Dedicated to Dr. Petr Marvan on the occasion of his 80th birthday

The pyrenoid ultrastructure in *Oocystis lacustris* Chodat (Chlorophyta, Trebouxiophyceae)

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Abstract: The fine structure of vegetative cells of *Oocystis lacustris* has been studied with special attention to the ultrastructure of the pyrenoid and its starch sheath. The TEM-investigation showed that the pyrenoid matrix is homogenous, not traversed by thylakoids and the surrounding starch sheath is continuous, horseshoe-shaped or fragmented in 2 starch plates. This starch sheath structure is regarded as a common feature within *Oocystis* and closely related genera *Eremosphaera* and *Neglectella.*

Key words: *Oocystis lacustris*, ultrastructure, pyrenoids, starch sheath

Introduction

Photosynthetic pigments, storage products and structure of plastids are some of the important features in the taxonomy of eukaryotic algae. In many types of algae within the chloroplast occurs a dense proteinaceous body, visible with light microscope and designated as a pyrenoid. The term "pyrenoid" was created by Schmitz (1882) who was the first to associate this structure with an effect on the accumulation of starch grains in the chloroplasts of green algae. Nowadays it is well known that pyrenoids contain the carbon fixing enzyme Rubisco and are commonly associated with formation of storage products (e.g. Graham & Wilcox 2000, Lee 2008). A remarkable number of morphological types of pyrenoids exists (e.g. Dodge 1973, Ettl 1980, Whatley 1993). The absence or presence of pyrenoids in vegetative cells was already used as a taxonomic criterion on the generic level of algae (Starr 1955, Hindak 1977-1990) whereas the morphology of the starch sheath itself, its structure, and location can assist in the identification of green algal species (Brown & McLean 1969, Ettl 1976, Komárek & Fott 1983, Ettl & Gärtner 1988a). The starch sheath of green algal pyrenoids is normally visible with light microscope (Ettl 1980) especially when stained with reagents such as Lugol's iodine solution. The main structure of the pyrenoid matrix (homogenous, perforated, lamellate or traversed by thylakoids) is also visible in LM with extraordinary optical equipment and when stained with reagents such as azocarmine-G solution (Ettl 1976, 1983, Gärtner 1985). The electron microscopy only cleared characteristic internal details of the pyrenoid matrix (Gibbs 1962, Dodge 1973, Pickett-Heaps 1975, Friedl 1989, Ingolić & Gärtner 2003). Among the members of the genus *Oocystis* A. Br. the ultrastructure of a pyrenoid with horseshoe-shaped starch sheath was first shown by Schnepf, Koch & Deichgräber (1966, p. 165, fig. 33) in a schematic graph of *Oocystis solitaria* Wittrock f. *maior* Wille. Later on, Robinson & White (1972, p. 112, fig. 5) presented a pyrenoid in one TEM-micrograph of *Oocystis apiculata* W. West, and recently Soldo et al. (2005, p. 314, fig. 2 A) documented the pyrenoid of *Oocystis nephrocytioides* Fott et Čado in a micrograph of an ultra thin cell section.

In this paper the ultrastructural details of the pyrenoid in cells of *Oocystis lacustris* Chodat are described for first time and comparison with the related genera *Neglectella* Vodeničarov

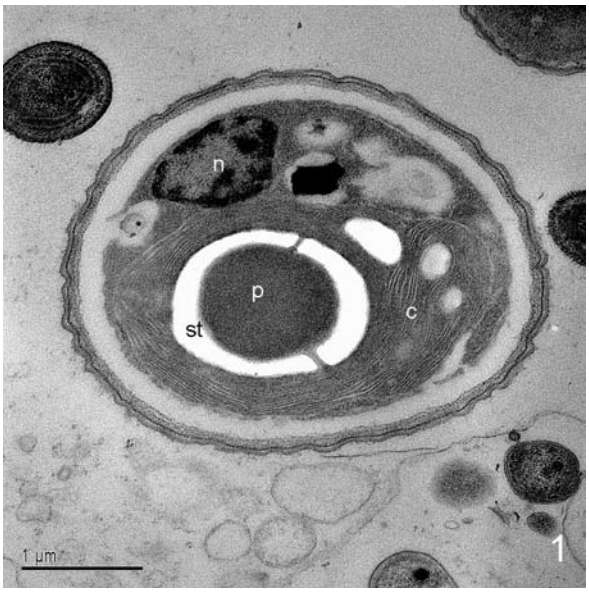
et BENDERLIEV, *Eremosphaera* DE BARY and *Siderocelis* FOTT is done.

