URIDINE DIPHOSPHATE ACETYLGLUCOSAMINE*

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Studies on partially purified preparations of uridine diphosphate glucose (1, 2) obtained from yeast revealed the presence of a similar compound containing a different sugar moiety (3). In the course of a systematic study on yeast nucleotides this substance, previously referred to as UDPX and now as UDPAG,[1] was obtained in larger amounts. The properties of the sugar moiety are those of acetylglucosamine, and therefore UDPAG is closely related to the compounds found by Park (4) in the cells of *Staphylococcus aureus* treated with penicillin, which contain uridine-5′-pyrophosphate combined with an unidentified amino sugar derivative.

Separation of Yeast Nucleotides

A nucleotide mixture obtained from yeast by extraction with 50 per cent ethanol, followed by precipitation with mercuric chloride and treatment with hydrogen sulfide, was run through an anion exchange resin (Dowex 1) and eluted with solutions of decreasing pH and increasing chloride concentration, following the procedure described by Cohn (5). The fractions corresponding to each peak were passed through small charcoal columns and the substances were subsequently eluted with ethanol-ammonia. The concentrated solutions thus obtained were analyzed so that the substances corresponding to each peak could be tentatively identified. The results appear in Fig. 1 and Table I.

It is interesting that several uridine compounds were found. One of them could not be identified and the others were UMP-5′, UDPG, and UDPAG. In view of the relatively large amount of UDPAG which could be obtained free from UDPG, most of the efforts were concentrated on the

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[1] The following abbreviations will be used: UDPG for uridine diphosphate glucose; UDPAG for uridine diphosphate acetylglucosamine, UDP for uridine diphosphate, UMP for uridine monophosphate, ADP for adenosinediphosphate, AMP for adenosinemonophosphate, DPN for diphosphopyridine nucleotide.

former substance. For this purpose a simplified scheme of elution from the resin was employed and, after adsorption on charcoal and evaporation under reduced pressure, the substance was fractionally precipitated as the calcium salt with ethanol.

The amount of UDPAG obtained from fresh bakers' yeast was variable, about 200 micromoles per kilo in one experiment and 50 μM in the others.

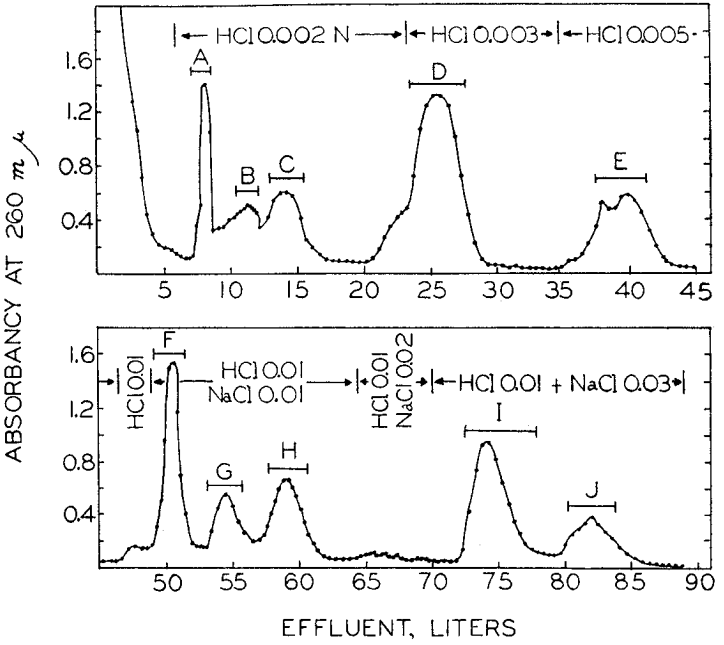


FIG. 1. Separation of yeast nucleotides by anion exchange. The lines above the peaks represent the fractions of each peak pooled for analysis. Peak B, mainly DPN; Peak C, a uridine derivative plus unknown substance; Peak D, AMP-5′; Peak E, UMP-5′ plus unidentified compound; Peak F, inosinic and guanylic acids; Peak G, an ADP-ribose compound; Peak H, ADP; Peak I, UDPAG; Peak J, UDPG.

The ratio UDPAG to UDPG in the extract before purification by anion exchange was about 2, except for some samples of toluene-treated yeast (1, 2) in which it was 0.3 or lower.

