, depicted using arrows and catalyzed by enzymes. In other words, an electron-poor atom or electrophile accepts electrons originating from a nucleophile or base rich in electrons.

2) An enzyme is a protein that binds or reacts with a particular substrate to bring the concerned chemical reactive entities close to one another so that they

can interact to generate bonds; consequently, the activation energy decreases.

3) In this conceptual study, extracellular organic compounds refer to carbohydrates, proteins, and lipids derived from the decomposition or hydrolysis of animal waste, which will be absorbed by plant roots as nutrients.

4) Plant absorption is the penetration process of extracellular chemical products, notably carbohydrates, proteins, and lipids, into plant cells, specifically into the plant cellular cytoplasm.

5) An analogous compound is a corresponding product generated from the combination of a single substrate with an appropriate reagent, which is similar to the parent substrate. Because of the similarity of an analogous product, it can diffuse or pass through the plant cellular membrane.

1.2. Objectives

In connection with our ongoing research on organic waste to produce fertilizers, I would like to report herein detailed reaction mechanisms, which I have applied to plant cellular chemical constituents and animal waste components such as carbohydrates, lipids or fatty acids esterified to glycerol including proteins [1]. Indeed, animal waste components or extracellular organic compounds react with plant cellular membrane components, namely glycoproteins, glycolipids, and phospholipids, so that they can easily be absorbed as substantial nutrients for plant development [2]. The descriptive enzymatic reaction mechanisms revealed in this article remain an added value for comprehensive plant nutritional absorption.

2. Plants

Enzymes are significantly involved in the degradation of carbohydrates, lipids, and proteins so that plants can easily utilize their derived molecules for growth and replenishment. In this context, farmers using organic fertilizers can better understand the enzymatic processes regarding nutritional cycling and chemical modifications because these processes are substantial for correctly fertilizing the soil or making it productive.

3. Animal Waste Organic Compound Constitution

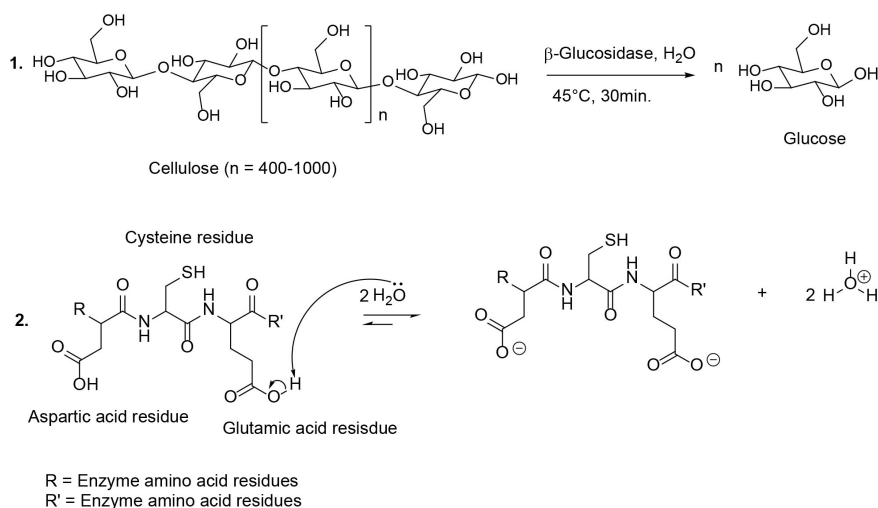
Animal waste mainly contains carbohydrates, lipids, and proteins as organic compounds. They are enzymatically decomposed into glucose, fatty acids, and amino acid units, respectively [3]-[9]. In other words, due to their functional groups, carbohydrates, proteins, and lipids behave as acids, bases, nucleophiles, or electrophiles toward soil microbial enzymes to achieve their hydrolysis. Their derivatives, notably glucose and amino acids, including fatty acids, will then react with glycoproteins, glycolipids, and phospholipids before entering plant cellular cytoplasm or being absorbed as nutrients by plants [3]-[9].

3.1. Enzymatic Animal Waste Carbohydrate Hydrolysis

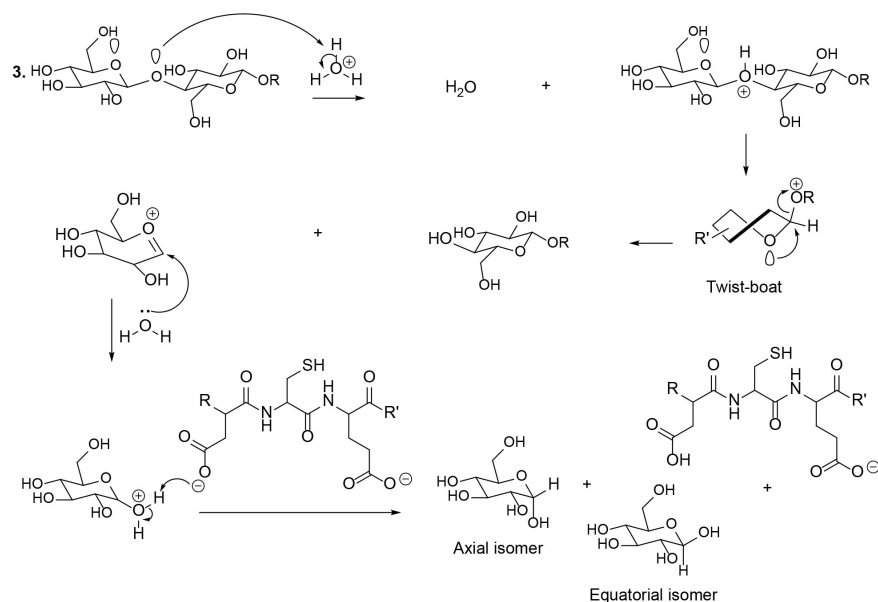
Soil microbes release enzymes into the intracellular medium, and these enzymes

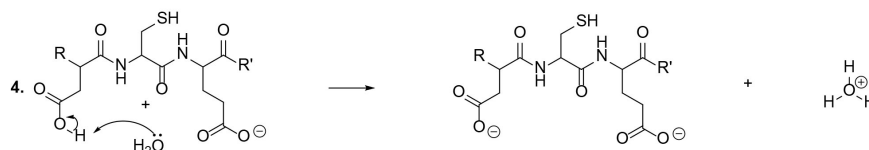
can pass through the cellular membrane to operate in an extracellular medium. Indeed, it has been reported that extracellular enzymes act as organic catalysts to accelerate the degradation of organic substances, including α -glucosidase, β -glucosidase, a specific enzyme that catalyzes the hydrolysis of glycosidic bonds and degrades complex carbohydrates such as cellulose into glucose [10]-[12].

The detailed carbohydrate hydrolysis mechanism will be particularly illustrated by utilizing the cellulose hydrolysis reaction mechanism under specific enzymatic catalysis [13]-[18]. Indeed, the experimental analysis revealed that at 45 °C the cellulase catalytic activity was still operational (Scheme 1, reaction 1). From the same perspective, it has been reported that aspartate, cysteine, and glutamate amino acid residues have been observed in the cellulase enzymes' active sites [13]-[18]. Indeed, amino acids are dissociated in the physiological medium due to its basicity (Scheme 1, reaction 2).



Scheme 1a

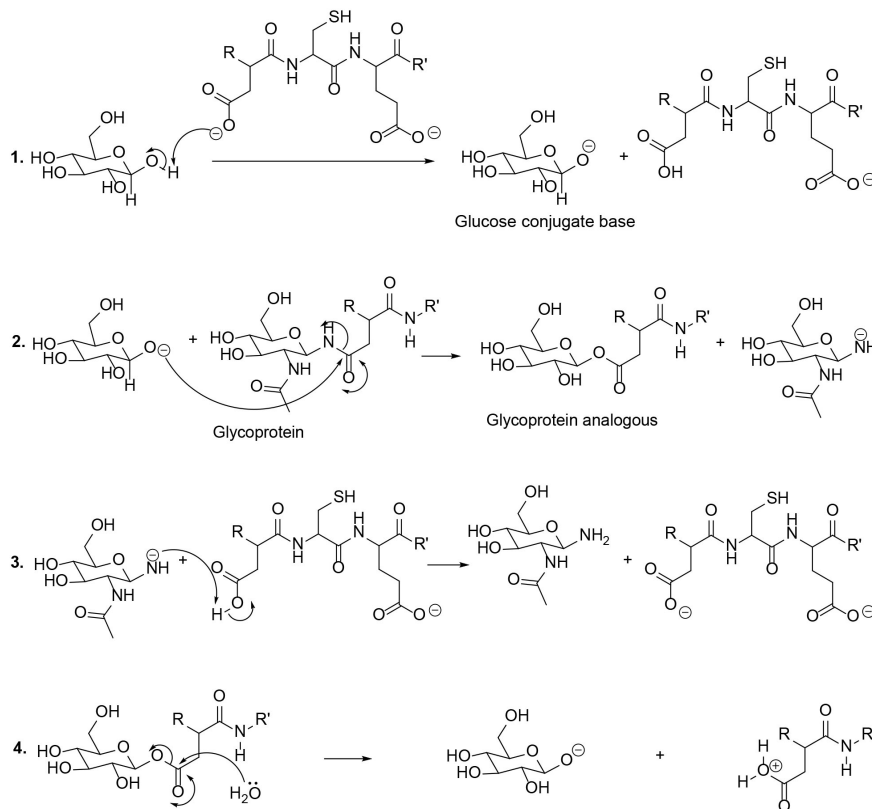


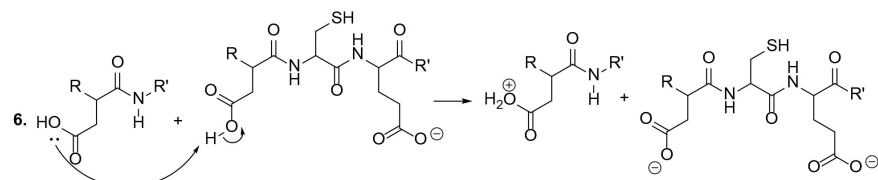
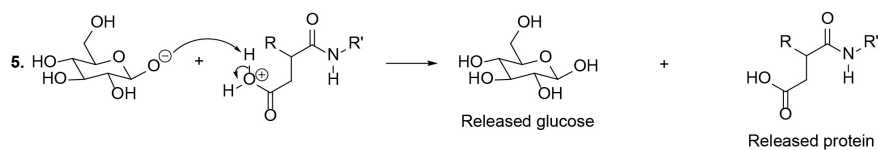