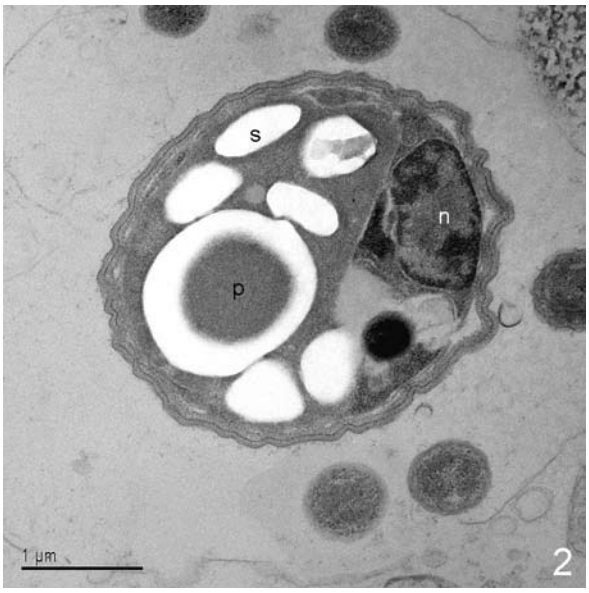
Material and methods

Oocystis lacustris material was obtained from selected phytoplankton samples from Lake Tanganyika dated June–July 2003 when it formed dense populations (STOYNEVA et al. 2007) and fixed in acid Lugol's solution. For detailed description of localities, sampling and methods refer to STOYNEVA et al. (2007). For TEM study cells were fixed a) in 3% glutaraldehyd in 0,1 M cacodylate buffer and b) in 1% aqueous O_sO_4 in 0,1 M cacodylatbuffer, dehydrated in acetone and embedded in Spurr's resine¸ ultrathin sections were stained with uranyl acetate and lead citrate (REYNOLDS 1963). Electron micrographs were taken with a Tecnai 12 (FEI) microscope equipped with a Gatan ccd camera.

Results

In ultra thin sections most of the vegetative cells of *Oocystis lacustris* contain one parietal chloroplast filling more than half of the cell size (Figs 1c, 3c). However, sometimes also two chloroplasts, and, occasionally, four or more of them have been observed. Their thylakoids occur in pairs. In each chloroplast one pyrenoid with a homogenous matrix is situated and surrounded by a thick starch sheath (Figs 1p, 2p, 3p). The diameter of the pyrenoid body is between 1 and 1.5 µm; the thickness of the starch sheath is about 0.25 µm. Thylakoids are not traversing the pyrenoid matrix. The starch sheath around the pyrenoid appears like a closed ring (Fig. 2) or as a horseshoe-shaped starch plate (Fig. 3). There can also be a sheath consisting of two starch plates, more or less regular in thickness (Fig. 1 st). Additionally, single lenticular starch grains, which are not in close association with the pyrenoid, are visible inside the chloroplast (Fig. 2s). These stroma starch grains may reach





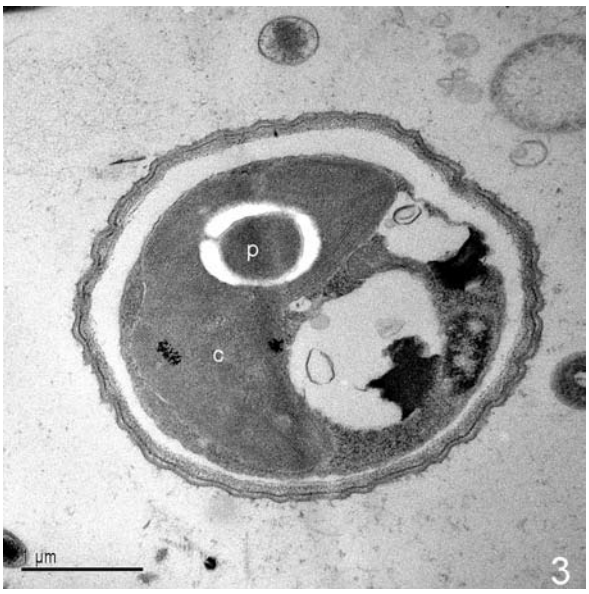


Fig. 1 Vegetative cell of *Oocystis lacustris* with 1 chloroplast (c) and a starch sheath (st) consisting of two starch plates around the homogenous matrix of the pyrenoid (p). n = nucleus. Scale bar 1 µm.