Identification of Sugar Moiety

UDPAG liberates by mild acid hydrolysis a substance with about half the reducing power of glucose (assuming 1 molecule of the substance per molecule of uridine) (3).

This substance was found to give a positive reaction for acetylhexosamines, and therefore the statement contained in a previous paper (3) on

the negative Elson and Morgan reaction was erroneous. The absorption spectra of the sugar moiety of UDPAG and acetylglucosamine after treatment with dilute alkali and *p*-dimethylaminobenzaldehyde, as described by Aminoff *et al.* (6), were compared and found to be identical (Fig. 2).

TABLE I

Analytical Data on Effluent Fractions

Substance	Spectrum type*	P-nucleoside ratio†	Labile P-total P ratio‡	$R_{adenosine}$ with ethanol-ammonium acetate, pH 7.5§	$R_{adenosine}$ with ethanol-ammonium acetate, pH 3.8‖	R_F with butanol-disodium phosphate¶	$R_{adenosine}$ of nucleosides with ethanol-ammonium acetate, pH 3.8‖	R_F of bases with ammonium sulfate-isopropanol**
B	DPN	2.1	0.17	0.37	0.24			
C	Uridine (atypical)	0.71	0.27	0.74	0.60, 0.73	0.90	1.18	
D	Adenosine	1.03	0.06	0.47	0.64	0.75	0.95	0.17
E	Uridine	0.92	0.12	0.55, 0.79	0.62, 0.80	0.88	1.17	
F	Inosine (atypical)	0.98	0.13	0.39	0.51, 0.62		0.95	
G	Adenosine	2.0	0.16	0.61	0.42		0.95	0.17
H	"	1.75	0.44	0.31	0.36	0.78	0.96	0.17
I	Uridine	1.8	0.49	0.79	0.61		1.18	
J	"	1.6	0.46	0.68	0.47			

* Determined in neutral, acid (0.1 N), and alkaline (0.1 N) solutions.

† Calculated from the total phosphate and the extinction coefficients of the substances as found in the literature.

‡ Labile phosphate is defined as the phosphate liberated by 15 minutes hydrolysis in 1 N acid at 100°.

§ $R_{adenosine}$ of adenosine-5′-phosphate, 0.47; inosine-5′-phosphate, 0.41; DPN, 0.38. For other data, see Paladini and Leloir (3).

‖ $R_{adenosine}$ of adenosine-5′-phosphate, 0.63; inosine-5′-phosphate, 0.63; DPN, 0.24; uridine-5′-phosphate, 0.79; uridine, 1.16; inosine, 1.02; AMP-3′ after phosphatase, 0.95. For other data, see Paladini and Leloir (3).

¶ R_F of adenosine-5′-phosphate, 0.75; adenylic acid a, 0.73; adenylic acid b, 0.64.

** R_F of adenine, 0.17.

By paper chromatography of the free sugar with pyridine-ethyl acetate-water (7), followed by spraying with aniline phthalate (8) or with the modified Elson and Morgan reagent (9), a single spot was obtained, with the same $R_{glucose}$ value and color as acetylglucosamine (Table II). More than twenty sugars have been tested with this solvent. Of these, only acetylgalactosamine and 3-methylgalactose migrated on the paper at a rate similar to that of acetylglucosamine. However, acetylgalactosamine can be easily differentiated from acetylglucosamine by using the same solvent,

but with borate-treated papers (Table II), while 3-methylgalactose gives different color reactions.

Identical $R_{glucose}$ values for acetylglucosamine and the sugar from UDPAG were also obtained by chromatography with butanol-ammonia.

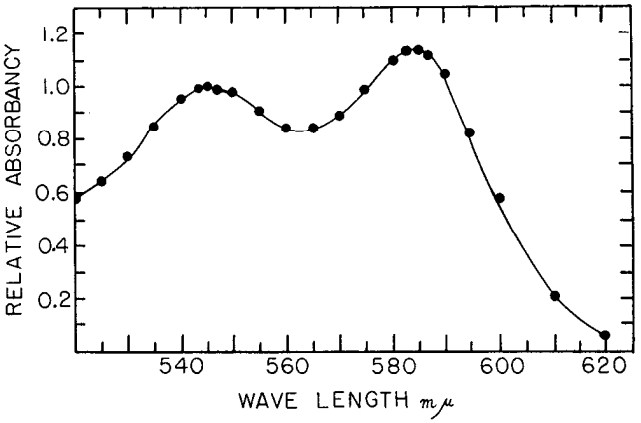


FIG. 2. Absorption spectra of the sugar moiety of UDPAG (●) and of acetylglucosamine (solid line) treated with alkali and *p*-dimethylaminobenzaldehyde according to Aminoff *et al.* (6). The absorbancy values were multiplied by a factor so as to make the absorbancy at 545 mμ equal to 1.0.

