THE PRESENCE OF URIDINE PYROPHOSPHOGALACTOSE AND URIDINE PYROPHOSPHOGALACTOSE-4-EPIMERASE IN NON-GALACTOSE ADAPTED YEASTS

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INTRODUCTION

In 1950 CAPUTTO, LELOIR, CARDINI AND PALADINI[1] isolated from bakers yeast the coenzyme for the enzymic conversion of galactose-1-phosphate (Gal-1-P) into glucose-1-phosphate (G-1-P) in galactose adapted *Saccharomyces fragilis*. They showed this coenzyme to be uridine pyrophosphoglucose (UPPG) and in 1951 LELOIR[2] demonstrated the presence in galactose adapted *S. fragilis* of the enzyme galactowaldenase which established the equilibrium:

$$\text{UPPGlucose} \rightleftharpoons \text{UPPGalactose} \qquad (1)$$

LELOIR[2] found the equilibrium point of this reaction to be 75% UPPG and 25% UPPGalactose (UPPGal), and postulated that the Gal-1-P to G-1-P conversion was brought about by the two step reaction:

$$\text{UPPG} + \text{Gal-1-P} \rightleftharpoons \text{UPPGal} + \text{G-1-P} \qquad (2)$$

$$\text{UPPG} \rightleftharpoons \text{UPPGal} \qquad (1)$$

This postulate was shown to be correct by KALCKAR, BRAGANCA AND MUNCH-PETERSEN[3] who demonstrated reaction (2) in extracts of galactose adapted *S. fragilis*.

UPPG may be estimated by means of the pyrophosphorolytic split (reaction 3), catalysed by the enzyme uridyl transferase from yeast[1,5,6].

$$\text{UPPG} + \text{pyrophosphate} \rightleftharpoons \underset{\text{Triphosphate (UTP)}}{\text{Uridine}} + \text{G-1-P} \qquad (3)$$

In this case the G-1-P is estimated spectrophotometrically by conversion to glucose-6-phosphate (G-6-P) with subsequent oxidation by the triphosphopyridine nucleotide (TPN) linked glucose-6-phosphate dehydrogenase. Alternatively, UPPG may be estimated by the diphosphopyridine nucleotide (DPN) linked dehydrogenase from liver[7,8].

$$\text{UPPG} + 2\,\text{DPN}^+ \rightarrow \underset{\text{(UPP glucuronic acid}}{\text{uridine pyrophosphoglucuronic acid}} + 2\,\text{DPNH} \qquad (4)$$

In both of these reactions (3 and 4) the ΔE_{340} of the pyridine nucleotide reduction gives an estimate of the UPPG.

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UPPGal may be estimated by a combination of either reactions 1 and 3 or 1 and 4, extracts of galactose adapted *S. fragilis* usually being used as a source of the galactowaldenase, now more correctly termed UPPGalactose-4-epimerase (*cf.* KALCKAR AND MAXWELL[9].)

Recent preparations of UPPG from yeast, made by the present authors by the method of CAPUTTO *et al.*[1], have given anomalous results when assays by reactions 3 and 4 were compared, and this has led to the demonstration of UPPGal in these UPPG preparations. At the same time it has been found that preparations of glucose-6-phosphate dehydrogenase from brewers yeast contain UPPgalactose-4-epimerase, as also do extracts of *S. fragilis* grown on a glucose medium.

MATERIALS AND METHODS

UPPGlucose was prepared from bakers yeast (Distillers Company Ltd.) by the method of CAPUTTO *et al*[1] and purified by ion exchange chromatography (CABIB, LELOIR AND CARDINI[10]).

TPN, 80% purity, adenosine triphosphate (ATP) (crystalline disodium salt), hexokinase (Type 2) and glucose-6-phosphate dehydrogenase (Practical, Type II, Lot No. 95–138, 0.82 K.U./mg) were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. The latter enzyme is free from 6-phosphogluconate dehydrogenase.

