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SYNTHESIS OF 4-CARBOXY-2-POLYPRENYLPHENOLS BY A PARTICULATE FRACTION OF BAKER'S YEAST

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Key Word Index—*Saccharomyces carlsbergensis*; *Saccharomyces cerevisiae*; baker's yeast; polyprenylpyrophosphate-*p*-hydroxybenzoate polyprenyltransferase; properties; intracellular distribution.

Abstract—The preparation of a cell-free homogenate and 10000 *g* particulate fraction with polyprenylpyrophosphate-*p*-hydroxybenzoate polyprenyltransferase activity from 0 to 7-day-old blocks of compressed baker's yeast is described. The synthesis of 4-carboxy-2-triprenylphenol from *p*-hydroxybenzoate and FPP by the particulate fraction has been studied in some detail. In particular it has been shown that the transferase catalysing the reaction is activated by Mg^{2+}, has a pH optima of 7 and is inhibited by phosphate buffer. Intracellular distribution studies have established that in freshly grown cells of *Saccharomyces carlsbergensis* the greater part of the polyprenyl transferase activity is present in the mitochondria.

INTRODUCTION

THERE are now several pieces of experimental evidence[1,2] to support the view that the nature of the pathway leading from *p*-hydroxybenzoate to ubiquinone in yeasts is similar to the pathways which operate in bacteria and mammals.[3,4] Thus Ah Law *et al.*[1] have isolated 5-demethoxyubiquinones from a number of yeasts and have shown them to be precursors of ubiquinones in *Saccharomyces cerevisiae* and *S. carlsbergensis* and, more recently, Thomas and Threlfall[2] have demonstrated that cell-free homogenates and mitochondrial preparations of *S. carlsbergensis* contain an enzyme (polyprenylpyrophosphate-*p*-hydroxybenzoate polyprenyltransferase) that is able to form 4-carboxy-2-polyprenylphenols from *p*-hydroxybenzoate and polyprenylpyrophosphates.

In the present paper we report on the preparation and properties of a particulate fraction with polyprenylpyrophosphate-*p*-hydroxybenzoate polyprenyltransferase (*p*-hydroxybenzoate polyprenyltransferase) activity from blocks of compressed baker's yeast and the intracellular distribution of *p*-hydroxybenzoate polyprenyltransferase activity in *S. carlsbergensis*.

Abbreviations: FPP, *trans-trans*-farnesylpyrophosphate; IPP, isopentenylpyrophosphate; MLE, *Micrococcus lysodeikticus* extract; MLE-IPP, MLE that has been preincubated with IPP.

[1] AH LAW, THRELFALL, D. R. and WHISTANCE, G. R. (1971) *Biochem. J.* **123**, 331.
[2] THOMAS, G. and THRELFALL, D. R. (1973) *Biochem. J.* **134**, 811.
[3] THRELFALL, D. R. and WHISTANCE, G. R. (1971) *Aspects of Terpenoid Chemistry and Biochemistry* (GOODWIN, T. W., ed.), pp. 357–404, Academic Press, London.
[4] MOMOSE, K. and RUDNEY, H. (1972) *J. Biol. Chem.* **247**, 3930.

RESULTS

Properties of preparations produced from blocks of compressed baker's yeast by mechanical grinding

In the preliminary study which established that *p*-hydroxybenzoate polyprenyltransferase activity is associated with yeast mitochondria, cell-free fractions were prepared by subjecting suspensions of freshly-harvested cells of *S. carlsbergensis* which had been ground with neutral alumina in a mortar to a scheme of differential centrifugation.[2] To obviate the relatively expensive and time-consuming procedure of growing cells and the wide variations in the quality and yields obtained by the above procedure of cell-disruption, it was decided to investigate the use of (a) blocks of compressed baker's yeast as a source of particulate preparations for experiments in which mitochondrial integrity is not essential and (b) a tissue disintegrator as a means of obtaining a more standard preparation. Accordingly suspensions of cells from a 4-day-old block of baker's yeast were subjected to different periods of mechanical grinding by Ballotini beads in a Mickle High Speed Tissue Disintegrator and the 10000 *g* particulate fractions assayed for protein content and ability to form $CHCl_3$ soluble ^{14}C-compounds from *p*-hydroxy-[7-^{14}C]benzoate and MLE that had been preincubated with IPP (MLE–IPP) to provide a source of protein bound polyprenylpyrophosphates (Table 1).* The results showed that exposure of the cells to mechanical grinding for 10 min at 0° gave a good yield of 10000 *g* particles with specific activities comparable to those obtained with the best preparations from fresh yeast cells. Subsequent studies showed that for the period 0–7 days from purchase the age of the yeast block had little effect on the specific activities of the preparations.

