Subcellular distribution of isocitrate dehydrogenase in early and term human placenta

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1. The activities of NAD-specific and NADP-specific isocitrate dehydrogenases were measured in early and term human placenta. In both tissues the activity of NADP-specific isocitrate dehydrogenase was severalfold higher than that of the NAD-dependent enzyme. 2. Subcellular distribution of these two enzymes in the placental tissue was estimated. About 60% of the total NADP-specific isocitrate dehydrogenase activity was found in the mitochondrial fraction and about 40% in the cytosol fraction. Insignificant amounts of the total activity were bound to the microsomal fraction. The whole of the NAD-specific isocitrate dehydrogenase activity was localized in the mitochondrial fraction. 3. The total mitochondrial NADP-specific isocitrate dehydrogenase activity in both early and term placenta was also estimated from the mitochondrial specific activity of this enzyme and the amount of mitochondrial protein in wet tissue, calculated from the activities of citrate synthase or cytochrome *c* oxidase assayed in the isolated mitochondrial fraction and in the tissue of early and term human placenta.

Most mammalian tissues contain both NAD-specific (EC 1.1.1.41) and NADP-specific (EC 1.1.1.42) isocitrate dehydrogenases (Hogeboom & Schneider, 1950; Kaplan *et al.*, 1956). The NAD-isocitrate dehydrogenase activity appears to be confined to the mitochondria (Plaut, 1970). The NADP-isocitrate dehydrogenase in various tissues of the rat and other animals is present in the mitochondria and the cytosol fraction of the cells (Plaut, 1963). Sung & Hsu (1957) presented evidence for the separation of NAD- and NADP-isocitrate dehydrogenase activities from an extract of acetone-dried powder of mitochondria isolated from human term placenta. They also found that the NADP-linked activity is 20-fold higher than the NAD-linked isocitrate dehydrogenase activity. The physiological role of NADP-isocitrate dehydrogenase is not fully understood. Some authors proposed that mitochondrial NADP-isocitrate dehydrogenase activity participates in the reduction of NAD⁺ to NADH by transhydrogenation (Smith & Plaut, 1979). Others have suggested that this activity is involved in lipid biosynthesis and the metabolism of tricarboxylic acid-cycle intermediates to amino acids (Amore & Bonevita, 1965; Loverde & Lehrer, 1973). In human placenta the regeneration of

NADPH plays an important role in the mitochondrial conversion of cholesterol into pregnenolone in the course of progesterone biosynthesis and in the microsomal biosynthesis of oestrogens from androgens (Simpson & MacDonald, 1981). This implies the generation of NADPH in different cell compartments. To investigate this we therefore undertook the present studies on the subcellular distribution of NADP-isocitrate dehydrogenase activity in human placental tissue.

Materials and methods

Chemicals

Acetyl-CoA (lithium salt), oxaloacetic acid, DL-isocitric acid (sodium salt), citric acid (sodium salt), cytochrome *c* (type IIA) and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). NAD⁺ and NADP⁺ were from Reanal (Budapest, Hungary). All other chemicals, of analytical grade, were from POCh (Gliwice, Poland).

Placental tissue

Early placental tissue (8–12 weeks of gestation) was obtained from pregnancies terminated for social

or therapeutic reasons. Term placentas were obtained from normal pregnancies.

Preparation of the tissue

Specimens of human placentas were prepared at 0–4°C. The blood was drained, membranes were removed and the tissue obtained was rinsed several times with 0.9% NaCl/5 mM-EDTA/10 mM-Tris/HCl buffer, pH 7.6, and then twice in 0.25 M-sucrose / 5 mM-EDTA / 10 mM-Tris / HCl buffer, pH 7.6. The tissue was passed through a meat grinder and finally was dried on blotting paper.

Extraction of enzyme activities from placental tissue

The extraction medium for NAD-isocitrate dehydrogenase consisted of 50 mM-Tris/HCl buffer, pH 7.6, containing 5 mM-MgCl₂, 2 mM-MnCl₂, 1 mM-EDTA, 2 mM-ADP, 0.2% bovine serum albumin, 2 mM-2-mercaptoethanol and 0.1% Triton X-100. The extraction medium for NADP-isocitrate dehydrogenase was the same except that ADP was omitted. For citrate synthase 0.1 M-potassium phosphate buffer, pH 7.4, containing 0.1% Triton X-100 was used. About 2 g of wet tissue was weighed out, homogenized manually in 5 ml of the appropriate medium and centrifuged at 20 000 g for 10 min, the supernatant was collected and the pellet was rehomogenized twice in the same medium. The volume of combined supernatants was measured (about 10 ml) and the enzyme activities were determined. For the extraction of cytochrome *c* oxidase about 1.5 g of wet placental tissue was weighed out and homogenized manually in 5 ml of 0.1 M-Tris/HCl buffer, pH 7.4, and then Triton X-100 was added (3 mg/g wet wt. of tissue) and the homogenate was sonicated at 0°C at 20 kHz with a 100 W MSE Ultrasonic Disintegrator for three periods each of 30 s duration separated by 30 s intervals. The material obtained was centrifuged at 20 000 g for 10 min, the supernatant collected and the pellet was extracted again in the same way.