DPN, 95% purity, and G-1-P were obtained from Boehringer and Soehne, Mannheim, W. Germany.

Gal-1-P was prepared by the method of KRAHL AND CORI[11].

Zwischenferment was prepared from dried brewers yeast (Kongens Bryghus, Copenhagen) by the method of LEPAGE AND MULLER[12]. This preparations contains, in addition to glucose-6-phosphate dehydrogenase, the enzymes uridyl transferase and nucleoside diphosphokinase[5,13]. It is free from 6-phosphogluconate dehydrogenase.

Uridyl transferase was prepared from dried brewers yeast (Kongens Bryghus, Copenhagen) by the method of MUNCH-PETERSEN[6].

UPPG dehydrogenase was prepared from calf liver by the method of STROMINGER, MAXWELL, AXELROD AND KALCKAR[8].

Phosphoglucomutase was prepared by the method of NAJJAR[14]. The preparation was taken as far as the 'second heat filtrate'.

Saccharomyces fragilis (National Collection of Yeast Cultures No. 100) was grown in the medium of DAVIES, FALKINER, WILKINSON AND PEEL[15] containing 2% glucose, harvested after 36 hours at 30° and lyophilised. The galactose adapted organism was subjected to two 24 hour cultures in the basal medium containing 2% galactose before growing for 36 hours in the galactose medium and harvesting as for the non-adapted organism.

Extract of *S. fragilis* were prepared by extracting 0.5 g of the dry powder with 2.5 ml 2.2% $(NH_4)_2HPO_4$ solution for 18 hours at 0°, followed by centrifugation and dialysis of the supernatant against running distilled water for 4 hours at 3°.

Paper chromatography of sugars was carried out on Whatman No. 1 paper in ethyl acetate–pyridine-water (2/1/2) and in *n*-butanol-acetic acid–water (4/1/5), the positions of the sugars being located by the aniline hydrogen phthalate reagent of PARTRIDGE[16].

Ionophoresis of sugars in borate buffer was carried out on Whatman 3 MM paper strips by the method of CONSDEN AND STANIER[17].

RESULTS

Comparison of the assay of various UPPG preparations by the pyrophosphorolytic and dehydrogenase reactions

Fig. 1 shows the results of the assay of UPPG preparation 5 by means of the pyrophosphorolytic reaction (curve b) and the UPPG dehydrogenase reaction (curve a). The theoretical ΔE_{340} for the TPN reduction in the first reaction is 0.62 for 0.1 μmole UPPG and for the DPN reduction in the second reaction is 1.24 for 0.1 μmole UPPG. It will be seen from the results presented that both reactions give a ΔE_{340} corresponding with the UPPG present.

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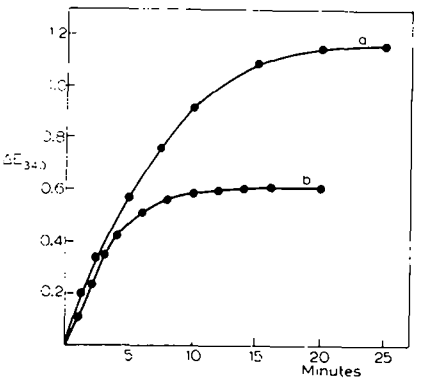

Fig. 1. The assay of UPPG preparations by the UPPG dehydrogenase reaction (a) and by the uridyl transferase reaction (b). Curve (a): 0.10 μmole UPPG/5 incubated with 20 μl UPPG dehydrogenase, 0.5 μmole DPN and 0.1 *M* Tris buffer pH 9.1 to 1.0 ml. Curve (b): 0.10 μmole UPPG/5 incubated with 10 μmoles $MgCl_2$, 2.5 μmoles cysteine, 50 μl phosphoglucomutase, 0.5 mg zwischenferment, 0.25 μmole TPN, 1.0 μmole pyrophosphate and 0.1 *M* Tris buffer pH 7.8 to 1 ml. Reaction started with pyrophosphate. Corrections made for controls run without UPPG an (a) and without pyrophosphate in (b).

