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MINI-REVIEW

Chemical and enzymatic routes to dihydroxyacetone phosphate

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Abstract Stereoselective carbon–carbon bond formation with aldolases has become an indispensable tool in preparative synthetic chemistry. In particular, the dihydroxyacetone phosphate (DHAP)-dependent aldolases are attractive because four different types are available that allow access to a complete set of diastereomers of vicinal diols from achiral aldehyde acceptors and the DHAP donor substrate. While the substrate specificity for the acceptor is rather relaxed, these enzymes show only very limited tolerance for substituting the donor. Therefore, access to DHAP is instrumental for the preparative exploitation of these enzymes, and several routes for its synthesis have become available. DHAP is unstable, so chemical synthetic routes have concentrated on producing a storable precursor that can easily be converted to DHAP immediately before its use. Enzymatic routes have concentrated on integrating the DHAP formation with upstream or downstream catalytic steps, leading to multi-enzyme arrangements with up to seven enzymes operating simultaneously. While the various chemical routes suffer from either low yields, complicated work-up, or toxic reagents or catalysts, the enzymatic routes suffer from complex product mixtures and the need to assemble multiple enzymes into one reaction scheme. Both types of routes will require further improvement to serve as a basis for a scalable route to DHAP.

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Introduction

Aldolases reversibly catalyze the enantioselective formation of carbon–carbon bonds, a key reaction in organic chemistry, and have become an indispensable tool in the preparative chemist's toolbox. Next to their stereoselectivity, they allow applying simultaneously several catalytic steps in one pot and circumventing or minimizing protective group chemistry. Their mild reaction conditions make them particularly suitable for multifunctional complex molecules, and together, these properties have led to a large number of applications of aldolases for preparative synthetic purposes, particularly in the production of carbohydrates and similar molecules (Wong et al. 1995; Gijsen et al. 1996). Recent industrial applications include the manufacturing of *N*-acetylneuraminic acid from pyruvate and *N*-acetylmannosamine for access to neuraminidase inhibitors (Mahmoudian et al. 1997), access to L-*threo*-*β*-(3,4-dihydroxyphenyl)serine (DOPS) in the treatment of parkinsonism (Liu et al. 2000) and novel routes to statin side chains in the manufacturing of cholesterol-lowering drugs (summarized in Panke and Wubbolts 2005).

Generally speaking, aldolases catalyze the reversible addition of a ketone donor to an aldehyde acceptor. While the range of acceptors that different aldolases can utilize is frequently broad, aldolases typically tolerate only a very small number of donors. The most important natural donors are pyruvate, phosphoenol pyruvate, acetaldehyde, glycine, and dihydroxyacetone phosphate (DHAP), giving rise to the synthetically most useful classification of aldolases (Fessner and Walter 1996). Glycine and acetaldehyde are easily accessible compounds, and excellent routes to

pyruvate have recently become available (Zelic et al. 2004). Correspondingly, the examples of industrial utilization mentioned above stem from these classes. In contrast, access to phosphoenol pyruvate and, in particular, to DHAP remains difficult, which is reflected in the prohibitively high prices for these compounds. This is in strong contradiction to the unique synthetic opportunities that have been elaborated over the last two decades based on DHAP-dependent aldolases. These opportunities rest on the fact that there are four types of enzymes that can provide access to a diastereomerically complete set of vicinal diols (Fig. 1; Wong and Whitesides 1983; Brockamp and Kula 1990; Ozaki et al. 1990; Fessner et al. 1991; Fessner and Eyrisch 1992). The corresponding diastereoselectivity is frequently excellent (Schoevaart et al. 2000b). Consequently, the DHAP-dependent aldolases offer the opportunity to carry out in a controlled fashion different stereochemically complementary carbon–carbon bond aldol reactions. This has been widely exploited on laboratory scale over the past two decades (Fessner and Walter 1996; Fessner 1998; Silvestri et al. 2003; Rowan and Hamilton 2006), in particular, in the area of iminocyclitols, which can inhibit glycosidases and are, therefore, studied for antiviral, anticancer, antidiabetic, and pesticidal properties (Whalen and Wong 2006).

Representatives from the required four classes of enzymes have been thoroughly developed—they have been cloned, overexpressed, and purified (von der Osten et al. 1989; Fessner et al. 1991; Eyrisch et al. 1993; Garcia-Junceda et al. 1995; Vidal et al. 2003; Durany et al. 2005; Ardao et al. 2006), and the 3D structures are available, so rational mutagenesis or directed evolution can be applied to attempt adapting the substrate specificity of a given enzyme (Dreyer and Schulz 1993; Cooper et al. 1996; Hall et al. 2002; Kroemer et al. 2003).

In summary, the DHAP-dependent aldolases are a particularly well-developed group of enzymes with enormous synthetic potential. However, in an industrial setting their applicability hinges on the availability of DHAP. Therefore, we summarize in this mini-review the routes that are available for the manufacturing of this compound. We will begin by summarizing the involvement of DHAP in metabolic pathways, as this has motivated the recruitment of the various enzymatic routes that will be discussed later. Subsequently, we will highlight synthetic chemical and enzymatic routes to DHAP and, finally, discuss a number of possible alternatives to the use of DHAP.

DHAP in metabolism

In metabolism, DHAP is involved in various pathways that have also inspired multi-enzymatic routes to its formation (see below). Firstly, it is an intermediate in the glycolysis and gluconeogenesis, formed in the reversible conversion of fructose 1,6-bisphosphate to glyceraldehyde 3-phosphate and DHAP. DHAP is then isomerized to glyceraldehyde 3-phosphate by triosephosphate isomerase. This enzyme proceeds by deprotonating DHAP to an enediolate intermediate, which is then re-protonated to give glyceraldehyde 3-phosphate (Fig. 2b).

Secondly, DHAP can be channeled into the lipid metabolism by NADH-dependent reduction to L-glycerol 3-phosphate by glycerol 3-phosphate dehydrogenase, or can be provided from glycerol by the reverse reaction or, more importantly in the present context, by the oxidation of L-glycerol 3-phosphate catalyzed by glycerol phosphate oxidase, which is a major route for glycerol assimilation in a number of microorganisms (Esders and Michrina 1979). Alternatively, a glycerol phosphate oxidase route can be

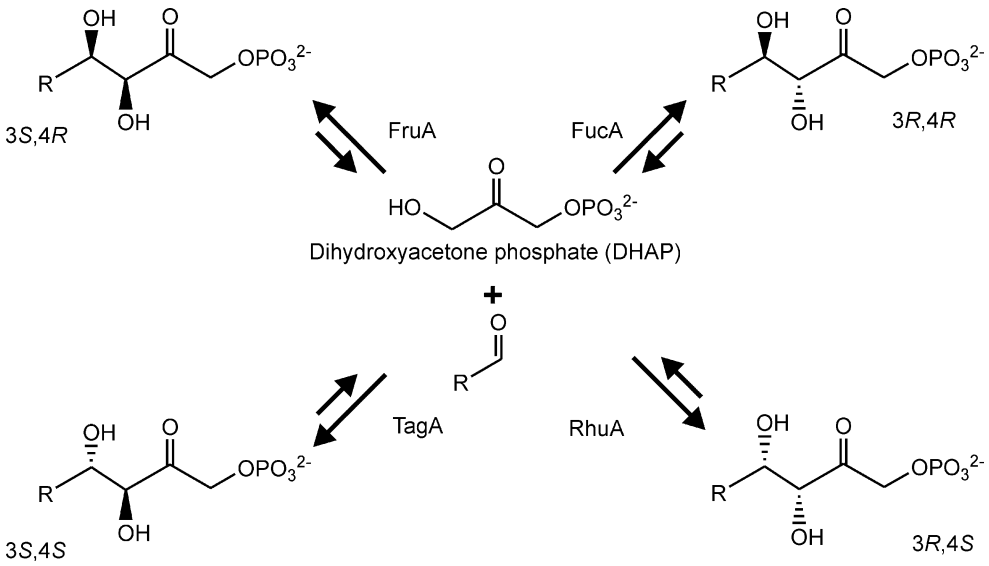

Fig. 1 Four DHAP-dependent aldolases provide access to a diastereomerically complete set of vicinal diols. *FruA* Fructose 1,6-bisphosphate aldolase, *FucA* fuculose 1-phosphate aldolase, *RhuA* rhamnulose 1-phosphate aldolase, *TagA* tagatose 1,6-bisphosphate aldolase

