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URIDYL TRANSFERASES, THEIR OCCURRENCE AND PHYSIOLOGICAL ROLE

BY

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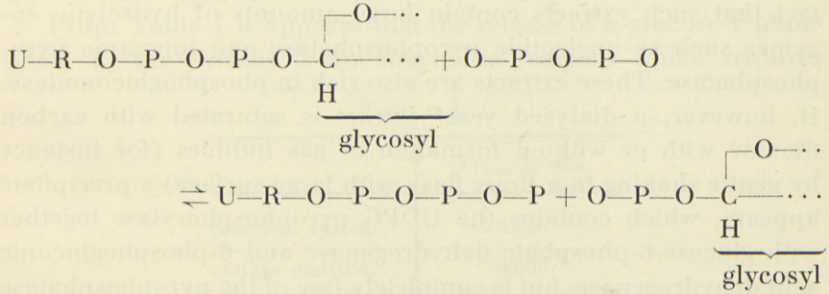
LELOIR *et al.* (1) in 1950 reported the isolation and identification of uridine diphosphoglucose as a coenzyme for the enzymic conversion of glucose-1-phosphate in extracts of galactose-adapted yeast (*Saccharomyces fragilis*). Since then increasing interest has centered around the uridine compounds, and the isolation of a number of other uridine compounds have already been reported (2), (3), (4), (5), (6), (7) as well as the demonstration of enzymic reactions in which these compounds participate (8), (9), (10), (11), (12). Mention will be made here only of one other uridine diphosphoglycosyl compound, which is present in yeast together with UDPG and was identified by LELOIR as uridine diphospho N-acetylglucose-amine (13).

In the present article we will describe an enzymic reaction which gives rise to the formation of uridine triphosphate from UDPG. The enzyme which we have classified as a uridyl transferase most likely brings about the following type of reaction:

$$\text{UDPG} + \text{PP} \rightleftharpoons \text{UTP} + \text{G-1-P} \quad (1)$$

or, more general



The following abbreviations are used: UDPG for uridine diphosphoglucose, UTP for uridine triphosphate, UDP for uridine diphosphate, UMP for uridine monophosphate, UDPAG for uridine diphospho N-acetylglucosamine, UDPGal for uridine diphosphogalactose, ATP for adenosine triphosphate, ADP for adenosine diphosphate, TPN for triphosphopyridine nucleotide, DPN for diphospho pyridine nucleotide, G-1-P for glucose-1-phosphate, gal-1-P for galactose-1-phosphate and PP for pyrophosphate.

This particular type of reaction has been observed as an important step reaction in the reversible biosynthesis of diphospho pyridine nucleotide from adenosine triphosphate and nicotinamide monoclucleotide (14), and has been called pyrophosphorolysis (14). In a similar manner flavine adenine dinucleotide can undergo pyrophosphorolytic cleavage, producing ATP and flavine mononucleotide (15). These enzymes bring about a transfer of the adenyl group and may consequently be termed adenyl transferases (16).

In this paper a detailed account will be given of the uridyl transferase reaction, and evidence will be presented that uridine triphosphate is a primary product of pyrophosphorolysis of UDPG and correspondingly that UTP incubated with α-glucose-1-phosphate in the presence of the same enzyme can give rise to the formation of UDPG. Methods for quantitative and highly specific determination of UDPG will also be presented.

Enzyme preparation.

The UDPG pyrophosphorylase is present in extracts from fresh baker's yeast as well as from dry yeast. Assay of the enzyme, as described in detail below, is based upon the determination of the glucose-1-phosphate liberated in reaction (1). If unfractionated extracts from yeast are used, observations are complicated by the fact that such extracts contain large amounts of hydrolytic enzymes such as nucleotide pyrophosphatase and inorganic pyrophosphatase. These extracts are also rich in phosphoglucomutase. If, however, a dialyzed yeast extract is saturated with carbon dioxide with or without formation of gas bubbles (for instance by gentle shaking in a Roux flask with large surface) a precipitate appears, which contains the UDPG pyrophosphorylase together with glucose-6-phosphate dehydrogenase and 6-phosphogluconic acid dehydrogenase, but is completely free of the pyrophosphatase as well as the phosphoglucomutase.

It is in general more advisable to use Warburg-Christian's Zwischenferment (17) (18), dried over phosphorus pentoxide and stored as a powder. This preparation is not only devoid of pyrophosphatase and phosphoglucomutase, but also of the 6-

phosphogluconate dehydrogenase. The pyrophosphorylase as well as the clucose-6-phosphate dehydrogenase are insoluble in water, but soluble in dilute salt solutions. Precipitation at 45 % saturation with ammonium sulphate removes large amounts of nucleoprotein and pigments, and further addition of ammonium sulphate to 60 % saturation accomplishes a precipitation of the active fraction. This fraction is dissolved in 0.05 M Tris-hydroxymethyl-aminomethane, pH 7.8; 10 mg. protein per ml. yields a clear solution which is stable with respect to the pyrophosphorylase for a couple of days when stored at 0°.