Scheme 1b

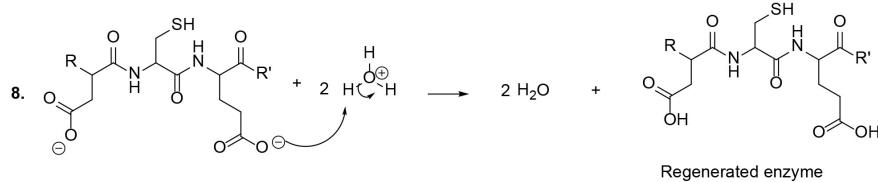
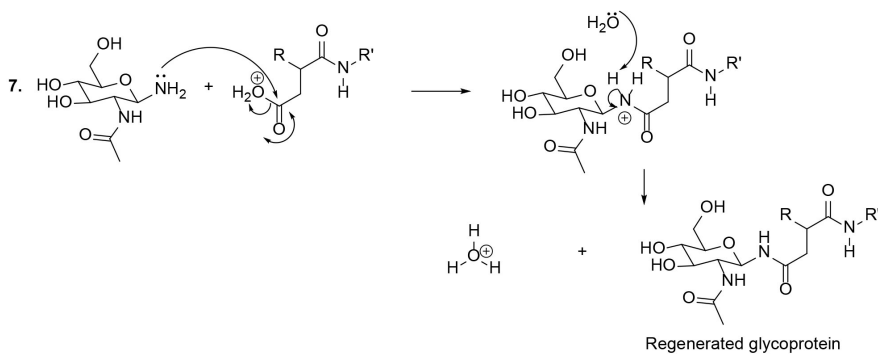
3.1.1. Glucose Addition to Plant Cellular Membrane Glycoprotein

Before entering the plant cellular cytoplasm or being absorbed by the plant roots, the glucose (axial isomer or equatorial isomer) will react with glycoprotein to generate the corresponding analogous product or glycoprotein analog, which is similar to the original glycoprotein. Therefore, the produced glycoprotein analog can easily pass through the plant cellular membrane and reach the cytoplasm, where it will be hydrolyzed to release the expected glucose for the benefit of the plant. In this regard, the plausible mechanism involves the reaction of the enzyme conjugate base with the equatorial isomer hydroxyl group to enhance its nucleophilic character (Scheme 2, reaction 1). This step is followed by the nucleophilic addition of the glucose conjugate base to glycoprotein to eject the leaving group and to produce the expected glycoprotein analog (Scheme 2, reaction 2). The following step is the hydrolysis of the glycoprotein analog within the plant cellular cytoplasm, and it will be metabolized to contribute to adequate plant growth (Scheme 2, reactions 4-5). The final steps of this reaction process are the regeneration of glycoprotein including the enzyme (Scheme 2, reactions 7-8).