Preparation of homogenate and mitochondrial, microsomal and cytosol fractions

The tissue specimens were homogenized manually in 0.25 M-sucrose / 2 mM-EDTA / 10 mM-Tris / HCl buffer, pH 7.6. For term placenta about 100 g of tissue was suspended in 400 ml of medium, and for early placenta about 25–30 g of tissue was suspended in 100 ml of medium. The homogenates were centrifuged at 600 g for 5 min, and the supernatant was filtered through cheesecloth. The volume of the supernatant was measured, and then it was assayed for enzyme activities. The mitochondrial fraction was sedimented from the supernatant by centrifugation at 13 000 g for 15 min; this supernatant was called 'crude cytoplasm'. Microsomal fraction and cytosol were obtained by centrifugation of 'crude

cytoplasm' at 105 000 g for 60 min. These fractions were also used for the assay of enzyme activities. The isolation of mitochondria from early (Żolnierowicz *et al.*, 1982) and term (Świerczyński *et al.*, 1976) placenta was as described previously.

Assays of enzyme activities

Mitochondria were suspended in 50 mM-Tris/HCl buffer, pH 7.6, to a concentration of 25 mg of protein/ml, and Triton X-100 was added to final concentration of 0.2% when either citrate synthase or isocitrate dehydrogenase activity was to be measured. When cytochrome *c* oxidase activity was to be measured mitochondria were suspended in 0.1 M-potassium phosphate buffer, pH 7.2, to a concentration of 2 mg of protein/ml, Triton X-100 was added (0.5 mg/mg of protein) and then the mitochondria were sonicated at 0°C at 20 kHz for two periods each of 30 s duration with a 30 s break. The activities of citrate synthase and NAD- and NADP-linked isocitrate dehydrogenases were measured spectrophotometrically at 30°C with a Specord UV-Vis recording spectrophotometer in 1 ml assay mixtures. The reaction was monitored at 340 nm for isocitrate dehydrogenase and at 412 nm for citrate synthase under conditions similar to those described by Alp *et al.* (1976). The assay medium for NAD-isocitrate dehydrogenase contained (final concentrations) 50 mM-Tris/HCl buffer, pH 7.2, 5 mM-MgCl₂, 2 mM-ADP, 1 mM-NAD⁺, 25 mM-citrate, 5 mM-DL-isocitrate and 5 µg of antimycin A/ml. The reaction was initiated by the addition of citrate plus isocitrate. The assay medium for NADP-isocitrate dehydrogenase contained (final concentrations) 50 mM-Tris/HCl buffer, pH 7.6, 5 mM-MgCl₂, 2 mM-MnCl₂, 1 mM-NADP⁺, 25 mM-citrate and 5 mM-DL-isocitrate. The assay medium for citrate synthase contained (final concentrations) 50 mM-Tris/HCl buffer, pH 7.6, 0.2 mM-5,5'-dithio-bis-(2-nitrobenzoic acid), 0.2 mM-acetyl-CoA and 0.5 mM-oxaloacetate. The reaction was started by the addition of oxaloacetate. Cytochrome *c* oxidase activity was measured polarographically at 30°C with a Clark oxygen electrode with a 2.5 ml reaction chamber. The reaction medium and assay conditions were as described by Schnaitman *et al.* (1967).

Protein concentration was determined by the biuret method (Layne, 1957), with bovine serum albumin as standard.

