Chapter 13
Biopolymers

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13.1 Introduction

The limited future of the fossil energy providers oil, natural gas and carbon represents one of the major future problems. Since additionally there are expectations that the worldwide energy requirement will increase by up to 60% by 2030 (Qaim 2006), there is an urgent need to identify alternative renewable energy sources based on wind, water or solar forces. The plants' use of solar energy via photosynthesis is extremely efficient; hence plants as renewable resources are surely an option. Nevertheless, any economic use still depends on governmental subsidies; in addition it seems to be unavoidable to intensify the agricultural land use in order to satisfy the energy demand, which might lead to a reduction of the food supply (Thrän et al. 2005). One of the main possibilities to increase harvest is plant breeding leading either to higher biomass production or to the formation of new and special ingredients of economic interest, like biopolymers. The combination of both to create a double-use plant might increase the outcome for the farmer, reduce the amount of arable land used for non-food purposes and in addition reduce CO_2 emission.

Naturally occurring polymers (e.g. polysaccharides, polyamides, polyesters) are produced by bacteria or plants. The most frequent polysaccharide is cellulose (glucose monomers connected by glycosidic bounds), the main component of plant cell walls, to date isolated mainly from wood, cotton, corn and wheat. Cellulose is the major constituent of paper and cardboard and also textiles made from cotton, linen and other plant fibres. Furthermore cellulose can be converted into cellophane, which is used in the packaging industry. Another important polysaccharide is starch (connected glucose–fructose dimers), the most important reserve substrate in plants, which is predominantly present as amylose and

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amylopectin. While amylase is used to created foils, amylopectin functions mainly as food additive, but also for technical applications, for example papermaking, as a thickener, glue or as raw material for biodegradable packing materials.

Polyamides are polymers containing monomers of amides joined by peptide bonds. They occur naturally as wool or silk. The best known type of silk is obtained from cocoons made by the larvae of the mulberry silkworm *Bombyx mori*, used for textile manufacture. A further type of silk naturally occurring in spiders like *Nephila clavipes* has a high tensile strength that is comparable to that of the synthetic superfibre Kevlar, but it additionally shows high elasticity (Tirrell 1996).

Polyesters occur in bacteria as a reserve substrate, having a functional ester group. They have thermoplastic, elastomeric and hydrophobic properties and are considered very suitable for consumer products such as bottles, fibres and films.

However all these groups have different drawbacks. Cellulose isolated from wood for the paper industry has some limitations, because costly and environmentally damaging processes are used to extract the lignin in order to obtain pure cellulose fibres. Therefore the paper industry is very interested in trees with lower lignin content or with modified lignin that can be more easily separated from the cellulose.

The usage of natural starch is limited due to its composition of amylose and amylopectin, components with very different characteristics and separate uses in industrial processes. Generally, only the thickening properties of amylopectin are required, while the amylose component is undesirable in many products and can additionally interfere with certain processes (Pickardt and de Kathen 2004). Unfortunately, the chemical modification or separation of amylopectin and amylose is associated with increased consumption of water and energy. However, see Chap. 11 for metabolic engineering of starch.

Naturally produced polyamids like spider silk cannot be obtained in large quantities from spiders, and the most commonly used polyamides, like nylons, aramids and sodium polyaspartate, do not even appear in nature.

The major problem for the commercial production and application of natural polyesters like polyhydroxyalkanoates (PHA) in consumer products is the high costs of bacterial fermentation, making it 5–10 times more expensive then the petroleum-derived polymers like polyethylene and polypropylene (Poirier 2002).

13.2 Transgene-Encoded Biopolymers

Envisioning both the drawbacks and the huge potential of plants as producers of cheap biomass, new production technologies are required to improve the competitiveness of plant-made biopolymers. Gene technology provides us with the tools to add new facilities to plant metabolic pathways, which should lead to the production of either high-quality or even new polymers in plants, possibly as a byproduct to traditional materials, such as starch, oil or sucrose. The following section discusses the production of four groups of polymers in plants in more detail: namely starch

and cellulose, PHAs, protein-based biomaterial and at least glucosyl glycerol used for cosmetics.

13.2.1 Starch and Cellulose

13.2.1.1 Starch

Starch is the major reserve carbohydrate in plants. Potato, maize, cassava and wheat provide the main sources of energy in the human diet, but also serve for many industrial processes like adhesives, cosmetics, detergents, paper, textiles and pharmaceuticals (Davis et al. 2003). Starch is also used for the production of biodegradable plastics as an alternative to petroleum-based products. However, native starches from various plant species have limited physiochemical properties, and thus are directly suitable for only a few specific end uses. For many industrial uses, enzymatic and chemical treatments, it is necessary to improve the usability of starch. The modification of starch is possible by using biotechnology to alter starch composition or to modify starch synthesis (Chap. 11). Out of many examples for starch modification, we describe one example for altered starch composition.

Starch is composed of amylopectin and amylose, which have different characteristics for industrial purposes. Amylopectin is used as a thickener, while amylose is undesirable for many products and can interfere with certain processes. Therefore a transgenic starch potato was developed which produces exclusively the amylopectin component of starch (Kull et al. 1995). In order to do so, the gene encoding the granule-bound starch synthase (GBSS) for the biosynthesis of amylose was inactivated by post-transcriptional gene silencing (PTGS). Two subgenomic fragments of the gene were expressed in antisense orientation under control of the CaMV 35S promoter. The resulting transgenic potato plants were effective in inhibiting amylose biosynthesis in tubers, thereby leading to an increase in the branched starch component amylopectin (>98%). The phenotype was stable during vegetative propagation. For commercial use the potato variety was named "Amflora" and has been analysed in field trials for several years to test yields and resistance to pests and disease. Furthermore the allergic and toxic potential of Amflora tubers was analysed, as well as potential other impacts on human health and the environment. No increased risk to humans, animals and the environment were shown in comparison to conventional potatoes (EFSA 2005).

