Phytochemistry, 1975, Vol. 14, pp. 1505–1506. Pergamon Press. Printed in England.

OPTIMUM CONCENTRATION AND pH OF PHOSPHATE BUFFER FOR ASSAY OF CYTOCHROME c OXIDASE IN INTACT PLANT MITOCHONDRIA

DIPANKAR SEN

"Ruchira", Purva-Palli, Santiniketan, West Bengal, India

(*Received* 22 *July* 1974)

Key Word Index—*Triticum*; *Hordeum*; *Zea*; *Phaseolus*; mitochondria; cytochrome c oxidase; phosphate buffer; effect on assay.

Abstract—For measurement of cytochrome c oxidase activity in intact plant mitochondria the optimum concentration of K–Pi buffer and pH in the reaction was found to be 75 mM and 7·4 respectively. The suitable concentration of K–Pi buffer for suspending and storing mitochondria, however, was found to be 20 mM or lower. These requirements applied equally well for mitochondria from wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), maize (*Zea mays* L.), and snap bean (*Phaseolus vulgaris* L.).

Cytochrome c oxidase is used as a marker enzyme for demonstrating the presence of mitochondria. Activities of this enzyme in extracts of plants from parental lines and their hybrids have been measured as an indicator of mitochondrial heterosis and complementation [1–4] as well as for identifying polymorphic plant mitochondria obtained by sucrose density gradient centrifugation [5]. Several methods have been used for assay of this enzyme [6–8]; the reaction has been run in a 20 mM [1,5], 30 mM [2] or 100 mM [3,4] K–Pi buffer at pH 7·2–7·4. In one report on plant cytochrome c oxidase, both in intact mitochondria and in purified form, a 0·1 mM K Pi buffer was used [9]. Yonetani found maximum activity of purified cytochrome c oxidase from beef heart at a pH 6·05 in 50 mM K Pi buffer [7]. This pH and concentration of K Pi buffer differed from the reports using plant mitochondria mentioned above.

In the present study, the optimum concentration and pH of the K Pi buffer in the medium for assaying cytochrome c oxidase in intact plant mitochondria has been determined. It was found that 75 mM K Pi buffer yielded the highest sp.act. for the enzyme. This was true for all the plant species used—namely wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), maize (*Zea mays* L.), and snap beans (*Phaseolus vulgaris* L.) (Fig. 1). Results obtained by varying the pH of the 75 mM K Pi buffer showed that pH 7·4 gave the highest activity for all species

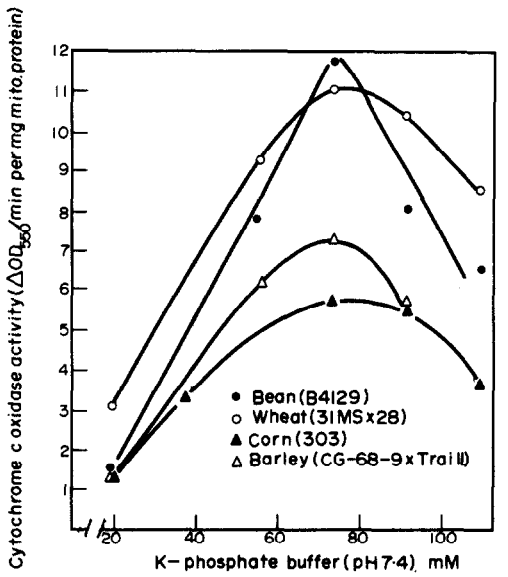

Fig. 1

studied with a marked fall-off at values greater or lesser than this.

It should be mentioned however, that suspending or storing mitochondria in 75 mM K Pi buffer yielded much lower values for the activity of the enzyme than when 10 mM or 20 mM K Pi buffers were used for the same purpose. The two latter concentrations yielded the same value. This suggests that the mitochondria should be suspended in a hypotonic buffer to enhance their swelling and subsequent ease of penetration by the substrate.

EXPERIMENTAL

Mitochondria were isolated from $2\frac{1}{2}$-day-old seedlings of wheat, barley, maize (entire shoots in all cases) and snap bean (the hook region). The tissues were ground lightly for 30–60 sec in a mortar using 10 ml/g of grinding buffer (0·5 M sucrose, 0·001 M EDTA, 0·067 M K Pi buffer with a final pH of 7·4). The homogenate was filtered through fine mesh nylon fabric and the filtrate was centrifuged at 2000 *g* for 2·5 min. The pellet containing cell debris and larger organelles was discarded and the supernatant was centrifuged at 40000 g for 2 min. The mitochondrial pellet thus obtained was suspended in 20 mM K Pi buffer (pH 7·4). All operations were carried out in a cold room (0–4°) using prechilled equipment and solutions. Centrifugations were done at 2° with 10 ml fluid per tube (Sorvall SS34). Mitochondria so prepared had ADP–O and respiratory control ratios indicating good coupling and thereby showing that the mitochondria were intact. Cytochrome c oxidase activities were measured by the method of Cooperstein and Lazarow [8] and mitochondrial protein was determined by the method of Lowry *et al.* [10].

This work was done as a part of research for a Ph.D. degree at Biology Department, Texas A and M University, College Station, Texas.

REFERENCES

1. McDaniel, R. G. (1967) Ph.D. Dissertation, West Virginia University.
2. Srivastava, H. K. (1970) Ph.D. Dissertation, Texas A and M University.
3. Sarkissian, I. V. and Srivastava, H. K. (1971) *Biochem. Genet.* **5,** 57.
4. Srivastava, H. K. and Sarkissian, I. V. (1972) *Biochem. Genet.* **7,** 347.
5. Sarkissian, I. V. and McDaniel, R. G. (1967) *Proc. Nat. Acad. Sci. U.S.* **57,** 1262.
6. Smith, L. (1955) in *Methods in Enzymology* (Colowick, S. P. and Kaplan, N. O., eds) Vol. 2. Academic Press, New York.
7. Yonetani, T. (1961) *J. Biol. Chem.* **236,** 1680.
8. Cooperstein, S. J. and Lazarow, A. (1950) *J. Biol. Chem.* **189,** 665.
9. Srivastava, H. K. and Sarkissian, I. V. (1971) *Phytochemistry* **10,** 977.
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265.