PRELIMINARY NOTES

BBA 61145

A single glyceraldehyde-3-phosphate dehydrogenase active with NAD and NADP in *Anabaena variabilis*

Glyceraldehyde-3-phosphate dehydrogenase is of especial interest because of its role in both the pentose phosphate cycle and the glycolytic pathway. The enzyme operative in the former is thought to use NADP as coenzyme (EC 1.2.1.13) whilst that of the latter uses NAD (EC 1.2.1.12); both enzymes have been reported in organisms carrying out green plant-type photosynthesis. The interaction of these two enzymic processes is of particular interest in procaryotic organisms, which lack intracellular functionally-specialised, membrane-bound organelles which could facilitate physical separation of metabolic pathways. The blue-green algae, like the bacteria, possess such a structure and this communication describes the purification from *Anabaena variabilis* of a glyceraldehyde-3-phosphate dehydrogenase active with either coenzyme.

A. variabilis was grown on a mineral salt medium and supplied with air:CO_2 (95:5, v/v) as previously described[1]. When larger quantities of microbial cells were required for enzyme purification 10-l batches were incubated at 32° in a round flask, illuminated by a circular, warm white strip light (60 W), and maintained in suspension by vigorous gassing through a sparger. A washed suspension of microorganisms was disrupted by ultrasonic treatment at 0° and a cell-free preparation obtained by centrifugation at 10 000 × *g* for 15 min at 0°.

In such an extract glyceraldehyde-3-phosphate dehydrogenase was present, and when measured near its optimum pH was nearly as active with NADP as coenzyme as it was with NAD. To find whether this was due to one enzyme protein, active with either coenzyme, or two separate enzymes each specific for one coenzyme, enzymic rates were measured in the presence of both coenzymes. The assay procedure was to follow the increase in $E^{1\,cm}_{340\,m\mu}$ due to coenzyme reduction under the conditions described by FULLER AND GIBBS[2]. At pH 10.0 an optimum, and approximately similar, rate was obtained with either coenzyme; at other pH values the rate obtained

TABLE I

GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE ACTIVITIES IN EXTRACTS OF *Anabaena variabilis*

The reaction mixture contains (μmoles); glycine–NaOH buffer, 100; sodium arsenate, 20; cysteine, 12; coenzyme, 0.6; glyceraldehyde-3-phosphate, 2.5; protein, 0.5 mg.

Extract	*pH*	*Rate (mμmoles/min per mg protein)*		
		NAD	*NADP*	*NAD + NADP*
Untreated	9.5	49	26	53
Untreated	10.0	63	53	63
Untreated	10.4	58	34	53
Extract heated at 62° for 3 min	10.0	29	29	—
Extract heated at 55° for 12 min	10.0	17	14	17

with NAD was rather greater than that with NADP. At each of three pH values there was no increase in enzyme rate when NAD and NADP were both present at saturating coenzyme concentrations (Table I). When the cell-free extract was heated at 55° for 12 min, or 62° for 3 min, the glyceraldehyde-3-phosphate dehydrogenase activity was reduced by over 50%. The activity with either coenzyme was equally affected, and as in the untreated extract, the rate obtained with both coenzymes present was not greater than that with NAD alone (Table I). These results suggest that the glyceraldehyde-3-phosphate dehydrogenase in this organism is capable of enzymic activity using either coenzyme. Purification of the enzyme was considered necessary to support this suggestion.

Cell-free extract of *A. variabilis* was treated with salmine sulphate (final conc. 0.28%) at 0°, and nucleic acids and chlorophyll containing fractions removed by centrifugation. The total protein in the supernatant was precipitated by 90% $(NH_4)_2SO_4$, centrifuged and taken up in small volume of buffer (Tris, pH 7.2, 50 mM) and loaded onto a Sephadex G-200 column (40 cm × 2,5 cm) arranged for upward flow. Elution with Tris buffer, pH 7.2, produced a peak of glyceraldehyde-3-phosphate dehydrogenase activity immediately following the void volume and ahead of the blue accessory pigment phycocyanin (Fig. 1). The enzymic activity obtained by gel

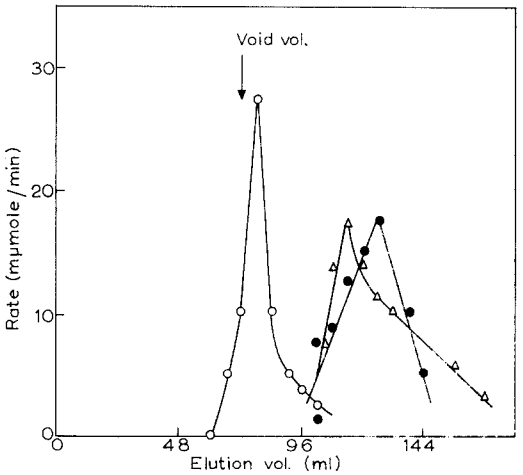

Fig. 1. Elution pattern of glyceraldehyde-3-phosphate dehydrogenase from *A. variabilis* and *Rhodomicrobium vannielii*. Proteins were eluted from the Sephadex G-200 column with Tris, pH 7.2, 50 mM. The blue accessory biliprotein was measured by $A_{620\ m\mu}$ and the enzyme assay was as described in Table I. ○, enzyme from *A. variabilis*; ●, enzyme from *Rhodomicrobium vannielii*; △, phycocyanin from *A. variabilis*.

filtration showed a purification of 100-fold, but, as before, had the same reaction rate with either coenzyme at pH 10.0. The mol. wt. of phycocyanin is 180 000 (ref. 3), so that of glyceraldehyde-3-phosphate dehydrogenase would be in excess of that figure. This is considerably greater than the molecular weight of the NAD specific glyceraldehyde-3-phosphate dehydrogenases from rabbit[4], yeast[5], or the photosynthetic bacterium *Chromatium*[6].

When the glyceraldehyde-3-phosphate dehydrogenase of the photosynthetic

bacterium, *Rhodomicrobium vannielii* was purified by the same procedure, it was eluted from the G-200 column, by a volume which indicated a mol. wt. of approx. 120 000 (Fig. 1). This purified enzyme was specific for NAD and the observed size is similar to that of the other NAD specific enzymes above.

The existence of an NAD and NADP dependant glyceraldehyde-3-phosphate dehydrogenase has not previously been reported, although FULLER AND HUDOCK[6] in examining the variation in levels of each enzyme in *Chlamydomonas reinhardii* noted that separation of the two activites had not been accomplished. The structural association of these two enzyme activities in *A. variabilis*, and the apparent possession of a common active site preventing the use of both coenzymes simultaneously, would offer a potential site of metabolic control.

We are indebted to the Science Research Council for support and W.H. acknowledges support of a Medical Research Council studentship.

Department of Biochemistry,
The University,
Liverpool (Great Britain)

W. HOOD
N. G. CARR

1 N. G. CARR AND M. H. HALLAWAY, *J. Gen. Microbiol.*, 39 (1965) 335.
2 R. C. FULLER AND M. GIBBS, *Plant Physiol.*, 34 (1959) 324.
3 E. SCOTT AND D. S. BERNS, *Biochemistry*, 4 (1965) 2597.
4 J. F. TAYLOR, *Federation Proc.*, 9 (1950) 237.
5 E. G. KREBS, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 1, Academic Press, New York, 1955, p. 407.
6 R. C. FULLER AND G. A. HUDOCK, in T. W. GOODWIN, *Biochemistry of Chloroplasts*, Vol. 2, Academic Press, New York, 1967, p. 181.

Received June 27th, 1967

Biochim. Biophys. Acta, 146 (1967) 309–311