13.2.1.2 Cellulose

All cell walls of higher plants contain: (i) cellulose, a homopolymer of β-1,4-linked glucose units, which is a flexible structural substance in the form of fibrils, (ii) hemicellulose, a heterogeneous polysaccharide, which represents a matrix in which

the cellulose fibrils are embedded, and (iii) lignin, a phenol polymer, which forms a bond between cellulose and hemicellulose. To date, cellulose is mainly isolated from trees. In order to obtain pure cellulose fibres (e.g. for the paper industry) lignins have to be eliminated. The chemical process is very expensive and pollutive under high energy consumption (Franke et al. 2000), hence reducing the lignin contents in trees might ease the isolation of cellulose.

Since the beginning of 1990 several strategies were investigated for plants to reduce lignin contents using gene technology. Mostly poplars were investigated, because there are fast-growing trees, relatively easy to modify and play an important role in paper manufacture. Up to now several effects have been achieved, due to the modification of biosynthetic pathway steps (Fig. 13.1; Pickardt and de Kathen 2004).

1. Decrease of complete lignin content by about 45%. Furthermore cellulose content was increased up to 14% (Hu et al. 1999).
2. Increase of the lignin compounds sinapyl to coniferyl units through overexpression of coniferaldehyde-5-hydroxylase. The total lignin content is equal.
3. Addition of either effects, when cumarat-CoA-ligase and coniferaldehyde-5-hydroxylase are overexpressed or downregulated in one transgenic plant. As a result the total lignin content is reduced about 52%, the part of cellulose

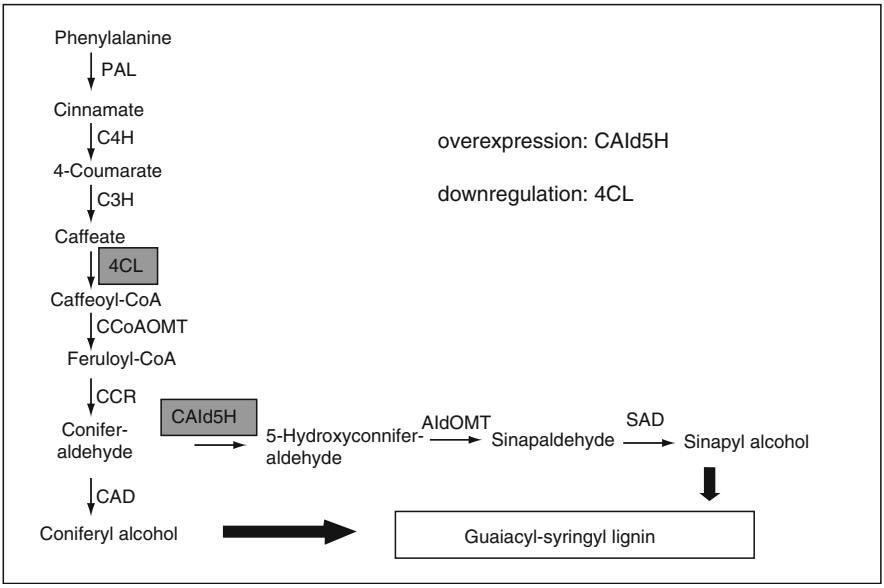

Fig. 13.1 Proposed biosynthetic pathway of lignin synthesis in trees. *PAL* Phenylalanine-ammoniumlyase, *C4H* cinnamate 4-hydroxylyase, *C3H* 4-coumarate 3-hydroxylase, *4CL* 4-coumarate-CoA ligase, *CCoAOMT* caffeoyl CoA O-methyltransferase, *CCR* cinnamoyl-CoA reductase, *CAld5H* coniferaldehyde 5-hydroxylase, *AldOMT* 5-hydroxyconiferaldehyde O-methyltransferase, *SAD* sinapyl alcohol dehydrogenase, *CAD* cinnamyl alcohol dehydrogenase

increased up to 30% and the proportion of sinapyl to coniferyl units increased up to 64% (Li and Quiros 2003).

4. The proportion of sinapyl to coniferyl units increased, when the coniferylaldehyd-5-hydroxylase gene is regulated by the promoter of the cinnamic acid hydrolase gene. The transgenic poplar trees show no phenotypical changes compared to control plants; furthermore their wood has a higher decomposition efficiency than normal poplar trees (Huntley et al. 2003).

The interest of industries in genetically modification of forest trees is extremely higher in the United States than in Europe, caused by geographical conditions and therefore expansion of the forest industry. Furthermore, commercialization of transgenic trees in Europe seems to have fewer chances, due to their risk assessment, e.g. durability and high spreading potential (Sauter and Hüsung 2005).

13.2.1.3 Glucoside

The ingredients for cosmetic creams, lotions, powder, perfumes, lipstick or make-up come from a variety of sources, for example antioxidants, alcohol, oil and also polymers. Polymers serve in hair-setting products, as binders in skin creams and to keep sunscreens from washing off. One example is α-D-glucosylglycerol (α-GG), which is used as an anti-aging agent and moisture-regulating compound (Da Costa et al. 1998). α-GG can be produced by chemical as well as by enzymatic methods and was naturally found in microorganisms as a compatible solute for providing some protection against stresses due to high salt concentrations, heat and UV radiation. It is also useful as an alternative sweetener in food stuffs, because of its low caloric value. The microbial synthesis of α-GG is presently not a mature process, because it does not allow the production of α-GG as a bulk chemical. The achievable concentrations are very low and also the productivity of three days is not advantageous for industrial production (Roder et al. 2005). However, α-GG is enzymatically synthesized (Gödl et al. 2008) by using sucrose phosphorylase to convert sucrose with glucosyl and glycerol into α-GG, which is isolated by chromatographic methods with a yield greater than 70%. Up to now, besides bacteria, GG accumulation has only been reported for the plants *Lillium japonicum* (Kaneda et al. 1984) and *Myrothamnus flabellifolia* (Bianchi et al. 1993); however nothing is known about the metabolic pathway of GG in these plants. In unpublished data GG accumulation was established in *Arabidopsis* by expression of the *ggpPS* (glucosylglycerol phosphate phosphatase/synthase) gene from the γ-proteobacterium *Azotobacter vinelandii*. Transgenic plants accumulated GG up to 30 μmol/g fresh weight. However, beside increased salt tolerance, plants with higher GG content also showed growth retardation, which is not observed in plants with low GG accumulation (1–2 μmol/g fresh weight; Klähn et al. 2008). The growth retardations might be caused by the interference of GG synthesis with trehalose biosynthesis and in turn also other carbohydrates. The improvement of the GG synthesis in plants needs more investigation by regulated gene expression.