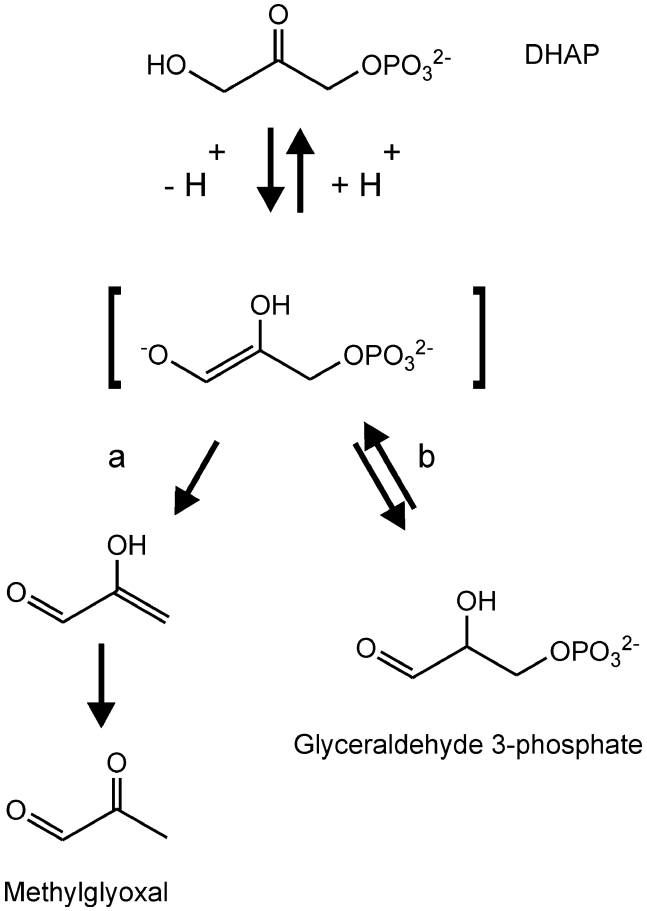

Fig. 2 Most prominent conversions of DHAP. DHAP is deprotonated and degrades chemically along route **a** to methylglyoxal. Alternatively, it is converted by triose phosphate isomerase along route **b** to glyceraldehyde 3-phosphate

obtained from eukaryotes that use the mitochondrial glycerol phosphate shuttle to regenerate NAD^+ instead of transferring reducing equivalents, such as in trypanosomes (Operdoes 1987).

Thirdly, DHAP is an important intermediate in the anaerobic dissimilation of glycerol, during which glycerol is oxidized to dihydroxyacetone and channeled into the glycolysis via phosphorylation to DHAP by dihydroxyacetone kinases (Sun et al. 2003). The latter class of enzymes is also important in the assimilation of formaldehyde in methylotrophic yeasts and in the detoxification of dihydroxyacetone in yeasts (Erni et al. 2006). These dihydroxyacetone kinases do not accept glycerol (in contrast to glycerol kinases that accept dihydroxyacetone, see below) and exist in two structurally related classes: one that uses adenosine triphosphate (ATP) as phosphate donor and has only one structural gene, and one that uses phosphoenol pyruvate and has typically three structural genes (Cheek et al. 2005). Only the ATP-dependent enzymes are relevant for the present discussion.

Fourthly, DHAP has been found to be part of the metabolism of various sugars (in *Escherichia coli*, these are L-fucose, L-rhamnose, and galactitol) and the enzymes from the corresponding pathways—fuculose 1-phosphate aldolase (Chen et al. 1987), rhamnulose 1-phosphate aldolase (Sawada and Takagi 1964), and tagatose 1,6-bisphosphate aldolase (Nobelmann and Lengeler 1996)—form, together with fructose 1,6-bisphosphate aldolase, the complete diastereomeric platform (Fig. 1).

Next to these metabolic pathways, the knowledge of which has inspired the various enzymatic synthesis routes (see below), DHAP is an intermediate in the regeneration of ribulose 1,5-bisphosphate from C6 and C3 sugars in the dark reactions of photosynthesis, where the fructose 1,6-bisphosphate aldolase catalyzes the interconversion of erythrose 4-phosphate, DHAP, and sedoheptulose 1,7-bisphosphate.

One important property of DHAP is its rather low chemical stability (Fig. 2): Degradation is initiated by the deprotonation of DHAP, just as it is the case for triosephosphate isomerase, but then the phosphate group is eliminated rather than the intermediate re-protonated to glyceraldehyde 3-phosphate. This elimination occurs under neutral and, in particular, under basic conditions and leads to the formation of methylglyoxal (Richard 1991). The typical chemical half-lives for DHAP under neutral-to-slightly-basic conditions are between 3 h (37°C) and 30 h (25°C; Hettwer et al. 2002; Suau et al. 2006). This degradation reaction is even a side reaction of triose phosphate isomerase catalysis (Richard 1991) that makes methylglyoxal an inevitable side product of glycolysis (Phillips and Thornalley 1993). As the latter compound is cytotoxic, it is then removed by conversion to D-lactate or pyruvate (Cooper 1984). The chemical stability of DHAP has a profound influence on the strategies that have been followed to produce the molecule for preparative purposes. One strategy is to produce a stable precursor of DHAP rather than DHAP itself. This precursor would need to be converted to DHAP shortly before the aldol reaction. Alternatively, DHAP can be produced in situ and immediately react further, so that the side reaction to methylglyoxal can be effectively suppressed. Both approaches benefit if the step in which the DHAP is actually produced is compatible—in terms of solvent and by-product profile—to the subsequent step in which the DHAP is consumed again. This poses specific additional requirements on the activation and in situ production procedures.

Chemical routes to DHAP

Due to the multiple functionalities of DHAP, most of its published chemical syntheses require complicated multistep

procedures including protection/deprotection steps. Essentially, synthetic routes towards three stable DHAP precursors have been described in the literature (Fig. 3): The cyclic dimeric $DDDP_2$, one of the series of monomeric phosphorylated ketals, or dibenzyl-3-benzylhydroxyacetone phosphate. The first intermediate is available in several steps from the cheap dihydroxyacetone dimer (Fig. 3a). Several reaction sequences have been described. They are based on ketalization, phosphorylation by diphenylphosphorochloridate followed by hydrogenolysis (Colbran et al. 1967), ketalization, phosphorylation with phosphorus oxychloride, and isolation of the free phosphate as its barium salt (Effenberger and Straub 1987), or ketalization, phosphorylation with dibenzyl *N*,*N*-diethylphosphoramidite followed by oxidation and hydrogenolysis (Pederson et al. 1991). In particular, the improved route via dibenzyl *N*,*N*-diethylphosphoramidite produced good yields of the intermediate in the order of 73% (Jung et al. 1994). From the stable precursors, DHAP can be obtained via hydrolysis by heating or acidification. These routes have the advantage that the stable intermediate can be produced efficiently, but the yield of the final step, the conversion to DHAP, has so far been only moderate, although there might be some room for improvement.

