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Mini-Review Article

Cyanophycin as a Cyanobacterial Granule Polypeptide: Potential Sources and Applications

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HIGHLIGHTS

- Cyanophycin is a biopolymer of a poly-aspartate with arginine side chains.
- Cyanophycin is synthesized by the enzyme cyanophycin synthetase (CphA).
- Cyanophycin is extracted from cyanobacteria and is heterologously produced in bacteria, yeasts, and plants.
- Cyanophycin has a wide range of industrial, biomedical, and cosmeceutical applications.

ABSTRACT

Cyanophycin is a distinctive biopolymer composed of a poly-aspartate backbone adorned with arginine side chains, produced by cyanobacteria and certain bacterial strains through the enzymatic activities of CphA1 and CphA2. The CphA1 enzyme engages aspartate and arginine in separate reactions, while CphA2 facilitates a more streamlined polymerization of β-Asp-Arg dipeptides, potentially enhancing its efficiency for biotechnological applications. Although cyanophycin is typically insoluble at neutral pH, it becomes soluble in highly acidic or alkaline environments, leading to the formation of large, inert granules that play essential biological roles. This biopolymer primarily acts as a storage reservoir for nitrogen, carbon, and energy, with its metabolic processes tightly regulated to enable organisms to adapt to fluctuating environmental conditions. Cyanophycin's versatility spans multiple sectors, including biomedicine, where it shows promise as a biocompatible material for drug delivery systems and tissue engineering scaffolds. In industrial contexts, it is being investigated as a biodegradable substitute for synthetic polymers and utilized in water treatment applications due to its high viscosity. In agriculture, cyanophycin-derived dipeptides are considered potential nutritional supplements because of their excellent bioavailability. Furthermore, recent advancements in the heterologous expression of cyanophycin synthetases in various host organisms, including bacterial hosts such as *E. coli,* yeasts, and plants, aim to improve production yields and create hybrid materials with optimized properties. Collectively, the multifunctionality and biodegradability of cyanophycin position it as a strong candidate for diverse applications, underscoring the necessity for continued research and development to enhance its practical use and commercial feasibility. This mini-review article summarizes the cyanophycin potential sources, chemical modifications, and potential applications.

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Introduction

Cyanophycin, a biopolymer composed of aspartic acid and arginine, was first discovered in cyanobacteria by the Italian scientist, Borzi in 1887 (Frommeyer et al., 2016). This discovery marked the beginning of a long history of research into this cyanobacterial storage polymer containing a poly-α-aspartic acid scaffold with arginine linked via their α-amino group to the β-carboxyl group of each aspartate, which is primarily used by cyanobacteria for nitrogen and energy storage (Kwiatos and Steinbüchel, 2021). Fig. 1 shows the structure and the metabolism pathways of cyanophycin.

Subsequently, their characterization showed that Cyanophycin was a polyamide consisting of aspartic acid and arginine and they are synthesized by the enzyme cyanophycin synthetase (CphA) through ATP-dependent polymerization of aspartic acid and arginine (Sharon et al., 2023a).

