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Time-dependent alterations in the antenna pigment–protein complex by mercury ions in the cyanobacterium *Spirulina platensis*

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We have shown that mercury affects energy transfer in *Spirulina platensis*. It inhibits energy transfer from phycocyanin to chlorophyll *a* by specifically bleaching the *β*-84 chromophore of the chromo protein, phycocyanin (PC), in the cyanobacterium. This effect is observed during short-term exposure of cells to Hg^{2+} ions. Upon long-term (12 h) exposure, mercury at low concentrations (1–2.5 μM) causes the gradual degradation of the *β* polypeptide (22 kDa) of the PC of phycobilisomes in this cyanobacterium. The effect of mercury on this polypeptide is significant compared with the other phycobiliproteins.

Keywords: energy transfer, fluorescence, mercury, phycobilisome, phycocyanin, *Spirulina platensis*

Introduction

The phycobiliproteins of phycobilisomes (PBsomes) constitute the major light harvesting pigments in cyanobacteria (Gantt 1975, 1981). The efficiency of energy transfer from phycocyanin (PC) to chlorophyll (Chl) *a* can be influenced by several environmental factors such as temperature (Singhal *et al.* 1981, Babu *et al.* 1992), nitrogen stress (Yamanaka & Glazer 1980) and heavy metal ions (Fujimori 1964, Pecci & Fujimori 1967).

The effect of organomercurial compounds on the spectral properties of phycoerythrin (PE) from cyanobacteria belonging to genus *Hydrocolium* has been studied in detail by Pecci & Fujimori (1968). The analysis of mercury-induced alterations in PE suggested that, depending on the period of incubation, mercury caused distinct conformational changes in the pigment–protein interaction. Also, upon long-term incubation, it led to the dissociation of apoprotein (Pecci & Fujimori 1967). We have previously shown that mercury at low concentrations (1–3 μM) inhibits the energy transfer from PC to Chl *a* by specifically affecting the pigment–protein interaction of PC in *Spirulina* (Murthy *et al.* 1989, Murthy & Mohanty 1991). In this communication we have investigated the time-dependent effect of mercury ions on this chromophoric protein, i.e. PC. Our results indicate that mercury ions cause a selective bleaching of the chromophore of PC during short-term exposure and lead to the dissociation of the *β* subunit of PC, upon long-term treatment, in the cyanobacterium *Spirulina platensis*.

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Materials and methods

S. platensis cells were cultured in Zarrouk's medium (Zarrouk 1966) in glass vessels at 25 ± 2°C under continuous illumination (~ 15 $W m^{-2}$) as described previously (Murthy *et al.* 1989). Vigorously growing log phase cultures were used as experimental material. Cells were harvested and washed once with fresh growth medium and suspended in it. Cells were then incubated in the presence and absence of mercury (6–15 μM) for 5 min in the dark (short-term treatment). For long-term treatment, the cells were incubated in the presence of low concentrations of mercury (1–2.5 μM) for 12 h under normal growth conditions. PBsomes were isolated from *Spirulina* according to Gantt *et al.* (1979). Absorption spectra were recorded using a Shimadzu UV-3000 spectrophotometer. Room temperature fluorescence spectra were recorded on a Perkin-Elmer LS-5 spectrofluorimeter. Chl was estimated according to MacKinney (1941). Protein content was measured by following the procedure of Lowry *et al.* (1951). The total cell protein or PBsome protein was precipitated by the addition of 10% cold trichloroacetic acid. The precipitates were washed with ethanol. The

pellet was dissolved in a buffer and electrophoresis was performed on SDS–polyacrylamide gels with 12% acrylamide (Laemmli 1970). After electrophoresis, the gels were stained. The low molecular weight calibration kit of Bio-Rad was used for the determination of molecular weights.

