Electrophoretically Distinct Forms of Uridine Kinase in the Rat

TISSUE DISTRIBUTION AND AGE-DEPENDENCE

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By the use of polyacrylamide-gel electrophoresis, uridine kinase from foetal rat liver was separated into four types designated I, II, III and IV in decreasing order of mobility towards the anode. The most anodic (type I) was found only in rapidly growing tissues, such as foetal liver and brain, postnatal spleen and tumour cells. In adult tissues, types II, III and IV were found in the kidney, and types III and IV in the spleen and the liver, whereas type IV was the sole form of uridine kinase present in the brain.

A number of reports have shown the importance of uridine kinase (EC 2.7.1.48) in cancer chemotherapy with uridine or cytidine analogues, and it was shown that the development of resistance towards these anti-metabolites is associated with changes in some characteristics of the enzyme (Skold, 1963; Vesely *et al.*, 1971; Keefer *et al.*, 1974). Uridine kinase exists in different forms in Novikoff hepatoma (Krystal & Scholefield, 1973; Keefer *et al.*, 1975), in foetal liver (Krystal & Webb, 1971; Roux & Tuilie, 1974) and rat kidney (Vesely & Smrt, 1977).

We undertook a study of the heterogeneity of rat uridine kinase, using polyacrylamide-gel electrophoresis and electrofocusing. The present paper describes the different patterns obtained with the enzyme from foetal, adult and tumoral tissues.

Materials and Methods

The enzyme was extracted from the liver, cerebrum, kidney and spleen of male Sherman rats aged 4 months. Foetal tissues came from 17-day fetuses of the same strain. Hepatoma cells, obtained from Dr. C. Frayssinet and Dr. C. Lafarge (Institut de Recherche sur le Cancer, Villejuif, France), were grown for 5 or 6 days in the peritoneal cavity of newborn rats of the Wistar Commentry strain (Frayssinet & Lafarge, 1966).

The enzyme extracts were prepared by the method described by Fulchignoni-Lataud *et al.* (1976). The stock enzyme was dissolved in a minimal volume of 20 mM-potassium phosphate (pH 7.4), containing 1 mM-dithiothreitol (Sigma), and remained stable for several months at -20°C .

Uridine kinase activity was measured as described by Skold (1960) in 0.2 ml of medium contain-

ing 0.64 mM-[2- ^{14}C]uridine (CEA, Saclay, France) (0.05 μCi per test), 30 mM-ATP (Sigma) and 20 mM-MgSO₄ (Merck). The radioactivity in the uridine nucleotides formed (UMP, UDP and UTP) was measured by the filter-disc method (Ives *et al.*, 1969) in a Nuclear-Chicago liquid-scintillation counter mark I. Protein was measured by the method of Lowry *et al.* (1951).

Polyacrylamide-gel electrophoresis was performed in glass tubes (5 mm diam. $\times$ 100 mm long) by using a multiphasic buffer system (Tris/HCl/Tris/glycine) as described by Rodbard & Chrambach (1971). The size of the gels was 5 mm $\times$ 80 mm. The polyacrylamide (Eastman/Kodak) gels were prepared with 1.5 M-Tris/HCl buffer (pH 9), stored overnight at 4°C and then pre-run at 4°C for 60 min at 2.5 mA/gel. Tris/HCl buffer (pH 9), containing 1 mM-dithiothreitol and 0.3 mM-ATP, was used in the cathode compartment.

The enzyme samples (100–200 μg of protein) were applied to the gel in 200 μl of 20 mM-potassium phosphate buffer (pH 7.4) containing sucrose (150 g/litre) and Bromophenol Blue (0.025 g/litre). Electrophoresis was carried out for 2 h with a constant current of 2.5 mA/gel. The gel was sliced into 2 mm discs, each disc being immediately immersed in 250 μl of standard reaction mixture for the assay of uridine kinase activity.

