THE URIDYL TRANSFERASE OF LIVER

by

G. T. MILLS, R. ONDARZA* AND EVELYN E. B. SMITH**

Biochemistry Department, The University of Glasgow (Scotland)

It has recently been shown[1,2] that isolated liver nuclei will catalyse the reaction***:

$$\text{UDPG} + \text{P–P} \longrightarrow \text{UTP} + \text{glucose–1–phosphate}$$

and also that UDPAG can be substituted for UDPG with the formation of UTP. The enzyme responsible for this reaction has been called uridyl transferase by MUNCH-PETERSEN *et al.*[3]. At the same time it was found[2] that liver nuclei break down both UDPG and UDPAG to UDP and UMP and also dephosphorylate UTP to UDP.

In the present work we report the extraction from isolated liver nuclei of the uridyl transferase activity and demonstrate that this enzymic activity of the nuclei extract can be followed spectrophotometrically. It has also been confirmed that yeast uridyl transferase (present in the yeast Zwischenferment preparation[3]) does not attack UDPAG whereas it rapidly pyrophosphorylates UDPG.

The principal methods and materials used have been previously described[2], the pyrophosphorylation of UDPG being followed by the glucose-1-phosphate[3] or UTP[4] production, and that of UDPAG by the UTP production.

In the present work on UDPG it was necessary to use a preparation of glucose-6-phosphate dehydrogenase free from uridyl transferase, and recourse was made to the preparation of glucose-6-phosphate dehydrogenase of liver according to GLOCK AND MCLEAN[5], which has been found to be free from uridyl transferase activity. This enzyme was standardised spectrophotometrically using pure glucose-6-phosphate.

The liver uridyl transferase extract was prepared in the following manner. Nuclei were isolated from perfused guinea pig liver by the method of SCHNEIDER[6], using 0.25 *M* sucrose containing 0.0018 *M* $CaCl_2$[7], and washed free from cytoplasmic particles. The nuclei from 25 g liver were suspended in 20 ml 0.02 *M* phosphate buffer, pH 8.0 and brought into solution, which was a stiff gel, by the addition of 2 or 3 drops of 2 *N* KOH. DNA was then precipitated by the addition of 2 *M* KCl

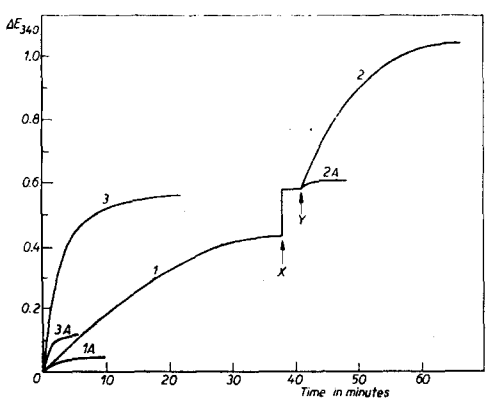

Fig. 1. The pyrophosphorylation of UDPG and UDPAG by liver nuclei extract. – *Curves 1 and 2.* 0.07 μmole UDPG was incubated with 120 μl nuclei extract, 1.0 μmole pyrophosphate, 10 μmoles $MgCl_2$ and 780 μl 0.1 *M* TRIS buffer pH 7.8 for 30 min at 20°. Glucose-1-phosphate liberated was estimated by the addition of 2.5 μmoles cysteine, 30 μl phosphoglucomutase, 100 μl liver glucose-6-phosphate dehydrogenase and 0.25 μmoles TPN. *Curve 1* indicates the change in E_{340} due to glucose-1-phosphate and *Curve 1A* is the control without UDPG. At point *X* the UTP produced was estimated by the addition of 1 mg yeast Zwischenferment preparation which contains nucleoside diphosphokinase, 20 μl yeast hexokinase, 10 μmoles glucose and 0.1 μmoles ADP, the ADP being added at point *Y*. The change in E_{340} (*Curve 2*) is due to the glucose-6-phosphate produced in the hexokinase reaction. *Curve 2A* is the control without UDPG. – *Curve 3.* 0.16 μmole UDPAG was incubated with 120 μl nuclei extract, 1.0 μmole pyrophosphate, 10 μmoles $MgCl_2$ and 780 μl 0.1 *M* TRIS buffer pH 7.8 for 30 min at 20°. The UTP produced was estimated as above. *Curve 3A* is the control without UDPAG.

* British Council Scholar (from Institute of Biology, University of Mexico City).

