

Electrophoretic Behaviour of Pea Formic Dehydrogenase

MARTTI KOIVUSALO and JAAKKO PISPA

Department of Medical Chemistry, University of Helsinki, Finland

Zone electrophoresis of partially purified formic dehydrogenase from green peas was carried out in polyvinyl chloride resin blocks.

The enzyme activity moved as a single symmetrical peak in all the preparations and was located between the two principal protein peaks, which presumably were vicilin and legumin. When normal human serum was run in the same block with formic dehydrogenase, the enzyme activity was found to have approximately the same mobility as the β -globulins.

A plant formic dehydrogenase was first discovered by Thunberg¹⁻³ in bean seeds. Pea seeds contain the same enzyme, which differs from the bacterial formic dehydrogenase requiring diphosphopyridine nucleotide as coenzyme⁴⁻⁷. The enzyme has been partially purified from peas and beans but has resisted many efforts for further purification⁸⁻¹⁰. During attempts in this laboratory to develop a reliable and sensitive enzymic micromethod for the determination of formic acid in blood and other biological materials, the pea formic dehydrogenase was taken under further study. In the present paper the results of studies on the electrophoretic behaviour of green pea formic dehydrogenase during various stages of purification are reported. Both the enzymic activity and the protein content of the electrophoretic fractions of the partially purified preparations were determined.

METHODS

The source of the enzyme preparations was green pea seeds (*Pisum sativum*, var. *Celvedon*).

The purification of the formic dehydrogenase was made principally by the methods of Nason and Little¹⁰ and Davison⁹ with only minor modifications. The purification steps mentioned in the present paper refer to the steps of Nason and Little¹⁰.

Diphosphopyridine nucleotide was purchased from Boehringer und Söhne (DPN, reinst). Calcium phosphate gel and alumina C_γ gel were prepared after instructions given by Colowick¹¹.

The preparative zone electrophoresis* was carried out using a rectangular perspex trough packed with polyvinyl chloride resin, Geon 426 (G. F. Goodrich Co.) moistened

* We are grateful to Dr. Rainer Pesola from this Institute for introducing this technique to us.

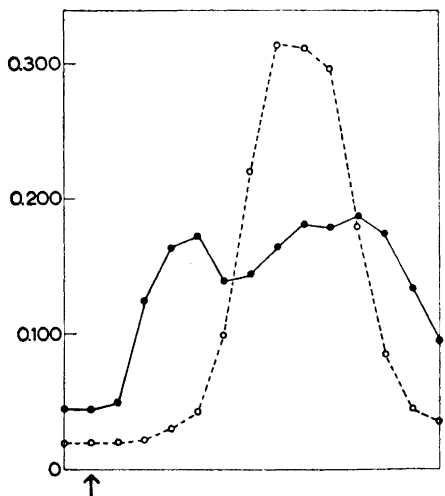


Fig. 1. Electropherogram of pea formic dehydrogenase preparation. Step 7. ○—○ enzyme activity, ●—● protein. Fractions of 0.8 cm. Place of application marked with arrow.

with barbital buffer, pH 8.6, ionic strength 0.05. To prevent evaporation melted paraffin was poured on the block and allowed to solidify. The substance under analysis was applied to the block through a narrow cut in the paraffin cover. The contact to the buffer vessels was effected from the ends of the block with strips of filter paper. During the run the block was in a vertical position in a perspex chamber¹².

The time of electrophoresis ranged from 48 to 72 h; a voltage of 400 V and a current of 10 mA were used per block. After the run the resin block was cut in 0.4 or 0.8 cm fractions, and each fraction was divided into two parts, from which the enzyme activity and the protein content, respectively, were determined.

The protein content of the fractions was determined by the method of Kunkel and Tiselius¹³ after elution by shaking for 30 min with 3 ml of 0.1 N NaOH.

The other part of each fraction was eluted with 3 ml of 0.5 M potassium phosphate buffer pH 6.5 and the formic dehydrogenase activity of the eluates was assayed in the following system: 1 ml of eluate, 1 ml of 0.5 M potassium phosphate buffer, pH 6.5, and 1 ml of assay mixture. The assay mixture consisted of 30 ml of 0.5 sodium formate, neutralised with hydrochloric acid, 10 ml of diphosphopyridine nucleotide solution (2 mg per ml) and 0.5 M potassium phosphate buffer pH 6.5 and 100 ml. The assay was carried out in test tubes which were held at room temperature for 2 h, after which the optical densities were read with a Beckman DU spectrophotometer at 340 m μ in 1 cm silica cuvettes.

Paper electrophoresis was run in the conventional apparatus using Whatman No. 1 filter paper strips. The time of the run was 6 h using a voltage of 500 V. Barbital buffer pH 8.6 and ionic strength 0.05 was used. After the run the proteins were stained with Lissamin Green and the papers were cut in fragments of 3 mm, which were eluted with 4 ml of 0.1 N NaOH each. The optical densities of the eluates were determined with Beckman B spectrophotometer at 635 m μ .

The free electrophoresis was carried out in the conventional Tiselius apparatus at pH 8.6.