The monomeric phosphorylated ketals (Fig. 3b) are available by multi-step reactions from 3-chloro-1,2-propanediol (Ballou and Fischer 1956), acetone (Valentin and Bolte 1995), 1,3-dibromoacetone (available by acidic bromination of acetone; Gefflaut et al. 1997), or dihydroxyacetone dimer (Ferroni et al. 1999; Charmantray et al. 2004). Again, the step from the precursor to DHAP can be made by acidic treatment. In comparison to the routes summarized in Fig. 3a, the final step to DHAP gives usually excellent yields, but overall yields in the steps to the precursor are only moderate. In summary, although it has been frequently shown that these routes lead to DHAP preparations that can be used directly in aldolase reactions, both sets of routes are multi-step, rather low yielding,

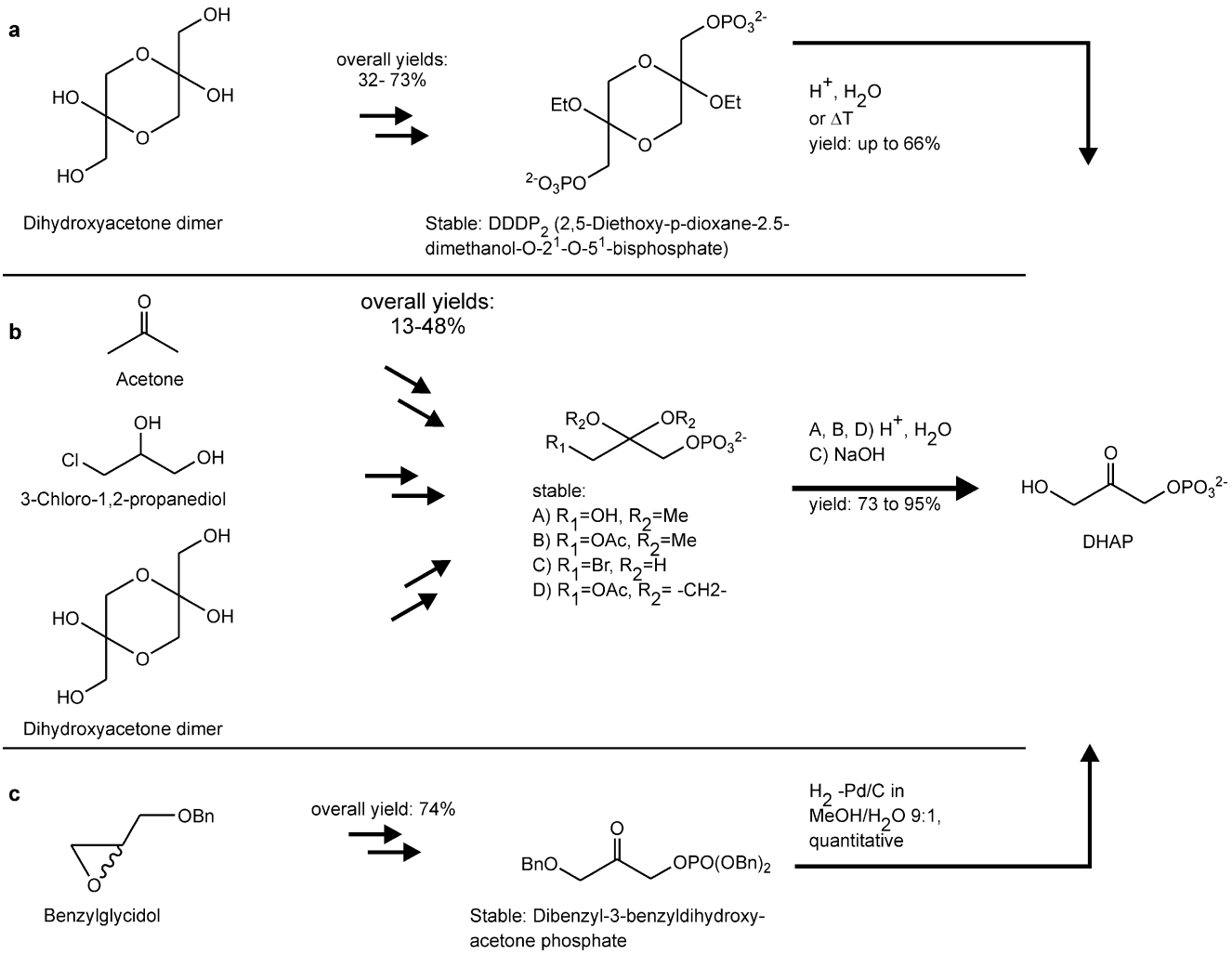

Fig. 3 Principal chemical routes to DHAP. **a** Routes to $DDDP_2$. **b** Route to monomeric phosphorylated ketals. **c** Route to dibenzyl-3-benzyldihydroxyacetone phosphate

include complicated purification procedures, and involve toxic and (in part) expensive chemicals.

Recently, a convenient gram-scale synthesis of a benzylated DHAP precursor starting from cheap racemic benzylglycidol was reported (Fig. 3c; Meyer et al. 2006). A Lewis acid-mediated regioselective epoxide ring opening with dibenzyl phosphate followed by the catalytic oxidation of the secondary alcohol by TPAP/NMO (tetrapropylammonium perruthenate/*N*-methylmorpholine *N*-oxide) led to dibenzyl-3-benzylhydroxyacetone phosphate as a stable stock material with an overall yield of 74%, which was then quantitatively hydrogenolyzed into DHAP that, in turn, was directly suitable for enzymatic aldol reactions (Meyer et al. 2004). However, this method requires stoichiometric amounts of the Lewis acid CuI and of NMO, which is inconvenient to prepare, and extensive use of the expensive catalyst TPAP. Furthermore, the starting material is not commercially available.

Enzymatic routes to DHAP

Enzymatic routes to DHAP follow one of three general routes from cheap unphosphorylated precursors: from dihydroxyacetone to DHAP, from glycerol via glycerol phosphate to DHAP, or via multi-step routes that mimic glycolysis. In general, these routes require at some point the transfer of phosphate residues from donors with a high transfer potential to either ADP and then to unphosphorylated starting material or to the unphosphorylated starting material directly. The majority of the syntheses discussed below rely on ATP as the phosphorylating agent. As ATP is expensive, regeneration becomes a point of prime concern in such routes. As this is of central importance for the following and applies to most of the routes discussed below, we will start with a brief discussion of ATP regeneration.

Regeneration of phosphate donors

An essential feature of the routes discussed below is the phosphorylation of a three-carbon unit that requires spending a high-energy phosphoester bond, unless reverse hydrolysis conditions are applied (see below). Recent advances in the regeneration of ATP as the most important phosphate donor have been discussed before (Zhao and van der Donk 2003). In the set of syntheses discussed in this paper, two regeneration systems have been of prime importance (Fig. 4a): ATP regeneration from acetyl phosphate by acetyl kinase or from phosphoenol pyruvate by pyruvate kinase. Although these systems are well established and have been discussed frequently before (Wong and Whitesides 1994), it might be useful for this mini-review to summarize the most important features: Acetyl phosphate is very easy to prepare but has the disadvantage that it hydrolyzes rather rapidly. In contrast, phosphoenol pyruvate is a rather stable compound, but it is more laborious and expensive to produce. Furthermore, the pyruvate that is formed during ATP regeneration acts as a competitive inhibitor of the kinase, for example, with a K_i of only around 10 mM in the case of the muscle enzyme (Hirschbein et al. 1982).