As will be described in another article by one of us (A. M-P) it is possible not only to obtain glucose-6-phosphate dehydrogenase which is free of uridyl transferase, but also to obtain highly active UDPG pyrophosphorylase without glucose-6-phosphate dehydrogenase and with very high specific activity. This was accomplished through ethanol fractionation (see (19)).

UDPGlucose assay.

The assay, as performed routinely, is an estimation of α-glucose-1-phosphate liberated from UDPGlucose upon addition of the UDPG pyrophosphorylase and inorganic pyrophosphate. The indicator system consists of TPN, phosphoglucomutase and glucose-6-phosphate dehydrogenase; the equimolar reduction of TPN is measured as an increase in density at 340 mμ.

From Table I it appears that the release of α-glucose-1-phosphate in the system is an enzymatic reaction which requires

Table I.

	ΔE_{340}/30 min.
complete system . .	0.080
mutase omitted . . .	0.003
UDPG omitted . . .	0.009
PP omitted	0.000

Participation of UDPG and PP in the uridyl transferase reaction.
Complete system: 1 ml 0.05 ml tris-hydroxymethyl aminomethane HCl, pH 7.5, 0.02 μ mol UDPG, 0.5 μmol PP. 0.5 μmol P, 0.5 μmol TPN, 10 μl mutase (0.5 mg/ml), 100 μl Zwischenferment (0.5 mg/ml).

UDPGlucose as an α-glucose-1-phosphate donor and inorganic pyrophosphate as an uridyl acceptor. Hence the reaction is decidedly a pyrophosphorolysis and not a phosphorolysis. For each 0.1 micromole/ml of UDPGlucose undergoing fission an increase of 0.622 (20) in density at 340 mμ should be observed if glucose-6-phosphate is oxidized only one step, i.e. to 6-phosphogluconic acid. This is the case in most Zwischenferment preparations as well as in assays performed with the more purified UDPGlucose pyrophosphorylase and glucose-6-phosphate dehydrogenase.

However, in unfractionated extracts from dry yeast such as are used in many experiments with *S. fragilis*, a more than one step oxidation of glucose-6-phosphate is encountered; this is also the case in the active CO_2-precipitate obtained by shaking as described above. Therefore, if a known amount of α-glucose-1-phosphate is added to the reaction mixture, it turns out that the molar increase in extinction at 340 mμ reaches a higher value than expected, but is not quite doubled. In order to get reproducible values under the latter circumstances it is advisable to add excess phosphogluconate dehydrogenase to bring about an almost complete oxidation of 6-phosphogluconic acid. In this case the average value of the factor is 1.75, i. e. for each micromole glucose-1-phosphate metabolized 1.75 micromoles of TPN is reduced (cf. (21)). The same applies if this indicator system is used in connection with the pyrophosphorolysis of UDPGlucose as seen in Table II.

As illustrated in a later section, the assay system here described has served as a specific and quantitative micromethod for deter-

TABLE II.

substrate added	ΔE_{340} calculated	ΔE_{340} found	factor	μmol true UDPG $\left(\frac{\Delta E_{340}}{0.622 \times 1.76}\right)$
0.1 μmol G-1-P	0.622	1.085	1.75	
0.04 μmol G-1-P ...	0.249	0.443	1.77	
0.04 μmol UDPG ..		0.405	(1.76)	36.8
0.02 μmol UDPG ..		0.203	(1.76)	18.4

Analysis of UDPG with crude enzyme solutions.

mination of UDPGlucose. With the recent isolation of UDPG-dehydrogenase (22) an independent and highly sensitive alternative specific method has become available.

Identification of UTP.

The fact that inorganic pyrophosphate is required in stoichiometric amounts points strongly towards the possibility of a pyrophosphorolysis (Table I). This is further supported by the fact that radioactive pyrophosphate could be shown to be incorporated gradually into a new compound which is adsorbable on norite. If the norite is eluted with 50 % ethanol, containing 0.1 % concentrated ammonia, and this eluate is subjected to paper