13.2.2 Polyhydroxyalkanoates

A wide variety of bacteria produce polyhydroxyalkanoates (PHAs) as a carbon reserve and electron sink (for a review, see van Beilen and Poirier 2008). These PHAs consist of 3-hydroxy fatty acids with a chain length of 4–16 carbons and have wide-ranging potential for applications such as the formation of plastic bags, fibres and films. Besides their CO_2 neutral production, PHA products can be decomposed, which is desirable for the environmental friendly dispersal of disposable items as well as for some medical products which otherwise have to be removed from the body. Poly-3-hydroxybutyrate (PHB) is the most widespread and best characterized PHA found in bacteria like *Ralstonia eutropha* (for a review, see van Beilen and Poirier 2008). In contrast to cyanophycin synthesis, three enzymes are necessary for PHB synthesis. The first enzyme, β-ketothiolase, catalyses the reversible condensation of two acetyl-CoA moieties to form acetoacetyl-CoA. The acetoacetyl-CoA reductase in turn reduces acetoacetyl-CoA to R-(–)-3-hydroxybutyryl-CoA, which is subsequently polymerized through PHA synthase to form PHB. As an alternative to petrochemicals, PHA production was established in plants, first in *Arabidopsis thaliana* by the expression of the PHB synthase in the cytoplasm leading to a maximum of 0.1% PHB present in the cytoplasm, nucleus or vacuoles (van Beilen and Poirier 2008). However, the plants showed strong growth retardation and reduced seed production. PHB synthesis in the cytoplasm of tobacco (0.01%), cotton (0.3%) and oilseed rape (0.1%; John and Keller 1996; Nakashita et al. 1999; Poirier et al. 1992) showed similar plant damage. The deleterious effects of PHB production in the cytoplasm of plants might be caused by the diversion of acetyl-CoA and acetoacetyl-CoA away from the endogenous flavonoid and isoprenoid pathways, which are responsible for the synthesis of a range of plant hormones and sterols (van Beilen and Poirier 2008). Due to their high metabolic flow of acetyl-CoA, chloroplasts might provide a more suitable production platform, although β-ketothiolase is not present. Therefore the required enzymes – including β-ketothiolase – were targeted to plastids, using signal sequences for plastid import. The highest PHB accumulation was observed in *Arabidopsis*, with a maximum of 14% of dry weight in leaves without significant effects on plant growth but visible leaf chlorosis (Nawrath et al. 1994). In seeds of oil rape up to 8% dry weight PHB accumulation was detected in leucoplasts after the transfer of all three genes (Houmiel et al. 1999), a strategy leading to even 30–40% of dry weight in leaves of *A. thaliana*. Nevertheless, in contrast to the intact canola seeds, these plants were heavily reduced in growth and did not produce any seeds. Slightly reduced amounts were detected in corn leaves (6% dry weight), sugar cane leaves (2% dry weight) and sugar beet hairy roots (5% dry weight), whereas expression of the PHB pathway in plastids of alfalfa and tobacco led to only low amounts (<0.5% dry weight; Arai et al. 2001; Saruul et al. 2002. Since nucleus-encoded proteins are expressed to a lesser extent than those encoded by plastidic genes, it was supposed that the direct expression of the PHB pathway in the plastid genome might increase the PHB yield without increasing the deleterious effects. Nevertheless, in tobacco

this strategy only leads to relatively low amounts up to 1.7% dry weight, accompanied by reduced growth and male sterility (Arai et al. 2001; Bohmert et al. 2002; Lössl et al. 2003).

In order to improve the physical properties of PHB, extensive efforts have made to synthesise co-polymers with better properties like poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(HB-co-HV)] and medium-chain-length PHA (mclPHA). P(HB-co-HV) is produced by the inclusion of 3-hydroxyvalerate in PHB, which is less stiff and tougher than PHB and also easier to process, making it to a good target for commercial application (Noda et al. 2005). Coexpression of a threonine deaminase from *E. coli* along with the three PHB biosynthetic proteins in plastids led to P(HB-co-HV) accumulation up to 2.3% dry weight in seeds of oil rape and 1.6% dry weight in *A. thaliana* (Slater et al. 1999; Valentin et al. 1999). However there is a constriction providing 3-hydroxyvaleryl-CoA to the PHA synthase, which is caused by the inefficiency of the pyruvate dehydrogenase complex in converting 2-ketobutyrate to propionyl CoA (for a review, see van Beilen and Poirier 2008).

MclPHA are described as elastomers and their physical properties depend on the monomer composition (for a review, see van Beilen and Poirier 2008) In *A. thaliana* mclPHA monomers were produced up to 0.4% dry weight in seedlings and consisted of 40–50 mol% of C12 and longer monomers. The production of mclPHAs with longer-chain monomers by using the conversion of the fatty acid biosynthetic intermediate 3-hydroxyacyl-ACP into 3-hydroxyacyl-CoA led to only low amounts (below 0.03% dry weight) of mclPHA in plastids of potato leaves (Romano et al. 2005).

The most useful PHA would be a polymer containing primarily 3-hydroxybutyrate with a fraction of longer-chain monomers of C6 and higher. In terms of PHA quantity, plastids are the best location for PHA synthesis. However, the synthesis, regulation of precursors (like acetyl-CoA, propionyl-CoA or 3-hydroxyacyl-ACP) and the efficacy to channel them towards PHA without deleterious effects on plant growth needs more investigation (for a review, see van Beilen and Poirier 2008).

13.2.3 Protein-Based Biomaterials

Protein-based biomaterials have a wide area of application, ranging from tissue engineering, drug carriers, coatings and glues to elastomers and fibres, dispersants, thickeners and additives to hydrogels. Two important target proteins are described here in detail: fibrous proteins (e.g. spider silk) and non-ribosomally produced polyamino acids like cyanophycin.

