Dihydroxyacetone Synthase: a Special Transketolase for Formaldehyde Fixation from the Methylophilic Yeast *Candida boidinii* CBS 5777

By M. J. WAITES AND J. R. QUAYLE*

Department of Microbiology, The University, Sheffield S10 2TN, U.K.

(Received 13 July 1982)

The transketolase synthesized by *Candida boidinii* CBS 5777 during growth on non-C₁ substrates has been purified and shown to have a molecular weight of approximately 135 000 and to consist of two subunits of approximate molecular weight 68 000. The enzyme is active with xylulose 5-phosphate as glycolyl donor and ribose 5-phosphate as acceptor; under the conditions of assay formaldehyde was inactive as acceptor. *Candida boidinii* CBS 5777 also synthesizes a second transketolase during growth on methanol. This enzyme, which is unstable, has been purified to homogeneity. It has a wide substrate specificity towards glycolyl donors and acceptors; formaldehyde is a good acceptor and in terms of its physiological role during growth on methanol the enzyme can be described as a dihydroxyacetone synthase. This enzyme has a molecular weight of 105 000–110 000, with two subunits of molecular weight 62 000–67 000. Its properties have been compared with analogous enzymes purified elsewhere from methanol-grown *Candida boidinii* KD1 and *Candida boidinii* (*Kloeckera* sp.) 2201.

INTRODUCTION

A key step in the xylulose monophosphate (XuMP) cycle of formaldehyde fixation which operates in methylotrophic yeasts during growth on methanol is a transketolase-type reaction involving the transfer of a glycolaldehyde fragment from XuMP to formaldehyde, producing dihydroxyacetone (DHA) and glyceraldehyde 3-phosphate (Kato *et al.*, 1979; O'Connor & Quayle, 1980; Waites & Quayle, 1980). During growth on methanol two transketolases are synthesized which can be separated by ion-exchange or hydroxylapatite chromatography. One is a relatively stable classical transketolase that is also found during growth on multi-carbon substrates, the other is an unstable special transketolase (DHA synthase) (O'Connor & Quayle, 1980; Waites & Quayle, 1980, 1981; Bystrykh *et al.*, 1981; Kato *et al.*, 1982). The classical transketolase shows little or no activity with formaldehyde as glycolaldehyde acceptor whereas the DHA synthases from *Candida boidinii* CBS 5777 and *C. boidinii* (*Kloeckera* sp.) 2201 have been shown to exhibit both classical and DHA synthase activities, i.e. they can use both aldose phosphates (such as ribose 5-phosphate) and formaldehyde as acceptors for the glycolyl group from the donor (Waites & Quayle, 1981; Kato *et al.*, 1982). By contrast, Bystrykh *et al.* (1981) have reported that the DHA synthase from *Candida boidinii* KD1 is more specific, with formaldehyde being the only active glycolaldehyde acceptor out of the six aldehydes and aldose phosphates tested.

In the present paper we describe the purification, substrate specificity and general properties of the DHA synthase from methanol-grown *Candida boidinii* CBS 5777, comparing it with transketolases in general and DHA synthases recently purified from closely related strains.

Abbreviations: DHA, dihydroxyacetone; DHAP, dihydroxyacetone phosphate; DTT, dithiothreitol; GAP, glyceraldehyde 3-phosphate; MTT, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide; PCMB, *p*-chloromercuribenzoic acid; PES, phenazine ethosulphate; PMSF, phenylmethane sulphonyl fluoride; RiMP, ribose 5-phosphate; TEMED, *N,N,N',N'*-tetramethylethylenediamine; TPP, thiamin pyrophosphate; XuMP, xylulose 5-phosphate.

METHODS

Organism and growth conditions. *Candida boidinii* CBS 5777 was grown in shake flasks at 30 °C in the vitamin-supplemented mineral salts medium of Van Dijken *et al.* (1976). Filter-sterilized methanol (0.5%, w/v) or ethanol (0.2%, w/v) and autoclaved glucose or xylose (0.2%, w/v) were used as carbon and energy sources. For all studies, cultures were harvested in late-exponential phase by centrifugation at 6000 g for 20 min.