Fig. 2 Pyrenoid (p) with homogenous starch sheath and additional stroma starch grains (s) in the chloroplast. n = nucleus. Scale bar 1µm.

Fig. 3 Chloroplast (c) with pyrenoid (p) and homogenous horseshoe-shaped starch sheath. Scale bar 1µm.

considerable dimensions (up to 0.25–0.75 μm). A single nucleus is embedded in the cell lumen (Figs 1n, 2n). The cell wall is multilayered (Figs 1–3). Its appearance in wavy structure, most probably, is a result of fixation and dehydration during preparation.

Discussion

The finding of the multilayered cell wall during this study is in conformity with all previous data, which showed that the cell walls in Oocystaceae Bohlin are composed of several layers and this diacritic criterion is in accordance with the molecular data (Komárek 1979, Hepperle et al. 2000).

The pyrenoids and their starch components are of great value among the main diagnostic features for identifying coccal green algae with light microscope. Their structure can be cleared up by using staining procedures and squashing-method (Ettl & Gärtner 1988b, 1995). For further taxonomic investigations of unicellular green algae on species level TEM studies of ultrastructural details of cell components are important. Among them the pyrenoid matrix (homogenous, with invaginations or traversing thylakoids) combined with details of the starch sheath composition are significant. Brown & Bold (1964) and Brown & McLean (1969) were the first who used the ultrastructure of the pyrenoid and number and position of starch grains to classify various species of the green algal genera *Chlorococcum* Meneghini and *Tetracystis* Brown et Bold. In the genus *Trebouxia* Puymaly tubular or ramified invaginations into the pyrenoid matrix and thylakoids traversing through the matrix were shown to be species-specific (Friedl 1989, Ingolić & Gärtner 2003).

Profound light microscopical investigations of some genera of the Oocystaceae family have been published previously (e.g. Playfair 1916, Skuja 1956, Fott & Kalina 1962, Fott & Řeháková 1963, Smith & Bold 1966, Řeháková 1969, Hindák 1977–1990, Komárek & Fott 1983) and recently LM observations on *Oocystis lacustris* from tropical Lake Tanganyika have been documented by Stoyneva et al. (2007). However, for a comprehensive cytomorphological and taxonomic study of the whole group (subfamilies Oocystoideae and Eremosphaeroideae in Komarek & Fott 1983) still more investigations of cell ultrastructure and the pyrenoid construction would be necessary.

The genus *Siderocelis* Fott was also placed into the Oocystoideae (Fott 1976) based on detailed light microscopy (Fott 1976, Hindák 1977-1990) but yet the fine structure of its cells in most of the species is unknown. Identical cell wall structures of *Amphikrikos nanus* (Fott et Heynig) Hindák = *Siderocelis nana* Fott et Heynig to *Oocystis* species were documented by Crawford & Heap (1978). Recent TEM-studies of *Siderocelis irregularis* Hindák from Lake Tanganyika revealed its pyrenoid organization: matrix traversed by single undulating thylakoids and starch sheath consisting of 2–10 plates (Stoyneva et al. 2008, p. 798, figs. 21, 22). This structure is clearly different from the pyrenoids of *Oocystis* and *Eremosphaera,* as they are discussed below. This could be accepted as additional prove for the exclusion of *Siderocelis* from Oocystaceae (Ettl & Komárek 1982, Komárek & Fott 1983). Nevertheless, the degree of relationship of *Siderocelis* to the Oocystaceae and its inclusion in the trebouxiophycean lineage (Tsarenko et al. 2006) need support of molecular investigations, which still are lacking.