13.2.3.1 Fibrous Proteins

Fibrous proteins contain short blocks of repeated amino acids and can be regarded as elaborate block co-polymers with unique strength-to-weight, adhesive or elastic

properties (Huang et al. 2007; Sanford and Kumar 2005; Scheibel 2005). Well known fibrous proteins are elastin, resilin, collagen, keratin, mussel adhesive proteins and wheat glutenin (Kiick 2007). A huge combinatorial range is available by combining repeated sequences of the various natural fibrous proteins, or even using synthetic gene sequences or changing the linker elements between the repeated sequences (Holland et al. 2007; Nagapudi et al. 2005).

Natural silk fibres are mainly produced by a variety of silkworms (Altman et al. 2003; Shao and Vollrath 2002) and spiders (Perez-Rigueiro et al. 2003; Rising et al. 2005; Vollrath 2000). The development of novel silk-based fibres has mainly focused on the silks produced by the golden orb-weaving spider *Nephila clavipes*, which synthesizes different kinds of silks for several purposes, e.g. weaving cocoons, as a dragline or constructing a web (Hinman et al. 2000; Vollrath and Knight 2001). The main focus lays on the silks of the dragline, the main structural web silk and the spider's lifeline, because of their high tensile strength. This is comparable to that of the synthetic superfibre Kevlar, but it additionally shows high elasticity (Tirrell 1996), useful for industrial and medical purposes. Dragline spider silk consists of repeated sequence blocks of various types (Huang et al. 2007). The GGX motif probably forms a β_{10} helix, while the GPGXX motif is thought to form a β-turn spiral. Effective high-level and stable expression of silk proteins in fast-growing micro-organisms such as yeast and bacteria leads to difficulties, like the formation of inclusion bodies or distinct codon usage. In addition, bacterial production is genetically unstable due to recombination, resulting from the highly repetitive genes encoding the repetitively composed spider silk proteins. Nevertheless synthetic spider silk genes have been successfully expressed in transgenic tobacco, potato and *Arabidopsis thaliana* plants (Barr et al. 2004; Scheller et al. 2001) under control of the cauliflower mosaic virus (CaMV) promoter and targeted to the endoplasmatic reticulum. Transgenic plants were cultivated in greenhouses and in field trails (Menassa et al. 2004; Scheller and Conrad 2005). In tobacco and potato leaves up to 2% of total soluble protein (TSP) was observed (Scheller et al. 2001). The expression only in leaf apoplasts of *A. thaliana* led to spider silk production of 8.5% TSP, whereas targeting to seed endoplasmic reticulum yielded 18% TSP (Yang et al. 2005). The expression levels in plants are close to the level of 10% and 30% TSP reported in *Escherichia coli* and *Pichia pastoris*. The combination of spider silk protein and elastin polymer leads to a new biomaterial, which is used for industrial and medical purposes. For that, the expression of synthetic collagen, made from repeats of a motif found in elastin, and also of a chimeric protein composed of silk and elastin domains has been expressed in tobacco or potato (for a review, see van Beilen and Poirier 2008). The extraction of the proteins from 1 kg tobacco leaf material leads to a yield of 80 mg pure recombinant spider silk-elastin protein (Scheller et al. 2004).

The enhancement of fibrous protein synthesis in plant requires several approaches, for example optimization of the amino acid and tRNA pools for those amino acids, which form the main part of the protein, like glycine and alanine in spider silk. Further possibilities are dislocation to compartments and tissues that are optimal for protein synthesis and storage and also the co-expression of several

fibrous proteins as found in natural silk (for a review, see van Beilen and Poirier 2008). In addition to the optimized production of fibrous proteins, the resulted fibre should have characteristics similar to the silk proteins from spider. The properties of silk fibres depend on correct assembly of the different types of proteins by spinning. Recombinant spider silk, obtained from mammalian cells, shows similar toughness to dragline silk, but with a lower tenacity (Lazaris et al. 2002).

13.2.3.2 Non-Ribosomally Produced Poly-Amino Acids

Polymers produced in transgenic plants include polyaminoacids such as poly-γ-glutamate, poly-α-aspartate, and poly-ε-lysine, which have a wide range of applications, e.g. as dispersants, thickeners or additives to hydrogels (Chang and Swift 1999; Lössl et al. 2003; Oppermann-Sanio et al. 1999; Oppermann-Sanio and Steinbüchel 2002).

Polyaspartate is a soluble, non-toxic and biodegradable polycarboxylate (Tabata et al. 2000) that could replace the non-biodegradable polyacrylates in many industrial, agricultural and medical applications (Joentgen et al. 2001; Oppermann-Sanio and Steinbüchel 2002; Schwamborn 1998; Zotz et al. 2001). Because no polyaspartate-producing organism has been identified up to now, the polymer is chemically synthesized (Schwamborn 1996). However, it can also be obtained from cyanophycin (multi-L-arginyl-poly-L-aspartic acid). Cyanophycin is a cyanobacterial reserve polymer composed of a poly-α-aspartic acid backbone with arginine residues linked via their α-amino group to the β-carboxyl group of each aspartate residue (Simon 1976, 1987; Simon and Weathers 1976). Mild hydrolysis of cyanophycin (Joentgen et al. 2001) results in homo- and copolymers of polyaspartate and L-arginine. The basic amino acid L-arginine has been suggested to be a regulator of some immunological and physiological processes, e.g. being an immune system stimulator (Cen et al. 1999; de Jonge et al. 2002; Li et al. 2007; Nieves and Langkamp-Henken 2002; Popovic et al. 2007; Taheri et al. 2001; Tapiero et al. 2002; Yeramian et al. 2006), agrowth inductor (Lenis et al. 1999; Roth et al. 1995; Wu et al. 2007) or a tumour cell growth inhibitor (Amber et al. 1988; Caso et al. 2004; Flynn et al. 2002). Alternatively, aspartate and arginine from cyanophycin could serve as a starting point for the synthesis of a range of chemicals (Fig. 13.2). Arginine can be converted to 1,4-butanediamine, which can be used for the synthesis of nylon-4,6. Aspartate is converted in several chemicals like 2-amino-1,4-butanediol, 3-aminotetrahydrofuran (analogues of high-volume chemicals used in the polymer industry), fumaric acid (used for polyester resins) and acrylamide (used as a thickener, in manufacturing dyes or in papermaking). Cyanophycin is synthesized via non-ribosomal polypeptide synthesis in many Cyanobacteria (Simon 1987) and some other non-photosynthetic bacteria (Krehenbrink et al. 2002; Ziegler et al. 2002). For cyanophycin synthesis, only one enzyme, the cyanophycin synthetase encoded by *cph*A, is necessary to catalyse the ATP-dependent elongation of a cyanophycin primer by the consecutive addition of L-aspartic acid and L-arginine (Ziegler et al. 2002). In cyanobacteria, the polymer is variable