TABLE 1. RELEASE OF PROTEIN AND *p*-HYDROXYBENZOATE POLYPRENYLTRANSFERASE ACTIVITY FROM BAKER'S YEAST BY MECHANICAL GRINDING

Period of mechanical grinding (min)	Cell-free homogenate Protein (mg/6·4 g fr. wt)	10000 *g* Particulate fraction	
		Protein (mg/6·4 g fr. wt)	Transferase activity (nmol/10 mg protein/30 min)
5	324	99	6·9
10	455	152	7·8
15	464	248	4·5
20	480	261	4·8

Incubations for the assay of transferase activity consisted of 3 ml of MLE–IPP (11·4 μmol), 2 ml of 0·05 M phosphate buffer, pH 7·1, 13·4 nmol of *p*-hydroxy-[7-^{14}C]benzoate (7·7 μCi/μmol), 10 mg of protein and 100 μmol of $MgCl_2$. The mixtures were incubated for 30 min.

The biosynthetic capabilities of the cell-free homogenates and 10000 *g* particulate fractions from blocks of baker's yeast were investigated by incubating them with *p*-hydroxy-[U-^{14}C]benzoate and either IPP or MLE–IPP, and determining the nature of any $CHCl_3$ soluble ^{14}C-products (Table 2). It was found that both the cell-free preparation and 10000 *g* particulate fraction gave rise to $CHCl_3$ soluble ^{14}C-products when MLE–IPP was used as a source of polyprenylpyrophosphates, but that only the cell-free preparation

* Evidence that the polyprenylpyrophosphates are protein bound in the MLE–IPP supplement has been provided by the demonstration that on chromatography of MLE–[1-^{14}C]IPP on Sephadex G100 most of the radioactivity is eluted co-incident with the protein in the void volume (THOMAS, G. and THRELFALL, D. R., unpublished observations).

TABLE 2. SYNTHESIS OF 4-CARBOXY-2-POLYPRENYLPHENOLS FROM *p*-HYDROXY-[U-^{14}C]BENZOATE AND MLE–IPP OR IPP OR FPP BY CELL-FREE PREPARATIONS OF BAKER'S YEAST

Preparation	Protein (mg)	Source of side-chain	4-Carboxy-2-polyprenylphenols (nmol/10 mg protein/hr) Di-	Tri-	Tetra-	Penta-	Hexa-	Hepta-	Octa-	Nona-	Deca-
Cell-free homogenate	10·6	IPP	—	2·4	—	—	trace	trace	—	—	—
Cell-free homogenate	10·6	MLE–IPP	0·04	0·08	0·77	0·25	0·07	0·09	0·72	2·4	0·52
Washed 10000 *g* particles	9·7	IPP	—	—	—	—	—	—	—	—	—
Washed 10000 *g* particles	9·7	MLE IPP	0·21	0·22	2·0	0·94	0·18	0·23	1·82	5·99	1·43
Washed 10000 *g* particles	8·9	FPP		2·2							

The first 4 preparations were obtained from the same yeast homogenate. Incubations 1, 3 and 5 consisted of 5 ml of 0·05 M phosphate buffer, pH 7·1, 16·2 μmol of *p*-hydroxy-[U-^{14}C]benzoate, 8·9–10·6 mg of protein, 100 μmol $MgCl_2$, and either 7·5 μmol of IPP or 1 μmol of FPP. Incubations 2 and 4 consisted of the components listed in Table 1. The mixtures were incubated for 1 hr.

(due to the presence of a FPP synthesizing system) could use IPP as a potential source of side-chains. As expected if the transferase activity is present in the mitochondria, the 10000 *g* particulate fraction had a higher specific activity than the cell-free preparation. Adsorptive TLC established that the radioactive compounds present in the $CHCl_3$ extracts had the properties expected of 4-carboxy-2-polyprenylphenols. Reversed-phase TLC of the radioactive phenols recovered from the adsorptive TLC step was used to resolve the homologues from each other (Fig. 1). The identities of the phenols were established by