Results

Table 1 presents the NAD-isocitrate dehydrogenase and NADP-isocitrate dehydrogenase activities calculated per g wet wt. of tissue. In early and term placenta the NAD- and NADP-isocitrate dehydrogenase activities were similar. However, in both tissues the NADP-isocitrate dehydrogenase

activity was severalfold higher than the NAD-isocitrate dehydrogenase activity. The subcellular distribution of NAD- and NADP-isocitrate dehydrogenase and citrate synthase activities in human placental tissue is presented in Table 2. The values for each fraction represent the percentage of the total NAD(P)-isocitrate dehydrogenase activity in the homogenate in the presence of Triton X-100. In the absence of the detergent the NAD-isocitrate dehydrogenase activity did not appear in the homogenate. The whole of the NAD-isocitrate dehydrogenase activity was found in the mitochondrial fraction treated with detergent after isolation. Similar results were obtained for citrate synthase. The NADP-isocitrate dehydrogenase activity measured in the homogenate in the absence of Triton X-100 represented about 40% of the total activity. This result indicates that about 60% of the NADP-isocitrate dehydrogenase activity is particle-bound. The results obtained with the isolated subcellular fractions support this conclusion. About 60% of the total activity of the NADP-isocitrate dehydrogenase was bound to the mitochondrial fraction. The subcellular distributions of these two enzyme activities in the early and term human placental tissue are

Table 1. Activities of NAD-isocitrate dehydrogenase and NADP-isocitrate dehydrogenase in early and term human placenta

Activities are expressed as mean values $\pm$ S.D. for five independent experiments each measured in duplicate. Activities were measured in the homogenate of the tissue prepared as described in the Materials and methods section.

Source	Enzyme activity [μ mol of NAD(P) ⁺ reduced/min per g wet wt. of tissue]	
	NAD-isocitrate dehydrogenase	NADP-isocitrate dehydrogenase
Early placenta	0.19 $\pm$ 0.11	5.3 $\pm$ 0.46
Term placenta	0.25 $\pm$ 0.10	4.1 $\pm$ 0.62

Table 2. Subcellular distribution of NAD-isocitrate dehydrogenase, NADP-isocitrate dehydrogenase and citrate synthase in human placenta

For experimental details see the Materials and methods section. The values represent percentages of the total activity measured in the original whole homogenate in the presence of Triton X-100. The data are from a typical one of five separate experiments, the differences between experiments being less than 5%.

Fraction	Subcellular distribution (%)					
	NAD-isocitrate dehydrogenase		NADP-isocitrate dehydrogenase		Citrate synthase	
	Early placenta	Term placenta	Early placenta	Term placenta	Early placenta	Term placenta
Homogenate	—	—	41	42	—	—
Mitochondria	102	98	58	59	97	96
Microsomal fraction	0	0	1	2	0	0
Cytosol	0	0	40	41	0	0

very similar. The amount of enzyme activity present in mitochondria could be evaluated by the method described elsewhere (Świerczyński *et al.*, 1983). In this method the mitochondrial protein content of the tissue has to be estimated. Tables 3 and 4 present the amount of mitochondrial protein per g of tissue calculated by dividing the estimated citrate synthase or cytochrome *c* oxidase activity in 1 g of tissue by the same enzyme activity present in 1 mg of mitochondrial protein. By multiplying the amount of mitochondrial protein present in 1 g of tissue by the mitochondrial NADP-isocitrate dehydrogenase specific activity, the total mitochondrial NADP-isocitrate dehydrogenase activity in 1 g of wet tissue was determined. This value may be used to calculate the proportion of mitochondrial NADP-isocitrate dehydrogenase activity.

The results of the experiments reported in Tables 2, 3 and 4 indicate that the distribution of mitochondrial NADP-isocitrate dehydrogenase activity is the same in early and term placental tissue. Very similar are the percentages of mitochondrial NADP-isocitrate dehydrogenase activity estimated by the classical fractionation method (Table 2) and by the determination of mitochondrial protein content calculated from the citrate synthase activity in mitochondria and in whole tissue (Table 3). Values different by about 10% were obtained when cytochrome *c* oxidase was used as a mitochondrial marker enzyme, since this gave a higher estimate for the mitochondrial protein content (Table 4).

Discussion

The results given in the present paper indicate that the NADP-isocitrate dehydrogenase activity in human placental mitochondria is severalfold higher than the NAD-dependent enzyme activity. Similar results have been reported for heart, liver and spleen (Plaut, 1970). Only in the brain mitochondria is NAD-isocitrate dehydrogenase activity higher than that of the NADP-linked enzyme (Plaut, 1970).