TABLE II

Paper Chromatography of Sugar Moiety from UDPAG

Solvent, ethyl acetate-pyridine-water (7, 21).

Substance	$R_{glucose}$ values of spots	
	Untreated paper	Borate-buffered paper
Acetylglucosamine	1.24	2.25
Acetylgalactosamine	1.14	1.7
Hydrolyzed UDPAG	1.23	2.24

When the sugar was submitted to prolonged hydrolysis under conditions which lead to deacetylation of acetylglucosamine, it was found to give a positive Dische and Borenfreund reaction for glucosamine (10). The hydrolysate was run on paper with a solvent mixture composed of ethyl acetate, pyridine, ammonia, and water. The chromatogram, sprayed with the modified Elson and Morgan reagent, showed a residual spot with the same $R_{glucose}$ as acetylglucosamine, and a new one with the same $R_{glucose}$ as glucosamine, as can be seen in Table III.

The isolation of acetylglucosamine was not attempted on account of the

relatively small amounts of UDPAG available, but the evidence outlined above leaves little doubt about its identity. As unhydrolyzed UDPAG is non-reducing and gives no color with the Morgan and Elson reagent (11), it may be concluded that acetylglucosamine is linked to the rest of the molecule through the carbon atom 1. The sugar is very easily liberated by dilute acid, as shown in Fig. 3.

TABLE III

Paper Chromatography of Deacetylated Sugar from UDPAG

Solvent, ethyl acetate-pyridine-ammonia water (see the text).

Substance	$R_{glucose}$ values of spots
Acetylglucosamine	1.20
Glucosamine	0.68
Galactosamine	0.53
Deacetylated acetylglucosamine	0.69, 1.20*
" sugar from UDPAG	0.70, 1.20*

* Very feeble.

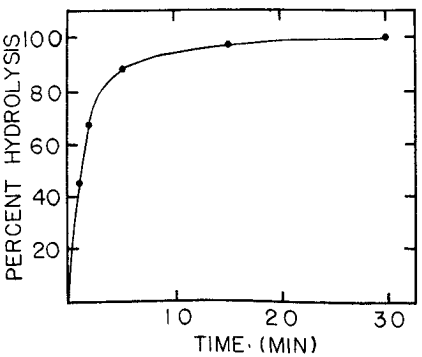


FIG. 3. Liberation of acetylglucosamine from UDPAG heated in 0.01 N acid at 100°. The value obtained after 30 minutes was taken as 100.

Presence of Uridine

The absorption spectrum of UDPAG was found to be identical with that of uridine (2) and to show the same changes with pH and upon the addition of bromine. Uridine could be identified also chromatographically as a breakdown product of UDPAG (see below).

Presence of Phosphate

As in UDPG, two phosphate groups per molecule of uridine were found. One of them is acid-labile and can be hydrolyzed in 20 minutes with 1 N

acid at 100°. Comparative hydrolysis data for UDPAG and UDPG are shown in Table IV.

Acid Hydrolysis of UDPAG

Uridine-5′-pyrophosphate and uridine-5′-monophosphate were identified as breakdown products of UDPAG, after acid hydrolysis, by paper chro-

TABLE IV

Phosphate Liberated from UDPAG and UDPG by Acid Hydrolysis

The figures represent the per cent phosphate liberated at 100°, the 30 minute value in 1 N acid being taken as 100.

Time	1 N acid		0.1 N acid	
	UDPAG	UDPG	UDPAG	UDPG
min.				
15	94	90.7	38.4	41
30	100	100	63	60.7
60	100	100	85	81.3
120			99	94.3

TABLE V

Paper Chromatography of Nucleotides Obtained by Acid Hydrolysis of UDPAG

Substance	$R_{adenosine}$ values of ultraviolet-absorbing spots	
	Ethanol-ammonium acetate, pH 7.5	Ethanol-ammonium acetate, pH 3.8
UDPG	0.43	0.45
UDPAG	0.58	0.60
UDP from UDPG	0.14	0.40
UDPAG heated 10 min. in 0.01 N acid at 100°	0.14	0.39
Synthetic UMP-5′	0.30	0.76
UDPAG heated 20 min. in 1 N acid at 100°	0.30	0.77
UMP-3′	0.40	0.88

matography in two different solvents (Table V). The standards of comparison were a synthetic specimen of UMP-5′ and UDP obtained from UDPG. This UDP has been shown by Anand *et al.* (12) to be identical with synthetic uridine-5′-pyrophosphate.