One additional system should be mentioned in this context: the regeneration of ATP from cheap polyphosphate by a polyphosphate kinase. This enzyme was overexpressed in *E. coli*, thereby reducing the problem of contaminating phosphatases that previously lead to phosphate accumulation (Noguchi and Shiba 1998). It was successfully used in ATP regeneration with polyphosphate to kinate nucleoside bisphosphates. Unfortunately, the degree to which the nucleoside bisphosphates can be phosphorylated seems to be limited to around 50% in the case of ADP for unclear reasons (Noguchi and Shiba 1998). When required, polyphosphate can also be used to regenerate ATP from adenosine monophosphate (AMP) by the combined activity of a polyphosphate: AMP phosphotransferase and an adenylate kinase (Resnick and Zehnder 2000). Although the discussed enzymes appear to be synthetically very useful, they have so far never been used for DHAP production, most probably because of the requirements for purified enzymes to avoid unproductive dephosphorylation of the poplyphosphate (Shiba et al. 2000).

From dihydroxyacetone to DHAP

The most straight forward enzymatic approach to DHAP starts at the dihydroxyacetone monomer (Fig. 4b) that forms from the cheap dimer in aqueous solution. This route has been pioneered by Wong and Whitesides (1983) who exploited the relaxed substrate specificity of glycerol kinases to produce DHAP from dihydroxyacetone and ATP (Crans and Whitesides 1985a). For example, glycerol kinase from *Saccharomyces cerevisiae* was used to produce DHAP on mole scale from dihydroxyacetone and ATP (Crans and Whitesides 1985b). Alternatively, dihydroxyacetone kinases have been employed as a substitute (Yanase et al. 1995; Itoh et al. 1999; Sanchez-Moreno et al. 2004). Although a number of these enzymes have been investigated [see Itoh et al. (1999) and references therein), it is difficult to assess the relative usefulness of the different species: The enzymes usually depend on divalent cations, the measured specific activities are in the range of 20 U mg^{-1} of purified protein, and the K_M values for dihydroxyacetone are generally below 100 μM, which makes these enzymes, in general, useful for DHAP production.

An alternative procedure for DHAP production that utilizes a cheap phosphate donor—and thus, has the potential to circumvent the issue of cofactor regeneration

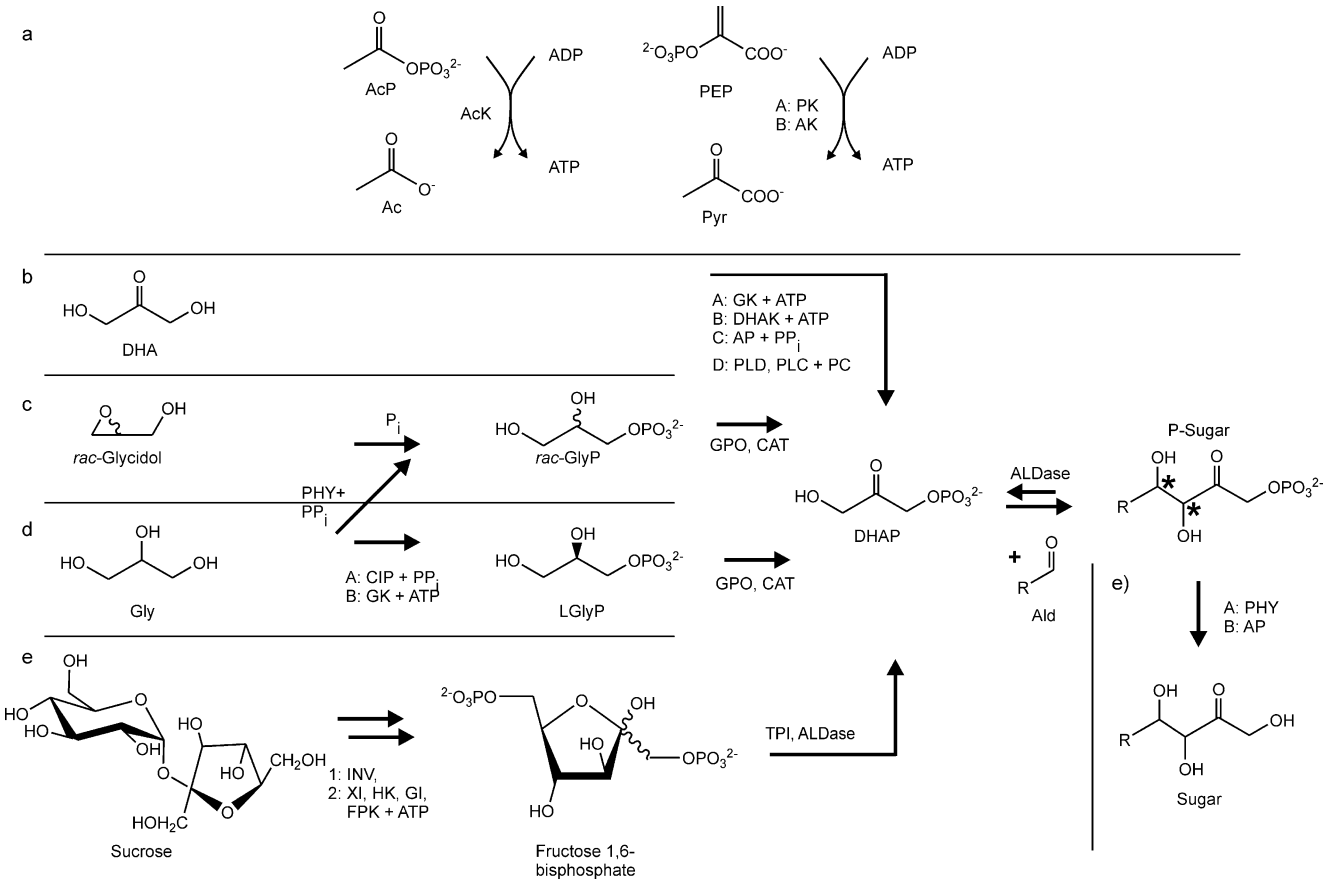

Fig. 4 Principal enzymatic routes to DHAP and the resulting sugar. Notation of enzymes and compounds as detailed for Table 1. **a** Routes for ATP regeneration that have been used in the experiments depicted in **b** to **d**. **b** Routes starting from dihydroxyacetone. **c** Routes passing through phosphorylated glycerol as intermediate. **d** Multi-enzyme route starting from sucrose. **e** Dephosphorylation of the sugar that resulted from the aldol reaction of DHAP and the aldehyde. For yields of the different routes, see Table 1. Capital letters indicate different enzymes that have been used for the same step. Numerals indicate that this step has been carried out in several subsequent steps

completely—is based on the kinetically controlled transphosphorylation of dihydroxyacetone from cheap pyrophosphate by an acid phosphatase from *Shigella flexneri* (van Herk et al. 2006). In an optimized procedure, the enzyme allowed the production of up to 100 mM of DHAP in 6 h with yields of 20% on dihydroxyacetone and pyrophosphate before the yields started to fall again because of the hydrolytic activity of the phosphatase on DHAP. These are rather high values for transphosphorylation reactions when compared to similar protocols for the phosphorylation of glycerol (see below), but of course, still far removed from preparative suitability. Furthermore, the enzyme has a large K_M value of 3.6 M for dihydroxyacetone, and its application leads to the accumulation of high concentrations of phosphate, which needs to be removed before application because it inhibits aldolases.

A similar, although more complicated, approach had been reported earlier when an excess of dihydroxyacetone was used for the transesterification of phosphatidylcholine to phosphatidyldihydroxyacetone in an organic phase by phospholipase D. The resulting compound was then isolated and hydrolyzed by phospholipase C to DHAP and 1,2-diacyclglycerol (Takami and Suzuki 1994; D'Arrigo et al. 1995). Again, the yields on dihydroxyacetone are low, as it is typical for transesterifications.

