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THE METABOLITE TRANSFER IN *CYANOPHORA PARADOXA* AND ITS CYANELLES

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Cyanophora paradoxa is an obligate autotrophic flagellate which possesses 2-4 organelle like inclusions, the cyanelles. These contain chlorophyll a, c- and allo-phycocyanin, but no chlorophyll b. The cyanelles perform photosynthetic CO_2-fixation and apparently supply the eukaryotic host with organic carbon. Cyanelles share features both with free-living cyanobacteria and chloroplasts of higher plants (for a review with the relevant literature see ref. 1). They possess a rudimentary peptidoglycan cell-wall which is indispensible for their division. Both subunits of ribulose-1-5-bis-phosphate carboxylase are encoded on the cyanelle DNA in contrast to the situation with chloro-plasts. On the other hand, the genome size is only 5 - 10 % of that of free-living unicellular cyanobacteria and thus similar to that of chloroplasts. In contrast to chloroplasts, cyanelles must possess the genes for the synthesis of phycobilins and for the peptidoglycan wall possibly indicating that other essential functions of chloroplasts are left out with cyanelles. Cyanelles do not respire and cannot utilize nitrate without the help of the eukaryotic host which clearly differentiates them from free-living cyanobacteria. From all these facts and others not mentioned here it is not clear as yet whether cyanelles represent a link in the evolution of free-living cyanobacteria to chloroplasts. They, however, can serve as models for the acquisition of plastids by eukaryotes.

The molecular biology of the cyanelles has extensively been studied particularly by the groups of Loeffelhardt (1), Bohnert (1), Schenk (2) and others. In contrast, informations about the metabolism of cyanelles is more scanty and scattered about the literature. This lab. (3) recently applied the silicone-oil centrifugation technique commonly used with chloroplasts (4) to study the exchange of metabolites from and to cyanelles. The measurements gave the somewhat surprising result that ^{14}C-labelled sorbitol freely penetrated the plasmamembrane of cyanelles to a similar extent as ^3H_2O. Maltose and sucrose were, however, virtually not accumulated by the cyanelles. The difference in the solute spaces for ^3H_2O and ^{14}C-labelled sucrose (or

maltose) grossly represents the internal space of cyanelles surrounded by the cytoplasmic membrane. The silicone oil centrifugation technique allows to determine the concentration of compounds inside the cyanelles and their uptake rates.

It had been published (3) that cyanelles do not take up ATP, ADP, malate, and oxaloacetate (the latter two either alone or in combination with each other) and therefore do not possess an ATP/ADP translocator and also not a dicarboxylic acid carrier. Newer experiments showed that cyanelles rapidly take up phosphate with an apparent K_m of 0.038 mM and a V_{max} of 1.77 µmol/ mg chlorophyll a x h. The utilization of phosphate is further enhanced by light approximately twofold. The phosphate uptake is inhibited by 3-phosphoglycerate (3-PGA), dihydroxy acetone phosphate (DHAP) and glucose-6-phosphate but not by phosphoenol pyruvate (PEP) or 2-PGA. Phosphate-utilization by the cyanelles is inhibited by 4,4'-isothiocyanostilbene-2,2'-disulfonic acid (DIDS) (K_i about 1 x 10^{-6} M) and pyridoxalphosphate (K_i about 0.3 mM). Thus cyanelles possess a phosphate translocator which transports phosphate, 3-PGA and DHAP (see Fig.1) similar to plastids of higher plants. The inhibition by glucose-6-phosphate could indicate that the carrier from cyanelles more closely resembles the phosphate translocator of amyloplasts of pea or of the chloroplasts in guard cells.

In contrast to chloroplasts and free-living cyanobacteria, isolated cyanelles prefer to utilize glutamine from the external medium, whereas glutamate is at best poorly taken up. The utilization of glutamine seems to be biphasic and possibly involves a high and a low affinity carrier. The uptake of glutamine is drastically stimulated by oxoglutarate and light and is severely affected by azaserine.

This inhibitor acts on the glutamine: oxoglutarate amidotransferase (GOGAT) reaction. It is, therefore, suggested that glutamine and oxoglutarate are taken up by the cyanelles and are then converted to 2 molecules of glutamate, in a reaction catalyzed by GOGAT inside the cyanelles. This enzyme had been shown to occur both in the cyanelles and the eukaryotic host (5). As glutamate does not or at best poorly penetrate the cytoplasmic membrane of cyanelles, the radioactive label accumulates inside the cyanelle space. The GOGAT reaction requires reduced ferredoxin in chloroplasts. This could well be the case also in cyanelles, since the uptake of glutamate and oxoglutarate is stimulated by light and is almost completely blocked by 5 x 10^{-6} M DCMU. The light-stimulated uptake of glutamine and oxoglutarate is also affected by 8 x 10^{-6} M FCCP indicating that the utilization of these compounds from the external medium might be energy-dependent. It is generally assumed in such studies that the exchange of metabolites across a membrane is freely reversible. Thus glutamine and oxoglutarate could well be cotransported across the cytoplasmic membrane of cyanelles, and both metabolites are either exported or imported, depending on the prevailing source-sink gradient. Glutamine and oxoglutarate could