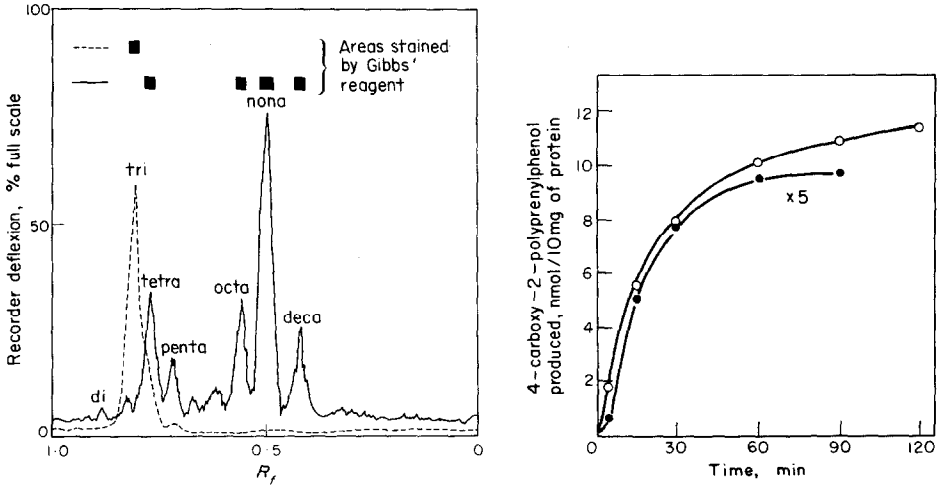

FIG. 1. RADIOCHROMATOGRAM SCANS OF REVERSED PHASE TL CHROMATOGRAMS OF PARTLY PURIFIED ^{14}C-PHENOLS.

Partly purified 4-carboxy-2-polyprenylphenols from cell-free homogenates that had been incubated with *p*-hydroxy-[U-^{14}C]benzoate and either MLE–IPP (——) or IPP (– – –) were chromatographed on paraffin-impregnated silica gel G developed with 80% aq. acetone.

FIG. 2. SYNTHESIS OF 4-CARBOXY-2-POLYPRENYLPHENOLS (○——○) AND 4-CARBOXY-2-TRIPRENYLPHENOL (●——●) BY A 15000 *g* PARTICULATE FRACTION.

Incubations for the assay of *p*-hydroxybenzoate polyprenyltransferase activity consisted of 3 ml of MLE–IPP (11·4 μmol), 2 ml of 0·05 M phosphate buffer, pH 7·1, 16·2 μmol of *p*-hydroxy-[U-^{14}C]benzoate (7·7 mCi/mmol), 10·5 mg of protein and 100 μmol of $MgCl_2$. Incubations for the assay of *p*-hydroxybenzoate triprenyl-transferase activity consisted of 3 ml of 0·05 M phosphate buffer, pH 7·1, 16·2 μmol of *p*-hydroxy-[U-^{14}C]benzoate (7·7 mCi/mmol), 1 μmol of FPP, 10·5 mg of protein and 100 μmol of $MgCl_2$. To aid in the comparison of the curves the values for the amounts of 4-carboxy-2-triprenylphenol produced have been multiplied by 5.

comparing their mobilities with that of authentic 4-carboxy-2-octaprenylphenol; the identifications were greatly helped by the facts that (a) in the incubations containing MLE–IPP the complete sequence of di- through to decaprenyl homologues was produced and (b) the principal products were present in sufficient amounts to be stained with Gibbs' reagent (Fig. 1). Confirmation of the chemical identities of the phenols produced was obtained by the same methods as those used to characterize the phenols produced by *S. carlsbergensis*[2] i.e. the demonstration that (a) they were decarboxylated to ^{14}C-compounds having the TLC properties of the appropriate 2-polyprenylphenols by cell-free preparations of *Rhodospirillum rubrum* and (b) they could be labelled from *p*-hydroxy-[7-^{14}C]benzoate and from MLE-[1-^{14}C]IPP.

Farnesylpyrophosphate as a source of side-chain

The experiments described above provided good evidence that the composition of the available polyprenylpyrophosphates determines which 4-carboxy-2-polyprenylphenols are synthesized by the yeast preparation. Confirmation that the enzyme uses preformed polyprenylpyrophosphates was provided by the demonstration that when a 10000 *g* particulate fraction is incubated with *p*-hydroxy-[U-^{14}C]benzoate and FPP the product is 4-carboxy-2-triprenylphenol (Table 2). At this stage it was decided to use this system for the rest of this part of the study, since it provided the most defined one that had been tested. Ideally, hexaprenylpyrophosphate should have been used since ubiquinone-6 is the constitutive ubiquinone of baker's yeast.[1] Unfortunately, the hexaprenol required for the chemical synthesis of hexaprenylpyrophosphate is not available from commercial sources.