From glycerol to glycerol phosphate and DHAP

An alternative route to DHAP is the phosphorylation of glycerol to L-glycerol 3-phosphate (*sn*-glycerol phosphate) and the subsequent oxidation to DHAP (Fig. 4c). Although this reaction sequence requires at least one enzymatic step more, it has the advantage that L-glycerol 3-phosphate is a stable compound that can be stored. The oxidation step is potentially very simple and requires only a glycerol phosphate oxidase and catalase (see below). Glycerol is somewhat more expensive than dihydroxyacetone, but this is poised to change at least to some extent with the increasing number of biodiesel installations that are coming on-stream and that produce glycerol as a side-product.

The first step in this route is the production of a phosphorylated glycerol. Glycerol phosphorylated at the 3-position is chiral, and although DHAP is not chiral, this chirality is important because the L-glycerol phosphate oxidase that converts glycerol phosphate to DHAP is selective for L-glycerol 3-phosphate. Consequently, racemic mixtures can maximally deliver a 50% yield of DHAP on glycerol. However, as glycerol is cheap, this might be tolerable if the phosphorylation is cheap as well.

One such procedure has been recently suggested to start from *rac*-glycidol, a common bulk chemical, the three-membered ring of which can be chemically opened with phosphate and gives then a mixture of the racemic terminal glycerol phosphate and the 2-glycerol phosphate (Charmantray et al. 2006). These conditions can be optimized for yields of racemic glycerol phosphate up to 55 or 28% for the required L-glycerol 3-phosphate.

Racemic glycerol phosphate without contaminating glycerol 2-phosphate is available enzymatically from glycerol and pyrophosphate by catalysis under reverse hydrolysis conditions with phytase (e.g., from *Aspergillus ficuum*; Schoevaart et al. 1999, 2000a). Conditions could be optimized to 95% glycerol and 150 mM pyrophosphate and resulted in quantitative conversion of pyrophosphate to racemic glycerol phosphate.

Replacing phytase by calf intestinal alkaline phosphatase, it was possible to use a similar scheme and produce L-glycerol 3-phosphate from glycerol and pyrophosphate with a ninefold excess over the D-enantiomer. The conditions were different with respect to pH and a reduced glycerol concentration (60% in water, pH 7.9), and pyrophosphate was not consumed completely (55%; Pradines et al. 1988).

Still, with enzymatic methods, it is also easily possible to direct the production of glycerol phosphate completely to the desired L-enantiomer. For example, the glycerol kinase from *S. cerevisiae* was exploited to produce exclusively L-glycerol 3-phosphate on a preparative scale (Crans and Whitesides 1985b) with ATP as the phosphate donor, and the enzyme from *Bacillus stearothermophilus* delivered L-glycerol 3-phosphate from ATP and glycerol up to concentrations of 300 mM (52 g l^{-1}, limited by substrate inhibition; Hettwer et al. 2002).

To obtain DHAP, the L-glycerol 3-phosphate has to be oxidized by a glycerol phosphate oxidase with consumption of oxygen and production of hydrogen peroxide. In this procedure, the inactivation of the glycerol phosphate oxidase by hydrogen peroxide was prevented by the addition of a catalase (Fig. 4c). Several L-glycerol phosphate oxidases have been investigated for their utility (Fessner and Sinerius 1994), and all show product inhibition from DHAP. Still, useful enzymes could be identified, in particular from *Streptococcus* sp., that still maintain 20% of the activity at DHAP concentrations of 100 mM (17 g l^{-1}), show hardly any substrate inhibition, tolerate relatively large concentrations of phosphate, are stable, and inactivate relatively slowly at elevated levels of hydrogen peroxide (Fessner and Sinerius 1994). When investigated more closely, L-glycerol phosphate oxidase preparations from *S. thermophilus* were shown to have inhibition constants in the order of 60 to 80 mM for L-glycerol 3-phosphate and were essentially insensitive against accumulating phosphate. However, the enzymes were sensitive against air bubbles in particular and against oxygen in general (Oldenburg 1998). Furthermore, oxidative conditions favored the production of polymers from DHAP. The authors evaluated several possibilities to provide mildly oxidative conditions and chose dosing of hydrogen peroxide in the presence of catalase to provide the required oxygen. Still, the glycerol phosphate oxidase showed significant inactivation over the process (60% remaining after 7 h) from the produced oxygen as well as from DHAP (Hettwer 1998; Oldenburg 1998). Remarkably, the procedure appears to be rather insensitive to substantial amounts of the 2-glycerol phosphate isomer, as the L-glycerol 3-phosphate in the corresponding mix of three glycerol phosphate isomers from the ring opening of the *rac*-glycidol (see above) could be quantitatively converted to DHAP. However, rather large concentrations of enzymes were employed (Charmantray et al. 2006).

Integration of steps

Due to the instability of DHAP, this molecule is usually not the desired product in any given reaction. Rather, it is a central intermediate that should be converted further with an appropriate aldehyde in the presence of an aldolase that provides one of the four possible stereochemistries. At the same time, for example, the route starting from glycerol requires at least two enzymatic steps to DHAP plus a cofactor regeneration and catalase—in short, it is highly desirable to integrate some or even all of these steps simultaneously into one pot, or if simultaneous reactions are impossible, then at least subsequently in one pot (Table 1). Enzyme-catalyzed reactions have here a unique advantage in that many of them have near-neutral pH, ambient temperature, and aqueous medium as their natural environment, so in principle, integration of several enzymatic steps should be possible. On the other hand, some of the single reactions take place under conditions that are rather removed from such "average conditions" and consume/produce substrates/products that by experience might cause problems in integration.

When starting from dihydroxyacetone, integration requirements are lower than for the other routes, as there is only one step from dihydroxyacetone to DHAP. In fact, this step, ATP regeneration, and aldolase reaction could be

Table 1 Enzymatic routes to DHAP

References	Route[a]	No. of steps[b]	No. of pots[c]	Overall no. of enzymes[d]	Max. no. of enzymes simultaneously	Phosphate donor[a]	Reported scale[e]	Yield[a,f]	Composition of final mixture[a,g]
Crans and Whitesides (1985a,b)	DHA to DHAP	1	1	2 (GK, AcK)	2	ATP, recycled from AcP	1.4 l or 66 g	98% on DHA, 87% on AcP	280 mM DHAP, 320 mM Ac, 40 mM Pi
D'Arrigo et al. (1995)	DHA to DHAP via PDHA	2	2	2 (PLD, PLC)	1	PC	9 ml or 0.3 g	3% on DHA, 52% on PC	Aq, 42 mM DHAP; org, 42 mM DAG, 16 mM PC [h)]
Sanchez-Moreno et al. (2004)	DHA to P-sugar via DHAP	1	1	3 (DHAK, AcK, ALDase)	3	ATP, recycled from AcP	30 ml or 0.1 g	84% on DHA, 84 % on Ald, 42 % on AcP	30 mM P-sugar, 36 mM Pi, 5 mM DHAP, 66 mM acetate
Eyrisch et al. (1993)	DHA to P-sugar via DHAP	1	1	4 (GK, PK, TPI, ALDase)	4	ATP, recycled from PEP	200 ml or 5.1 g	40% on DHA, 60% on PEP	75 mM P-sugar 1, 12 mM P-sugar 2, 125 mM DHA, 75 mM DHAP
van Herk et al. (2006)	DHA to sugar via DHAP and P-sugar	1	1	2 (AP, ALDase)	2	PP_i	10 ml or 0.2 g	95% on ald (53% isolated yield), 13% on PPi, 19% on DHA	95 mM sugar, 1.4 M Pi, 400 mM DHA
Pradines et al. (1988)	Gly to LGlyP	1	1	1 (CIP)	1	PPi	50 ml or 0.7 g	1% on gly, 55% on PPi (45% after isolation)	83 mM LGlyP, 8.1 M Gly, 217 mM Pi
Hettwer et al. (2002)	Gly to DHAP via LGlyP	2	1	4 (GK, PK, GPO, CAT)	2	ATP, recycled from PEP	350 ml or 6 g	84% on Gly, 84% on PEP	95 mM DHAP, 3 mM Pi, 103 mM Pyr, 3 mM glycerol, 5 mM Pi
Fessner and Sinerius (1994); Wong and Whitesides (1983); Crans and Whitesides (1985a,b)	Gly to P-Sugar via LGlyP and DHAP	2	1	5 (GK, AcK, GPO, CAT, ALDase)	3	ATP, recycled from AcP	10 ml or 0.2 g	90% on Gly, 78% on Ald, 41% on AcP	93 mM P-sugar, 26 mM Ald, 7 mM LGlyP, 3 mM Gly, 220 mM Ac, 130 mM Pi
Fesser and Walter (1992)	Sucrose to P-sugar via DHAP	2	1	8 (INV, XI, HK, GI, FPK, ALDase, TPI, PK)	7	ATP, recycled via PEP	200 ml or 1 g	92% on sucrose, 8% on PEP, 7% on Ald	18 mM P-sugar, 107 mM Pi, 107 mM Pyr, 220 mM Ald
Bednarski et al. (1989)	FDP to P-sugar via DHAP	2	1	2 (ALDase, TPI)	2	FDP	3.75 l or 400 g	73% on FDP, 49% on Ald	50 mM FDP, 310 mM Ald, 290 mM P-sugar
Schoevaart et al. (1999, 2000a,b)	Gly to sugar via *rac*-GlyP, DHAP, and P-sugar	4	1	4 (PHY, GPO, CAT, ALDase)	2	PPi	10 ml or 0.1 g	0.4% on Gly, 39% on PPi, (29% isolated yield), 34% on Ald	34 mM sugar, 7.5 M gly, 87 mM Pi, 9 mM DHAP, 66 mM Ald
Charmantray et al. (2006)	*rac*-Glycidol to sugar via *rac*-GlyP, DHAP, and P-sugar	4	1	4 (GPO, CAT, ALDase, AP)	2	Pi	10 ml or 0.3 g	28% on glycidol, (13% isolated yield), 28% on Pi, 70% on Ald	140mM sugar, 360 mM Gly, 500 mM Pi, 60 mM Ald