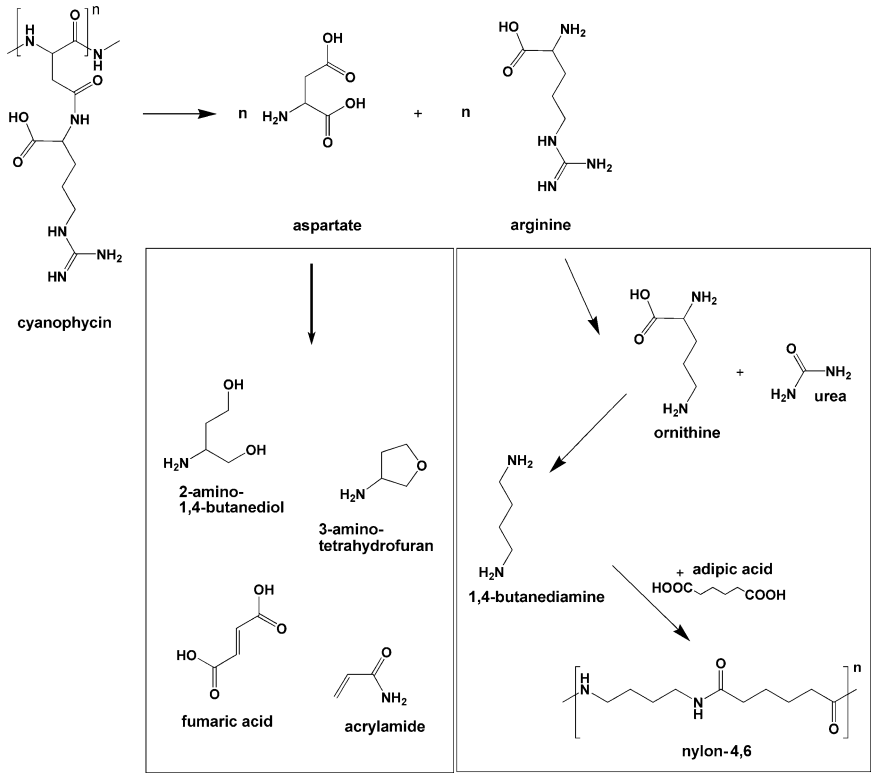

Fig. 13.2 Potential products derived from the polymer cyanophycin

in length (25–125 kDa), water-insoluble and stored in membrane-less granules (Allen 1984; Simon 1987). As a first step to establish a system for mass production of the polymer in plants, the cyanophycin synthetase gene from *Thermosynechococcus elongatus* BP-1 (*cph*A_{Te}) was incorporated into tobacco and potato plants, with mRNA expression under control of the constitutive cauliflower mosaic virus (CaMV) 35S promoter (Neumann et al. 2005). The maximum amount of cyanophycin in the cytosol of tobacco leaves was 1.14% of dry weight. However, the cyanophycin-producing plants exhibited phenotypical changes like thickened cell walls, variegated leaves and slow growth. The same was true for the transgenic potato plants containing maximal amounts of 0.24% of dry weight. The clone producing the most cyanophycin did not develop eyes and could not be propagated further. Moreover, in tubers, the presence of cyanophycin could only be demonstrated by electron microscopy. A much higher capacity to produce cyanophycin was achieved by targeting the cyanophycin synthetase to plastids of *Nicotiana tabacum* (Hühns et al. 2008). Yields of up to 6.8% dry weight in leaves were obtained, without significant disturbance of plant growth and development. However, the line producing the most cyanophycin produced fewer seeds.

When the *cph*A expression is restricted to tubers, the plant fitness and cyanophycin production in potato is further enhanced up to 7.5% dry weight, with minimal effects on growth and morphology of the plants.

The improvement of the cyanophycin accumulation in plants may require optimization of the gene sequence, adapted to the target plant and also the optimization of the pathways involved in supplying arginine and aspartic acid.

13.3 Conclusion

The production of biopolymers has proven to be feasible and might contribute to a sustainable agriculture. Nevertheless, application still lies in the far future. Before transgenic plants can be cultivated and used, they must undergo an authorization procedure based on a safety assessment to guarantee their safe usage. Currently, the approval of transgenic plants is an extremely cost and time-demanding process, specific for one event (one plant line derived from a single transformant with one insertion locus of the transgene). These efforts will only be undertaken if the expected gain can exceed the costs. The gain depends on: (i) the potential market of the biopolymer, (ii) the pureness and concentration of the biopolymer in the plants, (iii) the potential reduction of the primary value of the cultivar (e.g. reduction of biomass, starch content or processing quality) and (iv) the isolation costs. Except for the amylopectin potato Amflora, further investigations have to be done for all of the polymers described to optimize these parameters as far as possible, either by modification of the production compartment in the plant, selection of the production cultivar, support of plant health and biomass production, or by optimization of cultivation, harvest, storage and isolation strategies. Further, risk assessment strategies have to be optimized in order to reduce the cost and time for approval without any reduction in safety. This might be done by the development of new and specific techniques for analysis as well as by the acceptance of transgene- and cultivar-specific data to reduce effort for single events. In addition, analysis always has to be hypothesis-driven and the selection of topics addressed has to be restricted to risks specific for the event in question.

Due to the different legal frameworks in most parts of the world, it might be expected that biopolymer-producing transgenic plants will be on the market first in the United States and Canada since their regulations come under existing laws covering seed and pesticide approval as well as food and feed control and the basis for safety assessment of a new transgenic plant is by comparison to known and established plants and products. Most importantly, the final decision for approval is carried out by scientists. Nevertheless, no polymer-producing plants are close to the United States market. In contrast, in Europe, the assessment focuses on the process (i.e. genetic engineering) and the precautionary approach; and the final decision is made by the European Commission in consultation with the Member States. Therefore no transgenic polymer-producing plant has yet been authorized in the European Union, but the potato event Amflora is close to approval. Nevertheless,

although the safety assessment for cultivation and for food and feed use by the European Food Safety Authority (EFSA) was completed years ago, this event is still waiting for authorization.