The degree of binding of the 4-carboxy-2-triprenylphenol produced in the above reaction to the particulate components of the incubation mixture was investigated by subjecting incubation mixtures to 100000 *g* for 20 min before analysis. In all cases it was found that more than 90% of the incorporated activity was associated with the 100000 *g* pellet. This suggested that *in vitro* the product of the reaction is not released from the mitochondrial membranes.

Factors affecting the synthesis of 4-carboxy-2-triprenylphenol by a 10000 g particulate fraction from baker's yeast

Time. The first factor to be investigated was the effect of time on the rate of formation and yield of 4-carboxy-2-triprenylphenol (Fig. 2). As a comparison the effect of time of the formation of 4-carboxy-2-polyprenylphenols from *p*-hydroxy-[U-^{14}C]benzoate and MLE–IPP was also determined (Fig. 2).

The curves obtained for the amounts of 4-carboxy-2-polyprenylphenols produced with time were of the expected type. Rather surprisingly, however, since the amounts of polyprenylpyrophosphates present in the two types of incubation media were approximately the same, it was found that the synthesis of 4-carboxy-2-triprenylphenol in the incubation containing FPP ceased after 12% of the available *p*-hydroxy-[U-^{14}C]benzoate had been utilized, whilst the synthesis of 4-carboxy-2-polyprenylphenols in the incubation containing MLE–IPP continued until 72% of the available *p*-hydroxy-[U-^{14}C]benzoate had been utilized. The reasons for these differences are not clear, although one possibility is that the physical state in which the polyprenylpyrophosphates are presented is important.

For the rest of the work using FPP it was decided to use 10 mg or less of protein/3 ml of incubation mixture and to incubate for 30 min.

Concentration of FPP. Over the range 0–2 μmol of FPP/3 ml of incubation mixture the

expected type of relationship between [FPP] and reaction rate was obtained (Fig. 3). The apparent limiting rate observed at the highest [FPP] was, however, due in part to precipitation of some of the components of the incubation mixture during the course of the reaction: because of this 1 μmol of FPP/3 ml of incubation mixture was used for the remaining assays.

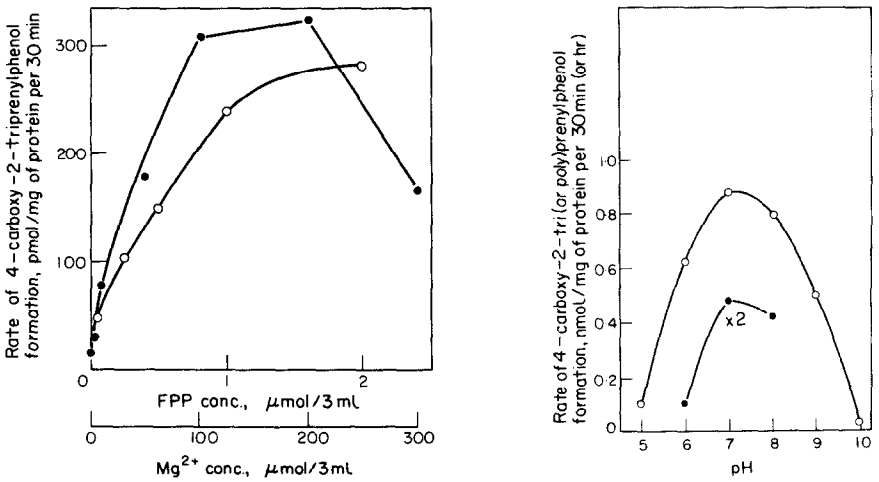

FIG. 3. EFFECT OF [FPP] (○——○) AND [Mg^{2+}] (●——●) ON THE RATE OF 4-CARBOXY-2-TRIPRENYLPHENOL FORMATION BY A 15000 *g* PARTICULATE FRACTION.

Incubations consisted of 3 ml of 0·05 M phosphate buffer, pH 7·1, 16·2 μmol of *p*-hydroxy-[U-^{14}C]benzoate (7·7 mCi/mmol), 4·3 mg of protein and either 100 μmol of $MgCl_2$ and variable amounts of FPP or 1 μmol of FPP and variable amounts of $MgCl_2$. The mixtures were incubated for 30 min.

FIG. 4. EFFECT OF pH ON THE RATE OF FORMATION OF 4-CARBOXY-2-POLYPRENYLPHENOLS (○——○) AND 4-CARBOXY-2-TRIPRENYLPHENOL (●——●) BY A 15000 *g* PARTICULATE FRACTION.