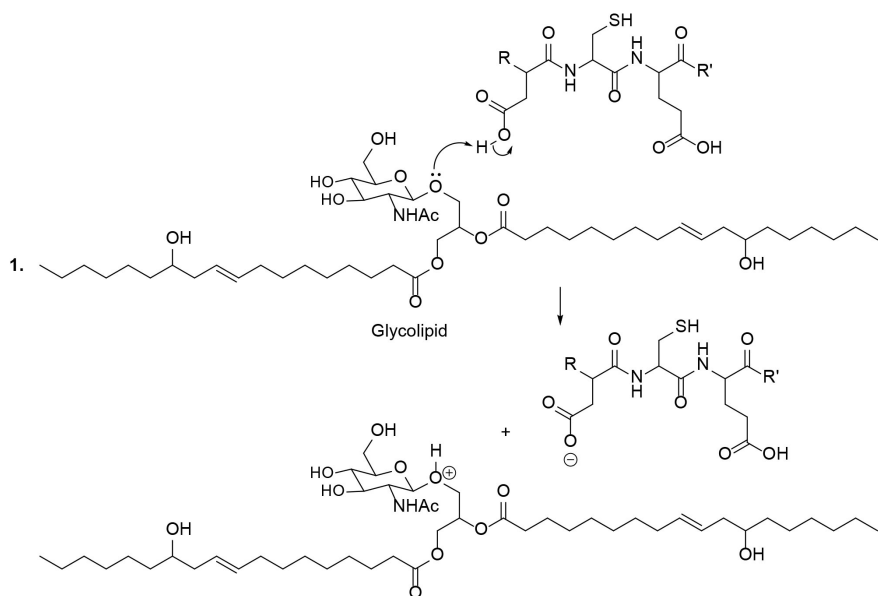


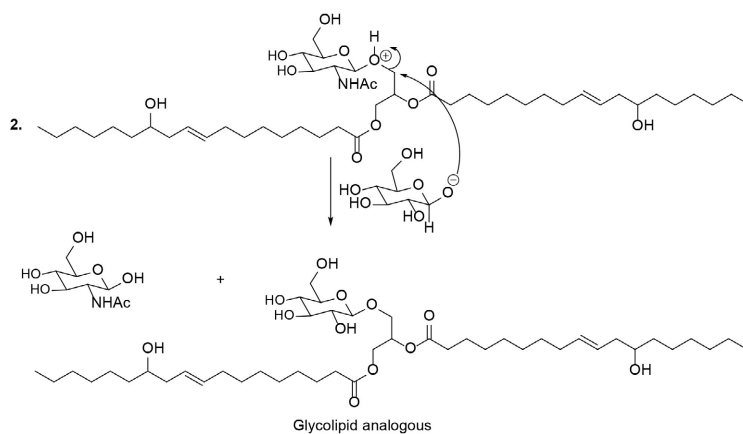
Scheme 2a



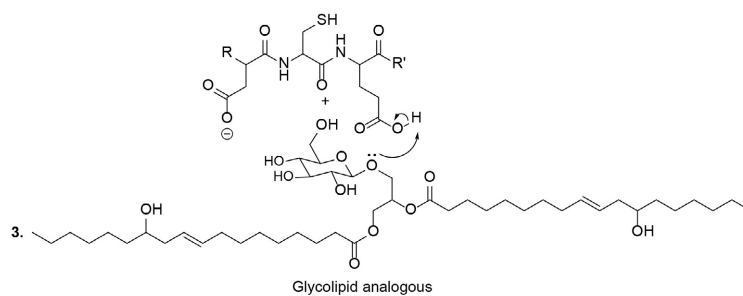
Scheme 2b

3.1.2. Glucose Addition to Plant Cellular Membrane Glycolipids

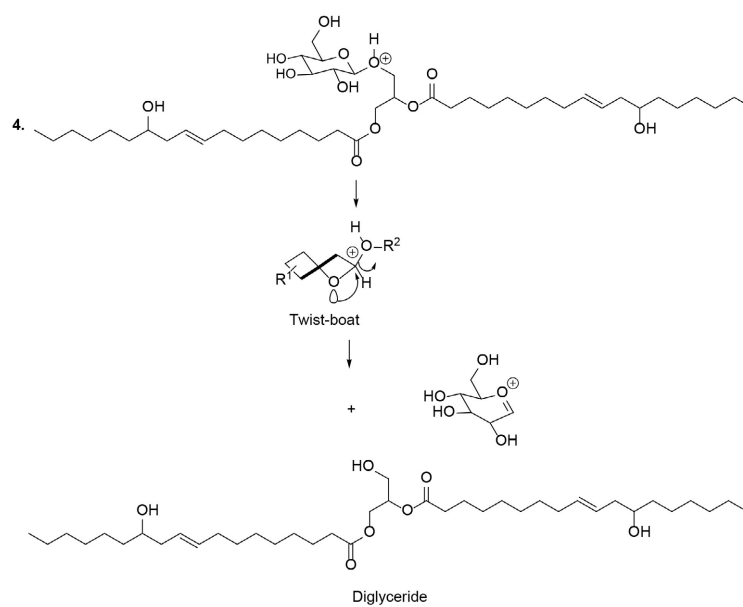


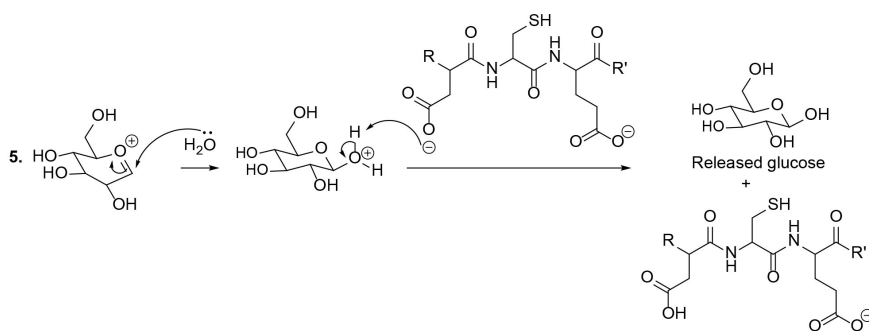


Scheme 3a

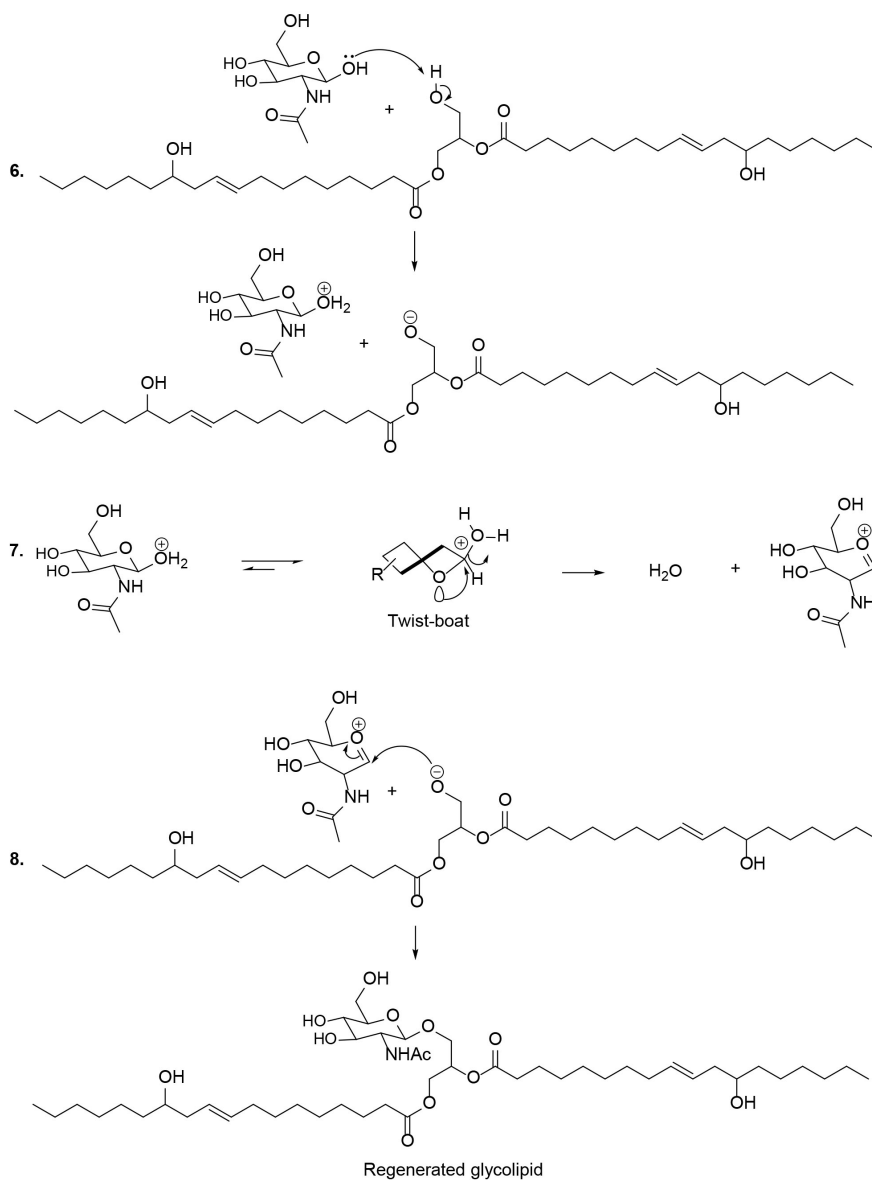


Scheme 3b





Scheme 3c



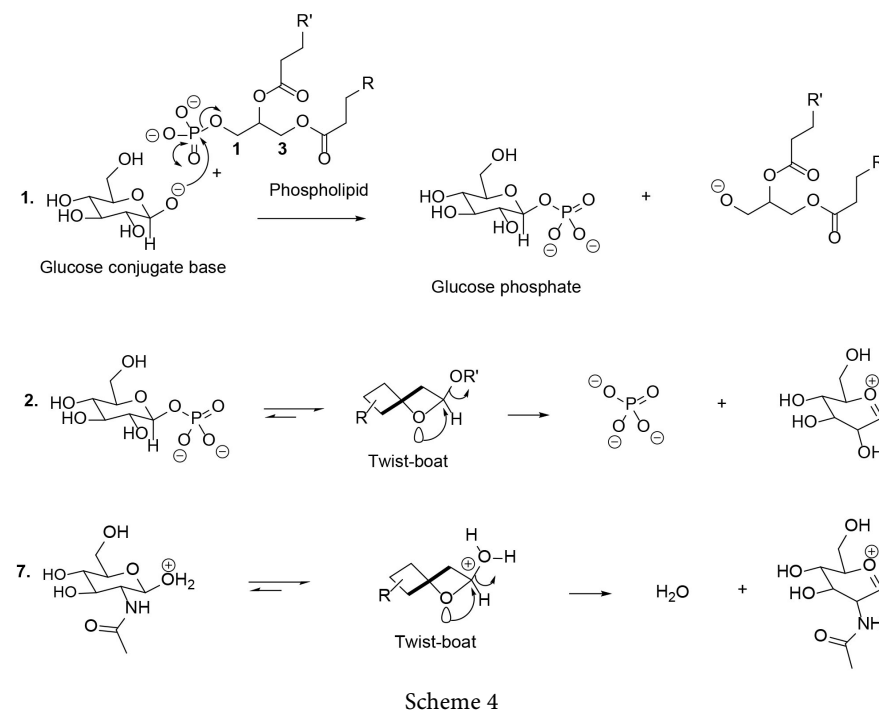
Scheme 3d

The glucose conjugate base as a powerful nucleophile will react with glycolipid to afford a glycolipid analog. In other words, it is a substitution reaction in which the

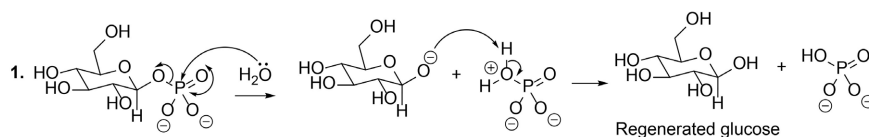
addition of the nucleophile to the substrate will displace the leaving group (Scheme 3, reaction 1). As previously stated, the enzyme will activate the substrate to bring it closer to the glucose conjugate base so that they can easily combine or react to substitute the leaving group and furnish the expected glycolipid analog (Scheme 3, reactions 1-2). When the glycolipid analog reaches the plant cellular cytoplasm, it will be disintegrated under the action of a molecule of water, producing or releasing the appropriate glucose as well as the corresponding diglyceride or diacylglycerol (Scheme 3, reactions 3-5). The final stage of this kind of reaction is the regeneration of the glycolipid substrate (Scheme 3, reactions 6-8).

3.1.3. Glucose Addition to Plant Cellular Membrane Phospholipid

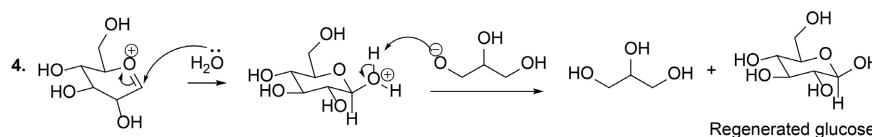
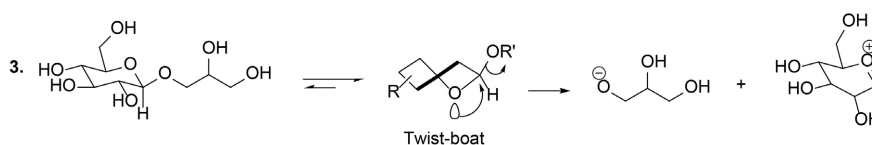
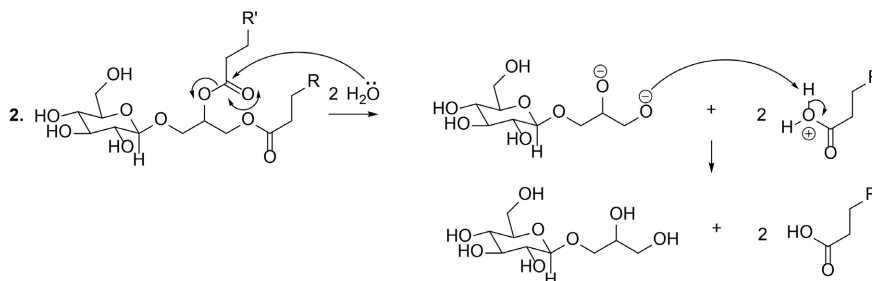
The addition reaction of glucose to phospholipid offers two possibilities. Undoubtedly, glucose can react with the phospholipid to remove the diglyceride conjugate base as a leaving group to generate the corresponding glucose phosphate, which is analogous to phospholipid (Scheme 4, reaction 1). Indeed, due to their similarity, the glucose phosphate will diffuse effortlessly through the plant cellular membrane and reach the cellular cytoplasm. In the same regard, the diglyceride ion (nucleophile) will react with the carbo-oxonium (electrophile) to afford the corresponding product that will also smoothly enter the plant cellular membrane (Scheme 4, reaction 2).



Glucose phosphate, due to its phosphate group electrophilic character, as well as its phospholipid analog, will therefore be hydrolyzed to release the expected glucose into the plant cellular cytoplasm, where it will be metabolized to fulfill its energetic role in the development of the desired plant (Scheme 5).



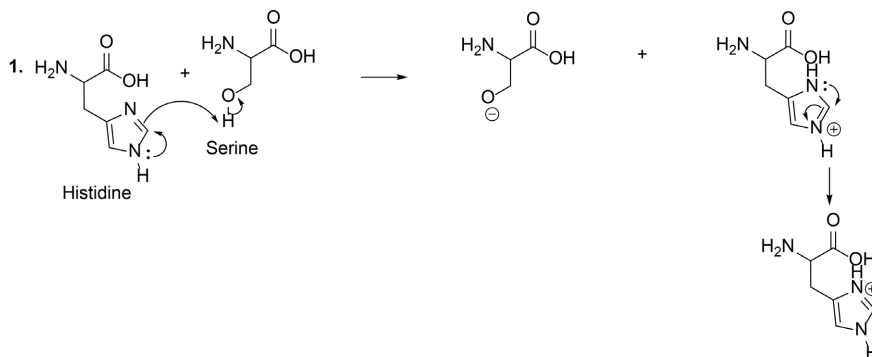
Scheme 5a

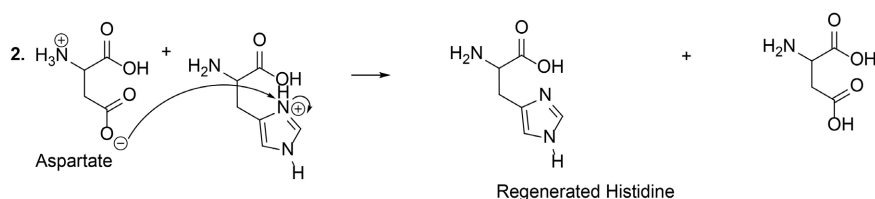


Scheme 5b

3.2. Enzymatic Animal Waste Lipid Synthesis

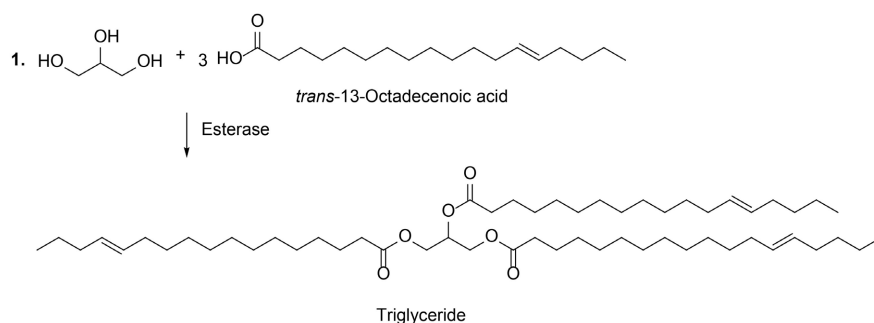
Lipids are organic compounds, which are constituted by fatty acids esterified to glycerol moisture. Indeed, glycerol is an alcohol bearing three hydroxyl groups that act as nucleophiles to displace three molecules of water from free fatty acids to produce triglyceride under an appropriate acid catalyst, which can be esterase or lipase because both enzymes catalyze the synthesis or the hydrolysis of esters [19]-[23]. This kind of synthetic reaction is known as the esterification of fatty acids, in which an acid catalyst favors the production of triglyceride or biodiesel. In this context, it has been reported that the essential active site amino acid residues of esterase or lipase are mainly comprised of aspartate, histidine, and serine that form the root of the enzymatic catalytic activities (Scheme 6) [19]-[23].



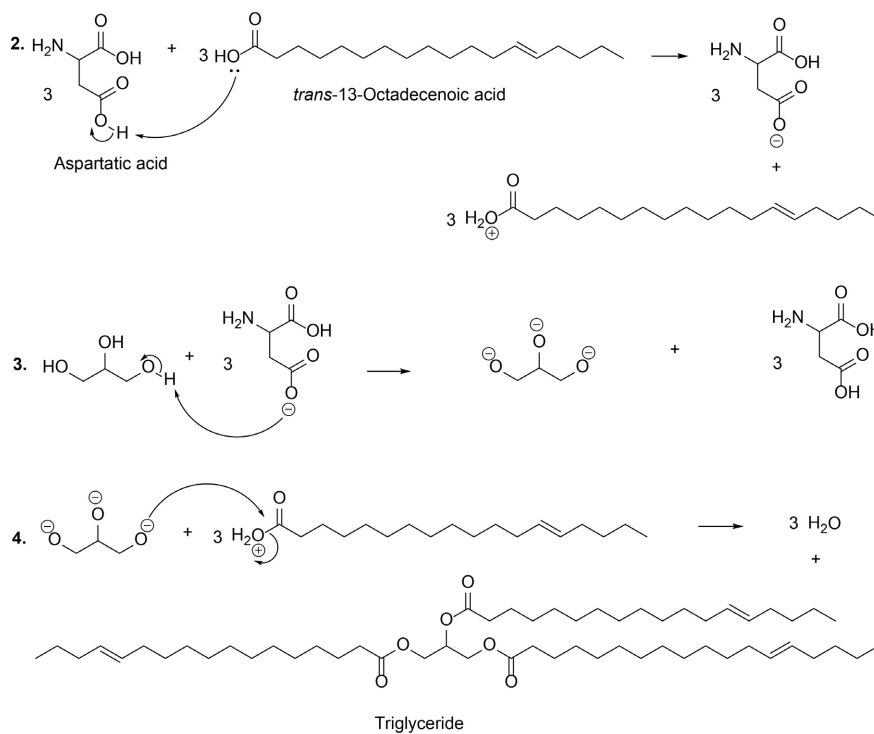


Scheme 6

Concerning the plausible mechanism of the esterification catalyzed by a specific enzyme, the first step is the protonation of the fatty acid by aspartic acid to generate aspartate, which will then deprotonate a molecule of glycerol to afford a corresponding conjugate base of glycerol and a regenerated aspartic acid (Scheme 7, reactions 2-3). The following step is the reaction between the glycerol conjugate base and the protonated fatty acids to remove 3 molecules of water and furnish the expected triglyceride (Scheme 7, reaction 4).