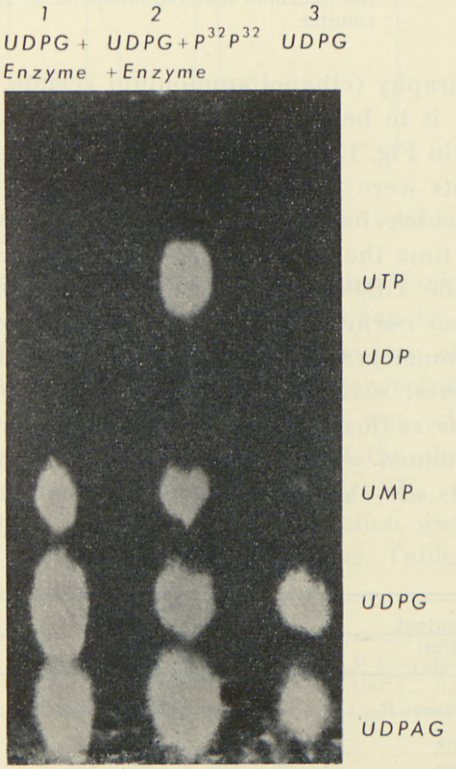


Fig. 1. Chromatogram, showing the formation of UTP from UDPG and PP. (Photographed in ultraviolet light).

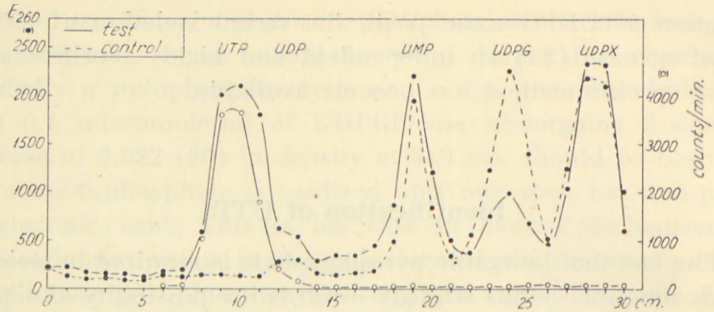


Fig. 2. Paper chromatogram of norite eluate from UDPG-pyrophosphorylase digest.
Reaction mixture: 0.2 μmol UDPG, 2 μmol inorganic pyrophosphate (8×10^4 cts./min.), 50 μl Zwischenferment (3 mg/ml), 3 ml tris-hydroxymethyl aminomethane HCl, pH 8.0, M/10, $MgCl_2$ M/100.
Control mixture: Same without pyrophosphate.
After 45 min. incubation the digest were acidified, adsorbed on norite and eluted with 50 % ethanol. Chromatographed 44 hours in neutral solvent (23). Chromatogram scanned in the Beckman spectrophotometer at 260 mμ and in the Geiger counter.

chromatography (ethanol/ammonium acetate, pH 7.5 (23); a 40 hours run is to be recommended), a chromatogram is obtained as shown in Fig. 1. It is seen that in the sample in which all the components were present during the incubation a marked spot appears which migrates slower than uridine diphosphate. At the same time the UDPGlucose spot has decreased in intensity whereas the UDPAG spot has essentially remained unaltered. This is also borne out by the results obtained by scanning the paper chromatogram with respect to ultraviolet absorption (Fig. 2). Moreover, scanning for radioactivity shows (cf. same Fig.) that all the radioactivity is confined to the new spot. The new spot was eluted with distilled water and subjected to analyses. The results are shown in Table III. The UTP-concentration of

Table III.

UTP-content μmol/ml	counts/min/μmol		P_7 μg/ml	
(calculated from E_{260})	UTP	P^{32}-P^{32} added	found	calculated from E_{260}
0.133	42×10^3	40×10^3	8.95	8.23

Analysis of UTP-solution, obtained from paper chromatogram (Fig. 1).

the solution was derived from the absorption value at 260 mμ, and it is seen that with respect to both radioactivity and 7 min.'s hydrolysable phosphate the values found agree with those calculated for UTP.

When larger amounts of UTP were prepared, the incubation mixture was adsorbed on an anion exchange column (Dowex

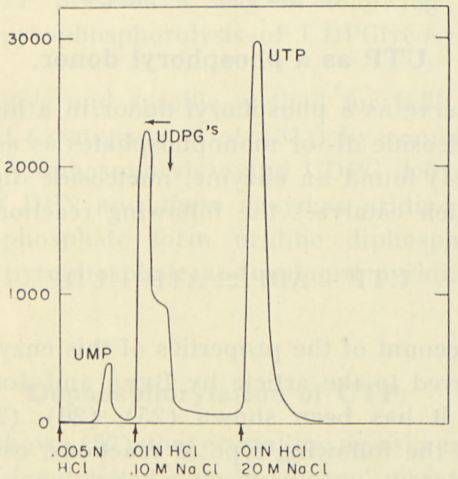


Fig. 3. Elution of UTP from a Dowex 2 Cl^- column.