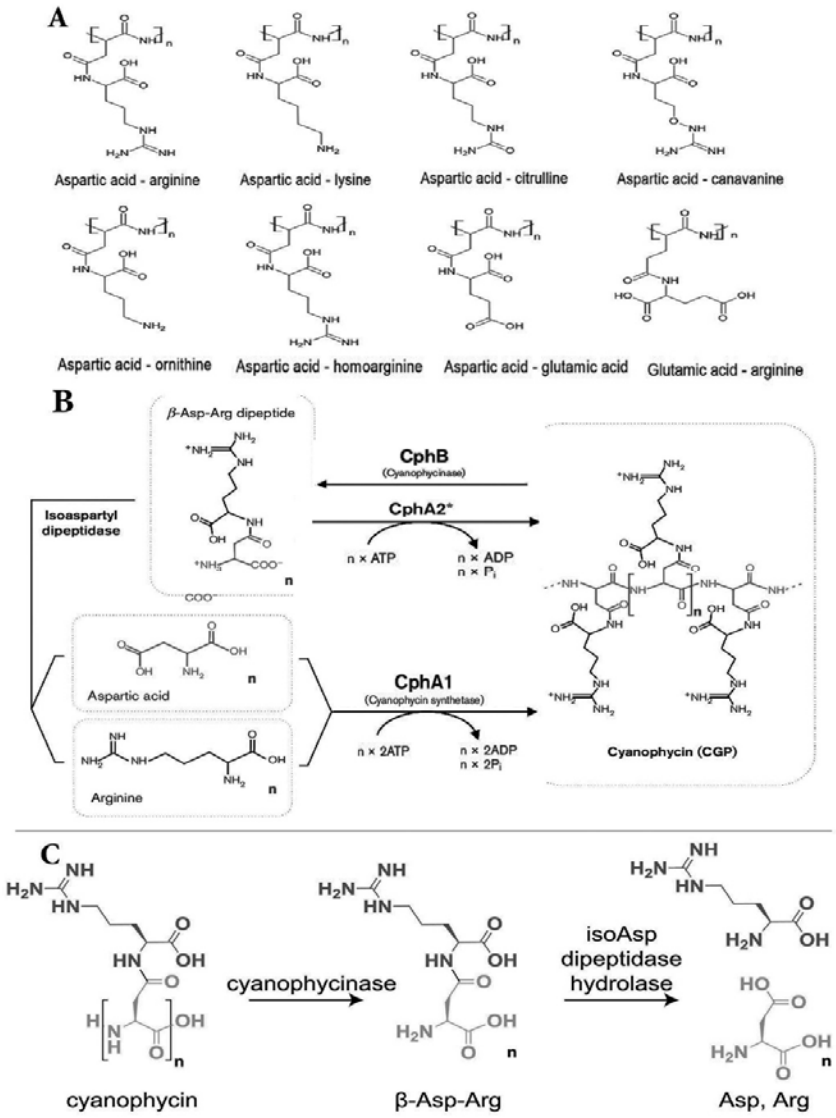

Figure 1. The structure (a) (Kwiatos and Steinbüchel 2021) [open access], biosynthesis pathway (b) (Watzer et al. 2016), and degradation pathway (c) of cyanophycin [(Sharon et al. 2023b), Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND)].

In following, the gene encoding cyanophycin synthetase was successfully expressed in various organisms, including *Escherichia coli* (Frey et al., 2002), *Pseudomonas putida* and *Ralstonia eutropha* (Diniz et al., 2006), yeast (*Saccharomyces cerevisiae*) (Steinle and Steinbüchel, 2010), and even, transgenic plants (tobacco and potato) (Neumann et al., 2005), enabling further studies on its synthesis and properties.

Recent advancements include detailed structural studies of cyanophycin synthetase using cryo-electron microscopy and X-ray crystallography, revealing insights into its catalytic mechanism and active sites (Sharon et al., 2021; Sharon et al., 2022a).

Cyanophycin is degraded into dipeptides by exo-cyanophycinases, which are further hydrolyzed into free amino acids by isodipeptidase enzymes (Obst and Steinbüchel, 2004; Sallam and Steinbüchel, 2008; Sharon et al., 2022b).

Cyanophycin is a natural polymer that is primarily produced by a wide range of bacteria, including cyanobacteria and some heterotrophic bacteria such as *Acinetobacter* sp., *Bordetella bronchiseptica,* and *Clostridium botulinum* (Aravind et al., 2016). It is composed of a poly-aspartate backbone with arginine attached to each of the aspartate side chains (Kwiatos and Steinbüchel, 2021; Markus et al., 2023). This mini-review discussed the key points regarding the sources, applications, production, and properties of cyanophycin.