The position of the phosphate group in uridylic acid was further confirmed by treatment with a 5′-nucleotidase from snake venom (13). As can be seen in Table VI, the uridylic acid obtained from UDPAG was hydrolyzed by this enzyme yielding inorganic phosphate and a substance with the same $R_{adenosine}$ as uridine, whereas UMP-3′ was not attacked.

Alkaline Hydrolysis

It was previously observed (3) that chromatography of UDPG and UDPAG mixtures with an alkaline solvent led to a decomposition of the former substance but to no observable change of the latter. The alkaline decomposition of UDPG was found to give rise to UMP-5′ and a cyclic phosphoric ester of glucose. No chromatographically detectable change has been observed after heating UDPAG during 5 minutes at 100° in concentrated ammonia. After heating 15 minutes at 100° in 0.15 N barium hydroxide, two substances could be detected by paper chromatography

TABLE VI

Action of 5′-Nucleotidase on Uridine Monophosphate from UDPAG

Each tube contained 0.1 ml. of *Crotalus adamanteus* enzyme, 5 μM of $MgCl_2$, 50 μM of glycine, pH 8.5, 1 μM of uridine monophosphate from UDPAG or UMP-3′. Total volume, 0.4 ml. The mixture was incubated at 37° and the reaction stopped by addition of 0.8 ml. of ethanol. After centrifuging, 0.5 ml. aliquots of the supernatant were taken for inorganic phosphate determinations and the rest was evaporated *in vacuo* and submitted to paper chromatography with ethanol-ammonium acetate, pH 3.8. The $R_{adenosine}$ of synthetic UMP-5′ and of uridine were 0.79 and 1.18 respectively.

Substrate added	Time of incubation	Inorganic phosphate in total sample	$R_{adenosine}$ of ultraviolet-absorbing substances
	min.	*μM*	
UMP from UDPAG	0	0.18	0.79
" " "	30	0.95	0.78,* 1.17
UMP-3′	0	0	0.89
"	30	0	0.87

* Feeble.

which appeared to be UMP-5′ and acetylglucosamine-1-phosphate (Table VII). The latter substance has been prepared synthetically and will be dealt with in future papers. Park (4) has commented on the different stability to alkali of UDPG and the UDP-amino sugar compounds. The cause of the greater stability would be that in UDPAG the hydroxyl at position 2 is unavailable for the formation of the cyclic phosphoric ester.

Linkage of Different Components

The analytical data for the calcium salt of UDPAG dried over phosphorus pentoxide are shown in Table VIII. The results correspond to a preparation of about 90 per cent purity of a compound containing one uridine, two phosphate groups (one of them being acid-labile), and one acetylglucosamine residue.

TABLE VII

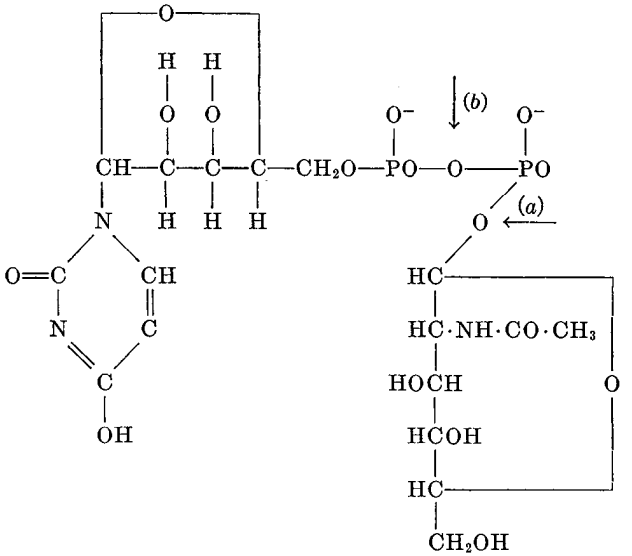
Paper Chromatography of Alkaline Degradation Products of UDPAG

After hydrolysis, the samples were neutralized with sulfuric acid and centrifuged. The supernatants were passed through a cation exchange resin (Dowex 50). The eluates were neutralized with ammonia, evaporated *in vacuo*, and used for chromatography. Solvent, ethanol-ammonium acetate, pH 3.8.

