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EFFECT OF SULPHITE ON ADENINE NUCLEOTIDES OF THE GREEN ALGA *TREBOUXIA*

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Key Word Index—*Trebouxia*; green alga; adenine nucleotides; sulfur dioxide; sulfite.

Abstract—The amounts of AMP, ADP and ATP were determined after sulfite incubation in photo-organotrophically cultivated cells of the green alga *Trebouxia*. Different concentrations of sodium sulfite (5×10^{-5}–5×10^{-3} M) cause characteristic alterations in the adenine nucleotide content. The ATP content decreases and the AMP content increases. This effect of sulfite on ATP content is reversible. The energy charge quotient [ATP] + 0.5 [ADP]/[ATP] + [ADP] + [AMP] used as a parameter of the energy status of the cells is inversely related to the sulfite concentration. Therefore, it might be a valuable bio-indicator at a physiological level.

INTRODUCTION

SO_2 is one of the most frequently occurring air pollutants. The toxicity of SO_2 to plant vegetation has been reviewed by several authors [1–3]. If the concentration of SO_2 increases beyond a critical point, fundamental cellular processes are altered or disrupted.

SO_2 and the products of its reaction with water, especially sulfite, inhibit (among others) oxidative processes in plants. Inhibition of photophosphorylation in isolated chloroplasts [4] and of oxidative phosphorylation in isolated plant mitochondria [5] has been demonstrated. SO_2 reduces photosynthetic CO_2 fixation by inhibiting the Calvin cycle enzymes, e.g. ribulose bisphosphate carboxylase [6].

The physiological processes which have been investigated are very complex and are easily attacked by toxic SO_2 concentrations. A decrease in metabolic activities appears before occurrence of visible symptoms of disease (e.g. chlorosis or necrosis). Therefore, distinct biochemical reactions can be used as highly sensitive indicators of air pollution.

Atkinson [7] and Chapman and Atkinson [8] proposed that the energy status of a cell might be best expressed in terms of the so-called energy charge [ATP] + 0.5 [ADP]/[ATP] + [ADP] + [AMP]. This quotient is an important regulatory control element and may actually determine whether a cell is viable, because all of the essential reaction sequences in living cells are energy-dependent. The ATP–ADP–AMP system is stabilized by means of effective regulatory mechanisms, and it strongly resists deviations.

The present paper describes a transient change in the ATP and AMP contents of the green alga *Trebouxia*, phycobiont of numerous lichen species, after exposure to sulfite. Lichens (e.g. *Hypogymnia physodes*) are known to be extremely sensitive to sulfurous pollutants and have thus disappeared from the areas around urban regions [1].

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RESULTS AND DISCUSSION

Figure 1 shows the amounts of the adenine nucleotides AMP, ADP, and ATP/mg dry wt in the perchloric acid extract after 3 days' treatment of algal cells with different concentrations of sulfite. The intracellular ATP was dependent on sulfite concentration in the medium. It was 1.20 nmol/mg dry wt in cells grown without sulfite or in the presence of 5×10^{-5} M sulfite. Concentrations above 5×10^{-4} M sulfite lead to a significant decrease of ATP content (0.93 nmol/mg dry wt in cells grown 72 hr with 5×10^{-4} M sulfite, and 0.57 nmol with 5×10^{-3} M sulfite). The decrease in ATP content was accompanied by an increase in AMP content, while the ADP level remained unaltered (Fig. 1).

The influence of sulfite on the total adenylate content is dependent on time of incubation (Fig. 2). Within 168 hr, the content was reduced to 65% of the control value.

The energy charge quotient is maintained constant at a value of *ca* 0.55 ± 0.03 in *Trebouxia* cells under control conditions. Figure 3 shows the effect of sulfite on the energy charge as a function of the time of incubation and

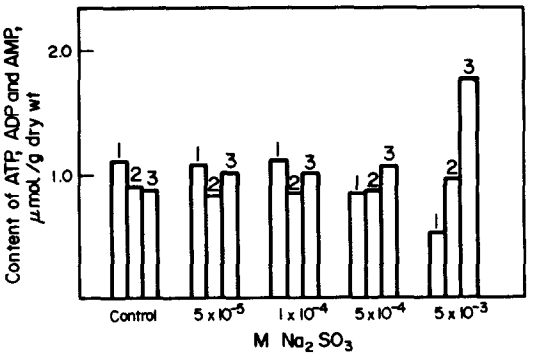

Fig. 1. Content of ATP, ADP and AMP in the perchloric acid extract after 72 hr incubation of *Trebouxia* cells with various concentrations of sodium sulfite. 1 — ATP; 2 — ADP; 3 — AMP.