Preparation of extracts. Cell-free extracts were prepared by ultrasonication (MSE model 150 W) at 0 °C for 6 × 1 min at 1 min intervals in 4 vol. 10 mM-KH₂PO₄/NaOH pH 7.0 containing 0.5 mM-TPP. For the breakage of methanol-grown cells the buffer contained the following additional compounds: MgCl₂, 5 mM; DTT, 1.0 mM; EDTA, 1.0 mM; PMSF, 0.024% (w/v) (Kato *et al.*, 1982); this is referred to hereafter as buffer A. Supernatants obtained following centrifugation at 38000 g for 20 min were used for all further enzyme studies.

Enzyme assays. All assays were carried out in 1 ml total volume at 30 °C using a Pye-Unicam SP1800 double-beam recording spectrophotometer.

Classical transketolase (EC 2.2.1.1) and DHA synthase were routinely assayed by the continuous methods described previously (Waites & Quayle, 1981). The DHA synthase assay system contained glycylglycine buffer pH 7.6, 50 mM; MgCl₂, 2 mM; NADH, 0.15 mM; TPP, 0.005% (w/v); ATP, 1 mM; triosephosphate isomerase (EC 5.3.1.1), 6 units; glycerol-3-phosphate dehydrogenase (EC 1.1.1.8), 0.8 unit; glycerol kinase (EC 2.7.1.30, from *Candida mycoderma*), 2 units; XuMP, 2.5 mM; formaldehyde, 2 mM. To study the substrate specificity for glycolaldehyde donors and acceptors the assay was slightly modified. In assays with various possible glycolaldehyde donors the acceptor was always formaldehyde; activity was measured by following only DHA production in the system described above but lacking triose phosphate isomerase. Activity with various glycolaldehyde acceptors was determined by monitoring only GAP production in the system described previously with XuMP as donor but lacking ATP and glycerol kinase.

Fructose-bisphosphate aldolase (EC 4.1.2.13) and catalase (EC 1.11.1.6) were determined by the procedures of Van Dijken *et al.* (1978) and Lück (1963), respectively. Lactate dehydrogenase (EC 1.1.1.27) was determined spectrophotometrically in an assay mixture containing 10 mM-Tris/HCl buffer pH 7.5, 0.15 mM-NADH and enzyme preparation; after assessing NADH oxidase activity the assay was started by adding sodium pyruvate to a concentration of 5 mM. Malate dehydrogenase (EC 1.1.1.37) was assayed by the same method except that the assay was started by addition of oxaloacetate to 0.5 mM instead of sodium pyruvate.

Purification of classical transketolase and DHA synthase and determination of their molecular weights. All procedures were carried out at 0–5 °C.

Step 1. Cell-free extracts obtained from 10 g cell paste were applied directly to a column (2 × 8 cm) of hydroxylapatite (Bio-Gel HTP) which had been equilibrated with sonication buffer, and eluted with a linear phosphate gradient (10–400 mM in 200 ml sonication buffer) essentially as described previously (Waites & Quayle, 1981). Extracts from cells grown on substrates other than methanol produced a single peak of classical transketolase activity. Those grown on methanol gave two peaks, the second also exhibited DHA synthase activity (cf. Waites & Quayle, 1981). The peaks were concentrated by ultrafiltration using an Amicon ultrafiltration unit fitted with a PM 10 Diaflo membrane (Amicon, High Wycombe, Bucks, U.K.).

Step 2. The concentrated preparation (2–4 ml) was applied to a Sephadex G-200 column (70 × 2.5 cm; bed volume, 375 ml; void volume, 147 ml), which had been equilibrated with buffer A and calibrated using the following molecular weight standards: ferritin (horse spleen), catalase (EC 1.11.1.6, beef liver), aldolase (EC 4.1.2.13, rabbit muscle), lactate dehydrogenase (EC 1.1.1.27, rabbit muscle), malate dehydrogenase (EC 1.1.1.37, pig heart, mitochondrial), ovalbumin (hen egg) and cytochrome *c* (horse heart). When purification alone was required the sample was run without accompanying standards, but for an accurate molecular weight determination samples were run with standards. Proteins were eluted with buffer A at a column flow rate of 15 ml h⁻¹ and collected in 3 ml fractions.

