Protocollagen Proline Hydroxylase of the Mouse Uterus during Pregnancy and Post-partum Involution

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1. The protocollagen proline hydroxylase in mouse uterus was found to be similar to that in other animal sources in its subcellular distribution and cofactor requirements. 2. The activities of this enzyme in uterine tissue from non-pregnant mice were comparable with those in various embryonic tissues. 3. In the second half of pregnancy the protocollagen proline hydroxylase activity increased markedly. 4. After parturition the activity of this enzyme decreased rapidly, reaching normal non-pregnant values at 24h *post partum*. The results suggest a good correlation between the synthesis of collagen and the activity of protocollagen proline hydroxylase.

Protocollagen proline hydroxylase is an enzyme that synthesizes the hydroxyproline in collagen by hydroxylating proline residues in protocollagen, the large proline-rich and hydroxyproline-deficient polypeptide precursor of collagen (Udenfriend, 1966; Prockop & Kivirikko, 1967). Partially purified preparations of this enzyme from chick embryo (Kivirikko & Prockop, 1967*a,b*; Hutton, Tappel & Udenfriend, 1967; Kivirikko, Bright & Prockop, 1968; Halme & Kivirikko, 1968) and newborn rat skin (Rhoads & Udenfriend, 1968) have an absolute requirement for atmospheric oxygen, ascorbate, α -oxoglutarate and Fe^{2+} . Protocollagen proline hydroxylase activity has been detected in a number of animal (Nordwig, Korble & Pfab, 1967; Hutton & Udenfriend, 1968; Takeuchi, Kivirikko & Prockop, 1967; Mussini, Hutton & Udenfriend, 1967; Fujimoto & Prockop, 1969) and human (Takeuchi & Prockop, 1969; Uitto, Halme, Hannuksela, Peltokallio & Kivirikko, 1969*a*) tissues, and appears to be high in tissues that actively synthesize collagen, e.g. in embryonic and granulomatous tissues (Mussini *et al.* 1967; Juva, 1968).

Mammalian uterine tissue is capable of a high rate of collagen biosynthesis (Kao, Hilker & McGavack, 1961*a,b*), and Kao, Treadwell, Previll & McGavack (1968) found evidence for the presence in pig uterus of protocollagen proline hydroxylase with the same cofactor requirements as enzyme preparations from other animal sources.

In the present study the characteristics of protocollagen proline hydroxylase in mouse uterus were determined and changes in its activity were investigated in pregnancy and post-partum involution.

MATERIAL AND METHODS

Inbred CBA mice were used throughout the study. Day 0 of pregnancy was determined by the presence of a vaginal plug. The uteri were dissected out and freed of membranes and placental tissue, cut with scissors in 0.1M-KCl-20mM-tris-HCl buffer, pH 7.8, at 0°C (1ml/100mg of tissue), then homogenized with a Teflon-glass homogenizer. The homogenates were centrifuged at 15000*g* for 30 min and portions of the supernatants were incubated for 60 min at 37°C with [^{14}C]proline-labelled protocollagen substrate (50000 d.p.m.), 50mM-tris-HCl buffer, pH 7.8 at 24°C, 2mM-ascorbic acid, 0.5mM- α -oxoglutarate, 0.08mM- FeSO_4 and 0.05mg of catalase (Sigma Chemical Co., St Louis, Mo., U.S.A.)/ml in a final volume of 4.0ml. After incubation 4ml of conc. HCl was added and the samples were hydrolysed overnight at 120°C. Total ^{14}C radioactivity (Prockop & Ebert, 1963) and hydroxy[^{14}C]proline (Juva & Prockop, 1966) were assayed in the hydrolysates. The [^{14}C]proline-labelled protocollagen used as substrate was prepared as described by Kivirikko & Prockop (1967*a*), boiled (Takeuchi *et al.* 1967) for 5 min and divided into portions (50000 d.p.m./portion) for storage.

Portions of the 15000*g* supernatants of uterine homogenates were hydrolysed in 6M-HCl at 120°C overnight and the hydrolysates were assayed for α -amino N (Rubinstein & Pryce, 1959).