Incubations for the assay of 4-carboxy-2-polyprenylphenol activity consisted of 3 ml of MLE–IPP (11·4 μmol) (prepared in the absence of buffer), 2 ml of Universal buffer (Britton and Robinson type),[5] 16·2 μmol of *p*-hydroxy-[U-^{14}C]benzoate, 15 mg of protein and 100 μmol of $MgCl_2$. The mixtures were incubated for 1 hr. Incubations for the assay of 4-carboxy-triprenylphenol activity consisted of 3 ml of Universal buffer, 16·2 μmol of *p*-hydroxy-[U-^{14}C]benzoate (7·7 mCi/mmol), 1 μmol of FPP, 4·3 mg of protein and 100 μmol of $MgCl_2$. The mixtures were incubated for 30 min. To aid in the comparison of the curves the values for the rates of formation of 4-carboxy-2-triprenylphenol have been multiplied by 2.

Concentration of Mg^{2+}. Mg^{2+} acted as an activator. Maximum activation was obtained at 100–200 μmol of Mg^{2+}/3 ml of incubation. The inhibitory effect of high [Mg^{2+}] was due in part to the precipitation of some of the components of the reaction mixture.

pH. The effect of pH on the rate of reaction was studied for incubations containing either FPP or MLE–IPP (Fig. 4). In both assay systems the pH optimum was found at or about pH 7.

Concentration of phosphate buffer. It had been reported that activity in rat liver preparations is inhibited by phosphate.[6] Similarly, it was found that in the yeast system [phosphate buffer] influenced the rate of reaction (Fig. 5); the reaction being some 10% inhibited (by extrapolation) under the assay conditions used.

[5] MCKENZIE, H. A. (1969) *Data for Biochemical Research* (DAWSON, R. M. C., ELLIOTT, D. C., ELLIOTT, W. H. and JONES, K. M., eds.), pp. 476–506, Oxford University Press, London.

[6] SCHECHTER, N., NISHINO, T. and RUDNEY, H. (1973). *Arch. Biochem. Biophys.* **158**, 282.

It is worth recording here that in incubations containing MLE–IPP, the rates were reduced when phosphate buffer was replaced by Tris–HCl buffer (Fig. 5).

KF. Momose and Rudney[4] found that the rate of synthesis of 4-carboxy-2-nonaprenylphenol from *p*-hydroxybenzoate and IPP by rat mitochondrial systems was markedly stimulated on the addition of KF. However, in none of the yeast systems tested was any stimulation observed.

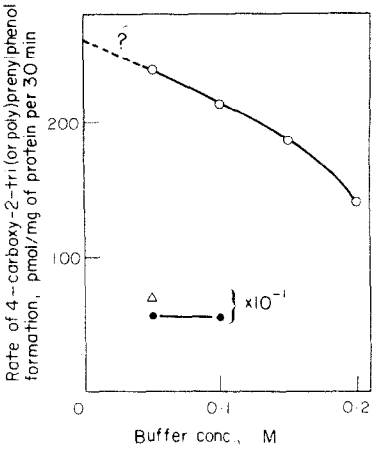

FIG. 5. EFFECT OF [PHOSPHATE BUFFER] ON THE RATE OF 4-CARBOXY-2-TRIPRENYLPHENOL (O—O) FORMATION BY A 15000 *g* PARTICULATE FRACTION.

Incubations for the assay of 4-carboxy-2-triprenylphenol activity consisted of the same components as those listed in Fig. 4, with the exception of the Universal buffer which was replaced by varying [phosphate buffer]. The mixtures were incubated for 30 min. Incubations for the assay of 4-carboxy-2-polyprenylphenol activity consisted of the same components as those listed in Fig. 4 with the exception of the Universal buffer which was replaced by either phosphate buffer (△) or Tris–HCl buffer (●—●). The values obtained in this part of the experiment have been multiplied by 10^{-1}.

Intracellular distribution of transferase activity in cells of S. carlsbergensis

Previous studies in which *S. carlsbergensis* homogenates containing no osmoticum were subjected to a scheme of differential centrifugation had suggested that the transferase activity was mainly confined to the mitochondria.[2] To confirm these findings suitably buffered and osmotically stabilized homogenates prepared from freshly-harvested cells of *S. carlsbergensis* by mechanical disintegration were fractionated by differential centrifugation, and the fractions analysed for transferase activity and two mitochondrial marker enzyme systems, NADH–oxidase and succinate–oxidase (Table 3).