No. 1) and eluted with solvents of increasing ionic strength. The results are shown in Fig. 3. In general it can be stated that UTP requires 0.2 M sodium chloride or potassium chloride for elution, while ATP is eluted at lower salt concentrations. The uridine polyphosphate present in the eluate was adsorbed on charcoal which was washed with water, and the charcoal was eluted with 50 % ethanol, containing 0.1 % NH_3. After evaporation of the ethanol the concentrated solution was analyzed for uracil and for total and labile phosphorus. Table IV shows

Table IV.

uracil/PP	uracil/P total	% P liberated per hour at 100° C., 0.01 M HCl
1.18	2.7	50

Analysis of uridine polyphosphate, eluted from Dowex 2 Cl^- with 0.01 M HCl, 0.2 M NaCl (Fig. 3).

that the new compound contains close to three phosphorus atoms per uracil molecule, and that the two phosphate groups are acid labile, although less so than those of ATP. It is known that UDP possesses an organic pyrophosphate linkage which likewise is more acid stable than the corresponding linkage in ADP (cf. LELOIR *et al.* (1)).

UTP as a phosphoryl donor.

UTP can serve as a phosphoryl donor in a number of reactions with nucleoside di- or monophosphates as acceptors. BERG and JOKLIK (24) found an enzyme, nucleoside diphosphokinase ('nudiki'), which catalyzes the following reaction:

$$UTP + ADP \rightleftharpoons ATP + UDP$$

For detailed account of the properties of this enzyme system the reader is referred to the article by BERG and JOKLIK (24).

Moreover, it has been shown (25), (26), (27), that UTP participates in the following type of reactions, catalyzed by enzymes present in yeast and liver extracts.

$$UTP + 5\text{-}AMP \rightleftharpoons ADP + UDP$$

$$ATP + UMP \rightleftharpoons ADP + UDP$$

These enzymes were named 5-nucleotide kinases (28).

In those reactions, i. e. the 'nudiki' and the 5-nucleotide kinases, UTP serves as a primary phosphoryl donor. However, in a number of other transphosphorylating reactions UTP seems to play a secondary role only as shown by BERG and JOKLIK (28). In the creatine kinase UTP is totally inactive without the ADP-'nudiki' system as participants, i. e. ATP is the primary phosphoryl donor. The same seems to be the case with yeast hexokinase although a very slow direct reaction seems not to be excluded.

UTP can be formed from UDP by phosphorylation with phosphopyruvate (KORNBERG (29)). It is not yet quite certain whether this phosphorylation is direct or goes through the ATP-ADP system.

Enzymic assay of UTP.

Assay of UTP can be carried out by the method of BERG and JOKLIK (24) using nucleoside diphosphokinase and catalytic amounts of ADP. The ATP formed is estimated by the hexokinase method of KORNBERG and PRICER (30). This method of determining UTP presents a way of following spectrophotometrically the pyrophosphorolysis of UDPGlycosyls other than UDPG.

A new sensitive and specific method for UTP estimation is being developed (ANDERSON *et al.* (31)) by combining two enzymes, UDPG pyrophosphorylase and UDPG dehydrogenase. In the presence of DPN and these enzymes uridine triphosphate plus glucose-1-phosphate form uridine diphospho glucuronic acid, inorganic pyrophosphate and reduced pyridine nucleotide.

Dephosphorylation of UTP.

It has been shown (32), that crystalline myosin and the 'heavy' fraction of trypsine-digested myosin ('heavy' meromyosin) bring about a dephosphorylation of UTP which is 3 to 5 times faster than that of ATP. The mechanism of this dephosphorylation must be more complex than one would anticipate, and will be the subject of discussion in a separate paper (33). STROMINGER *et al.* (34) have found recently that extract from acetone powder of pigeon liver brings about a dephosphorylation of UTP and UDP which is about 20 times faster than that of ATP to ADP. The mechanism of this reaction is definitely complex; it seems that UDP and not UTP serves as a phosphoryl donor in this system.

Action of UTP on various biological systems.

The reported action of UTP on the superprecipitation of actomyosin and on bioluminescence seems most likely to be indirect and to go through the ATP system by means of 'nudiki' although the mechanism of action in the case of actomyosin is still under discussion.

UTP as uridyl donor.