Substance	Time of heating in 0.15 N $Ba(OH)_2$	Distance of spots from starting line				
		Located with ultraviolet		Located with P reagent		
	min.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
UDPAG	0	18.6		18.6		
"	5	18.6	22.5	18.6	22.5	28.8
"	15	18.6*	22.8	18.6	22.8	28.7
Acetylglucosamine-1-phosphate	0					29.7
UMP-5′	0		23.7		23.7	

* Faint.

The fact that the intact compound is non-reducing and gives no Morgan and Elson reaction and the liberation of UDP and UMP-5′ by acid hydrolysis suggest a structure similar to that of UDPG. This structure is further supported by the results of the alkaline hydrolysis.



Uridine diphosphate acetylglucosamine

The compound represented by the accompanying formula would show

two primary phosphoric acid groups. Hydrolysis at the points marked *a* and *b* would yield one secondary phosphoric acid group in each case. Besides, hydrolysis at *a* and *b* would liberate acetylglucosamine and inorganic phosphate. Therefore, the electrometric data were compared with the values predicted from total phosphate, inorganic phosphate, and free acetyl glucosamine estimations in samples of the substance before and after hydrolysis. The results, which were exactly equivalent to those obtained previously (1, 2) with UDPG, are summarized in Table IX, and agree well with those predicted from the accompanying formula.

TABLE VIII

Analytical Data for Sample of Calcium Salt of UDPAG

Component	Found	Theoretical for Ca salt	Ratio, component-uridine
	μM per mg.	*μM per mg.*	
Uridine (from absorbancy at 260 mμ)	1.48	1.55	1
Total phosphate	2.75	3.1	1.86
Labile " (20 min. in 1 N acid at 100°)	1.31	1.55	0.885
Acetylglucosamine	1.49	1.55	1.01
Nitrogen	4.59	4.66	3.12

TABLE IX

Electrometric Titration of UDPAG

The technique was the same as described previously (2).

Time of heating at 100°; about pH 2	Base, μeq.			
	Calculated from analytical data		Observed on electrometric titration	
	Primary	Secondary	Primary*	Secondary†
min.				
0	9.6	0	9.6	0
10	9.6	5.0	9.6	5.2
60	9.6	8.0	9.6	8.2

* Titrated to pH 4.5.

† Titrated from pH 4.5 to 8.2.

Park's Compound I differs from UDPAG, since its sugar moiety contains an acid group and does not migrate on paper like acetylglucosamine (4).

Due to the close similarity in the structures of UDPAG and UDPG, and considering the coenzymatic activity of the latter, it may be suspected that UDPAG plays some rôle in the metabolism of hexosamines. However, preliminary experiments in this direction have been negative.

EXPERIMENTAL

Methods—Besides those employed in previous papers (1–3), the following analytical methods were used: those of Morgan and Elson (11) or Aminoff *et al.* (6) for acetylglucosamine, Dische and Borenfreund (10) for glucosamine, Johnson (14) for nitrogen, Albaum and Umbreit (15) for pentoses, Kalckar (16) for AMP-5′ deaminase, Schlenk and Schlenk for AMP-5′, using muscle enzymes (17), Colowick *et al.* (18) for DPN, and Cori and Green (19) for AMP-5′, using phosphorylase b.

Nucleosides were prepared from the nucleotides by treatment with a purified pig's kidney alkaline phosphatase (20).

The nucleotides were hydrolyzed to the free bases by heating at 100° in 1 N sulfuric acid during 30 minutes for the purines, and in 2.5 N sulfuric acid during 6 hours for the pyrimidines.

Paper chromatography of sugars was carried out with the ethyl acetate-pyridine-water mixture previously used (7, 21). Acetylglucosamine and acetylgalactosamine were separated by using papers which had been immersed in 0.2 M borate buffer of pH 8 and dried. Glucosamine and galactosamine showed a marked tailing with the above mixture, but gave well defined and separated spots after running with a more alkaline solvent prepared by mixing ethyl acetate, pyridine, concentrated ammonia, and water in the proportions 10:5:3:3 by volume. After equilibrating at 30°, the upper phase was used.