The results show that the distribution of transferase activity closely follows the distribution of the mitochondrial marker enzymes, the greater part of it being present in the 15000 *g* fraction. These findings serve to confirm that the transferase activity is almost completely confined to the mitochondria, although its presence in small amounts in other organelles cannot be excluded completely.

TABLE 3. INTRACELLULAR DISTRIBUTION OF *p*-HYDROXYBENZOATE POLYPRENYLTRANSFERASE ACTIVITY IN 2-DAY-OLD *S. carlsbergensis*

Fraction	Protein (mg)	Succinic oxidase (% total activity)	NADH oxidase (% total activity)	Transferase activity (nmol/10 mg protein/hr)	(% total activity)
Debris and nuclear	0·8	0·1	0·4	2·0	0·2
Mitochondrial	28·6	81·8	72·2	17·7	76·8
Microsomal	18·0	15·3	26·2	6·3	22·2
Supernatant	84·0	2·8	1·2	0·1	0·8

Incubations for the assay of transferase activity in the mitochondrial, microsomal and supernatant fractions each consisted of 3 ml of MLE–IPP (7·5 μmol), 2 ml of 0·05 M phosphate buffer, pH 7·0, 23·3 μmol of *p*-hydroxy-[U-^{14}C]benzoate (7·7 μCi/μmol), 10 mg of protein and 100 μmol of $MgCl_2$. The incubation for the assay of transferase activity in the supernatant fraction contained 1/5 of each of the standard components listed above and 0·6 mg of protein. The mixtures were incubated for 1 hr.

DISCUSSION

The first part of this study established that it is possible to prepare cell-free homogenates and particulate fractions having *p*-hydroxybenzoate polyprenyltransferase activity from 0 to 7-day-old blocks of compressed baker's yeast by mechanical grinding. The specific activities of the preparations are similar to those obtained with preparations from freshly grown yeast cells.

In a previous study it was found that *S. carlsbergensis* preparations supplemented with MLE–IPP as a source of protein bound polyprenylpyrophosphates produced the hexa- (17%), hepta- (11%), octa- (8%), nona- (52%) and deca- (12%) prenyl homologues of 4-carboxy-2-polyprenylphenol.[2] In the present study, however, both baker's yeast and *S. carlsbergensis* preparations when supplemented with MLE–IPP produced the di- through to deca-prenyl homologues with tetra (15%), penta- (7%), octa- (14%), nona- (46%) and deca- (11%) prenyl homologues as the predominant forms (Table 2 and Fig. 1). The reason for this change in pattern is not clear: although, it may be due to either the use of a different batch of MLE or some, as yet undetermined, variation in the conditions used for the preincubation of the MLE with IPP.

As expected the cell-free homogenate, due to the presence of a very active FPP synthesizing system, produced substantial quantities of 4-carboxy-2-triprenylphenol when supplemented with IPP (Table 2 and Fig. 1). Occasionally, in these incubations, small quantities of di-, hexa- and heptaprenyl homologues were produced. Thus establishing that the enzymes necessary for producing a polyprenylpyrophosphate with the same side-chain as the principal constitutive ubiquinone (ubiquinone-6) are present in the preparation. The 10000 *g* particles (which contain most of the transferase activity), however, could not make use of IPP as a side-chain precursor. This result is in contrast to the results obtained with animal mitochondria,[4] which can synthesize 4-carboxy-2-polyprenylphenols with the same side chains as the constitutive ubiquinones, and when considered in conjunction with the fact that cell-free homogenates are capable of producing 4-carboxy-2-hexaprenyl-pyrophosphate from *p*-hydroxybenzoate and IPP would suggest that hexaprenylpyrophosphate required by the transferase is not synthesized in yeast mitochondria. It may be of course that, as suggested previously, the 10000 *g* particles cannot use IPP due to the loss

or inactivation of one or more of the enzymes required for the synthesis of hexaprenylpyrophosphate.[2] This aspect is now the subject of further investigation. The above findings show that in common with *Rsp. rubrum*,[2] animal,[4] bean and *S. carlsbergensis* polyprenyltransferases,[2] the baker's yeast transferase shows little specificity with regard to the length of the polyprenyl side-chain it transfers to *p*-hydroxybenzoate.