Sources of cyanophycin

Cyanophycin is mostly produced by various heterotrophic bacteria and cyanobacteria, such as *Synechocystis* sp. Strain PCC 6803 (Canizales et al., 2021, Watzer and Forchhammer, 2018), *Aphanocapsa* 6308 (Allen et al., 1980), *Synechococcus* sp. strain MA19 (Hai et al., 2002), *Anabaena cylindrica* and *Synechocystis* 6308 (Mackerras et al., 1990), *Raphidiopsis raciborskii* (Lu et al. 2022), *Nostoc* sp. PCC 7120 (Trentin et al., 2023) and *Scytonema* sp. (Page-Sharp et al., 1998). In addition, *Acinetobacter* sp. has been found to have a high accumulation (46% of cell dry weight) of cyanophycin (Elbahloul et al., 2005, Elbahloul and Steinbüchel, 2006). A polydisperse copolymer of aspartic acid and arginine, with a small fraction of lysine, with MW ~ 30 kDa and good water solubility contrasting to cyanophycin, was discovered from *Desulfitobacterium hafniense*, which is a gram-positive, spore-forming anaerobis bacteria (Ziegler et al., 2002).

Genetic engineering of natural sources could influence the production yield and the solubility of the cyanophycin produced by them (Wiefel et al., 2019b). As an example, research has shown that the solubility of synthesized cyanophycin produced by *Corynebacterium glutamicum* was influenced by the cyanophycin synthetase used; for instance, CphADh produces exclusively soluble cyanophycin, while other synthetases yield both soluble and insoluble forms. A specific mutation in CphA6308Δ1_C595S led to increased lysine content. Under optimized growth conditions, cyanophycin content in *C. glutamicum* ATCC13032 reached 36% of the cell dry weight, with various alternative amino acids like lysine, ornithine, citrulline, and glutamic acid incorporated into the cyanophycin (Wördemann et al., 2021).

Heterologous production of cyanophycin in different prokaryotic hosts has been reported, including *Pichia pastoris* (Steinle et al., 2010), *Saccharomyces cerevisiae* (Steinle and Steinbüchel, 2010), *Sinorhizobium* (*Ensifer*) *meliloti* 1021(Abd-El-Karem et al., 2011), *Escherichia coli* (Du et al., 2017; K. Swain et al., 2023), *Corynebacterium glutamicum, Ralstonia eutropha* and in *Pseudomonas putida* (Aboulmagd et al., 2001). Table 1 summarizes some examples of the heterologous production of cyanophycin in *E. coli*.

As previously mentioned, transgenic plants have also been extensively studied for the large-scale production of cyanophycin (Neumann et al., 2005). In this regard, tobacco (Nausch et al., 2016; Nausch et al., 2020) and potato (Emmerling et al., 2012; Schmidt et al., 2017) are the most studied hosts (Neubauer et al., 2012).

Table 1: Some examples of the heterologous production of cyanophycin in *E. coli*

Cyanophycin synthetase	Yield	Reference
TmCphA1 (*Tatumella morbirosei*)	1.9 g/L	(Kyle Swain et al. 2023)
CphA49	1.72 g/L (soluble)	(Du et al. 2017)
CphA (*Synechocystis* sp. PCC6308)	26.6% (w/w) of cell dry mass	(Aboulmagd et al. 2001)
CphA (*Synechocystis* sp. PCC 6803)	In *E. coli* BL21-CodonPlus: up to 1.7 g/L	(Tseng et al. 2012)

Cyanophycin modification

Cyanophycin can be chemically modified to produce various derivatives, expanding its utility in the synthesis of bulk chemicals and specialty products. In fact, there are two methods of either chemical or enzymatic processes that have been successfully implemented for *in vitro* cyanophycin modifications. The chemical modification of cyanophycin was demonstrated for the first time. By employing a guanidination reaction, researchers successfully replaced 100% of the lysine side chains in cyanophycin with homoarginine (Frommeyer et al., 2014). Chemical treatments proved to be more effective, achieving higher conversion rates, which prompted a search for suitable reagents to facilitate reactions with cyanophycin. When CGP was reacted with methyl isocyanate, 50% of the lysine residues were converted, while only 3% of the arginine residues were modified. Methylation of CGP with formaldehyde resulted in an 84% conversion rate for lysine and 15% for arginine. Acetic anhydride was used for the acetylation of lysine residues, achieving a 100% conversion rate for single acetylation, with 63% of residues undergoing double acetylation. Arginine residues were acetylated at a rate of 89%, and diacetylation occurred in 80% of all arginine residues, while lysine was unaffected by this compound (Wiefel et al., 2019a).