Sugars were located as described by Partridge with aniline phthalate (8) or with a modified Elson and Morgan reagent (9).

For paper chromatography of nucleotides and nucleosides, the ethanol-ammonium acetate mixtures already described (3) were used, in addition to Carter's isoamyl alcohol-disodium phosphate solvent (22). The chromatography of free purine and pyrimidine bases was carried out with isopropanol-ammonium sulfate (23) or with butanol-ammonia (24). Standard substances were run in every chromatogram, since no precautions were taken to obtain reproducible R_F values. The position of ultraviolet-absorbing substances was ascertained with a Mineralight lamp and that of phosphorylated compounds according to Bandurski and Axelrod (25). The position of the substances on the paper is given relative to the position of glucose or adenosine. For instance, the ratio of the distance traveled by the unknown substance to the distance traveled by glucose is referred to as R_{glucose}.

Yeast Extract—To 10 kilos of bakers' yeast, 10 liters of 95 per cent ethanol were added and the mixture was heated with continuous stirring until it boiled. On the following day it was filtered through a 32 cm. Büchner funnel with a filter-aid. The filtrate was acidified with 5 N nitric acid until acid to Congo red paper. Then 30 ml. of mercuric acetate (2) per liter

were added. After mixing, the preparation was left overnight in the refrigerator. The suspension was filtered through a Büchner funnel. The precipitate was dried as much as possible by suction, and placed in a blendor with 1200 ml. of water, and decomposed with hydrogen sulfide in the cold. The mercuric sulfide was filtered off and washed with 100 ml. of water, and the combined filtrates were aerated and neutralized to pH 6. Prior to chromatography, the solution was brought to pH 7.5 by addition of concentrated ammonia.

Column Chromatography—A glass column (50 cm. high and 4.5 cm. inner diameter) was fed through a rubber tubing by a container hung about 1.5 meters above.

The fraction collector consisted of a row of forty flat bottles of 1 liter capacity over which the column was displaced on rails by an electrically driven motor, so that a distance of about 2 meters was covered in 24 hours. The tip of the column was fitted with a hanging funnel and an escape mechanism controlled by a row of suitably spaced nails which prevented the loss of effluent in the space between the bottles.

The strong base Dowex 1 anionic resin was employed throughout. The resin (200 to 400 mesh) was converted to the chloride form with 1 N hydrochloric acid and freed from fines by six or more decantations from water.

A smaller amount of coarser resin, obtained by further decantations and suspended in water, was poured into the column to form a layer 2 to 3 cm. thick, which prevented leakage of the smallest particles through the fritted glass disk. The rest of the resin, suspended in a small amount of water, was then added and allowed to settle. The column, which was 30 cm. high, was washed with 1 N hydrochloric acid until the absorbancy of the effluent dropped to a value of 0.03 to 0.04, followed by water until the pH of the effluent was about 5.

The solution of nucleotides (1500 ml., containing about 8000 μM calculated as uridine from the absorbancy at 260 mμ) was allowed to drain at a rate of 6 to 8 ml. per minute. From this point on, the procedure varied in different cases.

(*a*) For the type of experiment shown in Fig. 1, the column was first washed with water until the absorbancy of the effluent dropped below 0.1. Then the first eluent (0.002 N hydrochloric acid) was run through and the elution was followed by measurements of the absorbancy at 260 mμ. The rate of flow was maintained between 6 and 8 ml. per minute. Each eluent was replaced by the next one after the absorbancy of five to ten fractions (2.5 to 5 liters) had remained under 0.1.

The highest concentration of hydrochloric acid used was 0.01 N in order to prevent decomposition of labile nucleotides, and solutions of higher eluting power were prepared by addition of sodium chloride.

(*b*) When it was desired to isolate only UDPAG and UDPG, a simplified procedure was employed. After a washing with water (500 ml.), the following solutions were successively run through the column: 0.01 N hydrochloric acid, 0.01 N sodium chloride in 0.01 N hydrochloric acid, 0.02 N sodium chloride in 0.01 N hydrochloric acid. Each eluent was replaced by the next, the same criterion as in (*a*) being used. Finally, UDPAG and UDPG could be eluted separately with 0.03 N sodium chloride in 0.01 N hydrochloric acid. In extracts from toluene-treated yeast, which contained less UDPAG than UDPG, a complete separation between the two substances could be obtained only when a solution of 0.025 N sodium chloride in 0.01 N hydrochloric acid was used in the last step.

