by the hydrazone method gave values of 68 per cent for the *Anacystis* extract and 65 per cent for the crystalline aldolase of the expected triose from fructose diphosphate added by weight. The above data indicate a ratio of two triose per one fructose diphosphate split and support the assumption that the enzyme activity seen is that of aldolase.

All enzyme activity determinations were made with freshly made preparations. The aldolase activities from all three blue-green algae were very reproducible, with no instance of an inactive preparation. Stability of the crude enzyme from *Anacystis* was not examined except for an overnight storage of 4° C, when the loss of activity was less than 5 per cent.

There is nothing in the observations made during this work to suggest why both Richter and Fewson failed to find aldolase activity with *Anacystis*, or with other blue-green algae. The data presented here support the thesis that aldolase is present in blue-green algae and in sufficient amount to allow its participation in photosynthesis and also possibly in a glycolytic pathway of carbohydrate metabolism. However, the facile demonstration in blue-green algae of certain of the enzymes of the hexose monophosphate pathway (refs. 1 and 2, and unpublished observations) suggests the operation of this pathway also and no decision as to the relative importance of the two pathways can be made at this time.

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¹ Richter, G., *Naturwissenschaften*, **46**, 404 (1959).

² Fewson, C. A., Al-Hafidh, M., and Gibbs, M., *Plant Physiol.*, **37**, 402 (1962).

³ Van Baalen, C., and Marler, J. E., *J. Gen. Microbiol.*, **32**, 457 (1963).

⁴ Kratz, W. A., and Myers, J., *Amer. J. Botany*, **42**, 282 (1955).

⁵ Provasoli, L., McLaughlin, J. J. A., and Droop, M. R., *Arch. Mikrobiol.*, **25**, 292 (1957).

⁶ Van Baalen, C., *Botanica Marina*, **4**, 129 (1962).

⁷ Beck, W. S., in *Methods in Enzymology*, **3**, 291 (Academic Press, New York, 1957).

⁸ Peanasky, R. J., and Lardy, H. A., *J. Biol. Chem.*, **233**, 365 (1958).

⁹ Tausky, H. H., and Shorr, E., *J. Biol. Chem.*, **202**, 675 (1953).

¹⁰ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).

¹¹ Rutter, W. J., in *The Enzymes*, **5**, 341 (Academic Press, New York, 1961).

¹² Bard, R. C., and Gunsalus, I. C., *J. Bacteriol.*, **59**, 387 (1950).

Aldolase in *Anacystis nidulans* and *Rhodospseudomonas spheroides*

EXTRACTS of some Cyanophyceae¹⁻³, and *Rhodospseudomonas spheroides*^{2,4} appear to lack significant amounts of fructose-1,6-diphosphate aldolase. These findings were unexpected, at least with respect to the blue-green organisms, for two groups of authors^{5,6} found that the distribution of ¹⁴C in the products formed during photosynthesis was similar to that obtained with *Chlorella*, an alga which contains aldolase. This manuscript is concerned with the partial characterization of aldolase in *Anacystis nidulans* and *R. spheroides*. A similar enzyme was also detected in extracts of *Tolypothrix tenuis* and *Anabaenopsis* sp.

A. nidulans was grown autotrophically in medium 'C' (Kratz and Myers⁷) and *R. spheroides* was cultured as described by Szymona and Doudoroff⁸. The cells were suspended at a concentration of 0.5 g wet weight per 10 ml. of 0.1 M tris, pH 7.5, and were disrupted in a Mickel apparatus at 4° for 10 min under an atmosphere of air or N₂. Aldolase was assayed by the hydrazone procedure⁹ in an environment similar to the one in which the cells were broken.

The basic activity was markedly stimulated by N₂, cysteine or Fe⁺⁺ when added as ferrous sulphate or as ferrous ammonium sulphate (Table 1). Inhibition by ferric ammonium sulphate could be relieved by cysteine.