** Beit Memorial Research Fellow.

*** The following abbreviations are used throughout: UDPG = uridine diphosphoglucose, UDPAG = uridine diphosphoacetylglucosamine, UTP = uridine triphosphate, UDP = uridine diphosphate, UMP = uridine monophosphate, P–P = inorganic pyrophosphate, DNA = deoxyribonucleic acid, ADP = adenosine diphosphate, TPN = triphosphopyridine nucleotide.

to a final concentration of 0.14 *M* with adjustment of the pH to 7.0. The mixture was allowed to stand at 0° for 1 hour and the precipitate removed by centrifugation at 4000 g for 30 minutes. The supernatant (24 ml) was used as the uridyl transferase preparation.

The uridyl transferase activity of this preparation using UDPG and UDPAG as substrates was followed spectrophotometrically as shown in Fig. 1.

From curves 1 and 2 it was calculated that the quantity of nuclei extract used caused a complete transformation of UDPG into UTP and glucose-1-phosphate in 30 minutes, whereas in the case of UDPAG (Curve 3) only 50% had been pyrophosphorylated to UTP in the same time. This indicates that the reaction with UDPG is at least twice as fast as with UDPAG.

In addition it has been found that the nuclei extract does not cause any breakdown of UTP and that the breakdown of UDPG and UDPAG to UDP and UMP is very small. It would appear that the enzymes responsible for these reactions[2] are lost in the extraction procedure.

Further work is in progress on the purification of the uridyl transferase of liver.

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ISOLIERUNG VON FREIEN NUCLEOTIDEN AUS VERSCHIEDENEN GEWEBEN

II. ISOLIERUNG DER 5'-MONO-, DI- UND TRIPHOSPHATE VON ADENOSIN, GUANOSIN, CYTIDIN UND URIDIN AUS DEM ASCITESTUMOR DER MAUS

von

HANNS SCHMITZ

Institut für experimentelle Krebsforschung der Universität, Heidelberg (Deutschland)

In früheren Arbeiten[1-8] wurde gezeigt, dass in dem säurelöslichen Extrakt von normalen und Tumor-Geweben neben den bekannten Mono-, Di- und Triphosphaten von Adenosin auch die analogen Phosphorsäureester von Guanosin, Cytidin und Uridin vorkommen. Untersuchungen über den Einbau von ^{14}C-markierten Vorläufern für die Nucleinsäuren führten zu dem Schluss, dass die 5'-Mono-, Di- und Triphosphate von Adenosin, Guanosin, Cytidin und Uridin neben ihren bekannten — und zum Teil noch unbekannten — Funktionen als Coenzyme wichtige Intermediärprodukte im Rahmen der Nucleinsäuresynthese darstellen. Es erschien daher wünschenswert, weitere Tumoren auf ihren Gehalt an freien Nucleotiden zu untersuchen um festzustellen, ob sich bösartige Tumoren aufgrund ihres Gehaltes an diesen Verbindungen unterscheiden liessen.

Der bei 0° hergestellte perchlorsaure Extrakt aus den Zellen (4.2 g Frischgewicht) des Ascitestumors der Maus wurde mit KOH neutralisiert und nach Abscheidung des $KClO_4$ an einen Anionenaustauscher (Dowex-1, Formiatform, 0.8 × 19 cm) adsorbiert, von dem die verschiedenen Nucleotide nach einer bereits beschriebenen Methode[9-10] eluiert wurden.

Die Analyse des in Fig. 1 dargestellten Chromatogramms ergab, dass die freien 5'-Mono-, Di- und Triphosphate von Adenosin, Guanosin, Cytidin und Uridin auch im Ascitestumor vorhanden sind. Im Gegensatz zu anderen untersuchten Tumoren (Flexner-Jobling- bzw. Walker-Carcinom) wurden Uridindiphosphatderivate wie UDP-Acetylglukosamin, UDP-Glukose, UDP-Galaktose nur in sehr geringen Mengen gefunden. Die drei genannten UDP-Derivate entsprechen den zwei kleinen UV-Absorptionsmaxima, die in der Fig. 1 dem Guanosin-5'-Diphosphat vorangehen. Das in den anderen Tumoren, wie auch in Leber und Hirn, gefundene UDP-Derivat UDP-Glukuronsäure konnte im Ascitestumor überhaupt nicht sicher nachgewiesen werden. Im Vergleich zum Walker-Carcinom