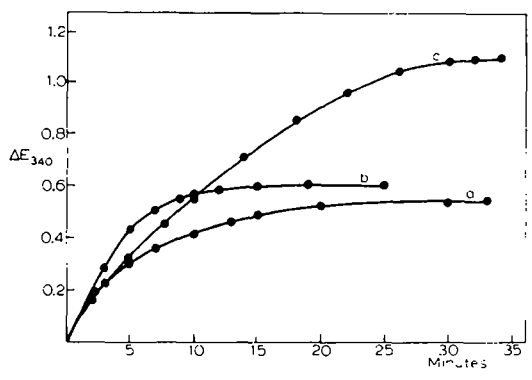

Fig. 2. The assay of UPPG/7 by the UPPG dehydrogenase reaction and the uridyl transferase reaction. Curve (a): 0.10 μmole UPPG/7 treated as in Curve (a) Fig. 1. Curve (b): 0.10 μmole UPPG/7 treated as in Curve (b) Fig. 1. Curve (c): 0.10 μmole UTP and 0.3 μmole G-1-P incubated with 0.5 units purified uridyl transferase[6], 20 μl UPPG dehydrogenase, 0.5 μmole DPN and 0.1 *M* Tris buffer pH 8.5 to 1.0 ml. Corrections made for controls run without UPPG in (a), pyrophosphate in (b) and UTP in (c).

Fig. 2 shows the results of the corresponding assays on UPPG preparation 7. In this case the ΔE_{340} in the pyrophosphorolytic reaction (curve b) corresponds with the expected UPPG content from the E_{260} of the preparation, but the ΔE_{340} of the UPPG dehydrogenase reaction indicates that only approximately 40% of this UPPG reacts with the UPPG dehydrogenase. Curve c (Fig. 2) shows the result of the simultaneous formation and dehydrogenation of UPPG in the presence of uridyl transferase, UTP, G-1-P and UPPG dehydrogenase. In this case the ΔE_{340} is that expected from the amount of UTP present.

The results of similar assays on UPPG preparation 8 are presented in Fig. 3. In these experiments, curves a and b correspond to curves a and b in Fig. 2; curve c in this case shows the result of the pyrophosphorolytic reaction using highly purified uridyl transferase[6] and glucose-6-phosphate dehydrogenase (Sigma).

Here again the results by the two methods do not give comparable values. The data presented in Figs. 2 and 3 indicate that there is present in UPPG preparations 7 and 8 a substance which reacts in the pyrophosphorolytic system to give G-1-P, but which will not react with the UPPG dehydrogenase. It was thought likely that this substance was UPPGal, and that the zwischenferment preparation and either the purified uridyl transferase or the glucose-6-phosphate dehydrogenase (Sigma) contained the enzyme UPPGalactose-4-epimerase.

The effect of UPPGalactose-4-epimerase on UPPG preparations

In order to test the possibilities indicated above, it was decided to react the UPPG preparations 7 and 8 with UPPGalactose-4-epimerase prior to assaying with UPPG dehydrogenase. Extracts of galactose adapted *S. fragilis* were used as a source of

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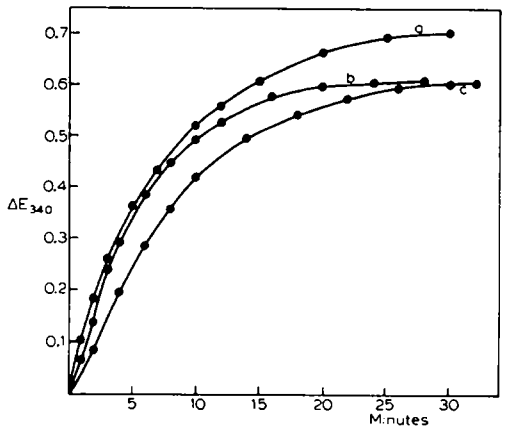