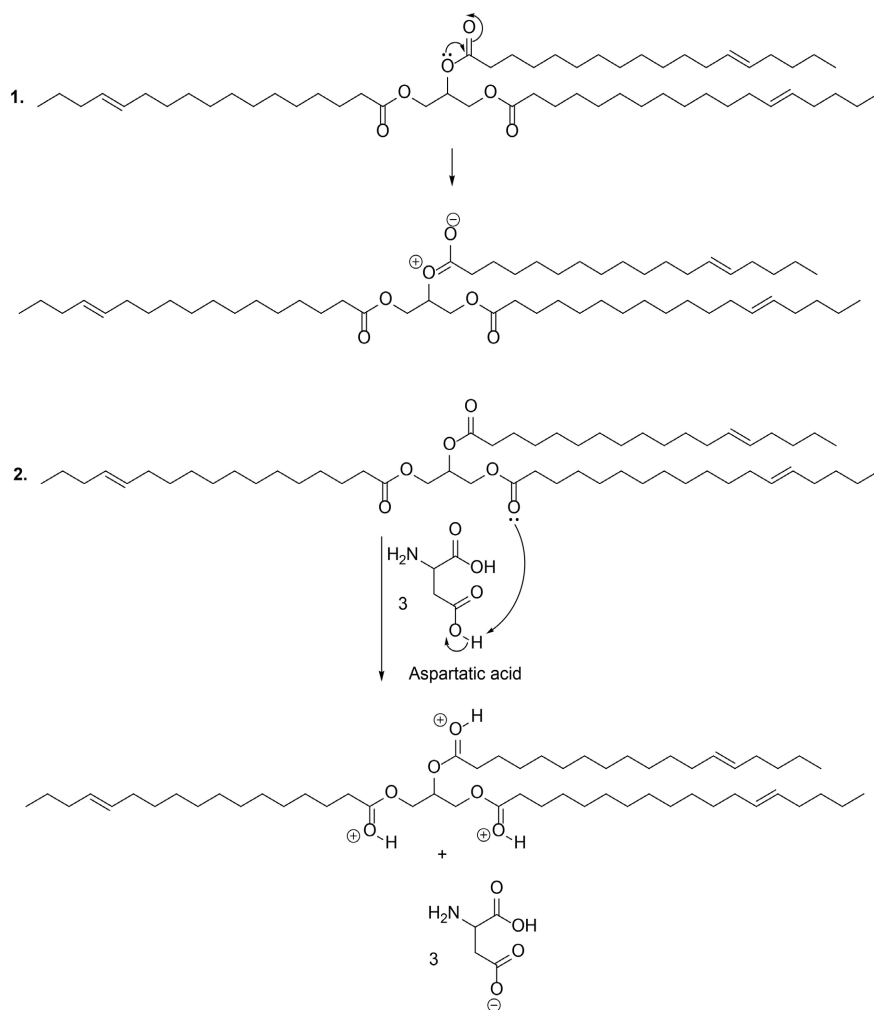
Scheme 7a



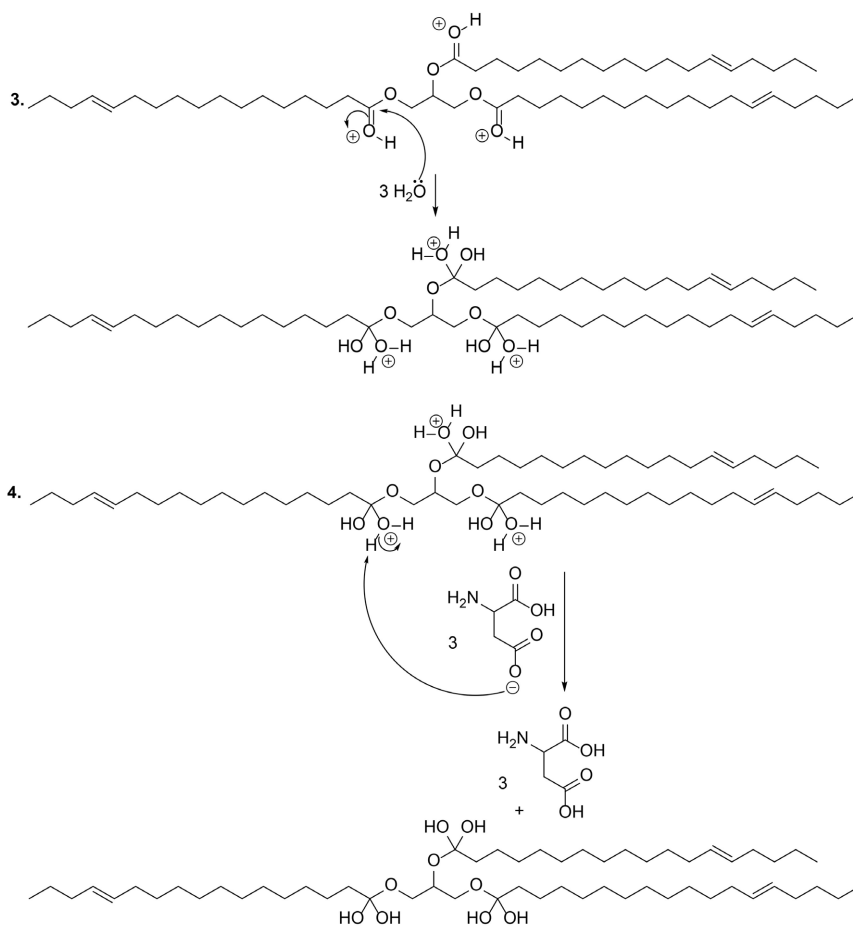
Scheme 7b

Enzymatic Hydrolysis of Animal Waste Lipids

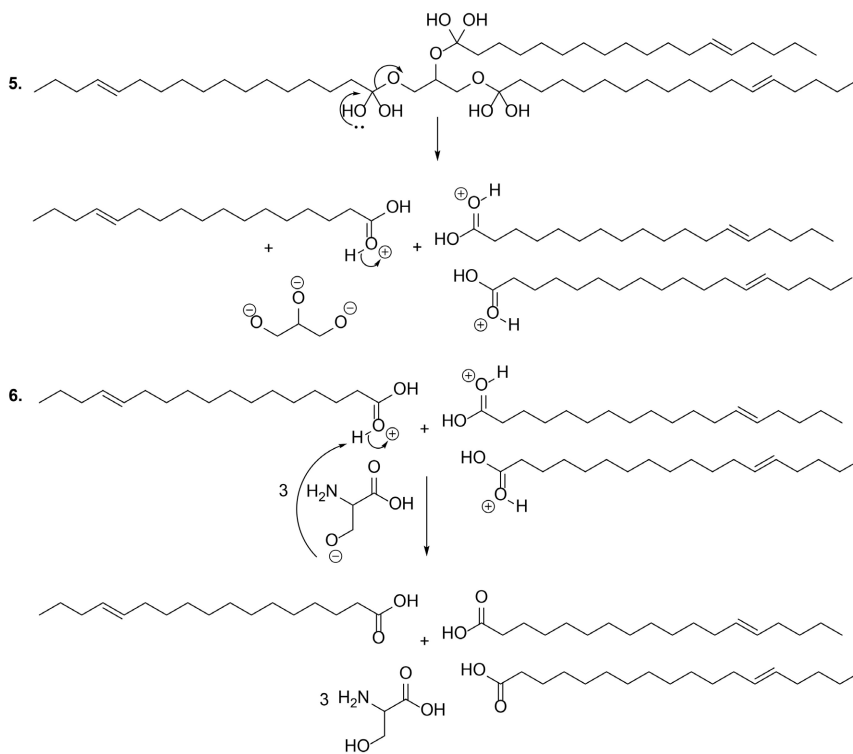
Having the synthesized lipid or triglyceride in mind, the bacterial enzymatic hydrolysis of lipids will therefore take place to release three molecules of fatty acids and a molecule of glycerol (Scheme 8). In this context, it has been reported that the essential active site amino acid residues of esterase are also applicable for the hydrolysis pathway [19]-[23]. Indeed, to illustrate animal waste lipid hydrolysis, *trans*-octadecenoic acids which have been observed in rats can be used for this purpose [19]-[23]. Regarding the reaction mechanism, the first stage is the protonation or activation of the ester moiety by the enzyme because the ester carbonyl group is less electrophilic due to the free electron pair on the oxygen atom, which can be delocalized toward the carbon atom of the carbonyl group, thereby decreasing its electrophilic character (Scheme 8, reactions 1-2). The ester carbonyl group being activated, three molecules of water will now hydrolyze the entire triglyceride with the support of the aspartic acid conjugate base and the serine conjugate base to furnish the expected three molecules of fatty acids and a molecule of glycerol (Scheme 8, reactions 3-7).