Non-equilibrium pH gradient electrophoresis (NEPHGE). The procedure used was based on the methods of O'Farrell *et al.* (1977). Gels (7.5%, w/v acrylamide) were prepared in glass tubes (7 × 0.5 cm) from a mixture which consisted of 6.25 ml stock acrylamide [28.4% (w/v) acrylamide and 1.6% (w/v) bis-acrylamide], 1.25 ml stock ampholines (LKB; 40%, w/v; pH 5–7) giving a final concentration of 2% (w/v), 35 µl TEMED and 17.5 ml distilled water. The mixture was degassed for 2 min and 50 µl freshly prepared 10% (w/v) ammonium persulphate added. The gels were poured, overlaid with distilled water and allowed to polymerize overnight. Gels were loaded into the electrophoresis apparatus which contained 0.02 M-NaOH in the lower reservoir with the cathode. The distilled water overlay was removed and samples of enzyme (40–60 µl) in buffer A containing in addition 10% (w/v) glycerol, 0.8% (w/v) ampholine pH 5–7 and 0.2% (w/v) ampholine pH 3–10 were placed on to the gels. Samples were overlaid to the top of the tubes with a solution containing a mixture of 0.8% (w/v) ampholine pH 5–7 and 0.2% (w/v) ampholine pH 3–10. The upper reservoir containing the anode was filled with 0.01 M-H₃PO₄ and the gels run without prerun at 400 V for 3 h at 5 °C.

Staining of NEPHGE gels for classical transketolase and DHA synthase activities. Gels were removed from the electrophoresis tubes, placed in test tubes and quickly washed in ice-cold 20 mM-glycylglycine buffer pH 7.6. The

buffer was decanted off and replaced with 2.6 ml of a solution containing glycylglycine buffer pH 7.6, 50 mM; MgCl_2 , 2 mM; sodium arsenate, 9.5 mM; NAD, 1 mM; TPP, 0.005% (w/v); MTT, 0.04% (w/v); PES, 0.01% (w/v); DTT, 0.5 mM. GAP dehydrogenase (EC 1.2.1.12), 10 units ml^{-1} , which was prepared immediately before use. The appropriate glycolaldehyde donor and acceptor substrates were added to the tubes in 0.4 ml to give a final concentration of each of 2 mM, followed by incubation in the dark at 30 °C for 30 min to 1 h. For the detection of DHA synthase XuMP or hydroxypyruvate was used as donor with formaldehyde or glycolaldehyde as acceptor. When hydroxypyruvate was used as glycolaldehyde donor 4 units of glycerol kinase (EC 2.7.1.30), 20 units of triose phosphate isomerase and 1 mM-ATP were also present. Classical transketolase activity was detected with XuMP as donor and RiMP as acceptor substrates. The DHA synthase band detected by the staining procedure was cut from the gel with a razor blade, placed in a vial with 0.5 ml distilled water and pulverized using a glass rod. Protein was extracted overnight at 5 °C; the overlying aqueous extract was then removed and stored at -15 °C until required.

SDS-disc polyacrylamide gel electrophoresis. The methods used were based on those of Laemmli (1970). Separation gels (5–10%, w/v acrylamide) were prepared without stacking gels in glass tubes (7 × 0.5 cm). Samples were prepared in the buffer mixture of Laemmli (1970) and then held at 90 °C for 3 min, cooled, and applied to the gels. Samples were overlaid with electrophoresis buffer and run at 5 mA for 3–4 h. Gels were stained with 2% (w/v) Coomassie brilliant blue G250 in 7% (v/v) acetic acid for 1–2 h, then destained in repeated changes of 7% (v/v) acetic acid containing 5% (v/v) methanol. Molecular weight standards used were cytochrome *c* (horse heart), trypsin inhibitor (soya bean), ovalbumin (hen egg), catalase subunit (beef liver), bovine serum albumin and RNA polymerase α , β and β' subunits (*Escherichia coli*).