The transfer of the uridyl moiety of UTP to acceptors like α-glucose-1-phosphate and α-galactose-1-phosphate seems to be a specific reaction for UTP.

$$UTP + \alpha\text{-glucose-1-phosphate} \rightarrow UDPGlucose + PP$$

If UTP is incubated with α-glucose-1-phosphate in the presence of the uridyl transferase described above, UDPGlucose is

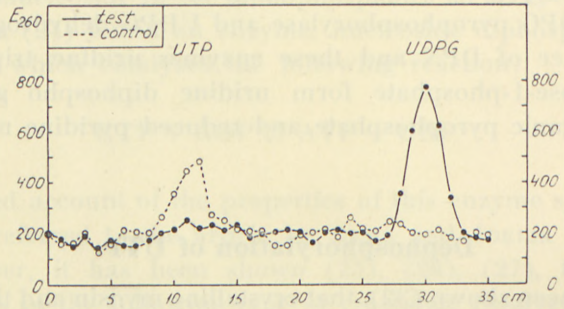


Fig. 4. Paper chromatogram of norite eluate from UTP-pyrophosphorylase digest.
Reaction mixture: 0.2 μmol UTP, 10 μmol α-glucose-1-phosphate, 100 μl inorganic pyrophosphatase, 50 μl Zwischenferment (3 mg/ml), 1 ml tris-hydroxymethyl aminomethane HCl, pH 8.0, M/10, $MgCl_2$ M/100.
Control mixture: Same without α-glucose-1-phosphate.
After 50 min. incubation the digests were acidified, adsorbed on norite and eluted with 50 % ethanol. Chromatographed 44 hours in neutral solvent (23). Chromatogram scanned in the Beckmann spectrophotometer at 260 mμ.

In the norite filtrates inorganic phosphate was precipitated as Mg-NH_4 salt, washed with dilute NH_3 and dissolved in 150 μl 0.2 M H_2SO_4. Counting of the samples in the Geiger counter gave the following results, expressed as counts per minute:

Control	Test
186	3660

formed as appears from the chromatogram scanned in U. V. (Fig. 4). It can be seen that in the sample which is incubated with all components present, the UTP spot has disappeared, and a U.V. absorbing spot located corresponding to UDPGlucose has appeared. The formation of UDPGlucose can also be demonstrated by enzymatic assay of the digest. It is seen from Table

TABLE V.

	% G-1-P consumed	μmoles present in norite-eluate		% UDPG formed
		UDPG	UV-absorbing compound	
control		0.00	0.200	
complete.....	46	0.135	0.315	43

Enzymatic synthesis of UDPG.

Equimolar amounts of UTP and G-1-P were incubated with the UDPG-pyrophosphorylase. After 30 min. the digest was acidified, spun and treated with norite. In the norite filtrate G-1-P was determined by addition of phosphoglucomutase, Zwischenferment and TPN. The norite eluate was analyzed for UDPG by means of pyrophosphorylase, phosphoglucomutase, Zwischenferment and TPN. In the control G-1-P was omitted.

V that the α-glucose-1-phosphate was consumed to an extent which corresponds to a reaction in which half of the UTP is consumed. The assay of the norite eluate with UDPGlucose pyrophosphorylase showed correspondingly that 43 % of the total uridine compounds appeared as UDPGlucose in the filtrates from the complete digest.

The application of a P^{32}-labeled uridyl acceptor was used as a third approach. In this case, illustrated in Fig. 5, P^{32}-labeled

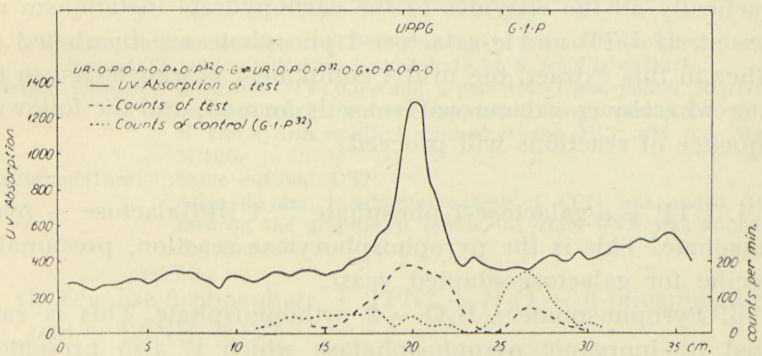


Fig. 5. Formation of P^{32}-labeled UDPG.

Paper chromatogram showing the reaction of UTP with G-1-P^{32}. Abscissa: Distance from starting line; ordinate: Extinction × 10^3 at 260 mμ. Reaction mixture: 0.5 μmol UTP, 8.5 μmol G-1-P^{32} (6500 cts./min.), 100 μl inorganic pyrophosphatase, 50 μl Zwischenferment (3 mg/ml), 1 ml tris-hydroxymethyl aminomethane HCl, pH 7.5, M/10. Control mixture: Same without UTP. After 45 min. incubation the digest were treated as described in Fig. 2. Chromatographed 22 hours in acid solution (23).