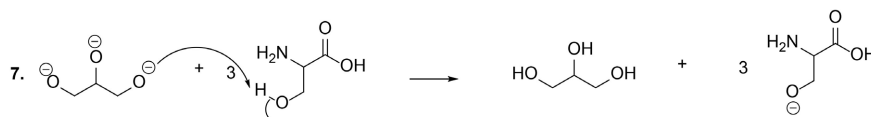


Scheme 8a



Scheme 8b

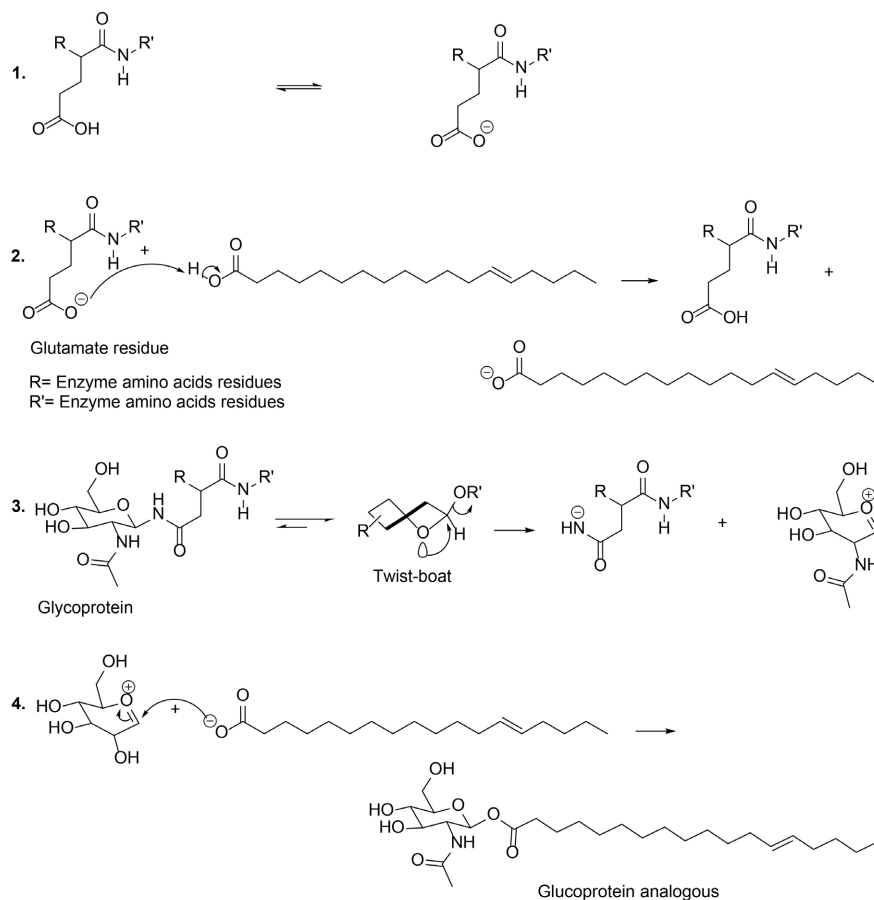




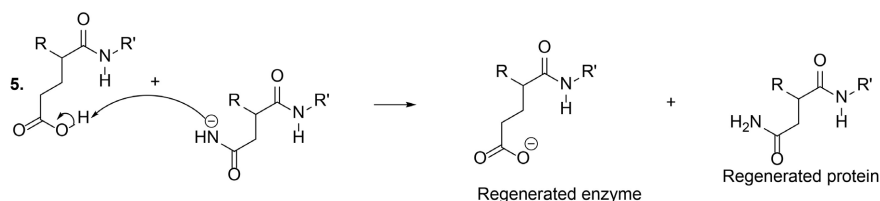
Scheme 8c

1) Enzymatic Addition of Fatty Acids to Plant Cellular Membrane Glycoprotein

To overcome the addition of fatty acid to glycoprotein, it is important to deprotonate the fatty acid to generate the corresponding acetate moiety derived from the fatty acid, which will then react with the oxonium ion generated from the twist-boat conformation adopted by the glycoprotein to facilitate the departure of the leaving group (Scheme 9, reaction 2). The following reaction stage is the combination of the oxonium ion with the fatty acid acetate moiety to achieve the formation of the glycoprotein-analogous product (Scheme 9, reaction 4). The final reaction step is the protonation of the protein conjugate base by the glutamic acid moiety of the enzyme to regenerate the enzyme as well as the protein containing an asparagine residue (Scheme 9, reaction 5). Indeed, the literature has revealed that glycosaminidase is specifically susceptible to achieving this kind of hydrolysis reaction and that the glutamic acid residue constitutes its catalytic action [14].



Scheme 9a



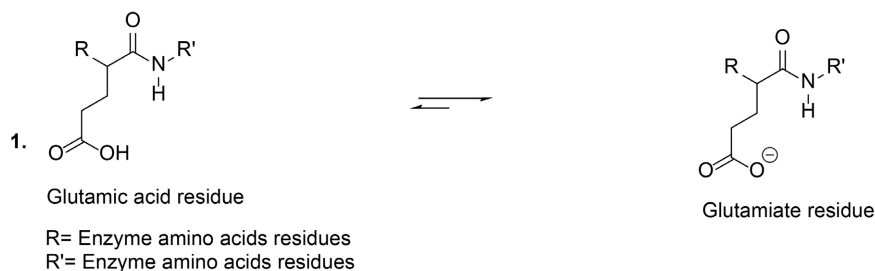
Scheme 9b

2) Enzymatic Addition of Fatty Acids to Plant Cellular Membrane Glycolipid

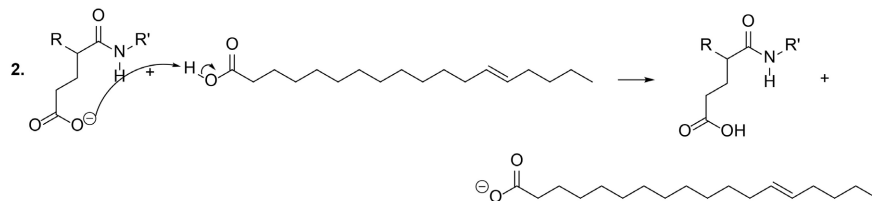
Transesterification is a suitable reaction to explain how animal waste fatty acids connect or react with plant cellular membrane glucolipids to afford the corresponding glucolipid analogues, which will then diffuse smoothly and fully through the plant cellular membrane to penetrate the plant cytoplasm for absorption purposes (Scheme 10). Indeed, the glutamate residue is more nucleophilic than the glutamic acid residue due to its free electron density, which allows it to easily remove the proton from the carboxylic group of the fatty acid to generate the corresponding carboxylate or the nucleophilic conjugate base (Scheme 10, reactions 1-2). In the following step, the generated nucleophilic conjugate base will decompose the plant membrane glucolipid to furnish the corresponding glucolipid analogue (Scheme 10, reaction 3). It is important to note that this process is called a transesterification reaction because the starting product or substrate possesses an ester moiety, which is maintained in the product after its transformation (Scheme 10, reaction 3). It is also important to mention that the structure of a compound determines its reaction behavior (Scheme 10).

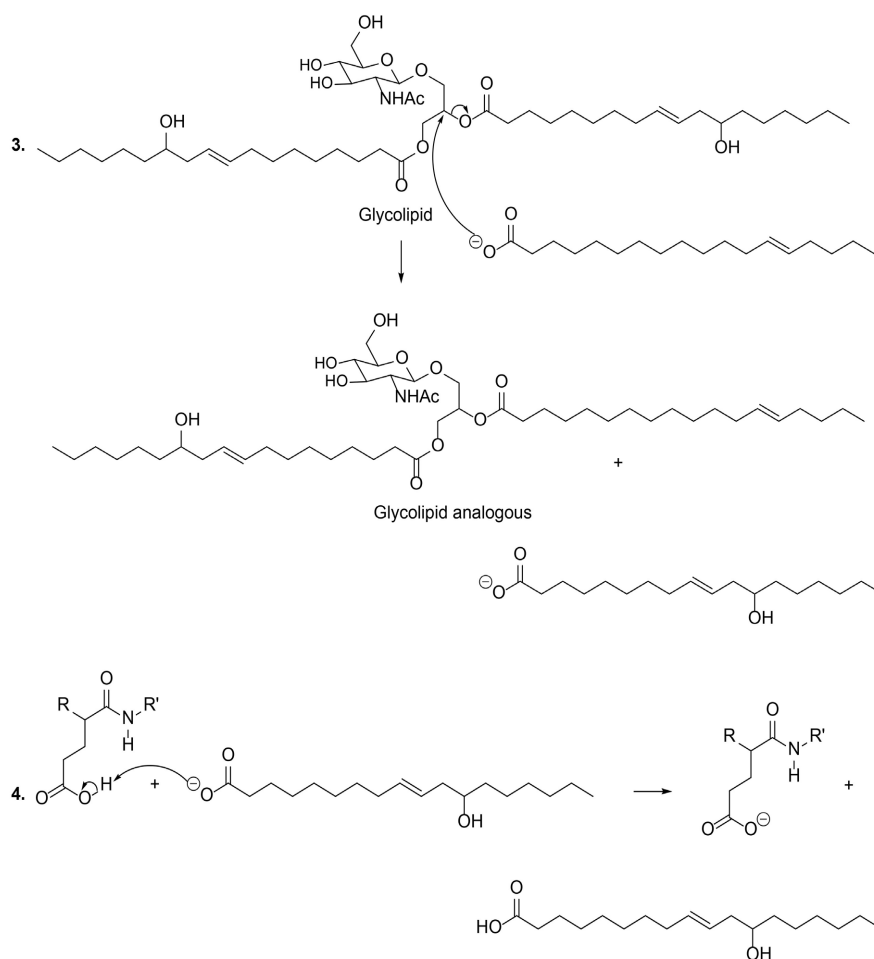
3) Enzymatic Addition of Fatty Acid to Plant Cellular Membrane Phospholipid

The transesterification chemical modification is appropriately applied to demonstrate the enzymatic catalysis of the addition of a fatty acid to a plant membrane