References

Allen MM (1984) Cyanobacterial cell inclusions. Annu Rev Microbiol 38:1–25

Altman GH, Diaz F, Jakuba C, Calabro T, Horan RL, Chen J, Lu H, Richmond J, Kaplan DL (2003) Silk-based biomaterials. Biomaterials 24:401–416

Amber IJ, Hibbs JB, Taintor RR, Vavrin Z (1988) The L-arginine dependent effector mechanism is induced in murine adenocarcinoma cells by culture supernatant from cytotoxic activated macrophages. J Leuk Biol 43:187–192

Arai Y, Nakashita H, Suzuki Y, Kobayashi K, Shimizu T, Yasuda M, Doi Y, Yamaguchi I (2001) Plastid targeting of polyhydroxybutyrate biosynthetic pathway in tobacco. Plant Biotechnol 18:289–293

Barr LA, Fahnestock SR, Yang JJ (2004) Production and purification of recombinant DP1B silk-like protein in plants. Mol Breed 13:345–356

Bianchi G, Gamba A, Limiroli R, Pozzi N, Elster R, Salamini F, Bartels D (1993) The unusual sugar composition in leaves of the resurrection plant *Myrothamnus flabellifolia*. Physiol Plant 87:223–226

Bohmert K, Balbo I, Steinbüchel A, Tischendorf G, Willmitzer L (2002) Constitutive expression of the beta-ketothiolase gene in transgenic plants. A major obstacle for obtaining polyhydroxybutyrate-producing plants. Plant Physiol 128:1282–1290

Caso G, McNurlan MA, McMillan ND, Eremin O, Garlick PJ (2004) Tumour cell growth in culture: dependence on arginine. Clin Sci 107:371–379

Cen Y, Luo X, Liu X (1999) Effect of arginine on deep partial thickness burn in rats. Hua Xi Yi Ke Da Xue Xue Bao 30:198–201

Chang CJ, Swift G (1999) Poly(aspartic acid) hydrogel. J Macromol Sci Pure Appl Chem A36:963–970

Da Costa MS, Santos H, Galinski EA (1998) An overview of the role and diversity of compatible solutes in bacteria and archae. Adv Biochem Eng Biotechnol 61:117–153

Davis JP, Supatcharee N, Khandelwal RL, Chibbar RN (2003) Synthesis of novel starches in planta: opportunities and challenges. Starch Starke 55:107–120

de Jonge WJ, Kwikkers KL, te Velde AA, van Deventer SJH, Nolte MA, Mebius RE, Ruijter JM, Lamers MC, Lamers WH (2002) Arginine deficiency affects early B cell maturation and lymphoid organ development in transgenic mice. J Clin Invest 110:1539–1548

EFSA (2005) Opinion of the scientific panel on genetically modified organisms on an application (reference EFSA-GMO-UK-2005-14) for the placing on the market of genetically modified potato EH92-527-1 with altered starch composition, for production of starch and food/feed uses, under regulation (EC) No 1829/2003 from BASF Plant Science. EFSA J 324:1–20

Flynn NE, Meininger CJ, Haynes TE, Wu G (2002) Dossier: free amino acids in human health and pathologies – the metabolic basis of arginine nutrition and pharmacotherapy. Biomed Pharmacother 56:427–438

Franke R, McMichael CM, Meyer K, Shirley AM, Cusumano JC, Chapple C (2000) Modified lignin in tobacco and poplar plants over-expressing the *Arabidopsis* gene encoding ferulate 5-hydroxylase. Plant J 22:223–234

Gödl C, Sawangwan T, Nidetzky B, Müller M (2008) Method for producing 2-O-glycerol-alpha-D-glucopyranoside. Patent WO/2008/034158

Hinman MB, Jones JA, Lewis RV (2000) Synthetic spider silk: a modular fiber. Trends Biotechnol 18:374–379

Holland C, Terry AE, Porter D, Vollrath F (2007) Natural and unnatural silks. Polymer 48:3388–3392

Houmiel KL, Slater S, Broyles D, Casagrande L, Colburn S, Gonzalez K, Mitsky TA, Reiser SE, Shah D, Taylor NB, Tran M, Valentin HE, Gruys KJ (1999) Poly(beta-hydroxybutyrate) production in oilseed leukoplasts of *Brassica napus*. Planta 209:547–550

Hu WJ, Harding SA, Lung J, Popko JL, Ralph J, Stokke DD, Tsai CJ, Chiang VL (1999) Repression of lignin biosynthesis promotes cellulose accumulation and growth in transgenic trees. Nat Biotechnol 17:808–812

Huang J, Foo CWP, Kaplan DL (2007) Biosynthesis and applications of silk-like and collagen-like proteins. Polymer Rev 47:29–62

Hühns M, Neumann K, Hausmann T, Ziegler K, Klemke F, Kahmann U, Staiger D, Lockau W, Pistorius EK, Broer I (2008) Plastid targeting strategies for cyanophycin synthetase to achieve high-level polymer accumulation in *Nicotiana tabacum*. Plant Biotechnol J 6:321–336

Huntley SK, Ellis D, Gilbert M, Chapple C, Mansfield SD (2003) Significant increases in pulping efficiency in C4H-F5H-transformed poplars: Improved chemical savings and reduced environmental toxins. J Agric Food Chem 51:6178–6183

Joentgen W, Groth TSA, Hai T, Oppermann FB (2001) Polyasparaginic acid homopolymers and copolymers, biotechnical production and use thereof. US Patent 61807572

John ME, Keller G (1996) Metabolic pathway engineering in cotton: biosynthesis of polyhydroxybutyrate in fiber cells. Proc Natl Acad Sci USA 93:12768–12773

Kaneda M, Kobayashi K, Nishida K, Katsuta S (1984) Glycerol glucosides in the *Lilium* genus. 3. Liliosides-D and liliosides-E 2 glycerol glucosides from *Lilium japonicum*. Phytochemistry 23:795–798