Fig. 3. The assay of UPPG/8 by the UPPG dehydrogenase reaction and the uridyl transferase reaction. Curve (a): 0.10 μmole UPPG/8 treated as in Curve (a) Fig. 1. Curve (b): 0.10 μmole UPPG/8 as in treated Curve (b) Fig. 1. Curve (c): 0.10 μmole UPPG/8 treated as in Curve (b) but with 0.05 units purified uridyl transferase and 0.1 mg glucose-6-phosphate dehydrogenase (Sigma) in place of the zwischenferment. Corrections made for controls run without UPPG in (a) and pyrophosphate in (b) and (c).

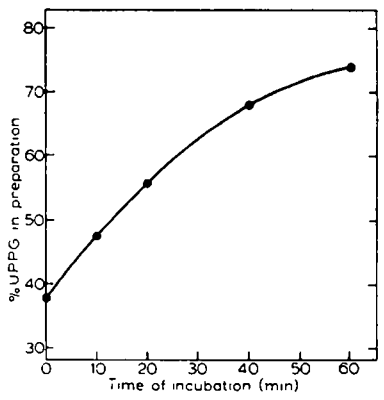

Fig. 4. The effect of extracts of galactose adapted *S. fragilis* on the UPPG content of UPPG/7. 0.10 μmole UPPG/7 was incubated with 5 μmoles $MgCl_2$, 20 μl. *S. fragilis* extract and 0.4 ml 0.025 *M* Tris buffer pH 7.8 for varying lengths of time at 25°. The reaction was stopped by heating in a boiling water bath for 30 seconds followed by cooling and centrifugation. The UPPG was then estimated with UPPG dehydrogenase and DPN as in Curve (a) Fig. 1.

UPPGalactose-4-epimerase. Fig. 4 shows the results of such an experiment where UPPG preparation 7 was incubated with the galactose adapted *S. fragilis* extracts and then assayed for UPPG by the dehydrogenase reaction.

It will be seen from Fig. 4 that treatment of UPPG preparation 7 with the *S. fragilis* extract produces, with time, a progressive increase in the UPPG reacting with the UPPG dehydrogenase and that the 60 minute figure approaches the 75% UPPG which is the equilibrium value for the UPPGalactose-4-epimerase reaction.

Fig. 5 shows the same experiment carried out with UPPG preparation 8 (curve a) and in addition (curve b) with the zwischenferment preparation replacing the *S. fragilis* extract. These results indicate that the zwischenferment is acting in the same manner as the extract of galactose adapted *S. fragilis*, which strongly suggests that it contains UPPGalactose-4-epimerase.

It will be noted from Fig. 3 that a combination of highly purified uridyl transferase and glucose-6-phosphate dehydrogenase gives the same ΔE_{340} in the pyrophosphorolytic reaction as does the zwischenferment preparation. This indicates that the former combination of enzymes als ocontains the UPPgalactose-4-epimerase. It was found that the uridyl transferase was free from the epimerase but that the glucose-6-phosphate dehydrogenase (Sigma) behaved as though it contained the epimerase. The results of an experiment in which UPPG preparation 7 was incubated with glucose-6-phosphate dehydrogenase for varying times before assay with UPPG dehydrogenase are shown in Fig. 6.

The data presented in Fig. 6 show quite clearly an increase in UPPG on incubation with the glucose-6-phosphate dehydrogenase preparation, which confirms the presence of UPPGalactose-4-epimerase. In addition however, it will be noted that the glucose-6-phosphate dehydrogenase causes a progressive breakdown with time of the UPPG.