Analytical methods and chemicals. Protein was determined by the Lowry method using bovine serum albumin as standard. Dextran blue, cytochrome *c*, ferritin and ovalbumin were estimated by extinction at 625 nm, 412 nm, 230 nm and 280 nm, respectively. Formaldehyde was prepared as described previously (Waites & Quayle, 1981). Biochemicals and enzymes were mainly purchased from Boehringer except transketolase, hydroxypyruvate, ovalbumin, glycolaldehyde, XuMP, ribulose 5-phosphate, SDS, MTT, PES and TPP, which were obtained from Sigma. DHA and glyceraldehyde were supplied by BDH. Ampholines were purchased from LKB.

RESULTS

Purification of DHA synthase

Previous attempts to purify DHA synthase have been greatly hindered by its extreme instability, and efforts to stabilize it by various means have had limited success (O'Connor & Quayle, 1980; Waites & Quayle, 1981). Neither the supernatant from boiled cell-free extracts nor peroxisome preparations from methanol-grown cells had appreciable stabilizing effect. Recently Kato *et al.* (1982) reported that the DHA synthase from *Candida boidinii* (*Kloeckera* sp.) 2201 could be stabilized by using a phosphate buffer containing MgCl_2 , TPP, EDTA, DTT and PMSF (buffer A), and that if any one component was omitted the stabilizing effect was greatly decreased. In our earlier studies we investigated all of these compounds, individually and in various pairs, but never all together. We have now examined the effects of buffer A on the thermal stability of DHA synthase in crude cell-free extracts of *C. boidinii* CBS 5777. During incubation at 35 °C there was an initial loss of approximately 50% of the activity. However, the remaining activity was stable for long periods at this temperature, which was a great improvement over the effect seen in the absence of the buffer A mixture (Fig. 1). The omission of individual components from buffer A was also studied (Table 1), and as Kato *et al.* (1982) found, the most effective stabilization was provided by the complete mixture. During our stability tests, omission of PMSF had relatively little influence, whereas stabilization was considerably lowered if any one of the other components, particularly EDTA, was removed. Thereafter buffer A was used in DHA synthase purifications where a long period (over 15 h) on a Sephadex G-200 column was the final step.

The purification procedure that we used, although involving only two steps, yielded apparently electrophoretically homogeneous preparations. In extracts of methanol-grown cells DHA synthase was separated from the classical transketolase by hydroxylapatite chromatography, a method which we have used extensively (Waites & Quayle, 1981; Lindley *et al.*, 1981). Previously, losses of DHA synthase at this stage were often over 90%, but the use of buffer A lowered this to approximately 50%. The final step was Sephadex G-200 gel filtration which we used both as a purification step (Table 2) and as a means of estimating the molecular weight.

Fig. 1. Thermal stability of DHA synthase in crude cell-free extracts of methanol-grown *C. boidinii*. The extracts were prepared from 1 g cell paste sonicated in 4 ml 10 mM-KH₂PO₄/NaOH buffer pH 7.0 (●) or 4 ml buffer A (○) (see Methods for details). Extracts were incubated at 35 °C and samples were withdrawn at intervals and assayed for DHA synthase activity.

Table 1. *Effects of the various components of buffer A on the stability of DHA synthase activity in crude extracts of methanol-grown Candida boidinii CBS 5777*

Cell-free extracts of methanol-grown *C. boidinii* were prepared in 10 mM-KH₂PO₄/NaOH buffer pH 7.0. The extract was divided into portions to which additional buffer containing various components was added to give a final protein concentration of 5 mg ml⁻¹ and component concentrations as specified below. Samples of these preparations were then assayed for DHA synthase activity, incubated at 35 °C for 15 min and re-assayed. Relative activity is based on activity with complete buffer A as 100%.

Incubation buffer	Relative DHA synthase activity remaining after 15 min incubation at 35 °C
Complete buffer A*	100
– MgCl ₂	63.9
– TPP	48.5
– EDTA	34.6
– DTT	79.2
– PMSF	96.2
– MgCl ₂ , TPP, EDTA, DTT, PMSF	1.5

* Buffer A contained MgCl₂, 5 mM; TPP, 0.5 mM; DTT, 1.0 mM; EDTA, 1.0 mM; PMSF, 0.024% (w/v).