In a study, the insoluble component of multi-L-arginyl-poly-L-aspartate (MAPA), cyanophycin, named iMAPA was conjugated with polyethylene glycol (PEG) at two different degrees, producing high (iMAPA(PEG)h) and low (iMAPA(PEG)l). Both conjugates displayed UCST (upper critical solution temperature)-type characteristics within a pH range of 3 to 10 at a concentration of 2 mg/mL. The cloud-point temperature increased with concentration in PBS, ranging from 20 to 58 °C for concentrations between 0.1 and 3 mg/mL. Hysteresis was noted during cycles of heating and cooling. It made the resulting derivatives good candidates for drug delivery (Tseng et al., 2018).

Following this, the next logical step was to explore enzymatic modifications of cyanophycin. One of the known enzymes capable of modifying cyanophycin is cyanophycinases, which are present in various strains, including those from *Anabaena* and *Synechocystis* (Obst et al., 2002). However, these enzymes do not alter the side chains, instead, they cleave cyanophycin into dipeptides. The aim of using enzymatic modification was to modify the side chains or the backbone of the polymer while keeping the overall structure of the molecule intact.

Cyanophycin could be modified by enzymatic reaction, such as peptidyl arginine deiminase, which in one study, was derived from *Oryctolagus cuniculus*, recombinantly produced and purified from *Escherichia coli*. It could convert about 10% (4% of the total side chains) of the arginine side chains to citrulline, which had a slightly different solubility (Wiefel and Steinbüchel, 2016).

Applications of cyanophycin

Cyanophycin holds significant promise across various industries due to its versatile properties and renewable nature. It also has shown valuable applications in nutraceutical, pharmaceutical, and cosmeceutical fields. In the following parts, different applications of cyanophycin are illustrated.

Industrial applications

Cyanophycin can be used as a renewable substitute for petrochemical-based products. It has potential applications in the production of biodegradable plastics. Cyanophycin demonstrates thermal stability in air up to 200 °C and does not experience a glass transition within this thermal stability range. The polypeptide is amorphous and lacks long-range order in its solid form. When in solution, the water-soluble cyanophycin is thermoresponsive, showing both upper and lower critical solution temperatures (Khlystov et al., 2017).

Moreover, it can be used as a precursor for poly-aspartic acid (PASP), which is used in water treatment and as a superabsorbent material (Maslova et al., 2018). Cyanophycin can be hydrolyzed into its constituent amino acids, arginine, and aspartic acid, or converted into a derivative with lower arginine content, such as PASP. The PASP has a wide range of applications, such as a substitute for polyacrylic acid in water treatment plants and low-temperature cooling towers, acting as a corrosion inhibitor due to its metal ion chelation properties (Adelnia et al., 2023). Additionally, cyanophycin-derived PASP is utilized in hydrogels (Solaiman et al., 2011) and functions as a super-swelling material in products like diapers, feminine hygiene items, and food packaging. It is also employed as a biodegradable detergent and dispersant in various applications. Polyaspartates (PAA), which are

condensation polymers made from aspartic acid, present alternatives to traditional polycarboxylates (Wang and Kim, 2018).

Investigations disclosed that through the base-catalyzed hydrolysis of cyanophycin, the rate at which L-arginine released, is considerably greater than that of L-aspartic acid. This difference allows for the selective hydrolysis of cyanophycin, resulting in a residue that possesses commercially valuable PASP functionality (Könst et al., 2011).

It was also suggested that cyanophycin' low molecular weight, along with its zwitterionic properties, is an amorphous and rigid polymer that could have potential applications similar to ionomers. Previous studies on the solubility of cyanophycin, particularly regarding the impact of increased lysine content, imply that it may also serve unique functions as a surfactant (Khlystov et al., 2017).

In wastewater treatment, a study showed that the synergy between algae and bacteria enhances the removal of N and phosphorus, by assimilating and covering these moieties into valuable chemicals, such as cyanophycin that can be further used for other industrial purposes (Liu et al., 2024).

Biomedical applications

Due to its biocompatibility and biodegradability, cyanophycin is being explored for use in drug delivery systems, and as a scaffold material for cell attachment and tissue regeneration.