[a] *Ac* Acetate, *AcP* acetyl phosphate, *Ald* aldehyde (together with DHAP substrate of the aldolase reaction), *DAG* diacylglycerol, *DHA* dihydroxyacetone, *DHAP* dihydroxyacetone phosphate, *FDP* fructose 1,6-bisphosphate, *Gly* glycerol, *GlyP* glycerol 3-phosphate, *LGlyP* L-glycerol 3-phosphate, *Pi* inorganic phosphate, *PC* phosphatidylcholine, *PEP* phosphoenol pyruvate, *PDHA* phosphatidyldihydroxyactone, *PPi* pyrophosphate, *P-sugar* monophosphorylated sugar (product of aldolase reaction of aldehyde and DHAP), *Pyr* pyruvate, *sugar* dephosphorylated P-sugar, *rac-PGly* racemic glycerol phosphate

[b] Clearly distinct processing steps; the addition of enzyme b after the reaction catalyzed by a is finished would be counted as two steps. Repeated addition of one agent (simulating a constant feed) was counted as one step.

[c] Indicates whether a purification step needs to be performed in-between.

[d] *AcK* Acetyl kinase, *ALDase* aldolase, *AP* acid phosphatase, *CAT* catalase, *CIP* calf intestinal alkaline phosphatase, *DHAK* dihydroxyacetone kinase, *FPK* fructose 6-phosphate kinase, *GI* glucose 6-phosphate isomerase, *GK* glycerol kinase, *GPO* L-glycerolphosphate oxidase, *HK* hexokinase, *INV* invertase, *PHY* phytase, *PK* pyruvate kinase, *PLC* phospholipase C, *PLD* phospholipase D, *TPI* triosephosphate isomerase, *XI* xylose isomerase

[e] Volume of reaction and mass of produced product. When there was more than one step, the volume of the final step is mentioned.

[f] Where the yield was a function of time, the maximum yield was chosen. Isolated yields based on the substrate mentioned are reported when available.

[g] The compositions have been deduced from the yields and under the assumptions that (1), at the end of the reaction, all phosphate donors, such as AcP, PPi, PEP, have been consumed and (2) that DHAP does not degrade to methylglyoxal. Buffer components (on the assumption that, in a real process, pH would be kept constant by the addition of acid or base), enzymes, ATP, and salts deriving from pH switches are not considered. When there were several examples for one route, the example with the larger scale has been selected.

[h] Two-step and two-phase reaction. The product was isolated after the first reaction. The phases of the second reaction are listed separately as aqueous (aq) and organic (org).

operated simultaneously in one pot at pH 7 (Sanchez-Moreno et al. 2004). The relatively broad pH tolerance of the rabbit muscle fructose 1,6-bisphosphate aldolase allowed even to integrate the operation of acid phosphatase with the aldolase operation: At pH 4.5, a compromise between the activities of the two enzymes, the dihydroxyacetone was phosphorylated, the DHAP converted to the phosphorylated sugar, and the product was even dephosphorylated once the pyrophosphate was consumed—a particularly nice example of the potential benefits of the integration of several reaction steps (van Herk et al. 2006).

The integration of routes passing through L-glycerol 3-phosphate is more challenging. The aldolase-catalyzed coupling of DHAP to an aldehyde can be integrated with the oxidation of glycerol phosphate, which has the added benefit that this is an effective way to prevent the inhibition of the glycerol phosphate oxidase by accumulating DHAP (Fessner and Sinerius 1994). However, glycerol phosphate production with glycerol kinase cannot be integrated because the kinase is rapidly inhibited by the oxygen required for the oxidation of the glycerol phosphate (Fessner and Sinerius 1994).

The substitution of ATP-regeneration by transphosphorylation catalyzed by phytase is also difficult to integrate. This step was optimized for a pH of 3.5 and a glycerol concentration of 95%, and both conditions are not compatible with the subsequent requirement for the oxidation of L-glycerol 3-phosphate by the glycerol phosphate oxidase and catalase. The aldolase step could most probably be integrated with the previous step. However, the final step, the dephosphorylation of the DHAP-adduct, requires a second shift of pH back to acidic conditions (Schoevaart et al. 2000a). Still, this procedure is also a one-pot procedure, and as a particular elegant feature, the phytase that has been added for the first step was reactivated in the last step by the second pH shift.

One exceptional example of the integration of multiple enzymatic steps is the in vitro reconstitution of an eight-enzyme pathway for simultaneous operation in one pot (Fig. 4d; Fessner and Walter 1992): To produce DHAP, an eight-enzyme system was set up that started from sucrose to produce glucose and fructose, proceeded to fructose 6-phosphate, fructose 1,6-bisphosphate, and finally, to glyceraldehyde and DHAP. The required ATP was regenerated from phosphoenol pyruvate. Finally, DHAP was then converted by the aldolase to the phosphorylated ketose. This system was operated on a scale of 23 g l^{-1} of phosphorylated sugar. This multi-enzyme approach also presented access to the relatively expensive fructose 1,6-bisphosphate, which can also serve as a convenient starting material for DHAP (Bednarski et al. 1989).

The different multi-step enzymatic routes are compiled in Table 1. Although nearly all of the routes mentioned there are one pot-routes and, therefore, rather convenient, please note that some of those routes cover only part of the overall sequence from cheap starting material to dephosphorylated sugar. Still, as the entries 8 and 9 in Table 1 illustrate, the chance for smooth integration into one pot is high. On the other hand, it becomes also clear that none of these methods—to the best knowledge of the authors—has ever been realized on more than lab scale. This suggests that many rather fundamental problems still remain to be discovered and solved, as indicated by the glycerol phosphate oxidase inactivation problem that appeared when the step from L-glycerol 3-phosphate was systematically investigated for scale up (Hettwer et al. 2002).