In the genus *Neglectella* Vodeničarov et Benderliev, generally regarded as close to *Oocystis*, a pyrenoid with massive, thick continuous and homogenous starch sheath is described (Benderliev 1971, Vodeničarov & Benderliev 1971). According to the comparative studies of Benderliev (1971) and the text in Vodeničarov & Benderliev (1971) the pyrenoid of *Neglectella* is of the same type as the pyrenoid of *Eremosphaera viridis* De Bary. This coincides extensively with the descriptions given by Fott & Kalina (1962) but is in opposition to Smith & Bold (1966, p. 25) where in *E. viridis* "a number of polygonal starch grains often surround the pyrenoids, especially in aging or nitrogen-deficient cells". Such discrepancies could be based on different cultivation conditions. Recently in the description of *Eremosphaera tanganyikae* Stoyneva, Gärtner, Cocquyt et Vyverman (Stoyneva et al. 2006) some diagnostic features of pyrenoid and starch sheath - visible with light microscope - were included. The starch sheath was shown to contain two plates (Stoyneva et al. 2006, figs. 37, 39). These results generally coincide with our LM observations on *Oocystis lacustris*, where continuous starch sheath was detected (Stoyneva et al. 2007, p. 587) and with our recent TEM

investigations, showing that starch plates do not exceed two in number.

The presence or absence of a pyrenoid is documented for most species of *Oocystis* (e.g. Skuja 1956, Bourrelly 1966, Philipose 1967, Hindák 1977–1990, Komárek 1983, Komárek & Fott 1983, John & Tsarenko 2002), but the organization of the starch sheath was often neglected. A special note about the continuous starch sheath was provided by Fott & Čado (1966) in their LM diagnosis of *Oocystis nephrocytioides*. The TEM photo in Soldo et al. (2005) reveals a pyrenoid with homogenous matrix and bipartite starch sheath. Observations with TEM of *Oocystis apiculata* showed a "bilenticular" pyrenoid type (Chadefaud 1941, Hori & Ueda 1967) traversed by thylakoids and enclosed by a starch sheath fragmented in two parts (Robinson & White 1972).

Our results on *Oocystis lacustris* coincide partially with the observations on *O. apiculata*. In *O. apiculata,* as well as in *O. lacustris,* the starch envelope around the pyrenoid appears continuous or fragmented in two parts. But *O. apiculata* pyrenoids are traversed by a simple (single) tubular thylakoid system as it is shown by Robinson & White (1972, p.112, fig. 5). This was never observed in cells of *O. lacustris* where the pyrenoid matrix appeared homogenous and was not traversed by thylakoids (Figs 1–3). Therefore it is clear that the type of pyrenoid matrix (homogenous or traversed by thylakoids) still needs further studies and, most probably, is not a diacritic feature in *Oocystis* and in Oocystaceae. However, it seems that continuous or slightly fragmented starch sheath with at most two starch plates is a common diagnostic feature in members of the genus *Oocystis* and its closely related genera *Neglectella* and *Eremosphaera*, but this still has to be verified by further observations with TEM on more species.

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References

Benderliev, K. (1971): Bau und Entwicklung des Pyrenoids bei *Eremosphaera viridis* und *Neglectella eremosphaerophila* Vod. et Benderl. – Nova Hedwigia 21: 789–800.

Bourrelly, P. (1966): Les algues d'eau douce. I. – 511pp., Boubée & Cie., Paris.

Brown, R. M., Jr. & McLean, R. J. (1969): New taxonomic criteria in classifications of *Chlorococcum* species. II. Pyrenoid fine structure. – J. Phycol. 4: 114–118.

Brown, R. M. & Bold, H. C. (1964): Phycological Studies 5. Comparative studies of the algal genera *Tetracystis* and *Chlorococcum*. – Univ. Texas Publ. 6417: 1–213.

Chadefaud, M. (1941): Les pyrénoïdes des algues et l' existence chez ces végétaux d'un appareil cinétique intraplastidial. – Ann. Sci. Nat. Bot. 11: 1–44.

Crawford, R. M. & Heap, P. F. (1978) : Transmission electron microscopy X-ray microanalysis of two algae of the genera *Scenedesmus* and *Siderocelis*. – Protoplasma 96: 361–367.

Dodge, J. D. (1973): The Fine Structure of Algal Cells. – 261 pp., Academic Press, London, New York.

Ettl, H. (1976): Die Gattung *Chlamydomonas* Ehrenberg. – Beih. Nova Hedwigia 49: 1–1122.