The requirement of the enzyme for preformed polyprenylpyrophosphates was confirmed by the demonstration that the 10000 *g* particulate fraction produces 4-carboxy-2-triprenylphenol when incubated with *p*-hydroxybenzoate and FPP (Table 2). It is of interest that this phenol is produced in only some 20–30% of the amounts of the phenols produced when MLE–IPP is used as a source of side chains, even though the concentrations of polyprenylpyrophosphates in the incubation mixtures are probably very similar (Fig. 2). A possible explanation for this difference is that in the case of the incubations containing FPP the alcoholpyrophosphate is either adsorbed onto the particulate fraction or precipitated out of solution during the course of the incubation, whilst in the case of the incubation containing MLE–IPP the protein bound alcohol pyrophosphates remain constantly available to the enzyme.

The factors affecting the synthesis of 4-carboxy-2-triprenylphenol from *p*-hydroxybenzoate and FPP by the 10000 *g* particulate transferase were studied in some detail. It was found that for a fixed concentration of *p*-hydroxy-[U-^{14}C]benzoate the rate of reaction was proportional to the concentration of FPP over the range 0–1 μmol/3 ml of incubation, above this range the rate plateaued due in part to the precipitation of some of the components of the reaction mixture (Fig. 3). The transferase was activated by Mg^{2+} ions (Fig. 3), had a sharp pH optima at or about 7 (Fig. 4), and was inhibited by phosphate buffer (Fig. 5). KF, a compound that had been reported to have a marked stimulatory effect on the synthesis of 4-carboxy-2-nonaprenylphenol from *p*-hydroxybenzoate and IPP in rat liver mitochondria,[4] had no effect on the rate of reaction or final yield of products.

The last part of the study established that in freshly grown cells of *S. carlsbergensis* the greater part of the transferase activity is associated with the mitochondria, although its presence in other cell organelles cannot be excluded (Table 3). This finding when considered in conjunction with the fact that 5-demethoxyubiquinone-6 is localized mainly in the mitochondria[7] would seem to provide evidence that the yeast mitochondria contain all of the enzymes required to convert *p*-hydroxybenzoate into ubiquinone.

This investigation, therefore, establishes that the properties of the yeast *p*-hydroxybenzoate polyprenyltransferase are almost identical to those that have been described for the animal transferase.[4,6] Throughout this study no evidence for the presence of 4-carboxy-2-polyprenylphenol decarboxylase, the next enzyme on the pathway leading from *p*-hydroxybenzoate to ubiquinone, in any of the preparations has been obtained. This is not entirely surprising, since it has been found that *Rhodospirillum rubrum*, *Chromatium* sp. and *Pseudomonas fluorescens* decarboxylases are very labile and rapidly lose their activity in the presence of O_2 (G. Thomas and D. R. Threlfall, unpublished observations).

EXPERIMENTAL

Radiochemicals. p-Hydroxy-[U-^{14}C]benzoic acid (7·7 mCi/mmol) was prepared by alkaline fusion[8] of L-[U-^{14}C]tyrosine hydrochloride obtained from The Radiochemical Centre, Amersham, Bucks., U.K. *p*-Hydroxy-[7-^{14}C]benzoic acid (7·7 mCi/mmol) was prepared by dilution of *p*-hydroxy-[7-^{14}C]benzoic acid (55 mCi/mmol)

[7] AH LAW and THRELFALL, D. R. (1972) *Phytochemistry*, **11**, 481.
[8] WHISTANCE, G. R., THRELFALL, D. R. and GOODWIN, T. W. (1967) *Biochem. J.* **105**, 145.

obtained from Schwarz–Mann, Orangeburgh, New York, U.S.A. Triammonium-[1-^{14}C]IPP (60 mCi/mmol) was purchased from The Radiochemical Centre, Amersham.

Synthesis of IPP and FPP. Trilithium IPP was synthesized from isopentenol (Aldrich Chemical Co., Milwaukee, Wisconsin, U.S.A.) by the method of Cornforth and Pópjak.[9] FPP was synthesized from *trans-trans*-farnesol (Ralph Emmanuel, Wembley, U.K.) by the method of Cramer and Böhm[10] as used by Cornforth and Pópjak.[9] The concn of FPP present in the stock solns used for incubation studies was determined by assaying the inorganic phosphate liberated on boiling in 5 M H_2SO_4 for 10 min by the method of Fiske and SubbaRow.[11]

Biological material. S. carlsbergensis Herb I.M.I. 80178 was grown under the conditions described by Ah Law and Threlfall.[7] Blocks of compressed baker's yeast (*S. cerevisiae*) (N.G. & S.F., Fermentation Products, Dock Road, Felixstowe, U.K.) were purchased from a local supermarket and stored at 3° until required.