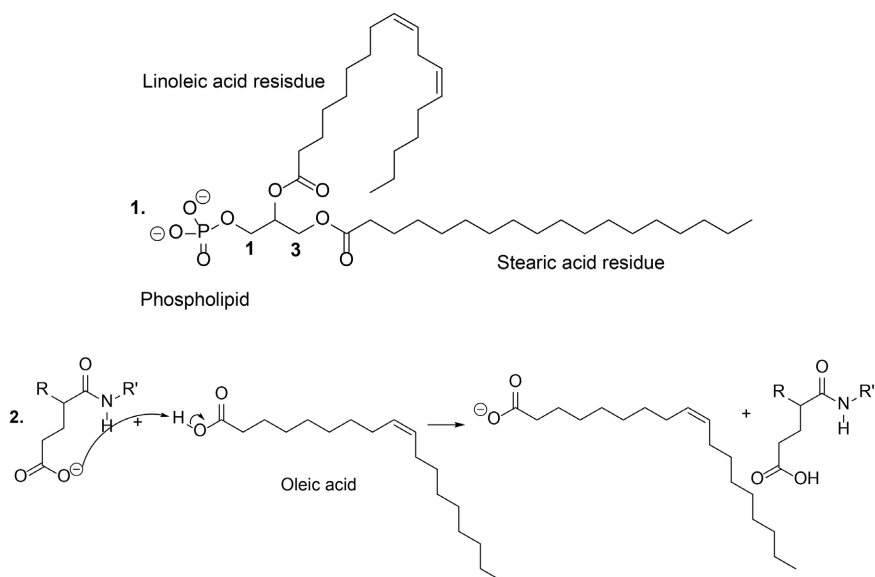
Scheme 10a



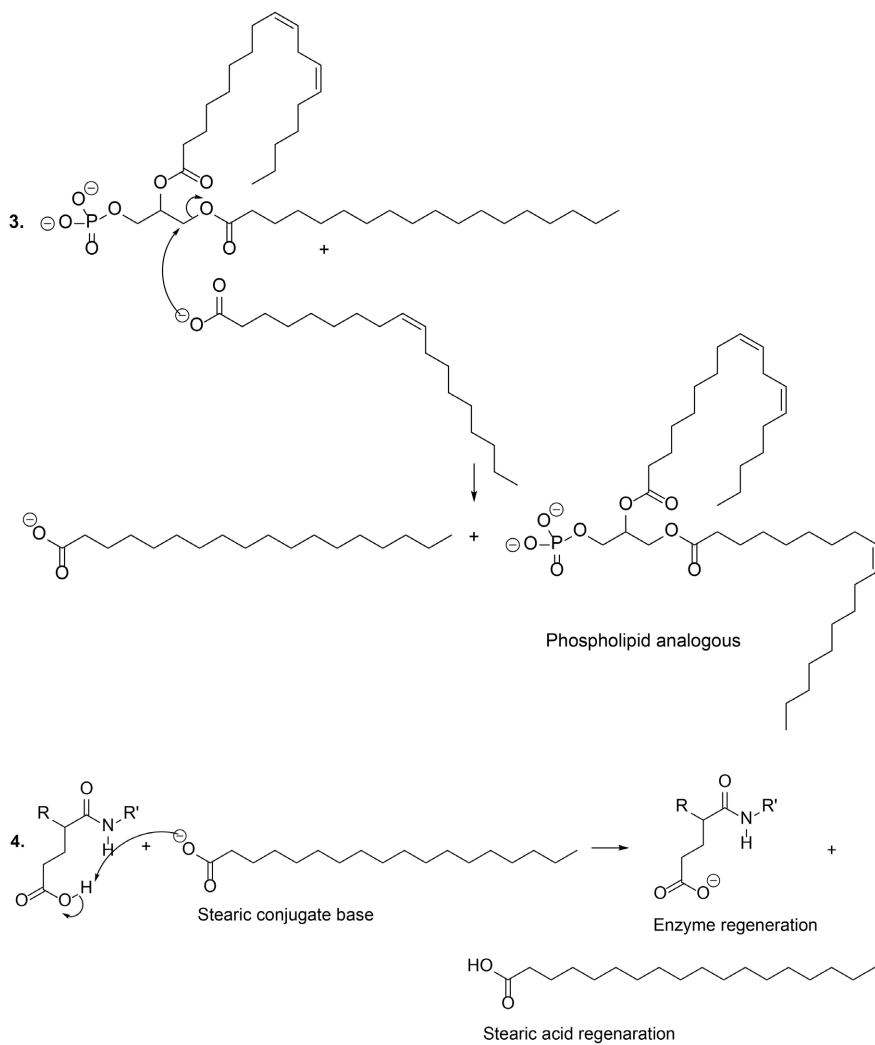


Scheme 10b

phospholipid. Indeed, it is notable to recall that a transesterification reaction is a chemical transformation of an ester as a starting material to afford another ester as a final product. Having that in mind, the connection of oleic acid, a fatty acid found in meat animals [19]-[23], to a phospholipid will displace a molecule of a linoleic acid residue or a molecule of a stearic acid residue as a leaving group to generate a corresponding analogous compound (Scheme 11, reaction 2). In other words, the plausible enzymatic mechanism of this kind of substitution reaction starts with the deprotonation of oleic acid to produce the acetate or the conjugate base of oleic acid and the conjugate acid of the glutamate residue (Scheme 11, reaction 2). The following step is the substitution of the stearic acid residue by the conjugate base of oleic acid, which behaves as a nucleophile, to furnish the corresponding phospholipid analog, capable of penetrating the plant cellular cytoplasm, and a conjugate base of stearic acid (Scheme 11, reaction 3). The final step concerns the protonation of the stearic acid conjugate base by the glutamate residue conjugate acid to regenerate the glutamate residue characteristic of the specific enzyme of this transesterification catalysis (Scheme 11, reaction 4).



Scheme 11a



Scheme 11b

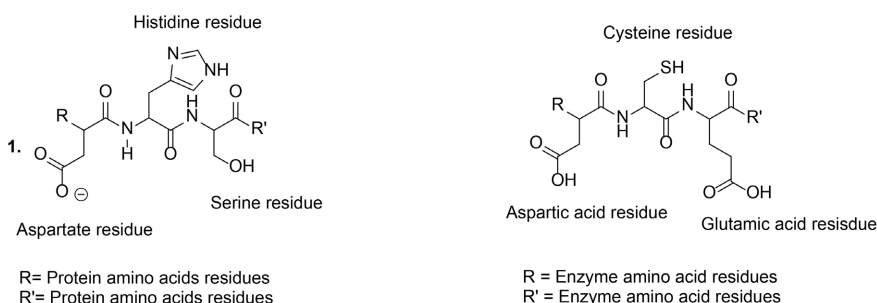
3.3. Enzymatic Animal Waste Protein Hydrolysis

Protein hydrolysis is defined as the disintegration of a protein into different amino acids under the action of an enzyme and a molecule of water. The hydrolysis reaction can take place in an acidic medium or a basic medium because of the nature or the chemical properties of the catalysis. In the current situation, due to the chemical properties of the lateral chains of the amino acid residues, the enzyme can behave as an acid catalyst or a base catalyst. In order to avoid confusion, it is meaningful to note that an enzyme is a particular protein, which catalyzes or accelerates the velocity or the speed of a specific reaction to achieve the desired product because of the polar amino acids of its active site, such as a glutamate residue (Scheme 12). This is a single region that constitutes the root of the catalytic activity of an enzyme.

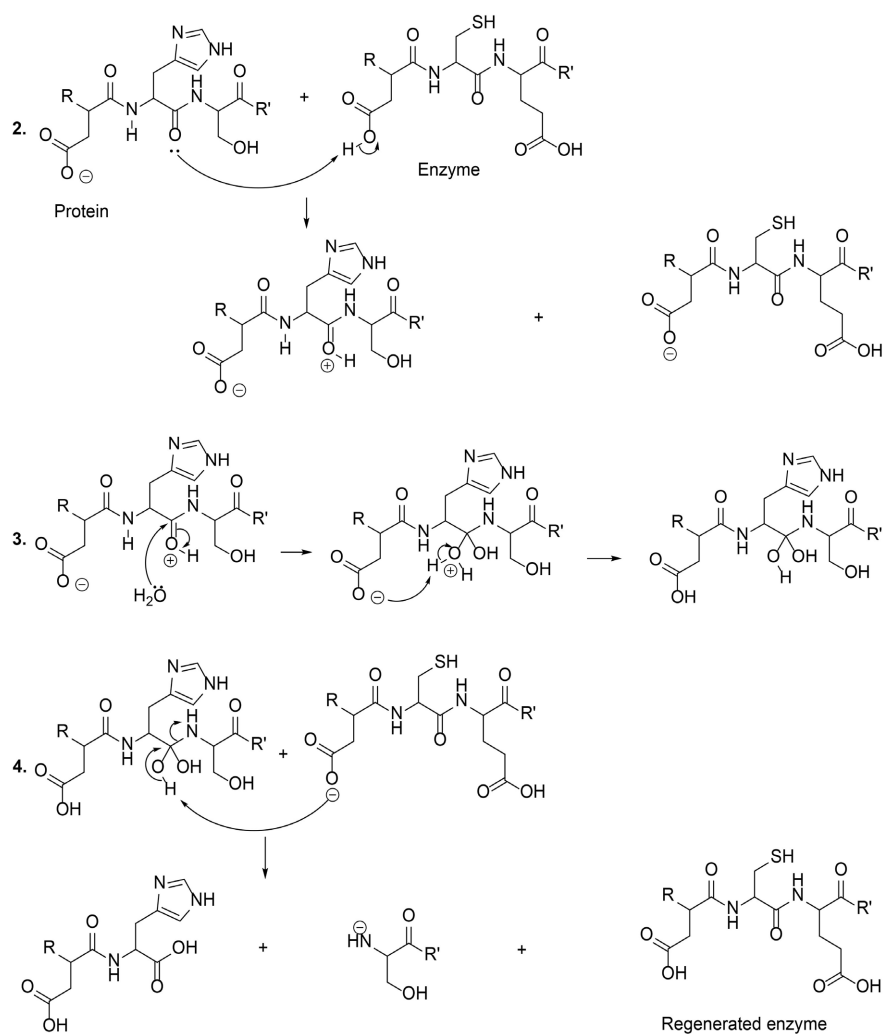
To accomplish the enzymatic animal protein hydrolysis, the enzyme protonates the protein carbonyl oxygen atom to increase the electrophilic character of the carbonyl group carbon and to afford the corresponding conjugate protein acid as well as the conjugate enzyme base (Scheme 12, reaction 2). In the following step, a molecule of water donates its free electron pair to the carbonyl group carbon previously protonated by the enzyme, cleaving the targeted peptide bond or amide bond and furnishing the expected amino acids as well as the regenerated enzyme (Scheme 12, reactions 3-5). The remaining protein portion is hydrolyzed following the same hydrolysis procedure (Scheme 12, reaction 6). The amino acids obtained from the enzymatic animal waste protein hydrolysis are therefore absorbed or incorporated into the plant cells through glycoprotein analog, glycolipid analog, or phospholipid analog synthesis (Scheme 12, reactions 5-9).

3.3.1. Enzymatic Addition of Amino Acids to Plant Cellular Membrane Glycoproteins

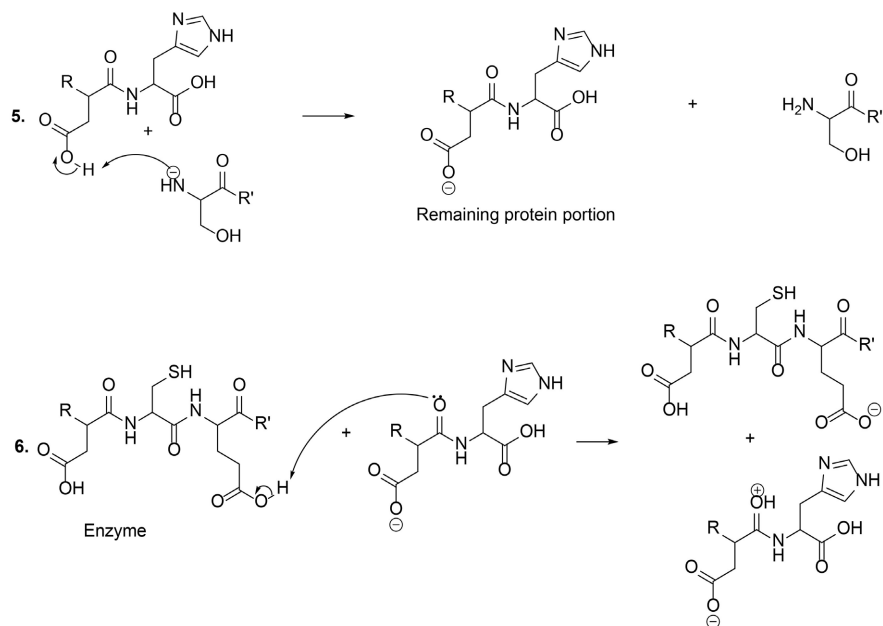
Considering the structure of the involved reactive entities, such as glycoprotein and histidine, the suitable addition of amino acids to glycoprotein is essentially a substitution reaction to substitute the protein moisture with histidine to generate a glycoprotein analog. In other words, the glycosyl fragment will remain in place or be conserved in the produced glycoprotein analog (Scheme 13, reaction 1). Indeed, the reaction mechanism will involve the protonation of the glycoprotein by the enzyme to obtain the corresponding glycoprotein conjugate acid as well as an

