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BRIEF COMMUNICATION

A Nicotinamide Adenine Dinucleotide Phosphate Phosphatase from *Chlorella*

R. CHOPOWICK* and G. F. ISRAELSTAM

Department of Botany, University of Toronto, Scarborough College, West Hill, Ontario, Canada

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Abstract. A phosphatase enzyme hydrolysing $NADP^+$ and NADPH to NAD^+ and NADH was found to be present in extracts of *Chlorella pyrenoidosa*.

The conversion of $NADP^+$ to NAD^+ by phosphatase has been shown to occur in yeast (von EULER and ADLER 1938); potato (KORNBERG and PRICER 1950); pig kidney (SANADI 1952) and more recently by FORTI *et al.* (1962) in pea leaves. GREEN and ISRAELSTAM (1970) suggested that this enzyme may be present in *Chlorella* and that it was involved in the hydrolysis of NADPH to NADH as part of a series of conversions of the reduced and oxidized forms of those coenzymes during a 30 s period after illumination. This work therefore, was undertaken to show the presence of NADP-ase in *Chlorella* in order to provide some additional support for the suggested reaction as well as to show that this enzyme is present in organisms other than those already mentioned.

NADP, NADPH and alcohol dehydrogenase were obtained from Boehringer and Son (New York). Cells of *Chlorella pyrenoidosa* (CHICK) CAMB. strain 211/8a, original culture from the Indiana University collection (STARR 1964) were grown in Bristol's medium (BOLD 1942) at 25 °C in gas washing bottles under an 8 h dark/16 h light regime. The cells were harvested at the end of the exponential phase, centrifuged at 7 500 × g for 10 min and suspended in 0.03 M $NaOH$-K_2HPO_4 buffer pH 6.7. About 2 g (dry weight) of cells were then suspended in 50 ml 0.03 M phosphate buffer containing 0.4 M sucrose together with 0.5 g pre-chilled 50 mm glass beads in a thick walled bottle. The mixture was shaken in a Braun MSK mechanical cell homogenizer agitating at 2 000 cycles/min for 3—5 min to break the cells. The bottle was cooled by a slow continuous stream of compressed liquid CO_2, passed into the chamber housing the bottle. The mixture was then centrifuged at 200 × g for 2 min to remove the glass beads. The supernatant containing

* *Present address:* Seneca College, 1750 Finch Avenue E., Willowdale, Ontario, Canada.

broken cells was centrifuged at 4 500 × g for 2 min to remove cell debris and gross chloroplasts. The resultant supernatant was recentrifuged at 18 000 × g for 30 min and the pellet suspended in 10 ml 0.05 M phosphate buffer pH 7.2. Centrifugation was carried out at 1°C. Chilled 75% acetone was then used to precipitate the protein, which was resuspended in 5 mM phosphate buffer pH 7, centrifuged at 18 000 × g for 20 min and dialysed overnight against the phosphate buffer to remove residual acetone. The enzyme was precipitated with solid $(NH_4)_2SO_4$ at a final concentration of 50% with constant agitation in the cold for 15 min.

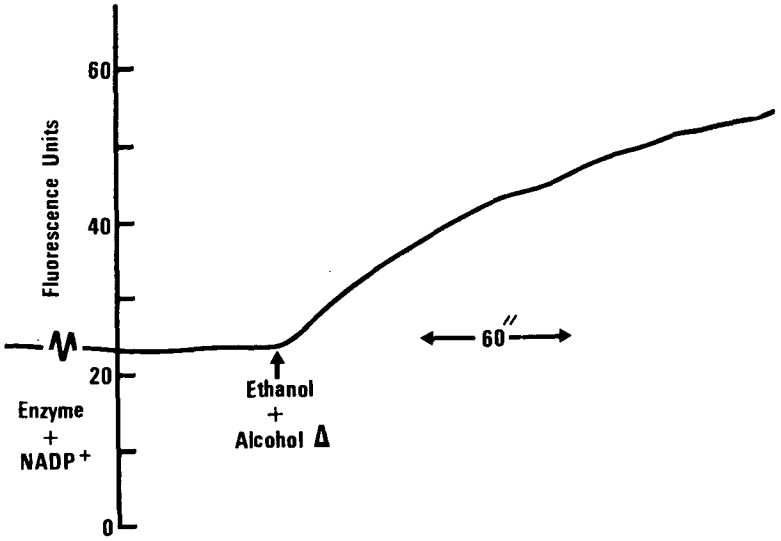

Fig. 1. Reduction of NAD+ formed from NADP+. See text for reaction medium. Time interval 60 s.

Enzyme analyses were carried out in 4 ml tubes in a Turner III fluorometer with a primary filter passing light at 360 nm and a secondary filter peaking at 460 nm. This instrument with its greater sensitivity to changes in pyridine nucleotide concentrations, compared to a spectrophotometer, was coupled to a Beckman 5" recorder set to provide continuous readings at 1" per min. 0.2 ml of the enzyme was mixed thoroughly with 0.1 mM NADP+ and 0.03 M phosphate buffer pH 6.7 to give a final volume of 3.5 ml and then incubated for 20 min at 24°C. To test for the presence of NAD+ 25 µl ethanol-semicarbazide and 25 µl of 0.3 mg ml^{-1} alcohol dehydrogenase was added at the end of the incubation period and the change in fluorescence recorded. Since the reduced form but not the oxidized form of the coenzyme fluoresces this reduction would appear as an upward deflection of the trace and was used as an indication that NADH was produced from NAD+.

As may be observed in the Fig. 1 the addition of ethanol-semicarbazide and alcohol dehydrogenase resulted in a marked increase in fluorescence, indicating the presence of NAD+ which must have been formed as a result of the action of a phosphatase on the NADP+. Similar results were obtained

if NADPH was used as substrate, the end product being NADH. The production of NADH was assayed with acetaldehyde in place of ethanol and on the addition of alcohol dehydrogenase a decrease in fluorescence was recorded. Attempts to reverse the reaction even with millimolar concentrations of NAD^+ and the addition of ATP were unsuccessful.

The presence of $NADP^+$ phosphatase in *Chlorella* suggests that the enzyme may occur in a fairly wide range of organisms. The fact that there is an enzyme, the activity of which results in the formation of NAD^+ and NADH from $NADP^+$ and NADPH respectively, supports the suggestion of GREEN and ISRAELSTAM (1970) that this interconversion probably does occur in *Chlorella*.

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