Isoelectric focusing was performed in polyacrylamide gels (5 mm diam. $\times$ 80 mm long; 38.8 g of acrylamide/litre plus 1.2 g of methylenebisacrylamide/litre) containing glycerol (150 ml/litre) and carrier ampholytes in pH range 5.4–7.5 obtained by mixing LKB Ampholine pH 4–6 (8 ml/litre), pH 5–7 (32 ml/litre) and pH 7–9 (8 ml/litre). The cathode tank contained 0.1 M-NaOH and the anode tank

0.02M- H_3PO_4 . After the gels had been prefocused for 1 h, the samples (100–200 μ g of protein) were applied in 200 μ l of 20mM-potassium phosphate buffer containing glycerol (150 ml/litre). Electro-focusing was performed at 4°C for 2 h to a final potential of 400 V. Gels were sliced into 2 mm discs, and each disc was immediately assayed for uridine kinase activity as described above. A duplicate gel was sliced and each disc was eluted with 1 ml of 0.5 M-NaCl overnight to determine the pH gradient.

Results and Discussion

The results of electrophoresis of uridine kinase extracted from foetal rat liver are shown in Fig. 1 and demonstrate four electrophoretically distinct forms of the enzyme, designated types I–IV in decreasing order of mobility towards the anode. In previous work we found that the enzyme from foetal liver could be separated into two active fractions by DEAE-cellulose chromatography (Fulchignoni-Lataud *et al.*, 1976). One of these forms was specific to the foetal tissue, the second being common to adult and foetal liver. These two fractions were

Fig. 1. Electrophoretic pattern of crude uridine kinase extracted from foetal rat liver (day 17 of gestation) Polyacrylamide-gel electrophoresis (gel concentration 40g/litre) was carried out as described in the Materials and Methods section. The 80 mm long gels were cut into 2 mm slices, and each was tested for uridine kinase activity. The activity is expressed as the radioactivity (c.p.m.) in the UMP formed.

submitted separately to polyacrylamide-gel electrophoresis. The foetal-specific form of the enzyme gave only one large fast-migrating peak, corresponding to the type-I enzyme. The second uridine kinase-active fraction was resolved into three peaks with R_m (i.e. mobility relative to that of the tracking dye) values identical with those found for the slow-migrating peaks of the crude extracts (i.e. types II, III and IV).

The results of isoelectric focusing the two active fractions separated by DEAE-cellulose chromatography are presented in Fig. 2. The fraction common to adult and foetal liver was again separated into three distinct peaks of activity, focusing at pH 7.66, 6.78 and 6.52 within a pH gradient of 5.4–7.5. On the other hand, the large fast-migrating peak I, which represented the foetal form of the enzyme, was divided into two bands, focusing at pH 6.72 and 6.50.

The relationship between mobility, gel concentration, molecular size and net charge has been well established by Chrambach & Rodbard (1971). When relative mobility is measured in each of three or more gel concentrations, a linear Ferguson (1964) plot ($\log R_m$ versus total acrylamide concentration) can be constructed. The slope of this curve is a measure of the molecular size, and the anti-log of

Fig. 2. Electrofocusing of uridine kinase from foetal rat liver

Polyacrylamide-gel electrofocusing was carried out for 2 h at 4°C in the pH range 5.4–7.5 (---). ----, Fraction specific to foetal liver; —, fraction common to foetal and adult liver. Uridine kinase activity is expressed as in Fig. 1.

the y intercept is a measure of the free electrophoretic mobility and therefore of the net charge. The Ferguson plots of the results obtained with polyacrylamide gels at six different concentrations from 75 to 35 g/litre are shown in Fig. 3. It is evident that form I is the least charged and the lightest molecule. The three other electrophoretically distinct molecules are more strongly charged and they migrate slowly in the gel because of their high molecular weights. Analysis of the data shows that the slopes for lines I, II and IV are simple multiples ($\times 1$, $\times 2$ and $\times 4$ respectively). Moreover, all three lines extrapolate to a common point at a low gel concentration

(approx. 15 g/litre). According to the work of Hedrick & Smith (1968), forms II and IV of uridine kinase are probably dimers and tetramers respectively of form I.

As shown above, type I can be separated from types II and IV by DEAE-cellulose chromatography, therefore polymerization is not consequent on polyacrylamide-gel electrophoresis. Forms III and IV are of similar size but different net charge, and consequently forms I and III are proteins of different molecular size and charge.