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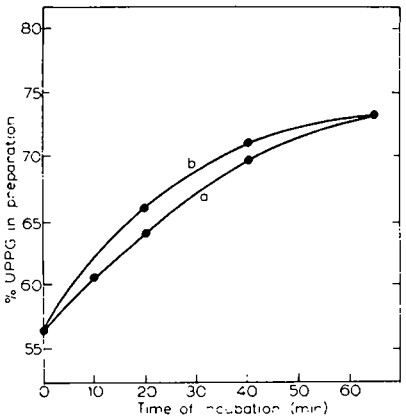

Fig. 5. The effect of an extract of galactose adapted *S. fragilis* and of zwischenferment on the UPPG content of UPPG/8. Experimental details as in Fig. 4. Curve (a): Incubation with 20 μl *S. fragilis* extract. Curve (b): Incubation with 0.1 mg zwischenferment.

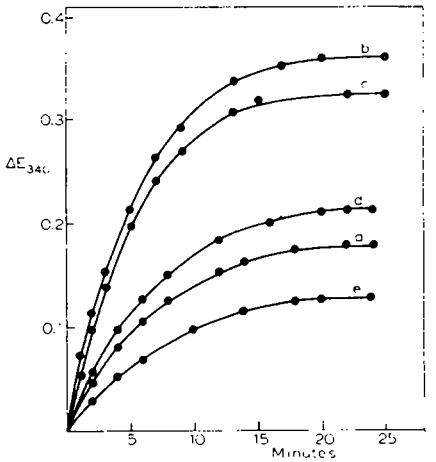

Fig. 6. The effect of glucose-6-phosphate dehydrogenase (Sigma) on the UPPG content of UPPG/7. 0.05 μmole UPPG/7 incubated at 25° with 0.1 mg glucose-6-phosphate dehydrogenase, 5 μmoles $MgCl_2$ in 250 μl 0.05 *M* Tris buffer pH 7.8 for various times. Reactions stopped by heating in a boiling water bath for 30 seconds followed by cooling and centrifugation. 200 μl samples were assayed for UPPG by incubation with 25 μl UPPG dehydrogenase, 0.3 μmole DPN and 0.1 *M* Tris buffer pH 9.1 to 1.0 ml. Curve (a) no incubation, curve (b) 10 minutes incubation, curve (c) 20 minutes incubation, curve (d) 40 minutes incubation and curve (e) 60 minutes incubation with glucose-6-phosphate dehydrogenase. Corrections made for controls run without UPPG.

The presence of UPPGalactose-4-epimerase in non-adapted and galactose adapted S. fragilis

The presence of UPPGalactose-4-epimerase in the zwischenferment and glucose-6-phosphate dehydrogenase preparations suggested that this enzyme is normally present in non-galactose adapted yeasts, and that the reaction induced by galactose adaptation is reaction (2)—namely the gal-1-P uridyl transferase.

Extracts of lyophilised *S. fragilis* grown on a glucose medium, and extracts of this organism adapted to galactose were assayed for UPPGalactose-4-epimerase by the method of MAXWELL, KALCKAR AND BURTON[18] (reactions 1 and 4) and for galactose-1-phosphate uridyl transferase[5].

The results of these assays are shown in Figs. 7 and 8.

It will be observed from Fig. 7 that incubation of the UPPG/UPPGal mixture with extracts of *S. fragilis* grown on a glucose medium causes an increase in the UPPG content of the mixture which indicates the presence of UPPGalactose-4-epimerase in this non-adapted yeast. The increase in UPPG content is not as great as in the case of the galactose adapted organism (*cf.* Fig. 4) and this is partially explained by the presence of an organic pyrophosphatase which splits UPPG and UPPGal. It was found that 10 minutes incubation with the unadapted *S. fragilis* extract raised the UPPG content of the mixture from 37% to 47% but more prolonged incubation caused considerable loss of UPPG.

Fig. 8 shows clearly that the Gal-1-P uridyl transferase is only present in the galactose adapted *S. fragilis*.