A lack in fundamental investigations regarding the scalability of the various procedures is also reflected in the rather poor yields, which in many cases, have simply not been optimized. However, a major drawback remains the complex nature of the product mixtures. The desired product, be it DHAP, the phosphorylated or even the dephosphorylated sugar, is never the most abundant species in the final mixture. This is particularly pronounced for the processes that utilize cheap phosphorylation protocols that typically require transesterification conditions and substantial excess of either pyrophosphate, dihydroxyacetone, or glycerol.

Viewed from such a process perspective, the ATP-dependent phosphorylations of dihydroxyacetone or glycerol appear still to be the most promising methods. Although the selected ATP regeneration process intrinsically leads to the accumulation of either acetate or pyruvate, this accumulation can most likely be kept to stoichiometric levels, which leaves the composition of the product mixture relatively simple, and after dephosphorylation, the separation of the charged products from the sugar should be relatively easy to achieve.

Alternatives to DHAP

The difficulties in DHAP production discussed above should have motivated the investigation of alternative approaches that would, on the one hand, allow exploiting the promising stereochemical possibilities of aldolases, but on the other hand, avoid the difficulties connected to the production of DHAP. Two principal options can be considered: (1) using the available set of enzymes but using a donor-analogue that is easier to produce, or (2) looking for similar enzymes that allow for a similarly attractive stereochemistry but do not require the phosphorylated donor.

However, as already indicated in the beginning, the DHAP-dependent aldolases do not tolerate substantial modifications of the donor. Still, some modifications are possible, in particular, at C1. This includes the use of phosphonates instead of a phosphate ester, which leads to a roughly tenfold reduction in relative activity. Motivated by the potential of

phosphonates to act as inhibitors for, e.g., glycolysis in human parasites, the 4-hdydroxy-3-oxobutylphosphonate, as the phosphonate analogue of DHAP, was also investigated as a donor in other DHAP-dependent aldolases. In fact, two of the four available stereochemistries could be produced with this donor (with FruA and RhuA variants, see Fig. 1; Arth and Fessner 1997). But as this donor leads to sugars that cannot be dephosphorylated easily and the production does not seem to give advantage over production routes to DHAP, the transfer of this approach into the area of preparative sugar synthesis is not helpful.

Another example for tolerated modifications at C1 is the substitution of the phosphoester of dihydroxyacetone by an arsenate ester. The arsenate ester forms spontaneously, can then be converted by the aldolase, and also hydrolyzes again spontaneously. In fact, using the arsenate method, a number of unphosphorylated sugars have been prepared (Drueckhammer et al. 1989). However, this method suffers from the required high concentrations of the highly toxic inorganic arsenate, which makes it highly unlikely that this method will be used preparatively.

Modifications at C3 of DHAP are even less tolerated, and those modifications with which some residual activity has been detected typically lead to an up to 1,000-fold reduction in relative activity (Bednarski et al. 1989).

Regarding the option of alternative enzymes, sources for those could be the environment or variants of the existing set of enzymes obtained by directed evolution. However, the phosphate binding site for the donor is the most conserved element in the family of $(\beta/\alpha)_8$-barrel proteins (Nagano et al. 2002) to which the DHAP aldolases belong, indicating that it might be a rather difficult undertaking to eliminate the donor-phosphate requirement by directed evolution. Furthermore, the phosphate group of DHAP has been suspected to play an important role in the catalytic mechanism of the aldolase catalyzed reaction (Periana et al. 1980). Correspondingly, no reports on the elimination of the phosphate requirement for the donor are available (to the best knowledge of the authors), although the preference for specific phosphorylated acceptors could be reduced for at least D-2-keto-3-deoxy-6-phosphogluconate aldolase (Fong et al. 2000). With respect to novel enzymes, a fructose 6-phosphate aldolase (FSA) that accepts dihydroxyacetone instead of DHAP as the donor has recently been discovered in *E. coli* (Schürmann and Sprenger 2001). Next to its natural acceptor, glyceraldehyde 3-phosphate (yielding fructose 6-phosphate instead of the bisphosphorylated fructose 1,6-bisphosphate), it also accepted non-phosphorylated acceptors and produced the same stereochemistry as FruA (3*S*,4*R*) (Schürmann et al. 2002). Although this is a very promising result, so far, only this stereochemistry is available for dihydroxyacetone-dependent aldolases, which limits the usefulness of the approach. Finally, it has to be mentioned that a number of successful attempts were made to produce catalytic antibodies that do not rely on phosphorylated donors, can mimic aldolase enzymes, and are useful on preparative scale (Schultz et al. 2002).

For the time being, these alternative routes do not possess the chemical versatility represented by the set of DHAP-dependent aldolases, and it might still take quite some time before DHAP as a strategically important intermediate can be substituted.

Conclusion

The synthetic potential of DHAP-dependent aldolases is so attractive that a variety of chemical and enzymatic routes have been devised to produce DHAP, either as the product itself, a precursor for it, or as an intermediate in a reaction sequence that continues, preferably, until the unphosphorylated sugar. Still, it remains doubtful whether most of the routes above can indeed serve as the reliable basis of a scalable route to DHAP. Many of them have not advanced beyond small scale, suggesting that, for example, high enzyme concentrations might have masked effects that will not become obvious before the influence of the various process parameters has been investigated in more detail.

The chemical routes suffer, above all, from the requirement for toxic and, in part, expensive chemicals, which makes it doubtful that any of the routes discussed here will be further developed.

Consequently, attention focuses on the enzymatic routes where no such problems exist. These routes are attractive, as they are all one-pot routes, and some of them even one-step routes to the phosphorylated sugar product. In principle, this should be an excellent foundation to start a useful preparative route. Nevertheless, a considerable number of issues remain: With most of the enzymatic routes, the final concentration of product is relatively low, and the respective product is part of a rather involved mixture of compounds. Part of this might be due to the fact that only little work for optimization has been invested for most routes, so the true potential for scale up is difficult to assess. Where the degree of integration has remained limited and well established ATP regeneration systems have been applied, the yields and composition of the final mixtures were promising, but the concentrations remained low (Crans and Whitesides 1985b; Hettwer et al. 2002).

Another point to consider for the enzymatic routes is the number of enzymes in one route. Although the different enzymes are available commercially to some extent or can be relatively easily overproduced in recombinant organisms, the multi-enzyme nature of the various routes remains a serious issue: Providing between two and eight enzymes for the manufacturing of an intermediate of a synthesis simply might be too many for a sustainable synthesis. Of

course, such a problem might be circumvented in the future by the construction of tailored strains.

Finally, the utilization of phosphoenol pyruvate as a phosphate donor on an industrial scale is currently rather unlikely due to its price and limited availability. Acetyl phosphate can function as a substitute. Still, the integration of novel technologies such as polyphosphate-dependent ATP regeneration might facilitate further the syntheses, as this would introduce a cheap, directly available phosphate donor but avoid introducing reverse hydrolysis conditions with the corresponding excess of some substrates. Of course, eliminating the requirement for ATP-regeneration altogether by recruiting the missing three stereochemistries for dihydroxyacetone-dependent enzymes might be the ultimate solution here.

It remains unclear how the unavoidable requirement to go to higher product concentrations will influence the operation of the multi-enzyme systems. So far, the achieved product concentrations have exceeded the physiological range somewhat, but for a truly attractive synthesis, this needs to be improved. This will exceed the concentrations for which enzymes are typically investigated, and consequently, there might be a number of so-far undiscovered consequences.

Finally, no adaptations aimed at process improvements has been reported with the enzymes involved in the different syntheses, for example, by directed evolution or rational enzyme engineering approaches. Consequently, here might lie quite some potential for process improvement.