Klähn S, Marquardt DM, Rollwitz I, Hagemann M (2008) Expression of the *ggpPS* gene for glucosylglycerol biosynthesis from *Azotobacter vinelandii* resulted in improved salt tolerance of *Arabidopsis thaliana*. J Exp Bot 60:1679–1689

Kiick KL (2007) Biosynthetic methods for the production of advanced protein-based materials. Polym Rev 47:1–7

Krehenbrink M, Oppermann-Sanio FB, Steinbuchel A (2002) Evaluation of non-cyanobacterial genome sequences for occurrence of genes encoding proteins homologous to cyanophycin synthetase and cloning of an active cyanophycin synthetase from *Acinetobacter* sp strain DSM 587. Arch Microbiol 177:371–380

Kull B, Salamini F, Rhode W (1995) genetic engineering of potato starch composition. Inhibition of amylose biosynthesis in tubers from transgenic potato lines by the expression of antisense sequences of the gene for granule-bound-starch synthase. J Genet Breed 49:69–76

Lazaris A, Arcidiacono S, Huang Y, Zhou JF, Duguay F, Chretien N, Welsh EA, Soares JW, Karatzas CN (2002) Spider silk fibers spun from soluble recombinant silk produced in mammalian cells. Science 295:472–476

Lenis NP, van Diepen HTM, Bikker P, Jongbloed AG, van der Meulen J (1999) Effect of the ratio between essential and nonessential amino acids in the diet on utilization of nitrogen and amino acids by growing pigs. J Anim Sci 77:1777–1787

Li G, Quiros CF (2003) In planta side-chain glucosinolate modification in *Arabidopsis* by introduction of dioxygenase *Brassica* homolog BoGSL-ALK. Theor Appl Genet 106:1116–1121

Li P, Yin YL, Li D, Kim SW, Wu GY (2007) Amino acids and immune function. Br J Nutr 98:237–252

Lössl A, Eibl C, Harloff HJ, Jung C, Koop HU (2003) Polyester synthesis in transplastomic tobacco (*Nicotiana tabacum* L.): significant contents of polyhydroxybutyrate are associated with growth reduction. Plant Cell Rep 21:891–899

Menassa R, Hong Z, Karatzas CN, Lazaris A, Richman A, Brandle J (2004) Spider dragline silk proteins in transgenic tobacco leaves: accumulation and field production. Plant Biotechnol J 2:431–438

Nagapudi K, Brinkman WT, Leisen J, Thomas BS, Wright ER, Haller C, Wu XY, Apkarian RP, Conticello VP, Chaikof EL (2005) Protein-based thermoplastic elastomers. Macromolecules 38:345–354

Nakashita H, Arai Y, Yoshioka K, Fukui T, Doi Y, Usami R, Horikoshi K, Yamaguchi I (1999) Production of biodegradable polyester by a transgenic tobacco. Biosci Biotechnol Biochem 63:870–874

Nawrath C, Poirier Y, Somerville C (1994) Targeting of the polyhydroxybutyrate biosynthetic-pathway to the plastids of *Arabidopsis thaliana* results in high-levels of polymer accumulation. Proc Natl Acad Sci USA 91:12760–12764

Neumann K, Stephan DP, Ziegler K, Hühns M, Broer I, Lockau W, Pistorius EK (2005) Production of cyanophycin, a suitable source for the biodegradable polymer polyaspartate, in transgenic plants. Plant Biotechnol J 3:249–258

Nieves C Jr, Langkamp-Henken B (2002) Arginine and immunity: a unique perspective. Biomed Pharmacother 56:471–482

Noda I, Green PR, Satkowski MM, Schechtman LA (2005) Preparation and properties of a novel class of polyhydroxyalkanoate copolymers. Biomacromolecules 6:580–586

Oppermann-Sanio FB, Steinbüchel A (2002) Occurrence, functions and biosynthesis of polyamides in microorganisms and biotechnological production. Naturwissenschaften 89:11–22

Oppermann-Sanio FB, Hai T, Aboulmagd E, Hezayen FF, Jossek S, Steinbüchel A (1999) Biochemistry of polyamide metabolism. In: Steinbüchel, A (ed) Biochemical principles and mechanisms of biosynthesis and biodegradation of polymers. Proceedings of the international symposium. Wiley-VCH, Weinheim, pp 185–193

Perez-Rigueiro J, Elices M, Guinea GV (2003) Controlled supercontraction tailors the tensile behaviour of spider silk. Polymer 44:3733–3736

Pickardt T, de Kathen A (2004) Gentechnisch veränderte Pflanzen mit neuen oder verbesserten Qualitäts-und Nutzungseigenschaften: Futtermittel- und rohstofffliefernde Nutzpflanzen, Pflanzen zur Bodensanierung und Zierpflanzen. Auftrag des Büros für Technikfolgenabschätzung De Kathen &Pickardt BiotechConsult GbR, Berlin

Poirier Y (2002) Polyhydroxyalknoate synthesis in plants as a tool for biotechnology and basic studies of lipid metabolism. Prog Lipid Res 41:131–155

Popovic PJ, Zeh HJ, Ochoa JB (2007) Arginine and immunity. J Nutr 137:1681S–1686S

Poirier Y, Dennis DE, Klomparens K, Somerville C (1992) Polyhydroxybutyrate, a biodegradable thermoplastic, produced in transgenic plants. Science 256:520–523

Qaim M (2006) The role of plant breeding for global food security. Ber Landwirtsch 84:198–212

Rising A, Nimmervoll H, Grip S, Fernandez-Arias A, Storckenfeldt E, Knight DP, Vollrath F, Engstrom W (2005) Spider silk proteins – mechanical property and gene sequence. Zool Sci 22:273–281

Roder A, Hoffmann E, Hagemann M, Berg G (2005) Synthesis of the compatible solutes glucosylglycerol and trehalose by salt-stressed cells of *Stenotrophomonas* strains. FEMS Microbiol Lett 243:219–226