After each run the column was regenerated with 1 N hydrochloric acid, followed by water. The same column was used several times, except for a small layer at the top which darkened during the run and was replaced each time by fresh resin.

Concentration—The method of Cohn (5), which makes use of small resin columns, was discarded in view of the conditions of high acidity to which the substances are exposed during the process of concentration. Instead, an alternative procedure was devised which yielded excellent results in most cases. The fractions belonging to each peak were pooled and adsorbed on a small charcoal column (3 gm. of Norit A in a fritted glass funnel 4 cm. in diameter). Elution from the charcoal was carried out with a water-ethanol-ammonia mixture (40 ml. of 95 per cent alcohol plus 1 ml. of concentrated ammonia, made up to 100 ml. with water).

Each fraction of 3 to 5 ml. was collected in the cold and immediately adjusted to pH 5 to 6 with hydrochloric acid. The fractions of high absorbancy were then pooled. It was thus possible to concentrate the solutions from several liters to 20 to 40 ml. The yield of this step was 70 to 80 per cent.

These solutions were further concentrated by evaporation under reduced pressure, and in some cases the nucleotides were precipitated as the calcium salt by addition of some drops of a saturated solution of calcium chloride in ethanol, followed by several volumes of ethanol until no more precipitation occurred.

Analysis of Effluent Fractions—Some of the results obtained from the study of the fractions isolated in the experiment of Fig. 1 are summarized in Table I. Besides the analytical data obtained for each compound, further information was provided by the composition of the eluent, since a lower pH and a higher anion concentration are needed for the elution of the ions with higher net negative charge (5).

A considerable amount of ultraviolet-absorbing material, representing about 30 per cent of the total absorbancy of the sample, was not adsorbed on the column and was recovered in the wash water. This frac-

tion, which contained no phosphate, probably consisted mainly of nucleosides and free bases. On standing, it gave rise to a crystalline precipitate which seemed to be hypoxanthine, as judged by its spectrum.

Peak A was not analyzed, as most of it was lost in the process of concentration.

Peak B gave an adenosine spectrum. A band at 340 mμ appeared in cyanide solution. The R_F values were those of DPN. It catalyzed the oxidation of alcohol and glyceraldehyde phosphate in the presence of yeast enzymes and dichlorophenol indophenol. The material contained in Peak B therefore appeared to be DPN, but some contaminant was present since the phosphate content was too high for the DPN values calculated from the absorbancy at 340 mμ.

Peak C—Two spots were obtained by paper chromatography with one of the solvents. After separation of the two substances, one (C_1) was found to give a spectrum similar to that of uridine, while the other (C_2) gave a spectrum which could not be identified (maximum 260 mμ in acid, 230 and 260 mμ in alkali).

The R_F of the nucleoside prepared from C_1 corresponded to that of uridine. Acid hydrolysis gave a substance of spectrum and R_F corresponding to uracil. In addition, two other substances appeared, one probably being UMP while the other could not be identified. Therefore, Peak C contained two substances, one of which is a uridine compound.

Peak D—The data obtained for this substance were as follows. The spectrum was typical for adenosine. There was one phosphate for each adenosine, 6 per cent of which was acid-labile. The R_F values in three solvents corresponded to AMP-5′, and the substance obtained after hydrolysis with phosphatase gave the R_F value of adenosine and that of adenine after acid hydrolysis.

The substance stimulated phosphorylase b and the dephosphorylation of phosphopyruvate as described by Schlenk and Schlenk (17). Muscle deaminase produced the same spectral changes as on AMP-5′. From this evidence it can be concluded that the substance in Peak D is AMP-5′.

Peak E gave two spots on paper chromatography, one of them (E_2) corresponding to UMP-5′, as judged by the R_F value and by the type of the spectrum. The other compound (E_1) gave a spectrum very similar to that of C_2. The results obtained after phosphatase or acid treatment of Peak E were very similar to those for Peak C. Therefore, Peak E appeared to contain UMP-5′ and an unidentified compound.

Peak F—The spectrum was somewhat similar to that of inosine. Analysis showed one phosphate per nucleoside residue. Two spots appeared on chromatography with one solvent and the R_F of one of these spots corresponded to that of inosinic acid. After elution of the substances in each spot, the faster gave a typical inosine spectrum and the slower a guanosine

type of spectrum. The bases liberated by acid hydrolysis were hypoxanthine and guanine, as judged by paper chromatography, followed by elution and spectrophotometry.