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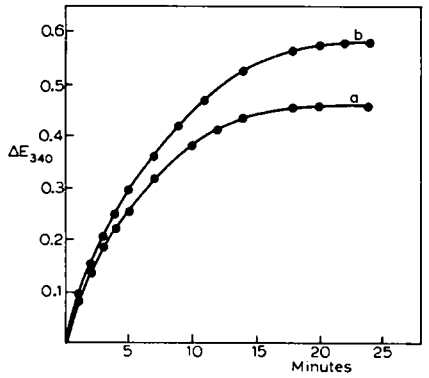

Fig. 7. The effect of an extract of *S. fragilis* grown on a glucose medium, on the UPPG content of UPPG/7. 0.10 μmole UPPG/7 incubated at 25° with 50 μl *S. fragilis* extract and Mg^{++} at pH 7.8, and the UPPG subsequently assayed using UPPG dehydrogenase and DPN as in Fig. 4. Curve (a): Zero time. Curve (b): After 10 min incubation. Corrections made for controls run without UPPG

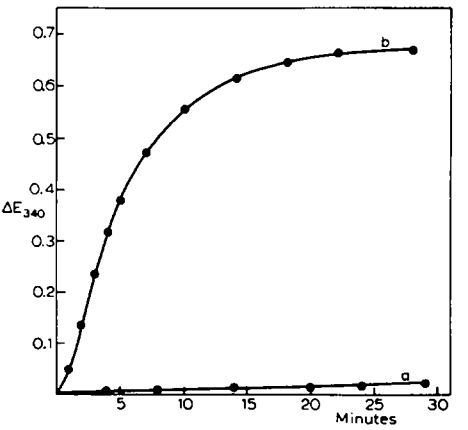

Fig. 8. The galactose-1-phosphate uridyl transferase activities of extracts of galactose adapted and non-adapted *S. fragilis*. Reaction mixtures contained 0.11 μmole Gal-1-P, 0.05 μmole UPPG, 5 μmoles $MgCl_2$, 2.5 μmoles cysteine, 50 μl phosphoglucomutase, 0.1 mg Glucose-6-phosphate dehydrogenase, 20 μl *S. fragilis* extract, 0.25 μmole TPN and 0.1 *M* Tris buffer pH 7.8 to 1.0 ml. Curve (a): *S. fragilis* grown on glucose medium. Curve (b): *S. fragilis* grown on galactose medium.

Analysis of the hexoses present in UPPG preparations

UPPG preparations 5, 7 and 8 were hydrolysed in 0.01 *N* HCl for 10 minutes at 100°, neutralised and sugar chromatography carried out in butanol–acetic acid–water and in ethyl acetate–pyridine–water. Preparation 5 showed the presence of only glucose, whereas preparations 7 and 8 showed the presence of both glucose and galactose. Ionophoresis on paper in borate buffer at pH 8.6 gave results for all three preparations which were identical with those found from paper chromatography.

Glucose and galactose were estimated in neutralised hydrolysates of UPPG preparations 7 and 8 by enzymic methods. For the glucose estimation, the reaction mixture contained ATP (1.0 μmole), hexokinase (1 mg dialysed free from contaminating glucose), TPN (0.5 μmole), glucose-6-phosphate dehydrogenase (0.5 mg), $MgCl_2$ (5 μmole), UPPG hydrolysate and 0.1 *M*-Tris buffer pH 7.8 to a final volume of 1 ml. The ΔE_{340} is a measure of the glucose present.

For a combined glucose and galactose estimate, use was made of the galactokinase and the Gal-1-P uridyl transferase present in extracts of galactose adapted *S. fragilis*. In this case the reaction mixture contained 25 μl of the galactose adapted *S. fragilis* extract, 50 μl phosphoglucomutase preparation, 2.5 μmole cysteine and 0.1 μmole UPPG in addition to the materials indicated above. In this case the ΔE_{340} gives a measure of the glucose plus galactose.

The results of these estimations showed that the glucose/galactose ratio for UPPG preparation 7 was 38/62 and for preparation 8 was 56/44.

These results correspond very closely with the UPPG/UPPGal ratios determined enzymically (Figs. 2 and 3).