α-glucose-1-phosphate was used as uridyl acceptor and UTP as uridyl donor. It can be seen that in the filtrate from the complete digest an intensive U.V. absorbing spot carrying radioactive P^{32} appears at the location corresponding to UDPGlucose. At the same time the radioactivity at the location corresponding to glucose-1-phosphate has decreased markedly. Experiments with various uridyl acceptors and in which the UTP phosphorus is P^{32}-labeled show that larger amounts of pyrophosphate were liberated when an active uridyl acceptor was present than in a control without acceptor. In this way it could be shown that a Zwischenferment from ordinary brewer's yeast with UTP as uridyl donor could not use only α-glucose-1-phosphate as acceptor, but also, although to a much smaller degree, α-galactose-1-phosphate; β-glucose-1-phosphate, however, was totally inactive. In *S. fragilis* the same technique can be used.

By means of the recently isolated UDPG-dehydrogenase (22) the *de novo* formation of UDPG can be followed spectrophotometrically.

The fourth approach was to see whether UTP could serve as precursor for co-galacto-waldenase in a dialyzed extract of galactose-adapted yeast. In such an unfractionated dialyzed extract from *S. fragilis* a number of co-enzymes are absent, but practically all the enzymes of the carbohydrate metabolism are present. If UTP and α-galactose-1-phosphate are incubated together in this extract, the uridyl component is transferred to the latter whereby co-galacto-waldenase is formed, and the following sequence of reactions will proceed:

i. UTP + α-galactose-1-phosphate $\rightleftarrows$ UDPGalactose + pyrophosphate. This is the pyrophosphorylase reaction, presumably specific for galactose-adapted yeast.

ii. Pyrophosphate + $H_2O \rightarrow$ 2 orthophosphate. This is catalyzed by inorganic pyrophosphatase which is also present in crude *S. fragilis* extracts.

iii. UDPGalactose $\rightleftarrows$ UDPGlucose. This is catalyzed by an inversion enzyme (Galacto-waldenase), specific for galactose-adapted yeast (35).

iv. UDPGlucose + α-galactose-1-phosphate $\rightleftarrows$ UDPGalactose

+ α-glucose-1-phosphate. This is catalyzed by a non-pyrophosphorolytic uridyl transferase presumably specific for galactose-adapted yeast (11).

v. α-glucose-1-phosphate ⇄ glucose-6-phosphate; catalyzed by phosphoglucomutase, which is also present in unfractionated *S. fragilis* extracts. Glucose-6-phosphate accumulates, and upon addition of TPN a very rapid reduction takes place corresponding to step vi.

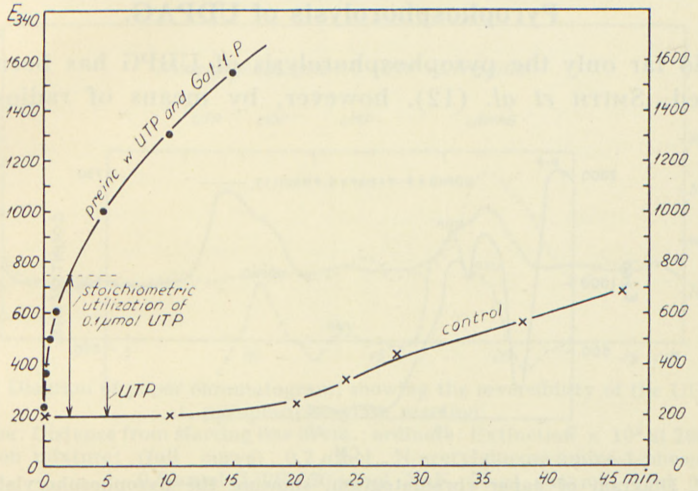


Fig. 6. Spectrophotometric analysis (TPN reduction) of glucose-6-phosphate formed from UTP and α-galactose-1-phosphate in *S. fragilis* extract.

Reaction mixture: 0.1 µmol UTP, 0.6 µmol α-galactose-1-phosphate, 50 µl dialyzed *S. fragilis* extract (approx. 2,5 mg protein), 500 µl 0.1 M tris-hydroxymethyl aminomethane HCl, pH 8.0, $MgCl_2$ M/100.

Control mixture: Same without UTP.

After 30 min. incubation excess of TPN was added (time zero on the graph). At 5 min. 0.1 µmol UTP was added to the control.

vi. Glucose-6-phosphate + TPN^+ + H_2O → 6-phosphogluconic acid + TPNH + H^+.

That the reaction, as measured by the reduction of TPN, proceeds beyond stoichiometric amount of UTP added (see Fig. 6), is caused by the non-pyrophosphorolytic uridyl transferase in step iv, which in conjunction with step iii tends to convert all the α-galactose-1-phosphate present into glucose-1-phosphate.

Incubation of extracts of *S. fragilis* with each component separately does not yield glucose-6-phosphate.

The presence of 'nudiki' in crude *S. fragilis* extracts in addition to the six other step enzymes mentioned above should account for the fact that ATP + UDP + α-galactose-1-phosphate also bring about formation of co-galacto-waldenase (36).