Table 2. *Purification of DHA synthase from methanol-grown Candida boidinii*

The crude extract was prepared from 10 g cell paste suspended in 40 ml buffer A (see Methods). This buffer was used at all stages of the purification. Values in parentheses are classical transketolase activities with XuMP and RiMP as glycolyl donor and acceptor, respectively. Activities are expressed as μmol min⁻¹, and specific activities as μmol min⁻¹ (mg protein)⁻¹. The values given in the 'Ratio' column refer to the activity ratio of classical transketolase to DHA synthase.

Step	Volume (ml)	Total activity	Specific activity	Yield (%)	Purification	Ratio
Crude extract	45	56.43 (36.18)	0.204 (0.131)	100	1	—
Hydroxylapatite chromatography	55	26.53 (17.68)	0.443 (0.295)	47.0	2.2	0.67
Sephadex G-200 chromatography	21	12.64 (9.35)	0.766 (0.567)	22.4	3.8	0.74

During purification, the activity ratio of the classical transketolase function (RiMP as acceptor) to DHA synthase function (formaldehyde as acceptor) of DHA synthase increased slightly from 0.67 in the peak from hydroxylapatite chromatography to 0.74 in the enzyme fraction from Sephadex G-200. Nevertheless the ratio remained constant enough to indicate that a single enzyme was responsible for both activities.

Table 3. *Substrate specificity of DHA synthase for glycolaldehyde acceptors*

Enzyme activity in preparations partially purified by hydroxylapatite chromatography was assayed by following the production of GAP in a continuous assay with XuMP as glycolaldehyde donor at an assay concentration of 2.5 mM and with an acceptor concentration of 2 mM (see Methods for details). Apparent kinetic constants were determined using the direct linear plot method of Eisenthal & Cornish-Bowden (1974). Relative activity is based on activity with formaldehyde as acceptor as 100%.

Acceptor substrate	Relative activity (%)	K_m (mM)
Formaldehyde	100.0	1.14
Glycolaldehyde	141.0	0.55
DL-Glyceraldehyde	60.9	2.60
Acetaldehyde	46.6	—
D-Erythrose 4-phosphate	127.2	1.68
D-Ribose 5-phosphate	72.4	0.45
D-Ribose	0	—
D-Glucose 6-phosphate	0	—
Glutaraldehyde	0	—
Dihydroxyacetone	0	—

Table 4. *Substrate specificity for glycolaldehyde donors of DHA synthase*

Enzyme activity in preparations partially purified by hydroxylapatite chromatography was assayed by following DHA production in a continuous assay. In each case the glycolaldehyde acceptor was formaldehyde at an assay concentration of 2 mM and with a donor concentration of 2.5 mM. Apparent kinetic constants were determined using the direct linear plot method of Eisenthal & Cornish-Bowden (1974). The values in parentheses are those obtained with a preparation further purified on a column of Sephadex G-200 and free from ribulose-5-phosphate 3-epimerase (see Methods for details). Relative activity is based on activity with XuMP as donor as 100%.

Donor substrate	Relative activity (%)	K_m (mM)
D-Xylulose 5-phosphate	100	0.72
Hydroxypyruvate	41.4	0.28
D-Ribulose 5-phosphate	(0)	(—)
D-Fructose 6-phosphate	25.2	1.56
D-Glucose 6-phosphate	0	—
D-Ribose 5-phosphate	0	—

Properties of DHA synthase

DHA synthase functioned with a range of both glycolaldehyde donor and acceptor substrates (Tables 3 and 4). Formaldehyde, glycolaldehyde and erythrose 4-phosphate appeared to be the best glycolaldehyde acceptors while glyceraldehyde, acetaldehyde and RiMP were less good. The enzyme was more specific as regards glycolaldehyde donor substrates (Table 4). Of those that we tested XuMP was the most effective, though hydroxypyruvate and fructose 6-phosphate also served in this capacity. RiMP and glucose 6-phosphate did not function as glycolaldehyde donors, nor did ribulose 5-phosphate unless ribulose-5-phosphate 3-epimerase (EC 5.1.3.1) was present.