Table 3. *Citrate synthase and mitochondrial NADP-isocitrate dehydrogenase activities in human placental tissue*
For experimental details see the Materials and methods section. Activities are expressed as mean values $\pm$ S.D. for four experiments with early and eight experiments with term human placenta. The amount of mitochondrial protein in 1 g wet wt. of tissue (c) was obtained by dividing the activity of mitochondrial marker enzyme (citrate synthase or cytochrome c oxidase) in 1 g wet wt. of tissue (a) by the mitochondrial specific activity of this enzyme (b). The mitochondrial NADP-isocitrate dehydrogenase activity in 1 g wet wt. of tissue (e) was obtained by multiplying the mitochondrial specific activity (d) by the mitochondrial protein content in 1 g wet wt. of tissue (c). The percentage of the total wet-tissue activity present in mitochondria (f) was calculated from the mitochondrial activity per 1 g wet wt. of tissue (e) and the total activity of the NADP-specific enzyme in 1 g wet wt. of tissue from Table 1.

Tissue	Citrate synthase activity		Amount of mitochondrial protein (mg/g wet wt. of tissue) (c = a/b)	Mitochondrial NADP-isocitrate dehydrogenase activity		
	($\mu\text{mol/min per g wet wt. of tissue}$) (a)	($\mu\text{mol/min per mg of mitochondrial protein}$) (b)		($\mu\text{mol/min per mg of mitochondrial protein}$) (d)	($\mu\text{mol/min per g wet wt. of tissue}$) (e = d $\times$ c)	(% of total activity in wet tissue) (f)
Early placenta	1.11 $\pm$ 0.12	0.105 $\pm$ 0.05	10.6	0.33 $\pm$ 0.06	3.50	66
Term placenta	1.24 $\pm$ 0.16	0.155 $\pm$ 0.08	8.0	0.30 $\pm$ 0.09	2.40	59

Table 4. *Cytochrome c oxidase and mitochondrial NADP-isocitrate dehydrogenase activities in human placenta*
See Table 3 for details of the values presented.

Tissue	Cytochrome c oxidase activity		Amount of mitochondrial protein (mg/g wet wt. of tissue) (c = a/b)	Mitochondrial NADP-isocitrate dehydrogenase activity		
	($\mu\text{mol/min per g wet wt. of tissue}$) (a)	($\mu\text{mol/min per mg of mitochondrial protein}$) (b)		($\mu\text{mol/min per mg of mitochondrial protein}$) (d)	($\mu\text{mol/min per g wet wt. of tissue}$) (e = d $\times$ c)	(% of total activity in wet tissue) (f)
Early placenta	1.51 $\pm$ 0.27	0.12 $\pm$ 0.02	12.6	0.33 $\pm$ 0.06	4.16	78
Term placenta	1.54 $\pm$ 0.31	0.145 $\pm$ 0.04	10.6	0.30 $\pm$ 0.09	3.18	78

Therefore some authors have proposed the participation of NADP-isocitrate dehydrogenase in tricarboxylic acid-cycle metabolism (Stein *et al.*, 1967; Londesborough & Dalziel, 1970). The distribution of NADP-isocitrate dehydrogenase activity varies with the tissue, from 80% in mouse (Hogeboom & Schneider, 1950) and rat (Pette, 1966) liver cytosol to about 90% in bovine (Fatania & Dalziel, 1980) and rat (Pette, 1966) heart mitochondria. Our results show that in human placenta about 60% of the total NADP-isocitrate dehydrogenase activity is localized in the rather small mitochondrial fraction. The estimated mitochondrial protein content in the placental tissue is comparable with that observed in mixed skeletal muscle and is low compared with heart (Idell-Wenger *et al.*, 1978), liver and kidney (Świerczyński *et al.*, 1983). In human placental mitochondria the utilization of isocitrate and its stimulatory effect on progesterone biosynthesis have been described (Klimek *et al.*, 1976). It has been concluded that NADP-isocitrate dehydrogenase is responsible for supplying the reducing equivalents utilized in progesterone biosynthesis (Bogusławski *et al.*, 1976). NADPH, as a highly specific electron donor in the hydroxylation system (Mason & Boyd,

1971), plays an important role, not only in the mitochondrial cholesterol side-chain-cleavage reactions (Klimek *et al.*, 1979), but also in the microsomal aromatase system (Fishman & Junichi, 1981). At term of a normal human pregnancy, the placenta produces 300 mg of progesterone and about 100 mg of oestrogens per day. This massive steroid biosynthesis implies a high rate of generation of NADPH in different cell compartments. The results given in the present paper indicate that the activity of NADP-isocitrate dehydrogenase and its distribution between mitochondrial and cytosol fractions are consistent with the view that it plays an essential role in supporting NADPH generation for the biosynthesis of steroid hormones in different cell compartments of human placental tissue.

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