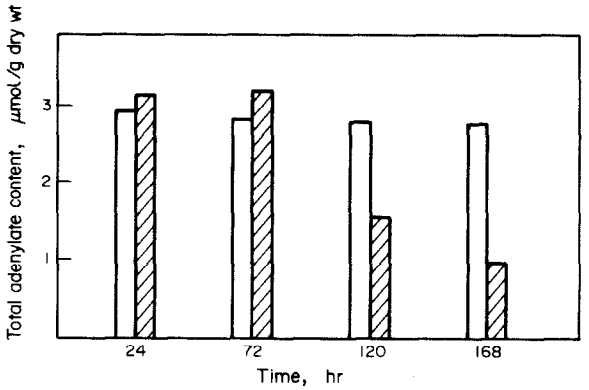

Fig. 2. Changes of total adenylate content [ATP + ADP + AMP] with time of incubation in cells treated with 5×10^{-3} M sodium sulfite. □—control cells; ▨—cells grown with 5×10^{-3} M Na_2SO_3.

of the concentration. Sulfite (5×10^{-3} M) causes a significant decrease in the energy charge (e.c.) already after the first 12 hr of exposure (from 0.55 to 0.41). A further decrease takes place between 24 and 36 hr (from 0.41 to 0.33). After 7 days treatment the e.c. was found to be 0.20 only. At a sulfite concentration of 5×10^{-4} M, e.c. was reduced from 0.55 to 0.45 within the first 24 hr. In cells grown with 5×10^{-5} M sulfite the e.c. quotient remained essentially unaltered in comparison with control (Fig. 3).

Concerning the effects of sulfite on micro-organisms, Schimz and Holzer [9] have demonstrated a rapid decrease in the ATP content after incubation of *Saccharomyces cerevisiae* with low concentrations of sulfite (1 mM). The depletion of ATP was reversible, depending on time of incubation and concentration of sulfite.

Field studies with *Pinus* hybrids demonstrated that ATP concentrations of needle cells declined as SO_2 concentration increased in the ambient air. An inverse linear relationship was found between ATP content and the measured SO_2 concentration [10].

To study the reversibility of the effect of sulfite on ATP content and e.c. quotient under conditions described in the Experimental in the alga *Trebouxia*, the cells were incubated 24, 48 and 72 hr with 5×10^{-3} M sodium sulfite. During this time the ATP content was lowered from 1.20 nmol/mg dry wt to 0.56 after 24 and 48 hr, and 0.3 nmol after 72 hr. An opposite relation was found for AMP content. The e.c. decreased remarkably during this treatment. The incubation was terminated by rapid sterile centrifugation, subsequent washing of the cells and resuspending in sulfite-free medium. After 24, 48 and 72 hr the contents of AMP, ADP and ATP were measured again. Figure 4 shows the time course of the regeneration of the e.c. value was not considered. At high sulfite adenine nucleotides and e.c. is possible. These results indicate that ATP can be resynthesized in *Trebouxia* cells if sulfite (5×10^{-3} M) is removed, also after long preincubation periods (3 days). No irreversible loss of ATP synthesis was observed.

The results indicate that different concentrations of sodium sulfite cause strong alterations of yield of ATP and on e.c. quotient in *Trebouxia*. In this case, the e.c. is more sensitive to sulfite (Fig. 3) than the total adenylate content (Fig. 2). In present studies the algae were exposed to sulfite for between 12 and 168 hr. Short time alteration of the e.c. value was not considered. At high sulfite concentrations the e.c. changes drastically to a level where its general regulation can be excluded.

EXPERIMENTAL

Organism and culture conditions. The green alga *Trebouxia* (Algal Collection Göttingen) was routinely grown photo-organotrophically in the medium according to ref. [11]. Composition: 980 ml Bold's Mineral Medium described in ref. [12]; 10 g casamino acids, 'vitamin free' (Difco Laboratories); 10 g glucose. Agar (2% w/v) was added if solid cultures were required.

Incubation. Cells of 1-month-old stock cultures were dropped into 100-ml-conical flasks (4 mg dry wt/ml medium) containing sterile Na_2SO_3 soln (final concn from 5×10^{-5} to 5×10^{-3} M), pH 5.6. Solns of Na_2SO_3 were prepared immediately before use. All incubations were done at 20 ± 1° under 360 lx illumination with shaking. After treatment for 12 to 168 hr cells were harvested by centrifugation, the dry wt was determined, aliquots (adequate to 100 mg dry wt algae) were centrifuged and washed once with NaPi buffer, 0.1 M, pH 5.6.

Extraction of samples. Samples were treated with 2 ml ice-cold 0.85 M $HClO_4$, 0.5 ml of a Tris–EDTA soln (5%; 72%; w/v) was added and extracted for 30 min at 0–2°. To get comparable

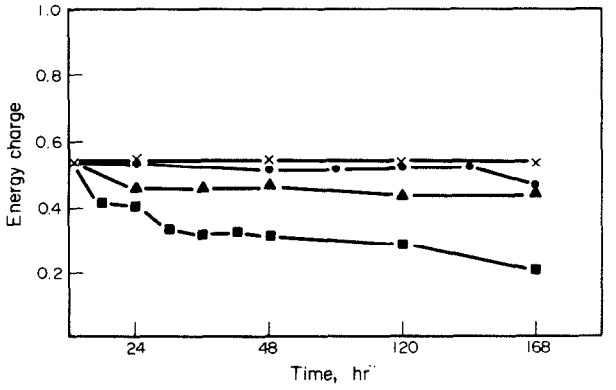

Fig. 3. Energy charge quotient of cells growing with different concentrations of sodium sulfite during 168 hr. ×—control; ●—5×10^{-5} M; ▲—5×10^{-4} M; ■—5×10^{-3} M Na_2SO_3.

