PURIFICATION OF 15-HYDROXY PROSTAGLANDIN DEHYDROGENASE FROM BOVINE LUNG

by

Sheikh A. Saeed and Ashim C. Roy
Research Division, Miles Laboratories Ltd.,
Stoke Poges, Slough, Buckinghamshire

Received February 28, 1972

SUMMARY

15-Hydroxy prostaglandin dehydrogenase has been isolated in a highly purified state from bovine lung by a simple 3-step procedure. Spectrophotometric studies have shown that this enzyme catalyzes the conversion of PGE_2(11α,15(S)-dihydroxy-9-ketoprost-5,13-dienoic acid) into 15-keto-PGE_2(11α-hydroxy19,15-diketoprost-5,13-dienoic acid).

INTRODUCTION

Prostaglandin dehydrogenase or NAD^+-15-hydroxy prostanoate oxidoreductase (EC 1.1.1) has been shown to catalyze specifically the oxidation of the 15(S)-hydroxy group of all known natural prostaglandins except the B and 19-hydroxylated types (Nakano, J. et al[1]). That this reaction is of considerable importance for the biological inactivation of prostaglandins has been shown by several in vivo studies on the metabolism of prostaglandins in lung tissue (Anggard, E. and Samuelsson, B.[2]; Anggard, E. et al.[3];

Anggard, E. and Samuelsson, B.[4]; and Anggard, E. and Samuelsson, B.[5]).

Prostaglandin dehydrogenase has been partially purified from swine lung (Anggard, E. and Samuelsson, B.[6]), but the method developed is tedious and results in an enzyme preparation of a very low specific activity. It is the purpose of this report to describe a simple and efficient method for the rapid purification of 15-hydroxy prostaglandin dehydrogenase from bovine lung. Particular emphasis has been placed on the experimental details of isolation because previous purifications have related to swine lung (Anggard, E. and Samuelsson, B.[5]).

MATERIALS AND METHODS

Bovine lungs were obtained immediately after slaughter and used either fresh or frozen; frozen lungs were stored for no longer than two weeks before use.

β-NAD^+.$3H_2O$ (yeast grade III) was obtained from Sigma Chemical Co., Ammonium Sulphate (ARISTAR) from British Drug Houses and Acetone (PRONALYS, Analytical Grade) from May and Baker Ltd., England. All other chemicals used were of analytical grade.

Buffer solutions:

A: 0.05M sodium phosphate, pH 7.4 also containing 1mM EDTA, 1mM β-mercaptoethanol and 1.4μM NAD.

B: Sodium pyrophosphate-semicarbazide buffer

(pH 8.7). This buffer contained 0.074M $Na_2P_2O_7$, 0.075M semicarbazide and 0.022M glycine. The final pH was adjusted to 8.7 with 2N NaOH.

Assay method of prostaglandin dehydrogenase: The enzyme activity was determined by the method of Anggard and Samuelsson[6] with slight modifications.

The standard reaction mixture contained 1.4μM (500μl) NAD, and 100μl of enzyme solution in a total volume of 2.9ml of buffer B. The reaction was started by the addition of 0.34μM (in 100μl of ethanol) PGE_2. The blank contained, except prostaglandin substrate, 100μl ethanol and all other reagents. Enzyme if necessary was diluted in buffer B. Protein was estimated by ultra violet absorption at 280mμ or by a micro Kjeldahl method for nitrogen determination.

RESULTS

The procedure was based on ammonium-sulphate fractionation of the bovine lung extract followed by precipitation with acetone. Interfering foreign matter precluded the spectrophotometric assay of prostaglandin dehydrogenase in the crude lung extract. The results of the later purification steps are summarized in Table 1. All steps were performed at 0-4°C and centrifugation at 3,5000 x g for 45 minutes, unless otherwise specified.

Step 1. Crude Extraction: About 1kg of bovine lungs were freed from fat, cut into smaller pieces and then homogenized in

Table 1. Purification of bovine lung 15-hydroxy prostaglandin dehydrogenase

	Specific activity (units/mg protein)	Total activity (units)	Yield (% age)
Ammonium sulphate fractionation	7	55,000	100
Acetone precipitation	70	30,500	55

These figures refer to purification of material first fractionated by ammonium sulphate precipitation.