Thus Peak F appeared to contain a mixture of inosinic and guanylic acids. Considering the acid stability of the phosphate, these substances probably corresponded to the 5′ isomers.

Peak G—A typical adenosine spectrum was obtained. No spectral change was observed after addition of cyanide. Two phosphate groups per adenosine were found. Paper chromatography of the nucleoside and base gave R_F values equal to those of adenosine and adenine respectively. Two ribose residues per each adenosine were found. The same data would be obtained for the product resulting from hydrolytic removal of the nicotinamide from DPN.

Peak H gave a typical spectrum for adenosine. The phosphate-adenosine ratio was 1.75, and 44 per cent of the phosphate was labile. Adenosine and adenine were obtained by hydrolysis. In the presence of crude hexokinase, phosphate was transferred to glucose. The bulk of the substance was probably ADP.

Peak I—The substance contained in the fraction is referred to as UDPAG and was studied in detail.

Peak J—The data shown in Table I, the chromatographic behavior and the coenzymic activity corresponded to those of UDPG.

Degradation Products of UDPAG

Identification of Acetylglucosamine—For the quantitative tests, UDPAG was hydrolyzed with 0.1 N sulfuric acid during 10 minutes at 100°, the mixture was neutralized with 0.3 N barium hydroxide, and the nucleotides precipitated by adding equal volumes of 5 per cent zinc sulfate and 0.3 N barium hydroxide. After centrifuging, acetylglucosamine was determined according to Aminoff *et al.* (6) in an aliquot of the supernatant solution.

To obtain a solution of the sugar suitable for chromatography, UDPAG was hydrolyzed as above and the liquid was treated successively with a cation exchange and an anion exchange resin (Dowex 50 and Amberlite IR-4B). This solution was evaporated *in vacuo* and submitted to paper chromatography (Table II).

The deacetylation of the sugar was carried out on an aliquot of the same solution, which was heated in a sealed tube with 0.1 N sulfuric acid 24 hours at 100°. The liquid was neutralized with barium hydroxide, centrifuged, and the supernatant solution used for chromatography. A control with an authentic sample of acetylglucosamine was run simultaneously (Table III).

Uridine-Diphosphate—UDPAG was hydrolyzed with 0.01 N sulfuric acid during 10 minutes at 100°. All the sugar but practically no phosphate is

liberated under these conditions. The acid was neutralized with dilute ammonia and the solution used for paper chromatography. For comparison, a sample of UDPG was submitted to the same treatment (Table V).

Uridine Monophosphate—UDPAG was hydrolyzed in 1 N sulfuric acid during 20 minutes at 100°. The acid was neutralized with barium hydroxide, and the supernatant solution used for chromatography (Table V).

Action of 5′-Nucleotidase on Uridine Monophosphate—For this experiment uridine monophosphate was prepared from UDPAG as described above, except that an excess of barium hydroxide (to pH 8 to 9) was added in order to precipitate most of the inorganic phosphate. After centrifuging, the supernatant fluid was neutralized with sulfuric acid and centrifuged again. The resulting solution was incubated with the enzyme, as described by Heppel and Hilmoe (13) (Table VI).

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SUMMARY

A preparation of nucleotides from bakers' yeast has been fractionated by chromatography on an anion exchange resin. The compounds contained in each fraction were tentatively identified and one of them was studied in more detail. This substance (UDPAG) gives the same spectrum as uridine at several pH values and after treatment with bromine. It contains two phosphate groups, one of which is acid-labile.

Mild acid hydrolysis liberates a substance identical with acetylglucosamine, as judged by color reactions and paper chromatography with different solvents. Moreover, by further acid treatment of the sugar moiety, a compound is released which shows the same chemical properties and chromatographic behavior as glucosamine.

The nucleotides set free from UDPAG by acid hydrolysis migrate on paper like the UDP and UMP obtained from UDPG.

Alkaline hydrolysis liberates substances behaving like UMP-5′ and acetylglucosamine-1-phosphate.

These facts, together with the analytical data and the results of the electrometric titration, lend support to a structure for UDPAG in which uridine-5′-pyrophosphate and acetylglucosamine are joined through a glycosidic link.

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