Romano A, van der Plas LHW, Witholt B, Eggink G, Mooibroek H (2005) Expression of poly-3-(R)-hydroxyalkanoate (PHA) polymerase and acyl-CoA-transacylase in plastids of transgenic potato leads to the synthesis of a hydrophobic polymer, presumably medium-chain-length PHAs. Planta 220:455–464

Roth FX, Fickler J, Kirchgessner M (1995) Effect of dietary arginine and glutamic acid supply on the N-balance of piglets. 5. Communication on the importance of nonessential amino acids for protein retention. Z Tierphysiol Tierernahr Futtermittelk 73:202–212

Sanford K, Kumar M (2005) New proteins in a materials world. Curr Opin Biotechnol 16:416–421

Saruul P, Srienc F, Somers DA, Samac DA (2002) Production of a biodegradable plastic polymer, poly-beta-hydroxybutyrate, in transgenic alfalfa. Crop Sci 42:919–927

Sauter A, Hüsung B (2005) TA-Projekt Grüne Gentechnik – transgene Pflanzen der 2. und 3. Generation. Büro für Technikfolgenabschätzung beim deutschen Bundestag, Berlin Arbeitsbericht 104

Scheibel T (2005) Protein fibers as performance proteins: new technologies and applications. Curr Opin Biotechnol 16:427–433

Scheller J, Conrad U (2005) Plant-based material, protein and biodegradable plastic. Curr Opin Plant Biol 8:188–196

Scheller J, Guhrs KH, Grosse F, Conrad U (2001) Production of spider silk proteins in tobacco and potato. Nat Biotechnol 19:573–577

Scheller J, Henggeler D, Viviani A, Conrad U (2004) Purification of spider silk-elastin from transgenic plants and application for human chondrocyte proliferation. Transgenic Res 13: 51–57

Schwamborn M (1996) Polyasparaginsäuren. Nachr Chem Technol Lab 44:1167–1179

Schwamborn M (1998) Chemical synthesis of polyaspartates: a biodegradable alternative to currently used polycarboxylate homo- and copolymers. Polymer Degrad Stab 59:39–45

Shao ZZ, Vollrath F (2002) Materials: surprising strength of silkworm silk. Nature 418:741

Simon RD (1976) Biosynthesis of multi-L-arginyl-poly(L-aspartic acid) in filamentous cyanobacterium *Anabaena cylindrica*. Biochim Biophys Acta 422:407–418

Simon RD (1987) Inclusion bodies in the cyanobacteria: cyanophycin, polyphosphate, polyhedral bodies. In: Fay P, van Baalen C (eds) The cyanobacteria, Elsevier, Amsterdam, pp 199–225

Simon RD, Weathers P (1976) Determination of structure of novel polypeptide containing aspartic acid and arginine which is found in Cyanobacteria. Biochim Biophys Acta 420:165–176

Slater S, Mitsky TA, Houmiel KL, Hao M, Reiser SE, Taylor NB, Tran M, Valentin HE, Rodriguez DJ, Stone DA, Padgette SR, Kishore G, Gruys KJ (1999) Metabolic engineering of *Arabidopsis* and *Brassica* for poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymer production. Nat Biotechnol 17:1011–1016

Tabata K, Abe H, Doi Y (2000) Microbial degradation of poly(aspartic acid) by two isolated strains of *Pedobacter* sp. and *Sphingomonas* sp. Biomacromolecules 1:157–161

Taheri F, Ochoa JB, Faghiri Z, Culotta K, Park HJ, Lan MS, Zea AH, Ochoa AC (2001) L-arginine regulates the expression of the T-cell receptor zeta chain (CD3 zeta) in Jurkat cells. Clin Cancer Res 7:958S–965S

Tapiero H, Mathe G, Couvreur P, Tew KD (2002) I. Arginine. Biomed Pharmacother 56:439–445

Thrän D, Weber M, Scheuermann A, Fröhlich N, Zeddies J, Henze A, Thoroe C, Schweinle J, Fritsche UR, Jenseit W, Rausch L, Schmidt K (2005) Sustainable strategies for biomass use in the european context. Analysis in the charged debate on national guidelines and the competition between sloid, liquid and gaseous biofuels. Institute for Energy and Environment GmbH, Leipzig

Tirrell DA (1996) Putting a new spin on spider silk. Science 271:39–40

Valentin HE, Broyles DL, Casagrande LA, Colburn SM, Creely WL, DeLaquil PA, Felton HM, Gonzalez KA, Houmiel KL, Lutke K, Mahadeo DA, Mitsky TA, Padgette SR, Reiser SE, Slater S, Stark DM, Stock RT, Stone DA, Taylor NB, Thorne GM, Tran M, Gruys KJ (1999) PHA production, from bacteria to plants. Int J Biol Macromol 25:303–306

van Beilen JB, Poirier Y (2008) Production of renewable polymers from crop plants. Plant J 54:684–701

Vollrath F (2000) Strength and structure of spiders' silks. J Biotechnol 74:67–83

Vollrath F, Knight DP (2001) Liquid crystalline spinning of spider silk. Nature 410:541–548

Wu GY, Bazer FW, Davis TA, Jaeger LA, Johnson GA, Kim SW, Knabe DA, Meininger CJ, Spencer TE, Yin YL (2007) Important roles for the arginine family of amino acids in swine nutrition and production. Livestock Sci 112:8–22

Yang JJ, Barr LA, Fahnestock SR, Liu ZB (2005) High yield recombinant silk-like protein production in transgenic plants through protein targeting. Transgenic Res 14:313–324

Yeramian A, Martin L, Arpa L, Bertran J, Soler C, McLeod C, Modolell M, Palacin M, Lloberas J, Celada A (2006) Macrophages require distinct arginine catabolism and transport systems for proliferation and for activation. Eur J Immunol 36:1516–1526

Ziegler K, Deutzmann R, Lockau W (2002) Cyanophycin synthetase-like enzymes of non-cyanobacterial eubacteria: characterization of the polymer produced by a recombinant synthetase of *Desulfitobacterium hafniense*. Z Naturforsch C 57:522–529

Zotz RJ, Schenk S, Kuhn A, Schlunken S, Krone V, Bruns W, Genth S, Schuler G (2001) Safety and efficacy of LK565 – a new polymer ultrasound contrast agent. Z Kardiol 90:419–426