ACTA CHEMICA SCANDINAVICA 9 (1955) 1537—1539

Note on the Transphosphorylation Reaction between Uridine Monophosphate and Adenosine Triphosphate

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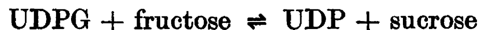
Spectrophotometric demonstration of uridine diphosphate is described. By use of adenosine triphosphate, ^{32}P-labelled in the terminal phosphate group, it is shown that the phosphorylation of uridine monophosphate by adenosine triphosphate, as catalyzed by yeast extract, is a transfer of one phosphate group, yielding uridine diphosphate and adenosine diphosphate.

A transphosphorylating reaction between mononucleotides and trinucleotides has at the same time been observed in several laboratories [1-4], and the appropriate enzymes have been demonstrated in yeast as well as in liver. So far it has been established that uridine-, cytidine-, guanosine-, and adenosine phosphates participate in reactions of this kind, but the specificity of the enzyme or enzymes is not yet known. In the following is described the demonstration of the following reaction *:

$$\text{UMP} + \text{ATP} \rightarrow \text{UDP} + \text{ADP}$$

It was noticed that incubation of UMP and ATP with yeast extract resulted in formation of UDP and UTP, which could be separated by ionophoresis from other nucleotides present. The ionophoresis was carried out in 0.02 M citrate buffer, pH 4.5, and a voltage of 220 volts was applied for 22 hours at 4° C. By this procedure the uridine di- and triphosphates are easily separated from the corresponding adenine nucleotides (*cf.* Ref. [5]).

UDP was identified by reversing the transglycosidic reaction, described by Leloir and Cardini [6], by which sucrose is synthesized from UDPG and fructose:



$$\text{UDPG} + \text{fructose} \rightleftharpoons \text{UDP} + \text{sucrose}$$

* The following abbreviations are used: UMP for uridine monophosphate, ATP for adenosine triphosphate, UDP for uridine diphosphate, ADP for adenosine diphosphate, UTP for uridine triphosphate, UDPG for uridine diphosphoglucose, TPN for triphosphopyridine nucleotide, G-6-P for glucose-6-phosphate, G-1-P for glucose-1-phosphate and PP for inorganic pyrophosphate.

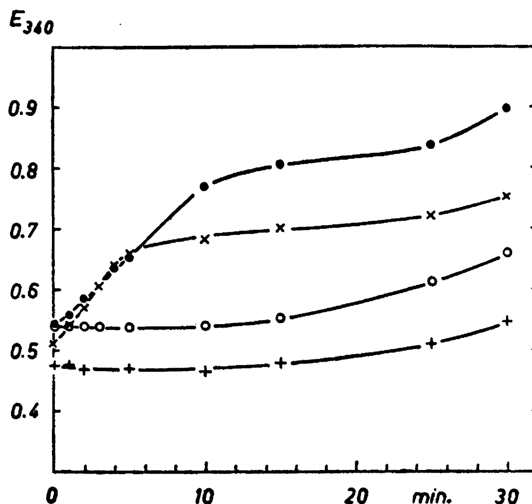


Fig. 1. Spectrophotometric demonstration of UDP.

The complete reaction mixture (in a 1 ml quartz cuvette) contained 1 ml 0.05 M tris-hydroxymethyl amino methane, HCl, pH 7.3, 200 ul wheat germ enzyme (0.3 mg protein/ml), 100 μl sucrose (0.2 M), and 0.2 μmole UDP. The mixture was incubated 20 min. at 30° C, and after cooling to room temperature the following were added (*cf.* Ref.[7]): 25 μl phosphoglucomutase (3 mg protein/ml), 25 μl cysteine (10 mg/ml), 10 μl G-6-P dehydrogenase, containing also the UDPG pyrophosphorylase, 20 μl TPN (0.01 M), and finally 10 μl PP (0.1 M) to start the reaction, which was followed spectrophotometrically at 340 mμ.

+ — + — + UDP omitted, ○ — ○ — ○ sucrose omitted,
× — × — × known UDP added, ● — ● — ● UDP from paper eluate (see text).