As a tissue scaffold, original cyanophycin needs to tailor its properties—such as biocompatibility, morphology, biodegradability, and mechanical strength—to meet the requirements of the targeted tissue. Crosslinking is a primary modification method used to enhance these biomaterial characteristics (Kwiatos et al., 2024).

A research group synthesized some derivatives of an octa-aspartic acid backbone with argininylated side chains derived from the biopolymer cyanophycin. They supposed that the structure of these unique guanidinium-rich oligomers with side chains on the Asp8 backbone that contain both a guanidine and a carboxylic acid group were anticipated to exhibit unusual cell-penetrating properties (Grogg et al., 2019).

The properties of cyanophycin that make it suitable for drug delivery (Sachin and Karn, 2021), are its biocompatibility and biodegradability (Sallam and Steinbüchel, 2009, Sharon, 2022), presence of soluble forms, which can be manipulated by cultivation conditions and fractionation processes (Füser and Steinbüchel, 2005, Tseng et al., 2017). These forms exhibit different physical properties, such as solubility and thermal response, which are crucial for drug delivery applications (Tseng et al., 2017).

In addition, it is possible to crosslink cyanophycin with other molecular moieties using various methods (e.g. chemical, physical, enzymatic), that can enhance its properties for scaffolding and drug delivery purposes. Crosslinked canophycins have been shown to be non-cytotoxic to certain cell lines, indicating their safety for biomedical use (Kwiatos et al., 2024).

Moreover, various hydrolysis products of cyanophycin have been reported with cell-penetrating activities that could be used as antimicrobial agents or delivery-enhancing agents in the pharmaceutical field. Cyanophycin's ability to form hydrogels and its solubility properties make it an excellent candidate for controlled drug release systems. For instance, its thermal response can be utilized to design drug delivery systems that release drugs at specific temperatures (Tseng et al., 2017). The polymer's structure allows for the encapsulation of therapeutic agents, ensuring targeted delivery and reducing side effects. Self-healing hydrogels derived from biopolymers offer significant benefits in bioengineering and bioscience due to their biocompatibility and biodegradability, particularly as carriers for drug delivery. A study developed a biodegradable hydrogel composed of poly(aspartic acid) (PAsp) and pectin for in vivo applications in cancer treatment, as a carrier for doxorubicin (DOX). Both the pectin and PAsp components provide biodegradability through enzymatic processes and in vivo conditions. The resulting hydrogel demonstrated effective tumor growth inhibition, reducing tumor weight by 80% compared to the control group, while also lowering the toxicity of DOX in vivo and holds great promise as a sustained drug delivery system (Jia et al., 2023).

In a study, as previously mentioned in the section of cyanophycin modification, Tseng et al. synthesized iMAPA (cyanophycin) conjugated derivatives with PEG Reversible core-shell vesicles of these derivatives were formed at room temperature in PBS, with sizes between 25 and 60 nm. This reversibility enabled the encapsulation of doxorubicin (Dox) at different weight ratios to iMAPA(PEG)h, achieving encapsulation efficiencies of up to 70% and a loading capacity of 1.5 mg Dox per mg of iMAPA(PEG)h. The Dox-loaded vesicles maintained stability at 4 °C for four weeks, with

leakage remaining below 2% and a slightly enlarged morphology. Temperature-triggered release of Dox was accomplished by gradually increasing the temperature, initiating with a 10% release at 37 °C and reaching complete release at 62 °C (Tseng et al., 2018).

Agricultural and nutritional applications

Cyanophycin and its derivatives can be used as dietary supplements and in the production of value-added agricultural products. Its high nitrogen content makes it a valuable component in fertilizers. In agricultural applications, it was reported that phosphate absorbed by cyanophycin granule polypeptide significantly enhanced the germination and growth of *Brassica napus*, altering rhizosphere microbial communities. Furthermore, transcriptomic analysis indicated a down-regulation of nitrate uptake and arginine synthesis, while arginine metabolism was up-regulated, likely due to cyanophycin degradation in soil. This degradation released nitrogen and phosphate, promoting plant hormone signaling and accelerating growth. Overall, the study suggested that cyanophycin granule polypeptide was effective for phosphate recovery and could serve as a nitrogen-phosphorus fertilizer (Zeng et al., 2023).