RESULTS

The activity of protocollagen proline hydroxylase in mouse uterus was studied by incubating different amounts of the 15000*g* supernatant of uterine homogenate under standard incubation conditions. The amount of hydroxy[^{14}C]proline synthesized in

Fig. 1. Assay of protocollagen proline hydroxylase activity in mouse uterus. The values show the synthesis of hydroxy $[^{14}\text{C}]$ proline with $[^{14}\text{C}]$ proline-labelled protocollagen (50000 d.p.m.) as substrate and various amounts of the 15000g supernatant of the uterine homogenate as the source of the enzyme. The incubation was carried out as described in the Materials and Methods section.

Fig. 2. Protocollagen proline hydroxylase activity in mouse uterus. (●), Enzyme activity/unit wet weight of uterus; (○), enzyme activity/mg of protein from the 15000g supernatant. Enzyme activity is expressed as the radioactivity (d.p.m.) of hydroxy $[^{14}\text{C}]$ proline synthesized in the $[^{14}\text{C}]$ proline-labelled protocollagen substrate. The incubation was carried out as described in the Materials and Methods section. The enzyme source was samples of 15000g supernatant of the tissue homogenate, each corresponding to 5–15 mg wet wt. of tissue.

Table 1. Subcellular distribution of protocollagen proline hydroxylase activity in mouse uterus

A homogenate of 500 mg of mouse uterus was centrifuged at 15000g for 30 min, then the supernatant was further centrifuged at 100000g for 60 min. The 15000g and 100000g sediments were washed three times and suspended in 0.1M-KCl-1mM-tris-HCl buffer, pH 7.8. The sediments and the 100000g supernatant were assayed for protocollagen proline hydroxylase activity as described in the Materials and Methods section.

Homogenate fraction	Hydroxy $[^{14}\text{C}]$ -proline formed (d.p.m./100 mg of tissue)	Activity in homogenate (% of total activity)
15000g sediment	1870	22
100000g sediment	70	1
100000g supernatant	6740	77

the $[^{14}\text{C}]$ proline-labelled protocollagen was proportional to the amount of 15000g supernatant protein added (Fig. 1), and in subsequent experiments the incubations were usually carried out with 0.1–0.3 mg of protein from the 15000g supernatant.

In animal tissues most of the activity of protocollagen proline hydroxylase is recovered in the 100000g supernatant of the tissue homogenates. Similarly (Table 1), about 80% of the total protocollagen proline hydroxylase activity in mouse uterus was found in the 100000g supernatant of the tissue homogenate. The enzyme in mouse uterus required the same cofactors or cosubstrates as enzyme preparations from other animal sources (results not shown).

The mouse uterus has considerable protocollagen proline hydroxylase activity per unit wet weight of tissue (Fig. 2) or per mg of protein from the 15000g supernatant (Figs. 1 and 2), and there seem to be no marked changes in activity (Fig. 2) during the first half of pregnancy. After day 11 of pregnancy protocollagen proline hydroxylase activity begins to increase, reaching a maximum of about a fourfold increase in activity at the end of pregnancy. After parturition the activity of this enzyme decreases rapidly to about normal values at 24 h post partum.

DISCUSSION

In the present work a soluble protocollagen proline hydroxylase was extracted from mouse uterus. Most of the enzyme activity was recovered in the 100000g supernatant of the uterine homogenate, and the enzyme resembled that from other animal sources in requiring atmospheric oxygen, ascorbate, α -oxoglutarate and Fe^{2+} . Protocollagen proline hydroxylase activity was determined under standard laboratory conditions (Uitto *et al.* 1969a; Uitto, Halme, Kahanpää, Karhunen & Lindy, 1969b; Halme, 1969). Uterine tissue from non-pregnant mice had activities of this enzyme comparable with those in various embryonic tissues. The amounts of 15000g supernatant added to the incubation medium were only about one-tenth of those used by Kao *et al.* (1968); this indicates that the method used here may be more sensitive.

Pregnancy was found to cause marked alterations in the activity of procollagen proline hydroxylase. In the first half of pregnancy the activity of this enzyme did not change notably, but an approximately fourfold increase, calculated per unit wet weight of tissue, occurred in the second half of pregnancy. The wet weight of the uterus increases manifold during pregnancy (Montfort & Pérez-Tamayo, 1961; Woessner & Brewer, 1963; Woessner, 1965), thus the increase in the total amount of the enzyme per uterus is even more conspicuous. This is in good agreement with previous studies showing an increase in the amount of collagen in the uterus late in pregnancy (Montfort & Pérez-Tamayo, 1961; Woessner & Brewer, 1963; Woessner, 1965; Morrione & Seifter, 1962).