DHA synthase required both TPP and $MgCl_2$, as activity was reduced by 28.0% and 62.4%, respectively, in their absence (Table 5). As with some classical transketolases, the order of addition of TPP and $MgCl_2$ also influenced activity (Datta & Racker, 1961*a*). If TPP was added to the enzyme before $MgCl_2$, activity was decreased by 37.1%. Manganese ions (2 mM) replaced magnesium ions (2 mM) to some extent but the same concentrations of zinc or nickel ions completely inhibited DHA synthase activity. Addition of PCMB (1 mM) also resulted in 100% inhibition. Reduced glutathione or ribose equimolar with formaldehyde (2 mM) produced 60% and 31.5% inhibition, respectively. Ammonium sulphate concentrations greater than 10 mM also inhibited DHA synthase activity; this effect was shown to be mainly due to the sulphate ion.

Table 5. *Effects of cofactors and inhibitors on the activity of DHA synthase*

Enzyme activity in preparations partially purified by hydroxylapatite chromatography was assayed by monitoring GAP production in a continuous assay with XuMP as glycolaldehyde donor and formaldehyde as acceptor at assay concentrations of 2.5 mM and 2.0 mM, respectively. The enzyme was dissolved in $\text{KH}_2\text{PO}_4/\text{NaOH}$ buffer (approx. 0.2 M, pH 7.0). See Methods for details.

Assay system	Relative activity (%)
Complete assay system	100
– TPP	72.0
– MgCl_2	37.6
Zn^{2+} replacing Mg^{2+}	0
Ni^{2+} replacing Mg^{2+}	0
Mn^{2+} replacing Mg^{2+}	72.6
Complete system, TPP added before Mg^{2+}	62.9
Complete system + PCMB (1 mM)*	0
Complete system + GSH (2 mM)	40.0
Complete system + D-ribose (2 mM)	68.5
Complete system + $(\text{NH}_4)_2\text{SO}_4$ (25 mM)	87.5
Complete system + $(\text{NH}_4)_2\text{SO}_4$ (100 mM)	56.0
Complete system + NH_4Cl (50 mM)	94.0

* Assay system contained 10-fold more linkage enzymes than the routine assay.

Table 6. *Molecular weights of classical transketolase and DHA synthase from Candida boidinii*

The enzymes were partially purified by hydroxylapatite chromatography and submitted to Sephadex G-200 chromatography with and without protein standards. The enzymes from the Sephadex column chromatography without standards were then submitted to SDS-disc-polyacrylamide electrophoresis in order to determine subunit molecular weights. Where molecular weights were determined in duplicate, the respective individual values are recorded. The value in parentheses is for the estimated subunit molecular weight of DHA synthase purified by non-equilibrium pH gradient electrophoresis, located on the gel by a specific activity stain, extracted and submitted to SDS-disc-polyacrylamide electrophoresis. See Methods for details.

Enzyme	Molecular weight	Subunit molecular weight
Classical transketolase		
Methanol-grown	142500	2×68000
Ethanol-grown	130000	—
Xylose-grown	132000	—
DHA synthase		
Methanol-grown	105000, 110000	2×64000 , 2×67000 (2×62500)

Molecular weight determinations

The molecular weights of classical transketolases from methanol-, ethanol- and xylose-grown and DHA synthase from methanol-grown *C. boidinii* CBS 5777 were determined by Sephadex G-200 chromatography. The classical transketolases from cells grown on each of the three above substrates gave similar results of approximately 135 000, whereas DHA synthase was somewhat smaller at approximately 107 500 (Table 6). Following gel filtration, samples of the peaks of classical transketolase and DHA synthase of methanol-grown cells were submitted to SDS-polyacrylamide gel electrophoresis. Single protein bands were obtained for each preparation and the subunit sizes were estimated to be approximately 65 500 (Fig. 2) and 68 000 for DHA synthase and classical transketolase, respectively, thus indicating that both native enzymes are probably composed of two identical subunits.