Results and discussion

Incubation of *Spirulina* intact trichomes for short durations (5 min) in the presence of a low concentration of mercury (6 μM) did not influence the absorption characteristics of PBsomes (particularly PC) appreciably. However, an increase in the mercury concentration to 15 μM induced a decrease in absorption by PC while no changes in Chl *a* absorption were observed (See Figure 1A). Unlike in the case of absorption studies, mercury at very low concentrations (3 μM) induced a large increase in the fluorescence emission intensity of PC. In untreated control cells an emission peak at 652 nm emanating from PC was clearly observed when cells were excited with 545 nm light (see Figure 1B; also see Murthy & Mohanty 1991). The addition of a low concentration of mercury (3 μM) caused not only a 34% increase in the fluorescence yield but also shifted the emission peak towards the blue region by 12 nm. An increase in the concentration of mercury chloride further induced an enhancement in the emission intensity as well as a shift in the peak position (data not shown). The increase in the peak emission in the presence of low concentrations of mercury in *Spirulina* suggests that there may be an alteration in PBsome structure which, in turn, causes an interruption of energy transfer. This interruption of energy transfer could arise either due to PBsome dissociation (uncoupling) between PC proteins with linkers and PC proteins without linkers (Glazer 1984) or could be due to the uncoupling between the PC hexamer and the allophycocyanin (APC) core, which is evident from the shift in the position from 657 to 645 nm. Thus it is clear that mercury affects the spectral properties of PBsomes in short-term incubations. Since the work of Pecci & Fujimori (1967) clearly showed time-dependent changes by organomercury (p-chloro mercuribenzoic acid) on PE (as discussed in the Introduction), we performed a similar study to investigate the effect of sublethal concentrations of mercury on PBsomes in *Spirulina*. As shown in Figure 2(A, lane 1) the major bands in the region 22–17 kDa represent the phycobiliproteins (PC and APC subunits) since 70% of the total protein in *S. platensis* is made up by PBsomes. In addition, other cellular proteins, such as the linker polypeptides of PBsomes and other Chl proteins, etc., occupy the positions above 22 kDa and below 97 kDa. To prove that the major bands in the electrophoretic profile of total cellular protein belong to PBsomes, the electrophoretic profile of intact PBsomes was also analyzed. Figure 2(B) shows the SDS–PAGE profile of PBsomes of *Spirulina*. Apart from the chromophore carrying

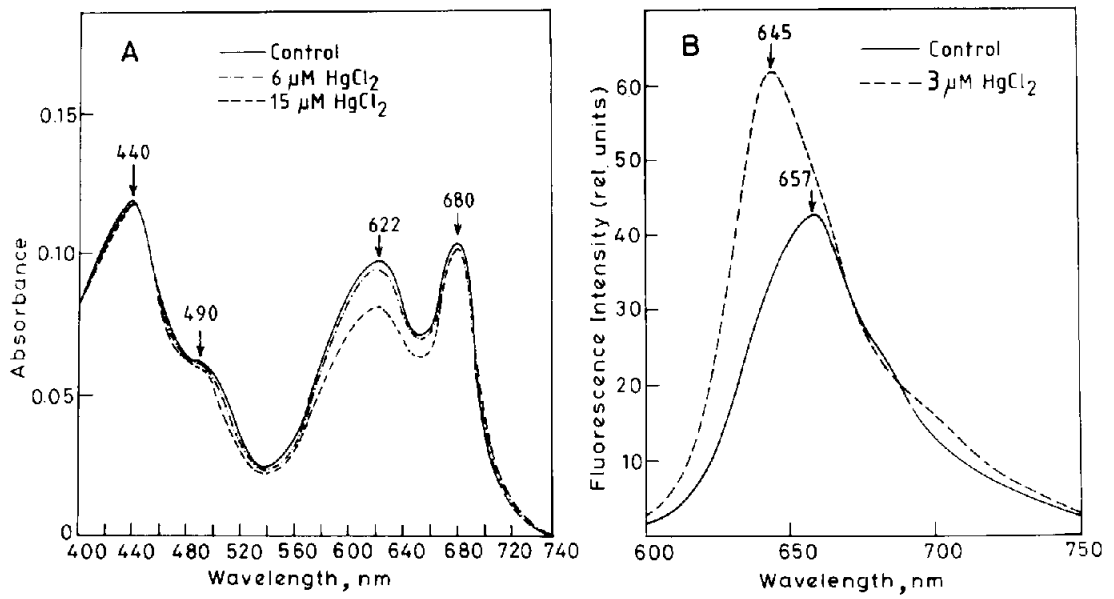

Figure 1. Effect of mercury ions ($HgCl_2$) on the absorption (**A**) and emission spectra (**B**) of intact cells in short-term (5 min) incubation. Cells were incubated in the presence and absence of $HgCl_2$. Cells were excited with 545 nm light to excite specifically PBsomes (slit width for both excitation and emission 5 nm). Cells equivalent to 5 μg of Chl *a* were used.