Pyrophosphorolysis of UDPAG.

So far only the pyrophosphorolysis of UDPG has been discussed. Smith *et al.* (12), however, by means of radioactive

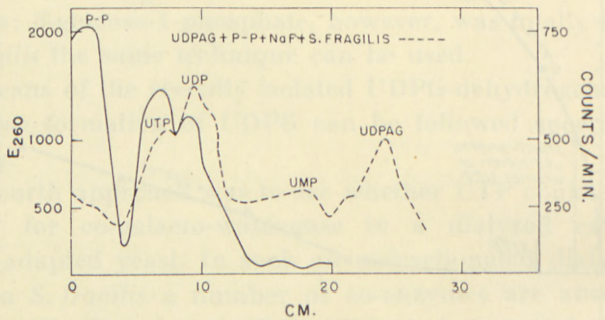


Fig. 7. Diagram of paper chromatogram, showing the pyrophosphorylation of UDPAG in extract of *S. fragilis*.
Abscissa: Distance from starting line; ordinate: Extinction $\times 10^3$ at 260 mμ.
Reaction mixture: 0.2 μmol UDPAG, 1 μmol PP (54 $\times 10^3$ cts./min.), 20 μmol NaF, 150 μl enzyme, 1 ml tris-hydroxymethyl aminomethane HCl, pH 7.5, 0.05 M.
After 30 min. incubation the digest was treated as described in Fig. 2.
Legend: - - - - - - - - - - Extinction $\times 10^3$ at 260 mμ
———— counts/min.

pyrophosphate reported the presence in liver nuclei of an enzyme which catalyzes the pyrophosphorylation of uridine diphospho (-N-acetyl)-glucosamine. This enzyme has later been isolated and purified, and the pyrophosphorolysis was demonstrated spectrophotometrically (37).

Furthermore, it is found that yeast extracts catalyze the formation of UTP from UDPAG and PP; if UDPAG and P^{32}-labeled pyrophosphate are incubated with extracts of *S. fragilis*, the formation of radioactive UTP can be demonstrated by paper

chromatography and subsequent scanning of the chromatogram (Fig. 7). Fluoride was added to the incubation mixture to suppress the activity of inorganic pyrophosphatase which is present in abundant amounts in the crude extracts of *S. fragilis*.

Also this reaction can be reversed. N-acetyl-glucosamine-1-phosphate was prepared from purified UDPAG in the following way: 0.5 μmoles of UDPAG were hydrolyzed with 0.1 M $Ba(OH)_2$ for 15 min. and neutralized with H_2SO_4. The centrifuged solution

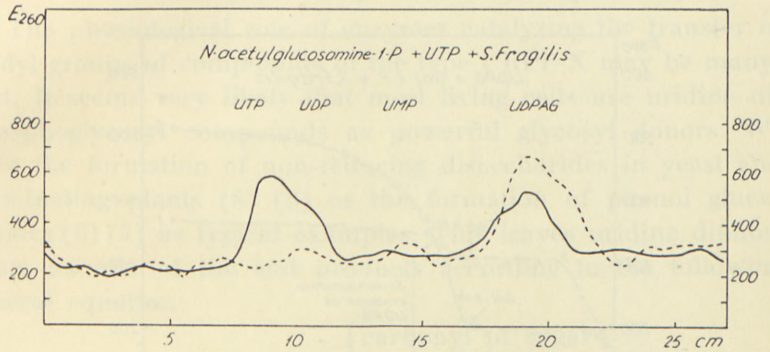


Fig. 8. Diagram of paper chromatogram, showing the reversibility of the UDPAG pyrophosphorylase reaction.
Abscissa: Distance from starting line in cm.; ordinate: Extinction $\times 10^3$ at 260 mμ.
Reaction mixture: (full curve) 0.2 μmol N-acetylglucose-amine-1-phosphate; 0.5 μmol UTP, 20 μmol NaF, 20 μl enzyme, 1 ml 0.05 M tris-hydroxymethyl aminomethane HCl, pH 7.5.
Control mixture: (dotted curve) 0.1 μmol UDPAG.
After 30 min. incubation the digest was treated as described in Fig. 2.

was passed through a column of Dowex 50 and evaporated to a small volume. The solution was chromatographed on paper for 20 hours in acid solvent (23). The N-acetyl-glucosamine-1-phosphate was localized by spraying a simultaneously run marker-chromatogram with $HClO_4$-molybdate mixture (38) followed by heating at 85° for 7 min. An aqueous eluate of the original spot contained no U.V. absorbing compounds. When the N-acetyl-glucosamine-1-phosphate was incubated with UTP in the presence of *S. fragilis* extract and sodium fluoride as above, formation of UDPAG could be demonstrated as seen in Fig. 8.