Ettl, H. (1980): Grundriß der allgemeinen Algologie. – 549 pp., VEB Gustav Fischer, Jena.

Ettl, H. (1983): Chlorophyta I. Phytomonadina. – In: Ettl, H., Gerloff, J., Heynig, H. & Mollenhauer, D. (eds): Süßwasserflora von Mitteleuropa 9. – 807 pp., Gustav Fischer, Stuttgart, New York.

Ettl, H. & Gärtner, G. (1988a): Chlorophyta II. Tetrasporales, Chlorococcales, Gloeodendrales. – In: Ettl, H., Gerloff, J., Heynig, H. & Mollenhauer, D. (eds): Süßwasserflora von Mitteleuropa 10. – 436 pp., Gustav Fischer, Stuttgart, New York.

Ettl, H. & Gärtner, G. (1988b): Eine einfache Methode zur Darstellung der Struktur der Stärkehüllen von Pyrenoiden bei Grünalgen (Chlorophyta). – Arch. Protistenk. 135: 179–181.

Ettl, H. & Gärtner, G. (1995): Syllabus der Boden-, Luft- und Flechtenalgen. – 721 pp., Gustav Fischer, Stuttgart, Jena, New York.

Ettl, H. & Komárek, J. (1982): Was versteht man unter dem Begriff "coccale Grünalgen"? – Arch. Hydrobiol. Suppl. 60, 4, Algol. Stud. 29: 345–374.

Fott, B. (1976): *Oocystis* und verwandte Gattungen aus der Unterfamilie der Oocystoideae; Namensänderungen, taxonomische Notizen und Bestimmungsschlüssel. – Preslia 48: 193–206.

Fott, B. & Čado, I. (1966): *Oocystis nephrocytioides*

sp. nov. – Phycologia 6: 47–50.

Fott, B. & Kalina, T. (1962): Über die Gattung *Eremosphaera* De Bary und deren taxonomische Gliederung. – Preslia 34: 348–358.

Fott, B. & Řeháková, T. (1963): Infraspecific variability in *Oocystis coronata* Lemmermann. – Nova Hedwigia 6: 239–243.

Friedl, T. (1989): Comparative ultrastructure of pyrenoids in *Trebouxia* (Microthamniales, Chlorophyta). – Pl. Syst. Evol. 164: 145–159.

Gärtner, G. (1985): Die Gattung *Trebouxia* Puymaly (Chlorellales, Chlorophyceae). – Arch. Hydrobiol. Suppl. 71, Algol. Studies 41: 495–548.

Gibbs, S. P. (1962): The ultrastructure of the pyrenoids of the green algae. – J. Ultrastruct. Res. 7: 262–272.

Graham, L. E. & Wilcox, L. W. (2000): Algae. – 640 pp., Prentice-Hall, Upper Saddle River, NJ.

Hepperle, D., Hegewald, E. & Krienitz, L. (2000): Phylogenetic position of the Oocystaceae (Chlorophyta). – J. Phycol. 36: 590–595.

Hindák, F. (1977–1990): Studies on the Chlorococcal algae (Chlorophyceae), I-V. – Biologické práce, Veda, Bratislava.

Hori, T. & Ueda, R. (1967): Electron microscope studies on the fine structure of plastids in siphonous green algae with special reference to their phylogenetic relationships. – The Science Rep., Tokyo Kyoiku Daigaku, Sec. B, 12/187: 225–244.

Ingolić, E. & Gärtner, G. (2003): Ultrastructure of pyrenoids in green algal taxonomy. – Performance Report 2001/2002. – pp. 90–91, Forschungsinstitut für Elektronenmikroskopie, Techn. Universität Graz, Austria.

John, D. M. & Tsarenko, P. M. (2002): Chlorococcales. – In: John, D. M., Whitton, B. A. & Brook, A. J. (eds): The Freshwater Algal Flora of the British Isles. An Identification Guide to Freshwater and Terrestrial Algae. – pp. 327–408, Cambridge, Cambridge University Press.

Komárek, J. (1979): Änderungen in der Taxonomie der Chlorococcalalgen. – Arch. Hydrobiol./Algol. Stud. Suppl. 56, 24: 239–263.