Preparation of cell-free homogenates and 10000 g *particulate fractions from baker's yeast.* Baker's yeast (9 g) was suspended in 12 ml of ice-cold 0·05 M phosphate buffer, pH 7·1, with gentle stirring. Fifteen ml of the suspension was then divided equally between two 15 ml Pyrex vessels each of which contained 1 g of Ballotini beads (no. 8) and shaken at max amplitude on a Mickle High Speed Tissue Disintegrator (Mickle Laboratory Engineering Co., Gomshall, U.K.) for 10 min. The resultant homogenates were transferred to a centrifuge tube which contained 15 ml of ice-cold 0·05 M phosphate buffer, pH 7·1, and fractionated by centrifuging twice at 1000 *g* for 5 min and, if required, once at 10000 *g* for 20 min. The 10000 *g* ppt. was washed by resuspension in 0·05 M phosphate buffer, pH 7·1 and resedimented at 10000 *g* for 5 min. The 1000 *g* supernatant and 10000 *g* ppt. were designated cell-free homogenate and 10000 *g* particulate fraction respectively.

Fractionation of S. carlsbergensis. Two-day-old cells (24 g fr. wt) from 2·5 l of growth medium were harvested by centrifugation, suspended in 12 ml of 0·05 M phosphate–EDTA buffer, pH 7·2, which was 0·25 M with respect to mannitol and disrupted by mechanical grinding in a Mickle High Speed Tissue Disintegrator under the same conditions as those described in the previous section. The homogenate was then fractionated by centrifugation at 500 *g* for 5 min, 1000 *g* for 5 min, 15000 *g* for 25 min and 120000 *g* for 40 min. The 1000 *g*, 15000 *g* and 120000 *g* ppt.s were designated cell debris and nuclear fraction, mitochondrial fraction and microsomal fraction respectively. The 120000 *g* supernatant was designated supernatant fraction.

Radiochemical assay of p-*hydroxybenzoqte polyprenyltransferase activity.* The details of the various incubation procedures used for the assay of transferase activity are given in the Results. The components in 0·05 M phosphate buffer, pH 7·1, were always added in the order: 0·05 M phosphate buffer, pH 7·1, MLE–IPP or Li_3 IPP or FPP, *p*-hydroxy-[^{14}C]benzoate, protein, Mg^{2+} (as $MgCl_2$). In those incubations supplemented with MLE–IPP the MLE was prepared and preincubated with IPP under the conditions described by Raman *et al.*[12] The mixtures were incubated in air for the appropriate period of time at 30° with gentle agitation. At the end of the incubation period the reaction was stopped by the rapid addition of 15 ml $CHCl_3$–MeOH (1:2) and, after allowing the mixture to stand for 2 hr, the lipids were extracted by the method of Galliard *et al.*[13] The resultant lipid extract was taken up in 2 ml petrol. (b.p. 40–60°) and 1 ml assayed for radioactivity. The radioactivity present in the lipid extracts was associated entirely with 4-carboxy-2-polyprenylphenols (see below) and was used to calculate the amounts of 4-carboxy-2-polyprenylphenols produced in the incubations.

Identification of radioactive compounds present in the lipid extracts. The nature and chain lengths of the ^{14}C-compounds present in the lipid extracts were determined by a combination of adsorptive and reversed phase TLC.[2] In all of the extracts examined the only ^{14}C-compounds present were 4-carboxy-2-polyprenylphenols.

Conversion of 4-*carboxy*-2-*polyprenylphenols to* 2-*polyprenylphenols by* R. rubrum *preparations.* This was carried out by the procedure described by Momose and Rudney.[4] The 2-polyprenylphenols produced were extracted from the incubation mixtures by the method described above and characterized by reversed phase TLC.[14]

NADH- and succinic oxidase activity. These were determined by a polarographic procedure.[7]

Protein estimation. Protein content was estimated by the method of Lowry *et al.*[15] Crystalline bovine serum albumin was used to prepare the standard curve.

Radioassay. Lipid samples were assayed for radioactivity in a Beckman Liquid Scintillation Spectrometer. The samples, after evaporation of any solvent, were taken up in 10 ml of toluene containing 0·05 g of 1,4-diphenyloxazole and 0·003 g of 1,4-bis-(4-methyl-5-phenyloxazole-2-yl)-C_6H_6. All counts were corrected for background and instrument efficiency. TL chromatograms were scanned for radioactivity in a Panax TL Chromatogram Scanner System.

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