DHA synthase was also purified by non-equilibrium pH gradient electrophoresis. The appropriate band was identified on the basis of its being the only one which appeared in an

Fig. 2. SDS-disc-polyacrylamide gel electrophoresis of purified DHA synthase from methanol-grown *C. boidinii*. A sample of DHA synthase eluted from a Sephadex G-200 column was added to a dissociation buffer mixture, heated at 90 °C for 3 min, cooled, and 30–80 μ l applied to the top of a 7.5% (w/v) polyacrylamide gel (7 $\times$ 0.5 cm) containing 0.1% SDS. Gels were run at 5 mA for 3 h and stained with Coomassie brilliant blue. See Methods for details.

Fig. 3. Non-equilibrium pH gradient electrophoresis (NEPHGE) of crude cell-free extracts of glucose-grown and methanol-grown *C. boidinii*. Cell-free extract (40–60 μ l) was applied to the NEPHGE gels pH 5–7 and run at 400 V for 3 h at 0 °C. Gels were then incubated in an activity stain mixture containing various combinations of glycolaldehyde donors and acceptors. See Methods for details. Band A is identified as DHA synthase and B may possibly be classical transketolase (see text).

activity-staining mixture in the presence of XuMP or hydroxypyruvate as donor substrates along with RiMP, formaldehyde or glycolaldehyde as acceptor substrates and also was only present in gels of methanol-grown cell extract (Fig. 3). The band was removed, extracted and submitted to SDS–polyacrylamide gel electrophoresis in order to obtain further confirmation of the subunit size. Using this procedure an estimated subunit molecular weight of 62 500 was obtained.

DISCUSSION

Candida boidinii CBS 5777 when grown on methanol, ethanol or xylose synthesizes a classical transketolase of molecular weight 130 000–142 500; there is no evidence that more than one enzyme is synthesized by the organism grown on these different substrates. Classical transketolases have previously been characterized from a variety of yeast species and their

Table 7. *Molecular weights and subunits of classical transketolase and DHA synthases from yeasts*

	Molecular weight	Subunit molecular weight
<i>Classical transketolase</i>		
<i>Saccharomyces cerevisiae</i>		
Datta & Racker (1961 <i>b</i>)	140000	2 × —
Cavalieri <i>et al.</i> (1975)	158000	2 × 76500
Heinrich & Wiss (1971)	—	2 × 70000
Saitou <i>et al.</i> (1974)	100000	2 × 50000
<i>Candida utilis</i>		
Klein & Brand (1977)	163000	2 × 78000
Specht (1977)	—	2 × 70000
Wood (1981)	—	2 × 81000
<i>Candida boidinii</i> CBS 5777		
Waites & Quayle	135000*	2 × 68000
<i>DHA synthase</i>		
<i>Candida boidinii</i> KD1		
Bystrykh <i>et al.</i> (1981)	145000	2 × 76000
<i>Candida boidinii</i> (Kloeckera sp.) 2201		
Kato <i>et al.</i> (1982)	190000	4 × 55000
<i>Candida boidinii</i> CBS 5777		
Waites & Quayle	107500*	2 × 65500*

* Mean of duplicate values from Table 6.

molecular weights range from 100000 to 163000 (Table 7); they are dimeric proteins, as is the enzyme from methanol-grown *C. boidinii* CBS 5777. Classical transketolases, in general, do not exhibit strict specificity towards donors or acceptors of glycolyl groups. Efficient donors include XuMP, fructose 6-phosphate, sedoheptulose 7-phosphate and hydroxypyruvate; RiMP, erythrose 4-phosphate and GAP are efficient acceptors, while aldehydes, including formaldehyde, are very poor acceptors (Racker, 1961). No detailed examination has been made of the substrate specificity of the classical transketolase from *C. boidinii* CBS 5777. It is active towards the XuMP/RiMP couple but, under the assay conditions used, no reaction between XuMP and formaldehyde could be observed (Waites & Quayle, 1981).