Komárek, J. (1983): Contribution to the chlorococcal algae of Cuba. – Nova Hedwigia 37: 65–180.

Komárek, J. & Fott, B. (1983): Chlorococcales. - In: Huber-Pestalozzi, G. (ed.): Das Phytoplankton des Süßwassers 7. – 1044 pp., Schweizerbart, Stuttgart.

Lee, R. E. (2008): Phycology. – 547 pp., Cambridge University Press, Cambridge.

Pickett-Heaps, J. D. (1975): Green Algae. – 606 pp., Sinauer Ass. Inc., Sunderland.

Philipose, M. T. (1967): Chlorococcales. – 365 pp., ICAR, New Delhi.

Playfair, G. I. (1916): *Oocystis* and *Eremosphaera.* – Proceedings of the Linnean Society of New South Wales 41: 107–147.

Printz, H. (1913): Eine systematische Übersicht der Gattung *Oocystis* Nägeli. – Nyt Magazin for Naturvidenskaberne 51: 165–203.

Řeháková, H. (1969): Die Variabilität der Arten der Gattung *Oocystis* A. Braun. – In: Fott, B. (ed.): Studies in Phycology. – pp. 145–196, Academia, Praha.

Reynolds, E. S. (1963): The use of lead citrate at high pH as an electron opaque stain in electron microscopy. – J. Cell. Biol. 17: 208–212.

Robinson, D. G. & White, R. K. (1972): The fine structure of *Oocystis apiculata* W. West with particular reference to the wall. – Br. Phycol. J. 7: 109–118.

Schmitz, F. (1882): Die Chromatophoren der Algen. – 180 pp., Max Cohen & Sohn, Bonn.

Schnepf, E., Koch, W. & Deichgräber, G. (1966): Zur Cytologie und taxonomischen Einordnung von *Glaucocystis*. – Arch. Mikrobiol. 55: 149–174.

Skuja, H. (1956): Taxonomische und Biologische Studien über das Phytoplankton Schwedischer Binnengewässer. – Nova Acta Reg. Soc. Scient. Upsaliensis, ser. IV, 16: 1–404.

Smith, R. L. & Bold, H. C. (1966): Phycological studies.VI. Investigation of the algal genera *Eremosphaera* and *Oocystis*. – Univ.Texas Publ. 6612: 7–121.

Soldo, D., Hari, R., Sigg, L. & Behra, R. (2005): Tolerance of *Oocystis nephrocytioides* to copper: intracellular distribution and extracellular complexation of copper. – Aquatic Toxicology 71: 307–317.

Starr, R. C. (1955): A comparative study of *Chlorococcum* Meneghini and other spherical, zoospore-producing genera of the Chlorococcales. – Indiana Univ. Publ., Sci. Ser. 20: 1–111.

Stoyneva, M. P., Cocquyt, C., Gärtner, G. & Vyverman, W. (2007): *Oocystis lacustris* Chod. (Chlorophyta, Trebouxiophyceae) in Lake Tanganyika (Africa). – Linzer. Biol. Beitr. 39: 571–632.

Stoyneva, M. P., Gärtner, G., Cocquyt, C. & Vyverman, W. (2006): *Eremosphaera tanganyikae* sp. nov. (Trebouxiophyceae), a new species from Lake Tanganyika. – Belg. J. Bot. 139: 3–13.

Stoyneva, M. P., Ingolić, G., Kofler, W. & Vyverman, W. (2008): *Siderocelis irregularis*. (Chlorophyta, Trebouxiophyceae) in Lake Tanganyika. – Biologia 63: 795–801.

Tsarenko, P. M., Wasser, S. P. & Nevo, E. (eds) (2006): Algae of Ukraine: Diversity, Nomenclature, Taxonomy, Ecology and Geography, Vol. 1. – 713 pp., A. R. G. Gantner, Ruggell, Liechtenstein.

VODENIČAROV, D. & BENDERLIEV, K. (1971): *Neglectella* gen. nov. (Chlorococcales). – Nova Hedwigia 21: 801–812.

WHATLEY, J. M. (1993): Chloroplast ultrastructure. – In: BERNER, T. (ed.): Ultrastructure of Microalgae. – pp. 135–204, CRC Press, Boca Raton, Ann Arbor, London, Tokyo.

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