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DISCUSSION

The mechanism of galactose utilisation in galactose adapted microorganisms has been attributed to the following pathway of reactions[2,5,19]:

$$\text{Galactose} + \text{ATP} \rightarrow \text{Gal 1 P} + \text{ADP (galactokinase)} \quad (5)$$

$$\text{Gal-1-P} + \text{UPPG} \rightleftharpoons \text{G-1 P} + \text{UPPGal (Gal-1 P uridyl transferase)} \quad (2)$$

$$\text{UPPG} \rightleftharpoons \text{UPPGal (UPPGalactose-4-epimerase)} \quad (1)$$

It was shown by CARDINI AND LELOIR[20] that the galactokinase activity of *S. fragilis* is increased twenty fold when this organism is grown on galactose and it has been assumed that reactions (1) and (2) are induced as a result of galactose adaptation[5]. It has been found in the present work however, that UPPGalactose-4-epimerase occurs in non-galactose adapted yeasts whereas reaction (2) only occurs in galactose adapted yeasts. The enzyme UPPGalactose-4-epimerase has been detected in a zwischenferment preparation from brewers yeast, a commercial glucose-6-phosphate dehydrogenase preparation and in extracts of *S. fragilis* grown on a glucose medium.

The presence of UPPGal in some UPPG preparations from bakers yeast, which is clearly established by the present results, is not surprising in view of the presence of UPPGalactose-4-epimerase in non-adapted yeasts. The present work indicates that the equilibrium point of the UPPGalactose-4-epimerase reaction is probably the same in non-adapted and galactose adapted yeasts, namely 75% UPPG and 25% UPPGal. The varying proportions of UPPGal in the UPPG isolated from yeast may be a reflection of the different metabolic states of the yeasts used, the UPPGal content depending upon the rate of its utilisation in some metabolic pathway, possibly polysaccharide synthesis.

It has not proved possible to determine the relative activities of UPPGalactose-4-epimerase in galactose adapted and non-adapted yeasts in view of the presence of organic pyrophosphatases causing a breakdown of the UPPhexoses.

The present work indicates the necessity of assaying UPPG preparations isolated from yeast (and other sources) by both the UPPG dehydrogenase reaction (4) and also by the pyrophosphorolytic reaction (3) in order to obtain a true assessment of the UPPG content and of any UPPGal contamination. Preparations of UPPG free from UPPGal may be made from UTP and G-1-P by the method of MUNCH-PETERSEN, KALCKAR AND SMITH[5] employing the purified uridyl transferase of MUNCH-PETERSEN[6].

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SUMMARY

Some preparations of uridine pyrophosphoglucose from bakers yeast have been found to contain considerable amounts (up to 60%) of uridine pyrophosphogalactose. The enzyme uridine pyrophosphogalactose-4-epimerase has been identified in non-galactose adapted yeasts as well as in yeast grown on a galactose medium. The significance of these findings is discussed.

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STRUCTURAL DIFFERENCES IN THE NUCLEIC ACIDS OF SOME TOBACCO MOSAIC VIRUS STRAINS*

I. MONOPYRIMIDINE NUCLEOTIDES IN RIBONUCLEASE DIGESTS

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Attempts to correlate the distinctive biological differences exhibited by the tobacco mosaic virus (TMV) strains with the chemical composition of their protein and nucleic acid components have been made[1]. The purine and pyrimidine composition of their nucleic acid part[2,3] and that of ribonuclease resistant residues (precipitable by 6% trichloroacetic acid) obtained after exhaustive digestion of each of the nucleic acids[4], have been found to be similar. If the nucleic acids represent the genetic material of viruses[5,6], it would seem that the nucleic acids of viral strains must differ in at least some structural features. Although there is no difference in the base composition of the strain nucleic acids and their ribonuclease resistant residues, it is probable that the way the individual nucleotides are arranged in each case might be different. Hence it was thought an examination of ribonuclease digests might reveal some differences in the intramolecular distribution of pyrimidine nucleotides in the nucleic acids of TMV strains.

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