Non-pyrophosphorolytic transfer of uridyl groups.

It has been shown (11) that extracts of galactose-adapted *S. fragilis* contain an enzyme which catalyze the following reaction:

$$\text{UDPGlucose} + \alpha\text{-gal-1-P} \rightarrow \text{UDPGal} + \alpha\text{-G-1-P}$$

An example of this reaction is given in Fig. 9. It is seen (curve 2) that upon addition of gal-1-P to UDPG and with no

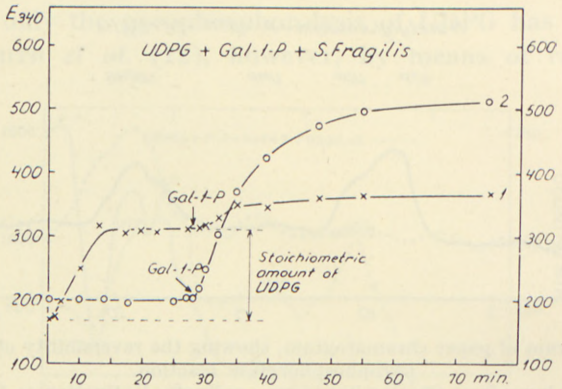


Fig. 9. Spectrophotometric demonstration of non-pyrophosphorolytic transfer of uridyl groups.
Abscissa: Time in min.; ordinate: Extinction $\times 10^3$ at 340 mμ.
Reaction mixture: 1. 0.025 μmol UDPG, 1 μmol PP, 30 μl dialyzed *S. fragilis* extract, 0.5 μmol TPN, 1 ml tris-hydroxymethyl aminomethane HCl, pH 8, 0.05 M.
2. same without PP.
After 28 min. incubation 0.5 μmol α-gal-1-P was added to each cuvette.

PP present a rapid reduction of TPN occurs, indicating that G-1-P is formed and subsequently converted to G-6-P and 6-phosphogluconic acid according to the sequence on page 14; here also the reaction will proceed beyond the stoichiometric amount of UDPG, due to the presence of galacto-waldenase. The stoichiometric amount of UDPG is derived from curve 2, where addition of PP causes pyrophosphorolysis of the compound with subsequent reduction of TPN. Addition of gal-1-P to this sample caused no appreciable further reduction of TPN, although a reaction between gal-1-P and the UTP formed was expected.

This must mean that either the proper uridyl transferase was not active in this particular *fragilis* extract, or, more likely, the UTP was rapidly broken down in the crude *fragilis* extract. Hence, at the time when gal-1-P was added, only small amounts of UTP were present, insufficient to yield significant amounts of UDPGal (and subsequent formation of UDPG) with gal-1-P.

Discussion.

The physiological role of enzymes catalyzing the transfer of uridyl groups of compounds of the type URPP-X may be manyfold. It seems very likely that most living cells use uridine diphosphoglycosyl compounds as powerful glycosyl donors. We have the formation of non-reducing disaccharides in yeast and germinating plants (8) (9) or the formation of phenol glucuronides (6) (7) as typical examples. This leaves uridine diphosphate as one of the end products according to the following general equation:

$$\text{U—R—P—O—P—O-glycosyl} + \left\{\begin{array}{c}\text{carbonyl of sugars}\\ \text{or}\\ \text{phenol groups}\end{array}\right. \rightleftharpoons \text{U—R—P—O—P—O} + \left\{\begin{array}{l}\text{nonreducing disac-}\\ \text{charides or}\\ \text{phenol glucuronides}\end{array}\right.$$

The 'discharged' uridine nucleotide (U-R-P-O-P-O, i.e. UDP) can be 'recharged' by the following enzymic mechanism operating in most cellular systems (24)

$$\text{U—R—P—O—P—O} + \text{A—R—P—O—P—O—P—O} \rightleftharpoons \text{U—R—P—O—P—O—P—O} + \text{A—R—P—O—P—O}$$

The UTP (U-R-P-O-P-O-P-O) reacts subsequently with α-glucose-1-phosphate (and presumably also with other glycosyl-1-phosphates) according to the equation:

$$\text{U—R—O—P—O—P—O} + \text{O—P—O-glycosyl} \rightleftharpoons \text{U—R—O—P—O—P-O-glycosyl} + \text{O—P—O—P—O}$$

Since the reaction is most adequately described as a transfer of the uridyl groups (U-R-O-P-) from pyrophosphate to a glycosyl-1-phosphate the class of enzymes was called uridyl transferases.

It can be seen that in the presence of ATP, the proper glycosylphosphate compound and glycosyl acceptor the uridine diphosphate acts catalytically, since it is formed through a cycle (the UDP cycle).

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