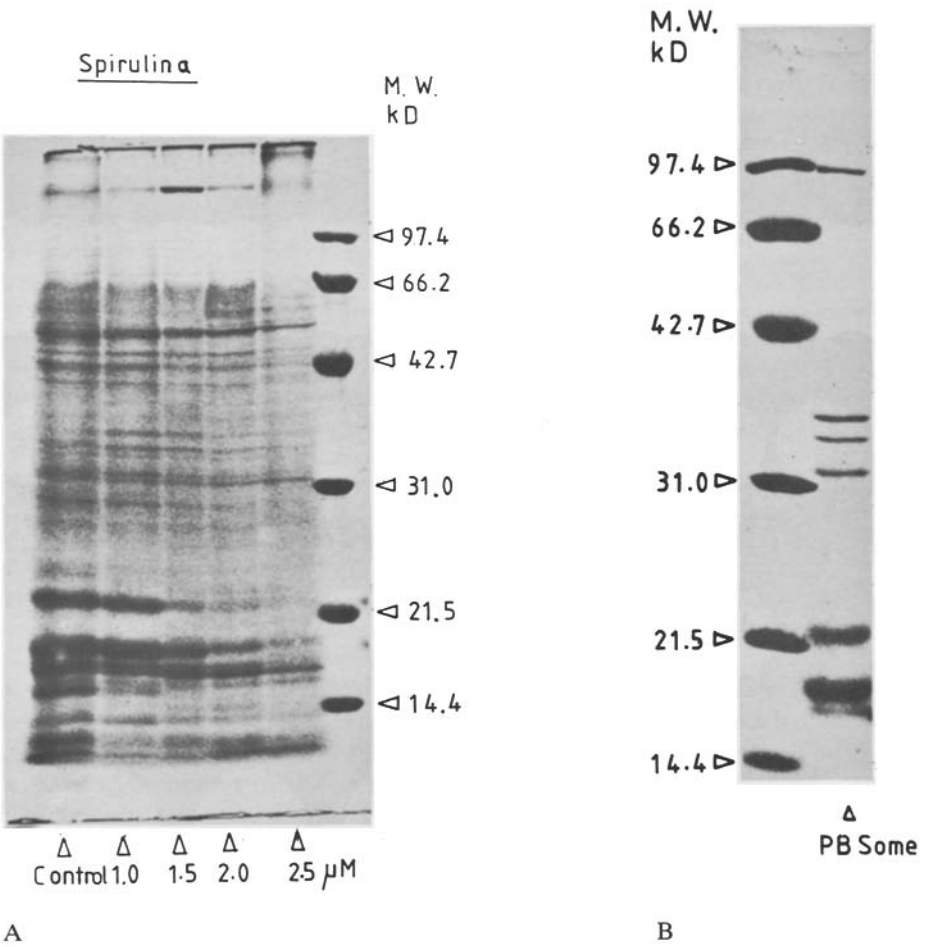

Figure 2. SDS–polyacrylamide gel profile of the total cellular protein (**A**) and PBsomes (**B**) of *Spirulina* cells. Panel A shows profile of total cellular protein from control and mercury-treated cells at indicated concentrations. Panel B indicates the polypeptide composition of the intact PBsomes. The α and β subunits of *Spirulina* PC have molecular weights of 17.5 and 22 kDa, respectively, whereas the α and β subunits of APC have molecular weights of 16 and 18 kDa, respectively. PBsome or total cell extract equivalent to 20 μg protein was used for electrophoretic measurements. For reference Bio-Rad markers having the following molecular weights were used: phosphorylase b, 97.4 kDa; bovine serum albumin, 66.3 kDa; ovalbumin, 42.7 kda; carbonic anhydrase, 31.0 kDa; soyabean trypsin inhibitor, 21.5 kDa; lysozyme, 14.4 kDa.

subunits of phycobiliproteins, five non-pigmented polypeptides are clearly seen (lane 2) to be associated with PBsomes. The α and β subunits of *Spirulina* PC have molecular masses of 17.5 and 22 kDa, respectively. Thus it is clear that the observed major bands in the total cellular protein electrophoretic profile are due to the presence of phycobiliproteins.

Figure 2(A, lanes 2–5) shows the effect of sublethal concentration of mercury on the total cellular protein electrophoretic pattern. It is clear that mercury specifically affects the 22.0 kDa polypeptide in a concentration-dependent manner. The increase in the concentration of mercury from 1 to 2.5 μM caused the complete disappearance of the 22.0 kDa polypeptide, which denotes the β subunit of PC. Similar observations have been made by Yamanaka & Glazer (1980) under nitrogen starvation conditions in *Synechocccus* 6301. The possible reasons for the disappearance of this β polypeptide of PC could be the protease mediated degradation of PC subunit(s), as previous hypothesized by Yamanaka & Glazer under nitrate starvation. Thus mercury in short-term treatment induces alterations in the pigment–protein interaction of PC, whereas in long-term incubation it causes the degradation of one of the PC subunits, most preferably the β-subunit. Further studies on the molecular aspects of PBsome degradation by mercury ions are required to substantiate this proposition.

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