During post-partum involution a rapid breakdown of uterine collagen occurs (Montfort & Pérez-Tamayo, 1961; Woessner & Brewer, 1963; Woessner, 1965; Morrione & Seifter, 1962), and the activities of acid hydrolases in uterine tissues increase markedly during the first 4 days after delivery (Woessner, 1965). In the present study the activity of procollagen proline hydroxylase decreased rapidly during post-partum involution, reaching normal non-pregnant values at 24h after parturition. This indicates rapid inactivation or catabolism of the enzyme protein in the early phases of post-partum involution.

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REFERENCES

- Fujimoto, D. & Prockop, D. J. (1969). *J. biol. Chem.* **244**, 205.
- Halme, J. (1969). *Biochim. biophys. Acta*, **192**, 90.
- Halme, J. & Kivirikko, K. I. (1968). *FEBS Lett.* **1**, 223.
- Hutton, J. J., jun., Tappel, A. L. & Udenfriend, S. (1967). *Archs Biochem. Biophys.* **118**, 231.
- Hutton, J. J., jun. & Udenfriend, S. (1968). *Proc. natn. Acad. Sci. U.S.A.* **56**, 198.
- Juva, K. (1968). *Acta physiol. scand. suppl.* 308.
- Juva, K. & Prockop, D. J. (1966). *Analyt. Biochem.* **15**, 77.
- Kao, K. Y. T., Hilker, D. M. & McGavack, T. H. (1961a). *Proc. Soc. exp. Biol. Med.* **106**, 121.
- Kao, K. Y. T., Hilker, D. M. & McGavack, T. H. (1961b). *Proc. Soc. exp. Biol. Med.* **106**, 335.
- Kao, K. Y. T., Treadwell, C. R., Previll, J. M. & McGavack, T. H. (1968). *Biochim. biophys. Acta*, **151**, 568.
- Kivirikko, K. I., Bright, H. J. & Prockop, D. J. (1968). *Biochim. biophys. Acta*, **151**, 558.
- Kivirikko, K. I. & Prockop, D. J. (1967a). *Proc. natn. Acad. Sci. U.S.A.* **57**, 782.
- Kivirikko, K. I. & Prockop, D. J. (1967b). *J. biol. Chem.* **242**, 4007.
- Montfort, J. & Pérez-Tamayo, R. (1961). *Lab. Invest.* **10**, 1240.
- Morrione, T. & Seifter, S. (1962). *J. exp. Med.* **115**, 357.
- Mussini, E., Hutton, J. J., jun. & Udenfriend, S. (1967). *Science, N.Y.*, **157**, 927.
- Nordwig, A., Korble, V. & Pfab, F. K. (1967). *Biochim. biophys. Acta*, **147**, 487.
- Prockop, D. J. & Ebert, P. S. (1963). *Analyt. Biochem.* **6**, 263.
- Prockop, D. J. & Kivirikko, K. I. (1967). *Ann. intern. Med.* **66**, 1243.
- Rhoads, R. E. & Udenfriend, S. (1968). *Proc. natn. Acad. Sci. U.S.A.* **60**, 1473.
- Rubinstein, H. M. & Pryce, J. D. (1959). *J. clin. Path.* **12**, 80.
- Takeuchi, T., Kivirikko, K. I. & Prockop, D. J. (1967). *Biochem. biophys. Res. Commun.* **28**, 940.
- Takeuchi, T. & Prockop, D. J. (1969). *Gastroenterology*, **56**, 744.
- Udenfriend, S. (1966). *Science, N.Y.*, **152**, 1335.
- Uitto, J., Halme, J., Hannuksela, M., Peltokallio, P. & Kivirikko, K. I. (1969a). *Scand. J. clin. Lab. Invest.* **23**, 241.
- Uitto, J., Halme, J., Kahanpää, K., Karhunen, P. & Lindy, S. (1969b). *Abstr. FEBS 6th Meet., Madrid*, p. 264.
- Woessner, J. F., jun. (1965). *Biochem. J.* **97**, 855.
- Woessner, J. F., jun. & Brewer, T. H. (1963). *Biochem. J.* **89**, 75.