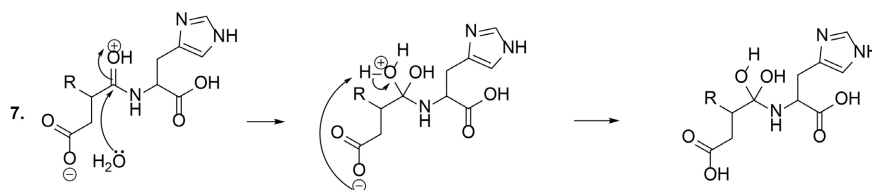


Scheme 12a

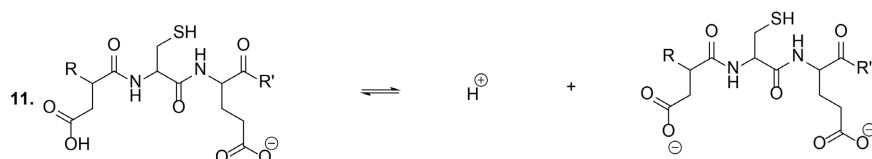
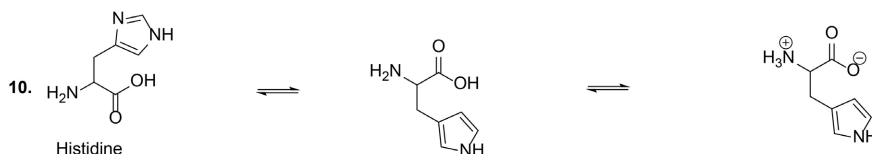
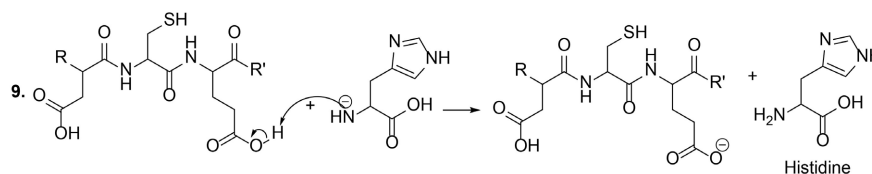
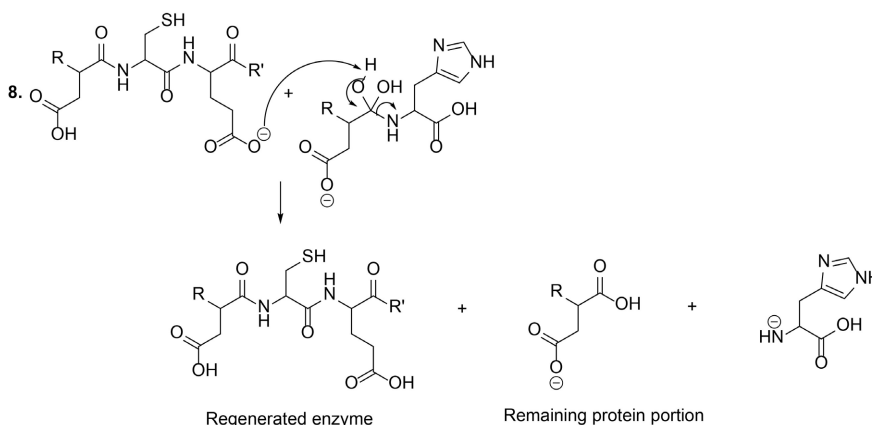


Scheme 12b



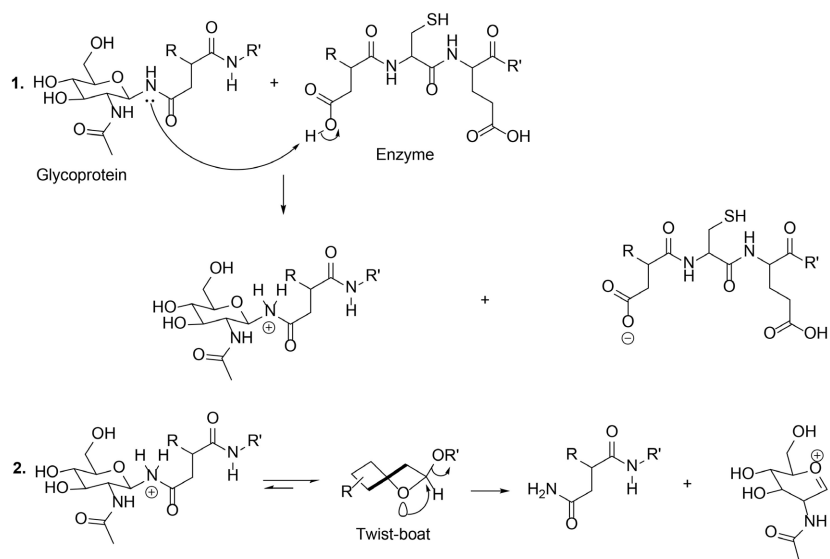


Scheme 12c

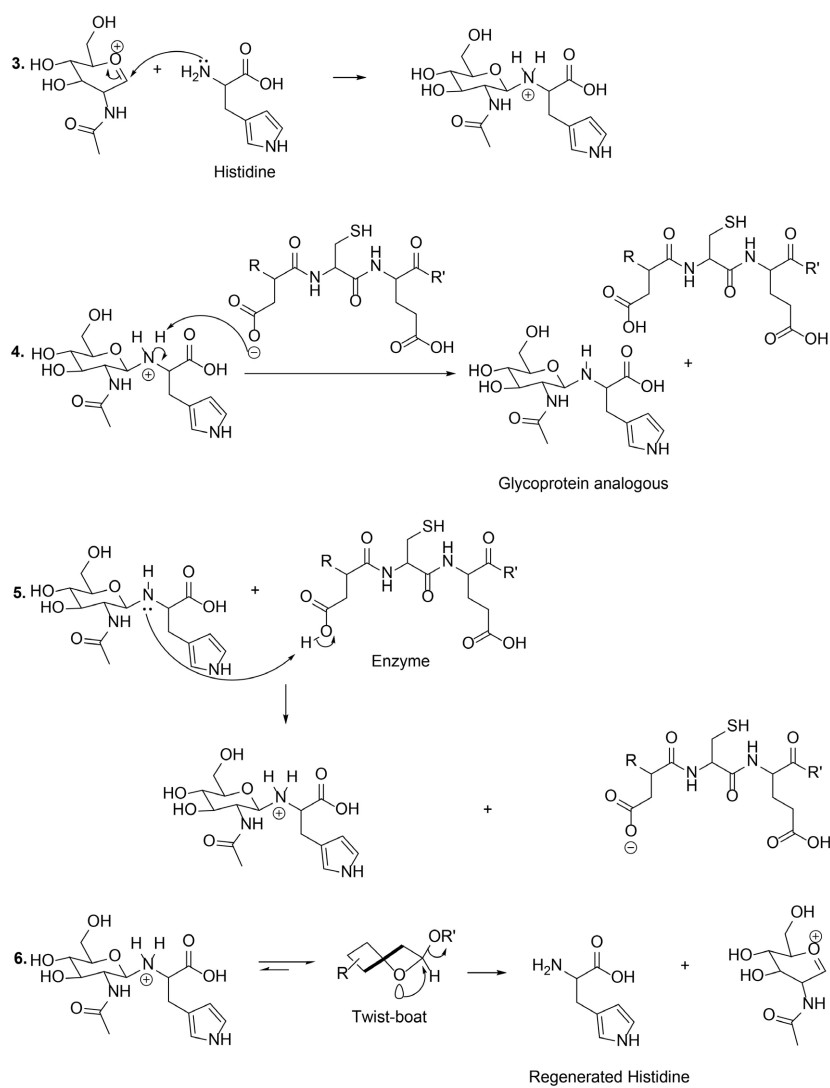


Scheme 12d

enzyme conjugate base (Scheme 13, reaction 1). In the following stage, the glycoprotein conjugate acid will adopt a twist-boat conformation to arrange the leaving group in a position opposite to the intracyclic oxygen atom of the glucosyl moiety, a structure that favors the departure of the leaving group (Scheme 13, reaction 2). The produced carbo-oxonium ion will receive the nitrogen's free double electrons from histidine to generate a protonated product, which will concede a proton to an appropriate conjugate base to produce a corresponding glycoprotein analog (Scheme 13, reactions 3-4). This type of analog will now be susceptible to penetrating the plant cytoplasm (Scheme 13, reactions 3-4). When the glycoprotein analog is now available in the plant cytoplasm, it will disintegrate to regenerate the corresponding histidine (Scheme 13, reactions 5-6).