---

a Waring Blendor for 2-3 minutes with one litre of standard buffer A. The homogenate was blended with another portion (one litre) of buffer A for an additional 10 minutes. The resulting suspension was clarified by first straining through cheese cloth and then by centrifugation. A red supernatant (crude extract) was obtained.

Step 2. Ammonium sulphate fractionation: To the supernatant

fluid obtained from the previous step solid ammonium sulphate was added in small portions each time, to 25% saturation. The precipitate was removed by centrifugation and discarded. Solid ammonium sulphate was then added to the supernatant to give a 65% saturation. Stirring was continued all the time and for 1 hour after the last addition of the salt. After standing overnight at 4°C the precipitate was centrifuged as before and dissolved in standard buffer A. The dissolved precipitate was then dialyzed exhaustively against the same buffer followed by a centrifugation at 35,000 x g to remove insoluble particulate material.

Step 3. Acetone Precipitation: All additions of acetone were made with the enzyme solution maintained between -5 and 0°C. The supernatant obtained from the previous step was diluted to a protein concentration of 10-15 mg/ml. Acetone precooled to -15°C was added slowly and dropwise with efficient stirring to the diluted enzyme solution until an acetone to buffer ratio of 1:3 (v/v) was reached. After standing at 0°C for 10-15 minutes the resulting inactive precipitate was removed by centrifugation and discarded. Acetone (-15°C) was again added dropwise to the supernatant until a final acetone to buffer ratio of 1:1 (v/v) was obtained. The suspension was allowed to remain at 0°C for 10 minutes and then centrifuged and the supernatant solution discarded. The precipitate was dissolved in a small volume of standard buffer A and dialyzed exhaustively against the same buffer. The enzyme was then stored frozen at -20°C.

DISCUSSION

In the present study bovine lung 15-hydroxy prostaglandin

dehydrogenase has been purified to a maximum specific activity of 70 in buffer B. The method of isolation is simple and the enzyme stability to acetone fractionation has facilitated further its purification. Anggard and Samuelsson[6] first described this enzyme from swine lung. Several studies since have implicated some potential uses of this enzyme in the study of naturally occurring prostaglandins, prostaglandin analogues and their metabolites, (Nakano, J., Anggard, E. and Samuelsson, B.[1]; Vonkeman, H., Nugteren, D.H. and VanDorp, D.A.[8]).

More recently another important aspect of the present work has originated from the work of Vane[9] on prostaglandin synthesis. Vane[9] using guinea pig lung homogenate has shown that aspirin-like drugs inhibit the synthesis of prostaglandins from arachidonic acid. In view of this, and the presence of both prostaglandin synthetase and 15-hydroxy PGDH in guinea pig lung, the interaction of the latter enzyme with aspirin-like drugs should be examined. Further work on this aspect is in progress.

ACKNOWLEDGEMENTS

We thank Dr. B.T. Warren for helpful discussions and Mrs. S. McDowell and Mr. N. Butt for technical assistance. We also thank Dr. J.E. Pike of the Upjohn Co., Kalamazoo for a gift of prostaglandin E_2.

1. Nakano, J., Anggard, E, and Samuelsson, B., Eur.J.Biochem., 11, 386 (1969).

2. Anggard, E., and Samuelsson, B., J.Biol.Chem, 239, 4097 (1964).

3. Anggard, E., Green, K., and Samuelsson, B., J.Biol.Chem. 240, 1932 (1965).

4. Anggard, E., and Samuelsson, B., Biochemistry, 4, 1864 (1965).

5. Anggard, E., and Samuelsson, B., Arkiv.Kemi, 25, 293 (1966).

6. Anggard, E., and Samuelsson, B., Methods Enzymol., 14, 215 (1969).

7. Anggard, E., Ann.N.Y. Acad.Science, 180, 200 (1970).

8. Vonkeman, H., Nugteren, D.H. and VanDorp, D.A., Biochem.Biophys.Acta., 187, 581 (1969).

9. Vane, J.R., Nature New Biology, 231, 232 (1971).