RESULTS AND DISCUSSION

Typical electrophoresis curves obtained are presented in Figs. 1 and 2.

The protein curve did not change much during the purification of the enzyme. There were two main peaks in all the curves obtained in various

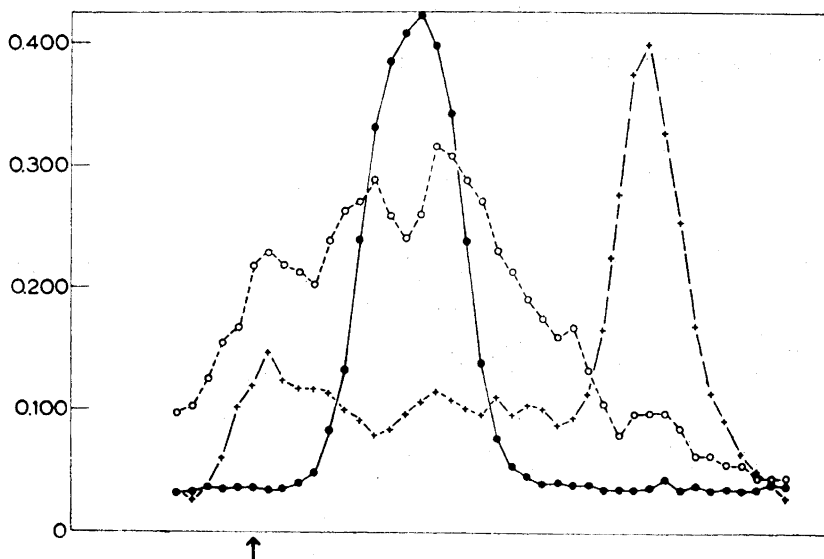


Fig. 2. Electropherograms of pea formic dehydrogenase and human serum run in the same block. +----+ serum protein. O----O enzyme protein, ●----● enzyme activity. Step 7. Place of application marked with arrow.

stages of purification. These peaks are most probably due to the two pea globulins, legumin and vicilin. Legumin and vicilin are two proteins differing in sulphur content, amino acid composition and molecular weight as determined by ultracentrifugation, and probably function principally as reserve proteins¹⁴⁻¹⁹.

With free Tiselius electrophoresis and paper electrophoresis similar protein curves with two peaks were also obtained.

The enzyme activity moved as a single symmetrical peak in all the electrophoretic runs made. It was always localised between the two protein peaks, a little nearer to the faster moving one. No clear peak was found in the protein curves to correspond to the enzyme activity even at the later stages of purification. However, all the preparations used were still rather crude.

To obtain some information of the relative electrophoretic mobility of formic dehydrogenase, normal human serum was run in the same block. When the curves obtained (Fig. 2) are compared, it is seen that the pea formic dehydrogenase activity has about the same mobility as the β -globulins.

Further purification was attempted with a technique in which the active fractions from several electrophoretic runs were pooled and lyophilised and submitted once again to electrophoresis. No increase in the purification was achieved, however, due to partial inactivation of the enzyme during lyophilisation.

REFERENCES

1. Thunberg, T. *Arch.intern.physiol.* **18** (1922) 601.
2. Thunberg, T. *Biochem.Z.* **206** (1929) 109.
3. Thunberg, T. *Skand.Arch.Physiol.* **74** (1936) 16.
4. Fodor, A. and Frankenthal, L. *Fermentforschung* **15** (1936) 44.
5. Lichtenstein, N. *Fermentforschung* **15** (1936) 44.
6. Andersson, B. *Z.physiol.Chem. Hoppe-Seyler.* **225** (1934) 57.
7. Adler, E. and Sreenivasaya, M. *Z.physiol.Chem.Hoppe-Seyler.* **249** (1937) 24.
8. Mathews, M. B. and Vennesland, B. *J.Biol.Chem.* **186** (1950) 667.
9. Davison, D. C. *Biochem.J.* **49** (1951) 520.
10. Nason, A. and Little, H. N. in Colowick, S. P. and Kaplan, N. O. *Methods in Enzymology*, Academic Press, New York, 1955, Vol. I, p. 536.
11. Colowick, S. P. In Colowick, S. P. and Kaplan, N. O. *Methods in Enzymology*, Academic Press New York, 1955, Vol. I, p. 90.
12. Nikkilä, E., Haahti, E. and Pesola, R. *Acta Chem.Scand.* **7** (1953) 1222.
13. Kunkel, H. G. and Tiselius, A. *J.Cen.Physiol.* **35** (1951) 89.
14. Osborne, T. B. *J.Am.Chem.Soc.* **20** (1898) 348.
15. Osborne, T. B. and Cambell, G. F. *J.Am.Chem.Soc.* **20** (1898) 410.
16. Danielsson, C-E. *Biochem. J.* **44** (1949) 387.
17. Danielsson, C-E. *Acta Chem.Scand.* **3** (1949) 41.
18. Danielsson, C-E. *Acta Chem.Scand.* **4** (1950) 762.
19. Danielsson, C-E. and Lis, H. *Acta Chem.Scand.* **6** (1952) 139.

Received August 28, 1958.