Regarding cyanophycin nutritional value, it is a polymer consisting of aspartic acid and arginine, functioning as a nitrogen and energy storage molecule for these microorganisms (Frommeyer et al., 2016). Its nutritional potential lies in its amino acid composition, particularly the presence of arginine, which is recognized for its health benefits. When cyanophycin degrades, it releases β-aspartic acid-arginine (β-Asp-Arg) dipeptides that can be absorbed in the intestine; however, studies indicate that the bioavailability of free arginine from cyanophycin is limited, which may affect its overall nutritional impact (Ponndorf et al., 2017).

Despite its challenges, such as limited bioavailability and economic feasibility for large-scale production, cyanophycin presents opportunities for food supplementation due to its rich amino acid profile. It could enhance the nutritional value of plant-based diets. Ongoing advancements in genetic engineering and biotechnological processes aim to improve the production and modification of cyanophycin, potentially increasing its suitability for various nutritional applications and facilitating the development of low-input production systems in plants (Nausch et al. 2016; Nausch et al., 2020).

Cosmeceutical applications

The polymer's properties make cyanophycin suitable for use in cosmetic formulations, providing benefits such as moisture retention and skin conditioning. Its ability to be modified chemically and enzymatically allows for the creation of tailored formulations that can enhance skin health and appearance. Studies have shown that cross-linked cyanophycin is non-cytotoxic, making it safe for use in cosmetic formulations (Kwiatos and Steinbüchel, 2021).

Given the various functions of melanins in the human body, extensive research has been carried out focusing on tyrosinase (TYR) as the rate-limiting enzyme of melanogenesis to develop its inhibitors. Through an in silico study, a new collection of compounds derived from cyanophycin was assessed for their potential to inhibit tyrosinase enzymes. Lig2 (aspartic acid – glutamate), which is composed of aspartic acid and glutamate, exhibited the lowest binding affinities with all the enzymes tested, except for TYRP1. In light of these findings, Lig2 has been recognized as a promising candidate (Krzemińska et al., 2022).

Cyanobacteria, the source of cyanophycin, produce compounds with moisturizing, photoprotective, antioxidant, and anti-inflammatory properties, all of which are desirable in cosmetics (Najar and Das, 2015).

Conclusion

In summary, cyanophycin is a biopolymer characterized by a poly-aspartate backbone with arginine side chains. It is synthesized by cyanobacteria and certain bacteria using two enzymes: CphA1, which utilizes aspartate and arginine in separate reactions, and CphA2, which polymerizes β-Asp-Arg dipeptides through a simpler mechanism. While cyanophycin is insoluble under neutral pH, it becomes soluble in acidic (pH < 2) or alkaline (pH > 9) conditions, forming large, inert granules during synthesis. Cyanophycin primarily functions as a storage compound for nitrogen, carbon, and energy in cyanobacteria, with its metabolism tightly regulated to adapt to varying environmental conditions. However, cynaohycin and its derivatives have diverse applications. In biomedicine, it serves as a biocompatible material for drug delivery systems and tissue engineering scaffolds; in industry, it is a biodegradable alternative to synthetic polymers and is useful in water treatment due to its viscosity; in agriculture, cyanophycin-derived dipeptides show

promise as nutritional supplements due to their high bioavailability. Furthermore, advancements in heterologous expression of cyanophycin synthetases in organisms like *E. coli* and plants aim to enhance production yields and develop hybrid materials with tailored properties. Overall, cyanophycin's versatility and biodegradability position it as a promising candidate for various applications across multiple industries, warranting further research and development for enhanced commercial viability.

Ethical Statement

This work did not contain any animal or human studies performed by the authors.

Competing Interests

The authors have no conflicts of interest to declare.

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Data Availability

All relevant data has been reported in the manuscript.

Authors Contribution

T. Hosseinabadi designed and supervised the project. M. Tabarzad and M.V. Tabarzad contributed to the manuscript drafting. The final manuscript was reviewed and confirmed by all authors.

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