The enzyme was prepared from wheat germ and purified to the extent described by Leloir. The resulting enzyme solution contained 0.3 mg protein per ml and was stable for several weeks when kept at —20° C. By incubating UDP with this enzyme and excess sucrose at pH 7.0, UDPG is formed, and by addition of TPN, UDPG pyrophosphorylase, G-6-P dehydrogenase, phosphoglucomutase and pyrophosphate the UDPG could be demonstrated spectrophotometrically at 340 mμ [7].

In Fig. 1 are shown the curves obtained with authentic UDP and with paper eluate of the suspected UDP. Controls omitting either of the reactants were run simultaneously. It should be emphasized that this assay cannot be used for quantitative determination of UDP, as the equilibrium of the reaction lies too far towards sucrose.

The UTP formed during the incubation of ATP and UMP with the yeast extract was identified together with catalytic amounts of ADP, according to the spectrophotometric method of Berg and Joklik [5].

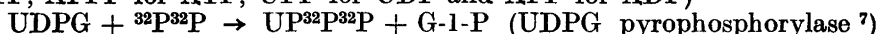
It was left to decide whether the reaction was a transfer of pyrophosphate with subsequent partial dephosphorylation of UTP to UDP, or a transfer of one phosphate group to form UDP which then by the action of nucleoside diphosphokinase was phosphorylated to UTP. For this purpose ATP, labelled

Table 1. Transfer of labelled phosphate from ATP to UMP.

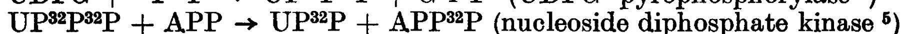
The reaction mixture contained, in a total volume of 1 ml, 0.5 μmole ATP, 8.1×10^3 cts/min/μmole, 0.6 μmole 5-UMP, 50 μl enzyme solution (8 mg protein/ml), 0.005 μmole $MgCl_2$, 0.05 M tris-hydroxymethyl amino methane, HCl, pH 7.3.

Nucleotide analyzed	cts/min/μmole
ATP	7 250
UDP	7 780
ADP	0

in the terminal phosphate group, was prepared from UDPG, ^{32}P-labelled pyrophosphate and ADP, according to the equations below: (UPPP stands for UTP, APPP for ATP, UPP for UDP and APP for ADP)



$$\text{UDPG} + {}^{32}\text{P}^{32}\text{P} \rightarrow \text{UP}^{32}\text{P}^{32}\text{P} + \text{G-1-P} \quad \text{(UDPG pyrophosphorylase [7])}$$

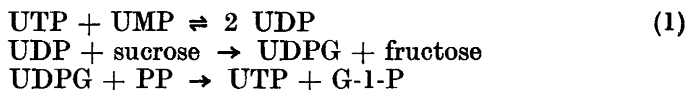


$$\text{UP}^{32}\text{P}^{32}\text{P} + \text{APP} \rightarrow \text{UP}^{32}\text{P} + \text{APP}^{32}\text{P} \quad \text{(nucleoside diphosphate kinase [5])}$$

As source of enzymes a G-6-P dehydrogenase preparation (LePage and Mueller [8]) was used, which contained abundant amounts of the UDPG pyrophosphorylase as well as of nucleoside diphosphate kinase. The ATP was isolated from the reaction mixture by adsorption on norite and elution with 50 % ethanol, followed by paper chromatography and elution of the ATP spot [7].

The labelled ATP was incubated with UMP in presence of the yeast enzyme. After the reaction was stopped, the nucleotides were isolated by paper ionophoresis, eluted and analyzed with respect to concentration and radioactivity. The results are presented in Table 1 and show clearly, that the reaction involves a transfer of only the terminal phosphate group of ATP to UMP, resulting in the formation of UDP with the same activity as the initial ATP.

Attempts to demonstrate, spectrophotometrically, by means of Leloir's enzyme and UDPG pyrophosphorylase, the following sequence of reactions:



$$\begin{aligned} &\text{UTP} + \text{UMP} \rightleftharpoons 2\ \text{UDP} \qquad (1) \\ &\text{UDP} + \text{sucrose} \rightarrow \text{UDPG} + \text{fructose} \\ &\text{UDPG} + \text{PP} \rightarrow \text{UTP} + \text{G-1-P} \end{aligned}$$

were negative, although reaction (1) has been reported to proceed in analogy with the myokinase reaction, in the presence of yeast extract [1].

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Received August 18, 1955.