Differences between 15-Hydroxyprostaglandin Dehydrogenase from Pig Placenta and Human Placenta

NICOLA BRADBEAR and JONATHAN JEFFERY

Department of Biochemistry, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, Scotland, U.K.

We have applied methods to pig placentae that allow the preparation of 15-hydroxyprostaglandin dehydrogenase (EC 1.1.1.141) from human placentae (Braithwaite & Jarabak, 1975; Schlegel & Greep, 1975). The sows (Sus scrofa) were Large White inseminated with Landrace semen (two animals), or Landrace × Large White F₁ hybrids inseminated with Landrace semen (one animal). In the former two animals, labour was spontaneous at term, and in the latter animal labour occurred at term after intramuscular injection the day before term of 175 μg of prostaglandin F₂α. In all three animals, normal litters (9–13 piglets) were delivered. The placentae were obtained at the moment of delivery from the sow and were immediately wrapped in aluminium foil and covered with chipped ice for transport to the laboratory, where they were worked up immediately. As far as possible, the placental (i.e. chorionic) tissue was dissected free from the umbilical cords, associated large blood vessels, and amniotic membranes, and the following steps were carried out at 0–5°C. Placental tissue was homogenized in buffer (10 mM potassium phosphate, pH 7.4, 1 mM-disodium EDTA, 1 mM-dithiothreitol and 20% v/v, glycerol) and the 77 800 g supernatant was either treated with (NH₄)₂SO₄ to precipitate the enzyme, or treated with cold 1 M-acetic acid to precipitate the enzyme at pH 5.2, or treated with glycerol to give 50% v/v glycerol and stored at -20°C. Prostaglandin E₂ was used as test substrate.

In the presence of the homogenate supernatant, NAD⁺ was reduced without addition of prostaglandin. This was probably because of the presence of an NAD⁺-dependent glycerol dehydrogenase activity; it did not occur after dialysis against glycerol-free buffer until glycerol was again added; and NADH was rapidly oxidized in the presence of D-glyceraldehyde. Similar findings were obtained using NADP⁺ and NADPH in place of NAD⁺ and NADH respectively.

Dialysis against glycerol-free buffer would be expected to result in loss of 15-hydroxyprostaglandin dehydrogenase activity. However, much of the activity that interfered with the assay of 15-hydroxyprostaglandin dehydrogenase in the homogenate supernatant was lost when the pH was decreased to 5.2, and most of what remained was located in the supernatant; 15-hydroxyprostaglandin dehydrogenase activity could be expected in the precipitate, according to the behaviour of the enzyme from human placenta.

No prostaglandin-dependent reduction of NAD⁺ was detected in any supernatant, or in any redissolved precipitate from any treatment of any of the three pig placentae. Control experiments with human placentae gave the expected results, with copious NAD⁺-dependent 15-hydroxyprostaglandin dehydrogenase activity. A relatively small amount of what appeared to be glycerol dehydrogenase activity was found to be mainly NADP⁺-dependent in the human placental preparations, and was probably caused by aldose reductase (EC 1.1.1.21) (Clements & Winegrad, 1972).

Our experiments allow the conclusion that at term NAD⁺-dependent 15-hydroxyprostaglandin dehydrogenase is either not present in pig placentae in amounts comparable with the large amounts present in human placentae, or is less stable than the enzyme from human placentae. The possible presence in vivo of small amounts of the enzyme, or of a highly unstable enzyme, is not excluded by this work.
The precise role of 15-hydroxyprostaglandin dehydrogenase in human placenta is not known. Pig placenta differs from human placenta in structure and in endocrinology (which is the reason why we examined it). It is therefore difficult to know whether our findings should be regarded as surprising, but it is noteworthy that Walker et al. (1977) reported the presence of 15-hydroxyprostaglandin dehydrogenase activity in pig conceptuses flushed from the uteri of 18–21-day-pregnant sows, and they indicated that the site of metabolism was the amniotic membranes. 15-Hydroxyprostaglandin dehydrogenase activity in numerous pig tissues (but not placenta) was described by Ånggård et al. (1971), and the relatively stable form of the enzyme in pig lung is well known (Ånggård & Samuelsson, 1966).

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**Preparation of 15-Hydroxyprostaglandin Dehydrogenase from Human Placenta and Estimation of its Molecular Weight**

OI TONG MAK and JONATHAN JEFFERY

*Department of Biochemistry, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, Scotland, U.K.*

Human placenta is the most abundant known source of NAD⁺-dependent 15-hydroxyprostaglandin dehydrogenase (EC 1.1.1.141). However, the enzyme is unstable in preparations from this source, and its isolation presents difficulties. The inclusion of 20% (v/v) glycerol and thiol-protecting agents (14mM-2-mercaptoethanol or 1mM-dithiothreitol) in the homogenization and other buffers achieves useful stabilization, but even when this is carried out there is considerable loss of activity during, for example, the hours required to carry out precipitation with (NH₄)₂SO₄ at 4°C, and subsequent dialysis.

Schlegel & Greep (1975) described the addition of cold acid to precipitate the enzyme, together with much other protein, at pH5.2, from the soluble supernatant of human placental homogenate. We have found that, although this procedure is accompanied by some loss of activity, it allows a partially purified preparation of the enzyme to be prepared quickly and conveniently. We have further found that when a solution in buffer [10mM-potassium phosphate, 20% (v/v) glycerol, 1mM-disodium EDTA and 1mM-dithiothreitol, pH7.4] is made immediately from this acid precipitate and applied to a column of Blue Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden), the enzyme is held up and elution with NAD⁺ (5–400µM) in 20% (v/v) glycerol results in selective elution of the enzyme in about 10µM-NAD⁺. Addition of glycerol to the appropriate fractions to give 50% (v/v) glycerol then gives a solution that can be stored at −20°C for several weeks with little loss of activity. By using this technique, we have obtained in this stable solution 68% of the activity present in the original homogenate supernatant, with 490 times the specific activity. This compares favourably with other preparations, such as: 21% yield, 336-fold enrichment (Braithwaite & Jarabak, 1975); 27% yield, 364-fold enrichment (Schlegel & Greep, 1975); 56% yield, 195-fold enrichment (Thaler-Dao et al., 1974); high purifications have been obtained only with very low yields (e.g. 6% yield, 3110-fold enrichment; Thaler-Dao et al., 1974).