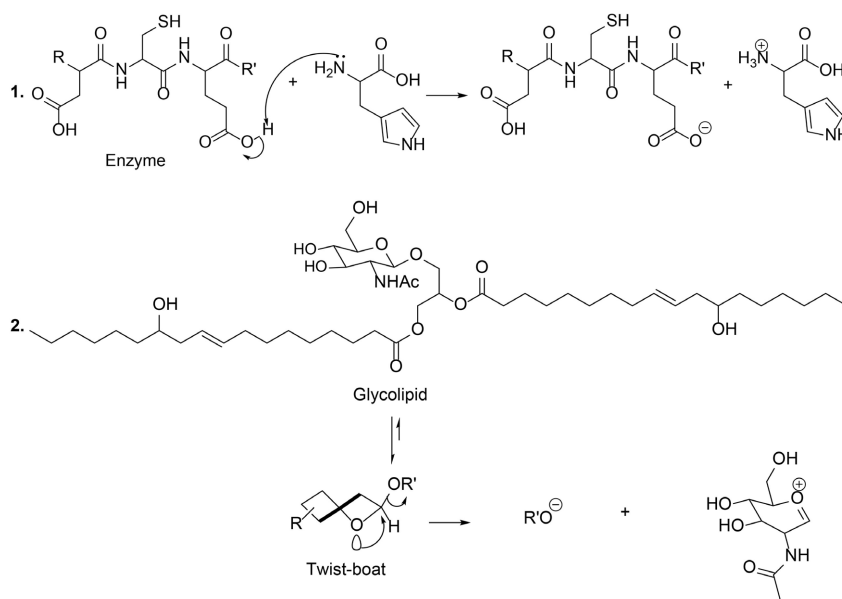
Scheme 13a



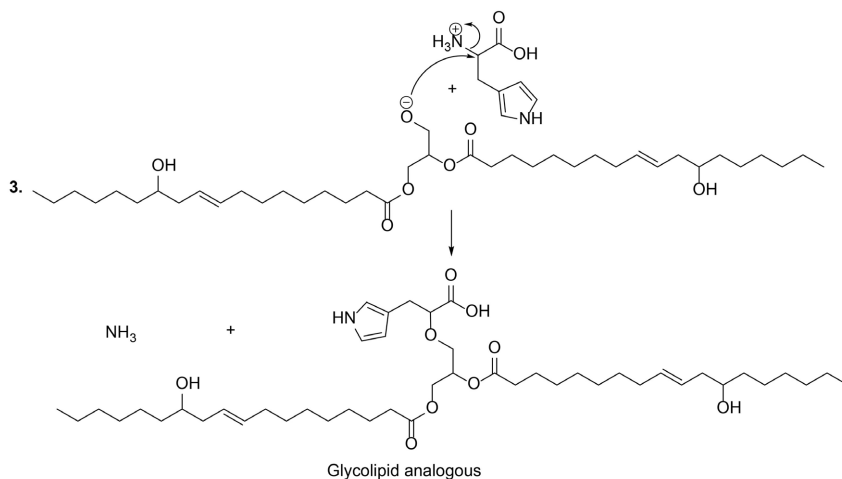
Scheme 13b

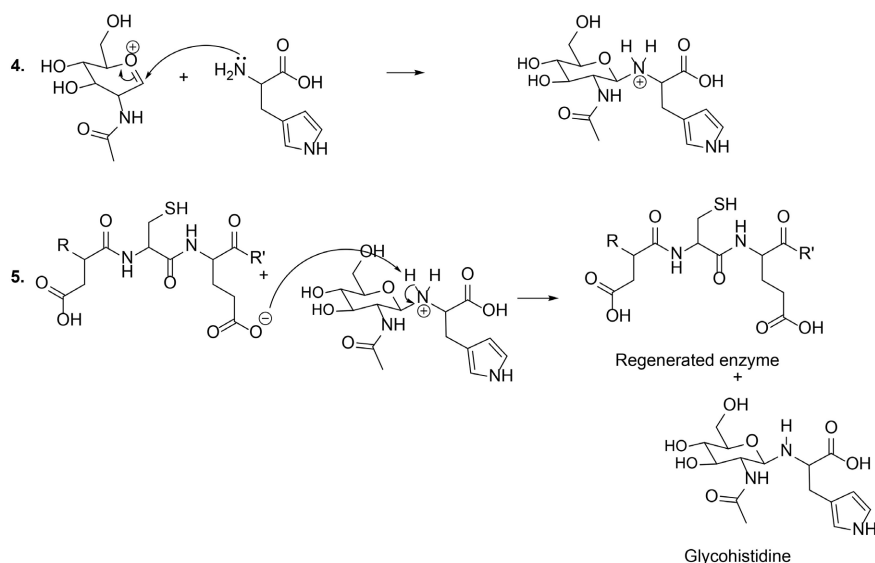
3.3.2. Enzymatic Addition of Amino Acids to Plant Cellular Membrane Glycolipids

To afford the enzymatic addition of an amino acid to a plant cellular membrane glycolipid, the amino acid, such as histidine, will react with the enzyme to furnish the enzyme conjugate base and the histidine conjugate acid (Scheme 14, reaction 1). In the following step, the glycolipid will adopt the twist-boat conformation to align the leaving group in a favorable orientation to produce the conjugate base of the leaving group as well as the carboxonium ion (Scheme 14, reaction 2). From the same perspective, the leaving group conjugate base, as a very good nucleophile, will react with the histidine conjugate acid to remove the histidine ammonium group, producing the glycolipid analog, which will penetrate the plant cytoplasm (Scheme 14, reaction 3). Another equivalent of histidine will react with the carboxonium ion to regenerate the enzyme and to produce glycol histidine. Bearing the glucosyl moiety, the glycol histidine will also diffuse through the plant membrane to reach the cytoplasm (Scheme 14, reactions 4-5).



Scheme 14a

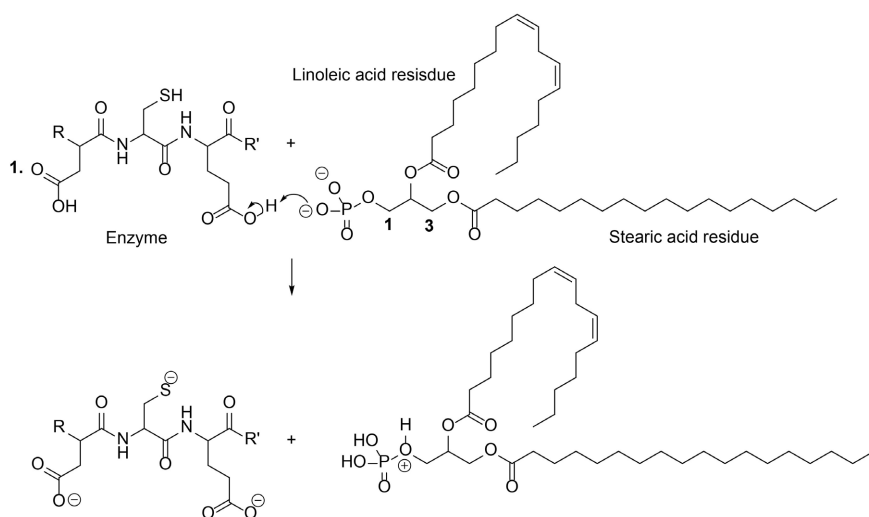




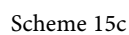
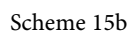
Scheme 14b

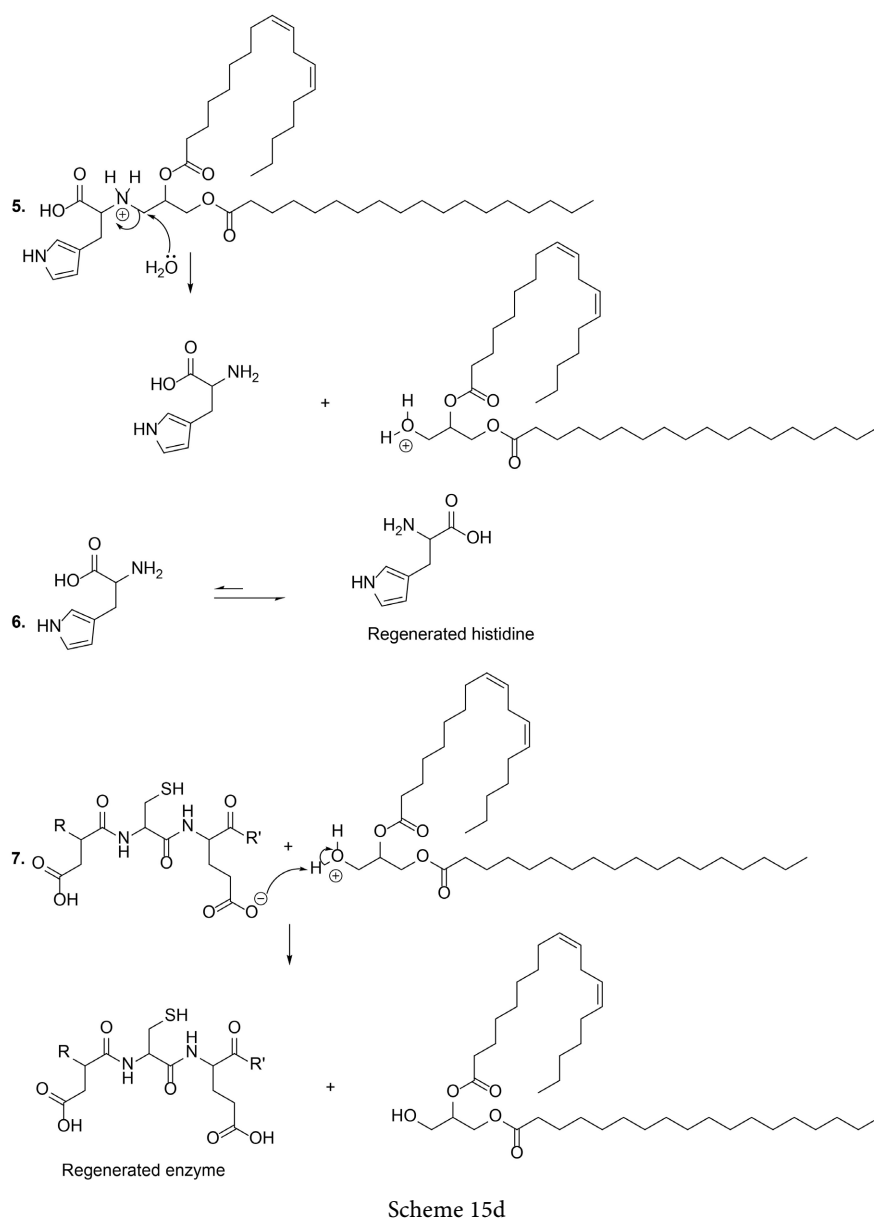
3.3.3. Enzymatic Addition of Amino Acids to Plant Cellular Membrane Phospholipids

In this kind of enzymatic addition reaction, the enzyme protonates the phospholipid phosphate group (Scheme 15, reaction 1). The protonated phosphate group is then removed by the nucleophilic addition of an adequate amino acid such as histidine to generate the corresponding intermediate compound (Scheme 15, reaction 2). This intermediate compound is subsequently deprotonated by the enzyme conjugate base to furnish the corresponding phospholipid analog (Scheme 15, reaction 3). Being similar to the parent phospholipid, the analog diffuses through the plant cellular membrane to reach the cytoplasm, where it is hydrolyzed to free the histidine for the benefit of the plant (Scheme 15, reactions 4-6). The enzyme is also regenerated in the plant cytoplasm (Scheme 15, reaction 7).



Scheme 15a





4. Conclusions

The current paper is an excellent demonstration of how extracellular organic compounds can diffuse through the plant cellular membrane. In fact, I have comprehensively detailed the mechanisms for the plant absorption of animal waste nutrients, particularly carbohydrates, lipids, and proteins. These animal waste substances are first decomposed or hydrolyzed by microorganisms through their enzymes, and then the resulting products, which are extracellular compounds, will then react or combine with the plant cellular membrane constituents such as glycoprotein, glycolipid, and phospholipid to acquire new shapes or structures that are similar or analogous to the plant cellular membrane constituents. The formation of glycoprotein, glycolipid, and phospholipid analogues is a subtle pathway to better understand the integration of animal waste compounds into the

plant cellular cytoplasm or their absorption by roots for the development of the plant.

Compared to the literature, the proposed plant uptake routes for organic nutrients are identical to the purpose of this conceptual study or theoretical elucidation, even if the used concepts are related to other scientific disciplines or research domains. For instance, it has been reported that plant roots interact with soil microbes to increase nutrient accessibility and plant health [24] [25]. In the same context, the experimental observations reveal that cellular membranes are constituted by glycoproteins, glycolipids, and glycerophospholipids [26]. In other words, plant root cellular constituents, particularly glycoproteins, glycolipids, and phospholipids, are plant root cellular membrane receptors, which interact with extracellular organic nutrients including carbohydrates, proteins, and lipids to generate the corresponding analogous products.

As I have explained previously, when extracellular organic compounds connect with intracellular receptors, they react or interact because they have functional groups at which organic reactions take place, and for that reason, they can behave as acids, bases, electrophiles, or nucleophiles depending on the reaction conditions, like enzymes acting as natural catalysts.

Conflicts of Interest

I declare that I have no conflict of interest regarding the publication of this paper.

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