The DHA synthases from *C. boidinii* CBS 5777, *C. boidinii* 2201 (Kato *et al.*, 1982) and *C. boidinii* KD1 (Bystrykh *et al.*, 1981) show a similar lack of strict specificity towards the glycolyl donor as do classical transketolases. The best donor substrates for the enzymes from the first two organisms are XuMP > hydroxypyruvate > fructose 6-phosphate. The greatest difference between the DHA synthases from *C. boidinii* CBS 5777 and *C. boidinii* 2201 and the classical transketolases is the greater reactivity of the former two enzymes towards aldehydes as acceptors. In the case of the enzyme from strain CBS 5777, the relative activity towards donors is: glycolaldehyde > erythrose 4-phosphate > formaldehyde > RiMP > glyceraldehyde > acetaldehyde. In the case of the enzyme from strain 2201, the following substrates were also tested and found to be good acceptors: butyraldehyde > propionaldehyde > heptaldehyde > glycolaldehyde > formaldehyde > D-erythrose > D-glucose > D-ribose. These two enzymes therefore encompass a remarkable range of efficient glycolyl donors and acceptors. The enzyme from *C. boidinii* KD1 is reported to show activity only towards formaldehyde as acceptor (Bystrykh *et al.*, 1981). Since RiMP is inactive as acceptor, presumably this enzyme displays no classical transketolase activity; this could have metabolic implications.

There are interesting differences in the molecular weights of the DHA synthases from *C. boidinii* CBS 5777, *C. boidinii* KD1 and *C. boidinii* 2201: the first two enzymes have approximate molecular weights of 107500 and 145000, respectively, and are dimeric, while the last enzyme has a molecular weight of 190000 and is tetrameric (Tables 6, 7 and 8). It is not known whether aggregation/disaggregation behaviour could explain these apparent differences, which are somewhat unexpected in three strains of the same species; multiple forms of classical transketolase or association of this enzyme with other proteins have frequently been observed (see Wood, 1981).

Table 8. Summary of the properties of DHA synthases from different strains of *Candida boidinii*

	DHA synthase from <i>C. boidinii</i> strain:		
	CBS 5777	KD1	2201
Co-factors			
TPP requirement	+	+	+
Mg ²⁺ requirement	+	+	+
Inhibitors			
PCMB	+	+	+
GSH	+	+	NT
Zn ²⁺	+	NT	+
Donor substrates			
XuMP	+	+	+
Hydroxypyruvate	+	NT	+
Fructose 6-phosphate	+	±	+
Ribulose 5-phosphate	—	—	—
Glucose 6-phosphate	—	NT	—
Acceptor substrates			
Formaldehyde	+	+	+
RiMP	+	—	+
Glycolaldehyde	+	NT	+
Glyceraldehyde	+	—	+
Acetaldehyde	+	NT	+
Glucose 6-phosphate	—	—	+
pH optimum	7.4–7.6*	6.8	7.0
Stability	very unstable	very unstable	very unstable
Molecular weight	107500†	145000	190000
Subunits (size)	2 (65500)†	2 (76000)	4 (55000)

NT, Not tested; —, no activity.

* O'Connor & Quayle (1980).

† Mean of duplicate values in Table 6.

The DHA synthases purified from the three different sources all show a requirement for TPP and Mg²⁺ and are inhibited by thiol reagents. As with many classical transketolases, DHA synthase from CBS 5777 is inhibited by sulphate ions at concentrations in excess of 10 mM. This fact needs to be borne in mind when using coupling enzymes suspended in ammonium sulphate solutions.

The question as to whether the high classical transketolase activity of DHA synthase plays any supplementary metabolic role in the pentose phosphate rearrangement phase of the XuMP cycle has been raised previously (Waites & Quayle, 1981). In the absence of mutant data this question still remains open, although the apparent lack of classical transketolase activity of the DHA synthase from *C. boidinii* KD1 would eliminate such a possibility in this particular organism.

We thank Dr C. E. Furlong (Seattle, U.S.A.) for helpful discussions regarding procedures for activity staining of polyacrylamide gels and the Science and Engineering Research Council for support under grant number GR/A/65966.

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