Uridine kinase extracts from various adult rat tissues were submitted to analytical polyacrylamide-gel electrophoresis as described above. Table 1 summarizes the results obtained with kidney, spleen, liver and cerebrum. Type-I uridine kinase was not found in any of the adult tissue extracts investigated. The kidney was the sole adult organ to contain all of the other three forms (II, III and IV). The spleen and liver both contained type-III and type-IV uridine kinase, although type IV was scarce and sometimes lacking altogether in the liver. The cerebrum exhibited a single large band of activity, identified by its R_m as type IV. Because of the different patterns obtained for foetal and adult liver, we investigated the electrophoretic behaviour of liver extracts taken at different stages of development (Table 2). Type I was present in the liver until the 20th day of gestation, the intensity of the peak decreasing rapidly till birth. This form was absent from the liver extracts taken from newborn rats killed within the first hours of postnatal life. Type II was present up to the third or fourth postnatal day. By day 5 the mature pattern was seen. Uridine kinase extracts from a rapidly growing undifferentiated hepatoma had the same pattern as obtained with foetal liver (Table 2). Assuming that the presence of type-I uridine kinase is related to rapidly dividing cells, we studied the fast-growing period specific to spleen (day 5 after birth) and to cerebrum (day 15 of gestation). The electrophoretic pattern obtained for both was similar to that obtained for foetal liver, where type I is present. Regenerating liver can also be used as a model for actively dividing liver cells,

Fig. 3. Plot of $\log R_m$ versus gel concentration (Ferguson plot)

I, II, III and IV refer to the four electrophoretically distinct forms of uridine kinase. The negative slopes of the lines are noted on the Figure.

Table 1. Tissue distribution of uridine kinase types in the rat

This was determined by polyacrylamide-gel electrophoresis (gel concentration 40 g/litre) as described in the Materials and Methods section. The results are expressed as the percentage of the total activity recovered in the 40 fractions of the gel.

		Activity			
Uridine kinase types		IV	III	II	I
(R_m)		0.15	0.22	0.34	0.50
Kidney		50.2	31.1	18.7	0.0
Spleen		79.1	20.9	0.0	0.0
Liver		18.0	82.0	0.0	0.0
Cerebrum		100.0	0.0	0.0	0.0

Table 2. *Distribution of uridine kinase types in liver extracts during development of the rat*
 The results are compared with extracts of hepatoma cells, foetal brain and postnatal spleen. Experimental conditions were the same as in Table 1.

Uridine kinase types (R_m)	Activity			
	IV	III	II	I
17 days gestation	17.9	14.2	17.3	50.6
18 days gestation	16.2	19.7	23.9	40.2
20 days gestation	9.1	42.9	25.0	23.0
Newborn	12.5	51.6	35.9	0.0
3 days postnatal	24.0	37.3	38.7	0.0
5 days postnatal	75.3	24.7	0.0	0.0
Hepatoma cells	28.7	28.4	28.5	14.4
Foetal brain	25.3	38.8	24.9	11.0
Postnatal spleen	8.5	9.8	20.0	61.7

but we did not find type-I uridine kinase after partial hepatectomy. The presence of this form of uridine kinase in the foetal liver and its disappearance on the 20th day of gestation could be related to the decrease in the haemopoietic activity in the latter organ at this stage of development.

The results reported above show that type-I uridine kinase resolved by polyacrylamide-gel electrophoresis is the foetal-specific form described previously (Roux & Tuilie, 1974; Fulchignoni-Lataud *et al.*, 1976) and could be related to the embryonic low-molecular-weight form isolated by other authors (Krystal & Webb, 1971; Krystal & Scholefield, 1973; Keefer *et al.*, 1975). The presence of three distinct enzyme forms in the kidney is confirmed by the findings of Vesely & Smrt (1977). As yet, the term isoenzyme cannot be used for these four species of uridine kinase. However, types I, II and IV are related by multiples of their molecular weight, the molecular weights of types II and IV being respectively 2 and 4 times that of type I. On consideration of such physical factors as molecular weight and charge, type III is distinctly different from the other three forms, and hence a tentative explanation could be that it is an isomer of type I. The technique described here is a useful tool for the analysis of uridine kinase activity in different tissues and could be applied, in our opinion, to the study of variations in the enzyme in different pathological conditions.

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