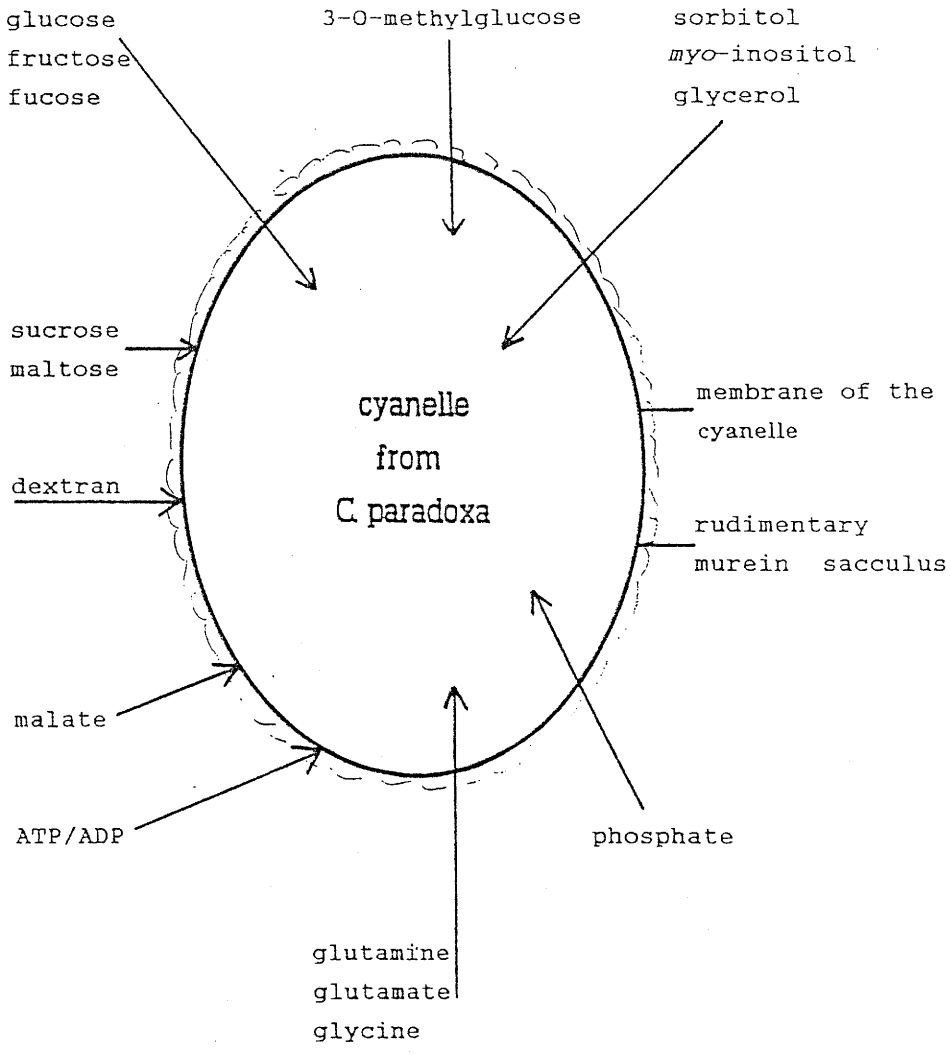

Fig . 1: Schematic representation of the compounds which penetrate across the cytoplasmic membrane of cyanelles and which do not pass it.

be converted to glutamate both in the eukaryotic host and in the cyanelles. The nature of the reductant in the eukaryotic host (reduced ferredoxin?) remains to be shown. It is noteworthy in this context that both the eukaryotic host and the cyanelles possess glutamine synthetase (GS) (5). The major part of the glutamate and glutamine formed might be synthesized within the cyanelles, since the further substrate of the reaction, ammonia, is produced inside the cyanelles (5). This is concluded from the observation

that nitrite reduction by intact *Cyanophora* is strictly light-dependent (6) and that nitrite reductase catalyzing the reduction of nitrite to ammonia resides inside the cyanelles in contrast to nitrate reductase which is exclusively in the eukaryote (5).

The previous investigation (3) indicated that cyanelles take up glucose from the external medium and that the solute space for glucose is almost as high as that for ^3H_2O (3). Newer experiments show that the apparent K_m for glucose is 0.8 mM and thus fairly high as it is in the case of chloroplasts. The uptake of glucose is severely inhibited by the substrate analogue 3-O-methyl-glucose. Thus a permease with similar properties as the glucose carrier of chloroplasts appears to exist at the cytoplasmic membrane of cyanelles. As with chloroplasts, details of the properties of this carrier remain to be investigated. It should be mentioned that despite of the now advanced knowledge about the carrier composition it it still not yet possible to isolate cyanelles which show sustained CO_2-fixation rates. It had been noted in the the previous study (7) that cyanelles carry on CO_2-fixations with approximately 20 % of the in vivo rate which, however, proceeds only for 3 min. The sudden decrease thereafter in activity cannot be explained as yet. The studies on the exchange of metabolites are always finished within the first three min.

In summary, the experiments clearly indicate that cyanelles possess a phosphate translocator and a glucose carrier as it is present in plastids of higher plants. A phosphate translocator has now been described in an organism of a low evolutionary scale. In their carrier composition, cyanelles are apparently more closely related to plastids than to free-living cyanobacteria. They, however, do not possess a malate/oxaloacetate shuttle, possibly indicating that they do not need to transport reducing equivalents out of the cyanelles by such a carrier. Cyanelles can apparently discriminate glutamine from glutamate which distinguishes them from both cyanobacteria and chloroplasts.

The current status of knowledge about the carrier composition is schematically presented in Fig. 1. More detailed accounts of the experiments with the relevant literature are in press (R. Schlichting, H. Bothe: The cyanelles of *Cyanophora paradoxa* (organelles of low evolutionary scale) possess a glucose-carrier and a phosphate translocator and K. Kloos, R. Schlichting, W. Zimmer, H. Bothe: The transport of glutamine and glutamate into cyanelles of *Cyanophora paradoxa*, both Botanica Acta, accepted).

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