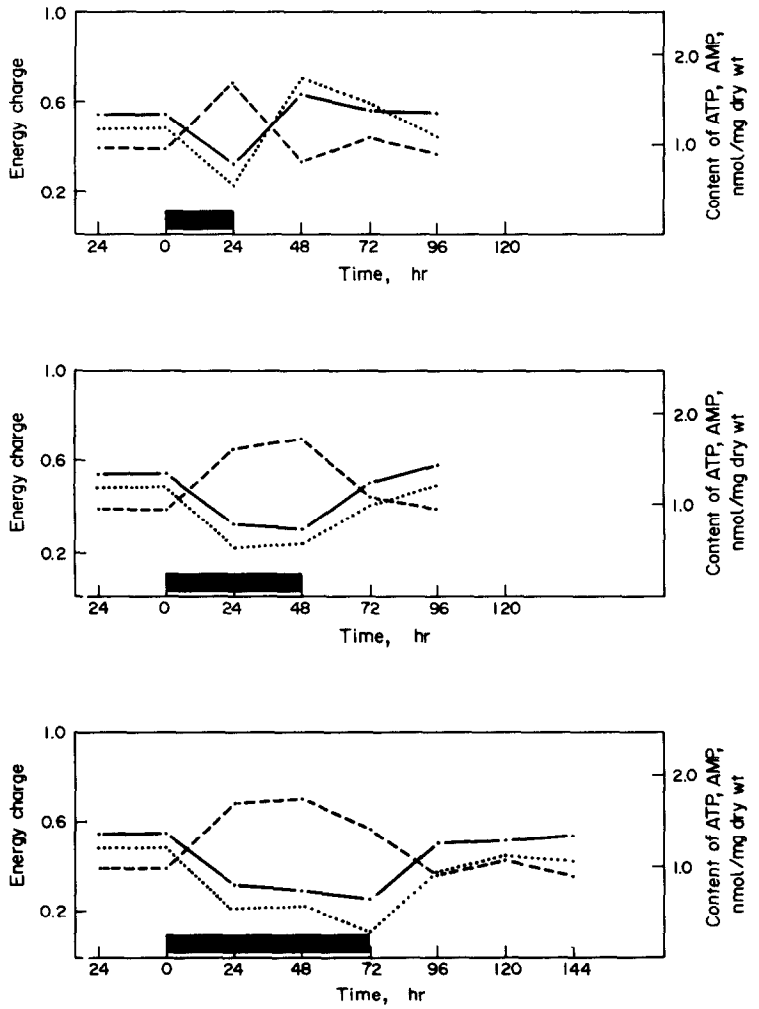

Fig. 4. Regeneration of energy charge after incubation of algal suspension for 24, 48 or 72 hr with 5×10^{-3} M sodium sulfite, pH 5.6, and subsequent culture in sulfite-free medium. ■—incubation periods; · — · —energy charge; · · ·—content of ATP; – – – –content of AMP.

results the cells were frozen twice in liquid air. After centrifugation for 10 min at 6000 g the supernatant was neutralized to pH 7.0–7.4 with 2 N $KHCO_3$ and used for adenine nucleotide determinations.

Determination of ATP. The assay mixture contained in a total vol of 2.41 ml: 1 ml sample; triethanolamine 250 mM, pH 7.6; $MgSO_4$ 2 mM; glycerate-3-phosphate 3 mM; NADH 0.2 mM.

The reaction was started by addition of 10 μl of enzyme soln containing glyceraldehyde-3-phosphate dehydrogenase (560 U/ml), phosphoglycerate kinase (450 U/ml), glycerol-1-phosphate dehydrogenase (80 U/ml), and triosephosphate isomerase (1000 U/ml) (Biochemica Test Combination, Boehringer).

Determination of ADP and AMP. The enzymatic assay system comprising lactate dehydrogenase (500 U/ml), pyruvate kinase (100 U/ml), and myokinase (720 U/ml) (Biochemica Test Combination, Boehringer) allows determination of ADP and AMP in a single procedure. The assay mixture contained in a total vol of 2.38 ml: 1 ml sample; triethanolamine–HCl 168 mM, pH 7.4; phosphoenolpyruvate 0.84 mM; KCl 110 mM; $MgSO_4$ 33.6 mM; NADH 6.21 mM; 30 μl enzyme soln (10 μl of each enzyme). The concns of adenine nucleotides were calculated from changes in the E at 366 nm (Photometer DB-G, Beckman) according to ref. [13]. All ATP, ADP and AMP values were expressed as nmol/mg dry wt. The H_2O content of cells (80.5 % and 0.37 ml of samples) was considered in calculations.

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