Table 1. ALDOLASE ASSAYS OF *R. spheroides* AND *A. nidulans*

Organism	Growth conditions	μmoles FDP split/mg protein/h							
		Control	Cysteine	Fe ⁺⁺	Cysteine and Fe ⁺⁺				
<i>R. spheroides</i>	Dark, 0.25% glucose	Air N ₂	Air N ₂	Air N ₂	Air N ₂	Air N ₂	Air N ₂	Air N ₂	Air N ₂
	Light, 0.25% glucose	0.2	0	0.5	1.3	0.1	0.7	0.4	0.5
<i>A. nidulans</i>	Light, 1% CO ₂	1.0	0.8	3.0	8.8	3.6	4.7	5.0	9.9
	Light, 1% CO ₂	0.5	0.3	3.6	17.0	3.4	11.4	5.5	13.0

In a final volume of 2.5 ml. the complete reaction mixture consisted of: 100 μmoles tris buffer, 140 μmoles hydrazine sulphate, 20 μmoles cysteine, 20 μmoles ferrous ammonium sulphate; extract containing 0.2 to 0.4 mg protein, and 5 μmoles fructose-1,6-diphosphate. The final pH was 7.5. After 20 min at 38° the reaction was stopped by addition of cold trichloroacetic acid. Further details are given in ref. 8.

BAL was able to replace cysteine while reduced glutathione was inactive. Frozen extracts prepared under air or N₂ and kept in the presence of cysteine lost roughly half their activity in 24 h. Rapid removal of cell debris after rupture of the cells was essential for obtaining high activity; this may account for the failure of others to detect the enzyme. *p*-Chloromercuribenzoate (10⁻⁴ M), *o*-phenanthroline (10⁻⁴ M), and α - α -dipyridyl (10⁻⁴ M) were effective inhibitors. Iodoacetamide (10⁻⁴ M) was ineffective.

The maximal activities obtained with *A. nidulans* and *R. spheroides* were more or less equal to those reported for higher plants³ and for photosynthetic bacteria⁹. These findings indicate that aldolase of the Cyanophyceae and photosynthetic bacteria, in contrast to that of higher plants and green algae³, is a metallo-sulphydryl enzyme, or type II according to the classification of Rutter¹⁰.

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¹ Richter, G., *Naturwiss.*, **46**, 604 (1959).

² Richter, G., *Biochim. Biophys. Acta*, **45**, 606 (1961).

³ Fewson, C. A., Al-Hafidh, M., and Gibbs, M., *Plant Physiol.*, **37**, 402 (1962).

⁴ Szymona, M., and Doudoroff, M., *J. Gen. Microbiol.*, **22**, 167 (1960).

⁵ Kandler, O., *Naturwiss.*, **48**, 604 (1961).

⁶ Kindel, P. K., and Gibbs, M., *Nature*, **200**, 260 (1963).

⁷ Kratz, W. Z., and Myers, J., *Amer. J. Bot.*, **42**, 282 (1954).

⁸ Sibley, J. A., and Lehninger, A. L., *J. Biol. Chem.*, **177**, 859 (1949).

⁹ Fuller, R. C., Smillie, R. M., Sisler, E. C., and Kornberg, H. L., *J. Biol. Chem.*, **236**, 2140 (1961).

¹⁰ Rutter, W. J., in *The Enzymes*, edit. by Boyer, P. D., Lardy, H. A., and Myrback, K., **5**, 341 (Academic Press, New York, 1961).

Lack of Uptake and Oxidation of Chylomicron Triglyceride to Carbon Dioxide and Ketone Bodies by the Perfused Rat Liver

FATTY acids concerned with supplying the caloric requirements of the body are transported in the blood primarily as free fatty acids (FFA) or as triglyceride fatty acids (TGFA). The liver is thought to play a central part in the metabolism of both chemical forms¹. Investigations have shown that injected FFA labelled with carbon-14 are taken up by the liver², where they may be catabolized by β -oxidation to carbon dioxide or ketone bodies³, or be synthesized into TGFA, which give rise to the very-low-density ($d < 1.006$) lipoproteins of the plasma². Chylomicron-TGFA labelled with carbon-14 are also reported to be taken up by the liver⁴ and to be oxidized to carbon dioxide or ketone bodies in certain experimental conditions⁵. However, previous experiments *in vivo* have demonstrated that the magnitude of ketogenesis in fat-fed rats is not related to the extent of the influx of chylomicrons⁶ but rather to the level of circulating FFA⁶. In perfused livers from fasted rats, Morris³ found that 75 per cent of chylomicron-TGFA were removed