In summary, a rather broad foundation of possible enzymatic routes to DHAP is available. Urgent issues to address are the design of engineered strains that eliminate the problem of multi-enzyme system assembly, the increase of the product concentrations, and the optimization of the composition of the final mixture for purification.

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References

Ardao I, Benaiges MD, Caminal G, Alvaro G (2006) One step purification-immobilization of fuculose-1-phosphate aldolase, a class II DHAP dependent aldolase, by using metal-chelate supports. Enzyme Microb Technol 39:22–27

Arth HL, Fessner WD (1997) Practical synthesis of 4-hydroxy-3-oxobutylphosphonic acid and its evaluation as a bio-isosteric substrate of DHAP aldolase. Carbohydr Res 305:313–321

Ballou CE, Fischer OL (1956) The synthesis of dihydroxyacetone phosphate. J Am Chem Soc 78:1659–1661

Bednarski MD, Simon ES, Bischofberger N, Fessner WD, Kim M-J, Lees W, Saito T, Waldmann H, Whitesides GM (1989) Rabbit muscle aldolase as a catalyst in organic synthesis. J Am Chem Soc 111:627–635

Brockamp H, Kula MR (1990) *Staphylococcus carnosus* aldolase as catalyst for enzymatic aldol reactions. Tetrahedron Lett 31:7123–7126

Charmantray F, El Blidi L, Gefflaut T, Hecquet L, Bolte J, Lemaire M (2004) Improved straightforward chemical synthesis of dihydroxyacetone phosphate through enzymatic desymmetrization of 2,2-dimethoxypropane-1,3-diol. J Org Chem 69:9310–9312

Charmantray F, Dellis P, Samreth S, Hecquet L (2006) An efficient chemoenzymatic route to dihydroxyacetone phosphate from glycidol for the in situ aldolase-mediated synthesis of monosaccharides. Tetrahedron Lett 47:3261–3263

Cheek S, Ginalski K, Zhang H, Grishin NV (2005) A comprehensive update of the sequence and structure classification of kinases. BMC Struct Biol 5:6

Chen Y-M, Zhu Y, Lin ECC (1987) The organization of the *fuc* regulon specifying L-fucose dissimilation in *Escherichia coli* K-12 as determined by gene cloning. Mol Gen Genet 210:331–337

Colbran RL, Jones JKN, Matheson NK, Rozema I (1967) A synthesis of dihydroxyacetone phosphate from dihydroxyacetone. Carbohydr Res 4:355–358

Cooper RA (1984) Metabolism of methylglyoxal in microorganisms. Annu Rev Microbiol 38:49–68

Cooper SJ, Leonard GA, McSweeney SM, Thompson AW, Naismith JH, Qamar S, Plater A, Berry A, Hunter WN (1996) The crystal structure of a class II fructose-1,6-bisphosphate aldolase shows a novel binuclear metal-binding active site embedded in a familiar fold. Structure 4:1303–1315

Crans DC, Whitesides GM (1985a) Glycerol kinase: substrate specificity. J Am Chem Soc 107:7008–7018

Crans DC, Whitesides GM (1985b) Glycerol kinase: synthesis of dihydroxyacetone phosphate, *sn*-glycerol-3-phosphate, and chiral analogues. J Am Chem Soc 107:7019–7027

D'Arrigo P, Piergianni V, Pedrocchi-Fantoni G, Servi S (1995) Indirect enzymatic phosphorylation: preparation of dihydroxyacetone phosphate. Chem Commun 2505–2506

Dreyer MK, Schulz GE (1993) The spatial structure of the class II L-fuculose-1-phosphate aldolase from *Escherichia coli*. J Mol Biol 231:549–553

Drueckhammer DG, Durrwachter JR, Pedersen RL, Crans DC, Daniels L, Wong C-H (1989) Reversible and in situ formation of organic arsenates and vanadates as organic phosphate mimics in enzymatic reactions: mechanistic investigation of aldol reactions and synthetic applications. J Org Chem 54:70–77

Durany O, de Mas C, Lopez-Santin J (2005) Fed-batch production of recombinant fuculose-1-phosphate aldolase in *E. coli*. Process Biochem 40:707–716

Effenberger F, Straub A (1987) A novel convenient preparation of dihydroxyacetone phosphate and its use in enzymatic aldol reactions. Tetrahedron Lett 28:1641–1644

Erni B, Siebold C, Christen S, Srinivas A, Oberholzer A, Baumann U (2006) Small substrate, big surprise: fold, function and phylogeny of dihydroxyacetone kinases. Cell Mol Life Sci 63:890–900

Esders TW, Michrina A (1979) Purification and properties of L-a-glycerophosphate oxidase from *Streptococcus faecium* ATCC 12755. J Biol Chem 254:2710–2715

Eyrisch O, Sinerius G, Fessner WD (1993) Facile de novo synthesis and NMR spectroscopic characterization of D-tagatose 1,6-bisphosphate. Carbohydr Res 238:287–306

Ferroni EL, DiTella V, Ghanayem N, Jeske R, Jodlowski C, O'Connell M, Styrsky J, Svoboda R, Venkataraman A, Winkler BM (1999) A three-step preparation of dihydroxyacetone phosphate dimethyl acetal. J Org Chem 64:4943–4945

Fessner WD (1998) Enzyme mediated C–C bond formation. Curr Opin Chem Biol 2:85–97

Fessner W-D, Eyrisch O (1992) One-pot synthesis of tagatose 1,6-bisphosphate by diastereoselective enzymatic aldol addition. Angew Chem Int Ed 31:56–58

Fessner W-D, Walter C (1992) "Artificial metabolisms" for the asymmetric one-pot synthesis of branched-chain saccharides. Angew Chem Int Ed 31:614–616

Fessner W-D, Walter C (1996) Enzymatic C–C bond formation in asymmetric synthesis. Top Curr Chem 184:97–194

Fessner WD, Sinerius G (1994) Synthesis of dihydroxyacetone phosphate (and isosteric analogues) by enzymatic oxidation-sugars from glycerol. Angew Chem Int Ed 33:209–212

Fessner WD, Sinerius G, Schneider A, Dreyer M, Schulz GE, Badia J, Aguilar J (1991) Diastereoselective enzymatic aldol additions: L-rhamnulose and L-fuculose 1-phosphate aldolases from *E. coli*. Angew Chem Int Ed 30:555–558

Fong S, Machajewski TD, Mak CC, Wong CH (2000) Directed evolution of D-2-keto-3-deoxy-6-phosphogluconate aldolase to new variants for the efficient synthesis of D- and L-sugars. Chem Biol 7:873–883

Garcia-Junceda E, Shen G-J, Sugai T, Wong CH (1995) A new strategy for the cloning, overexpression and one-step purification of 3 DHAP-dependent aldolases-rhamnulose-1-phosphate aldolase, fuculose-1-phosphate aldolase and tagatose-1,6-diphosphate aldolase. Bioorg Med Chem 3:945–953

Gefflaut T, Lemaire M, Valentin ML, Bolte J (1997) A novel efficient synthesis of dihydroxyacetone phosphate and bromoacetol phosphate for use in enzymatic aldol syntheses. J Org Chem 62:5920–5922

Gijsen HJM, Qiao L, Fitz W, Wong C-H (1996) Recent advances in the chemoenzymatic synthesis of carbohydrates and carbohydrate mimetics. Chem Rev 96:443–473

Hall DR, Bond CS, Leonard GA, Watt I, Berry A, Hunter WN (2002) Structure of tagatose-1,6-bisphosphate aldolase—insight into chiral discrimination, mechanism, and specificity of class II aldolases. J Biol Chem 277:22018–22024

Hettwer J (1998) Kinetik aldolasekatalysierter Aldolkondensationen und Prozessoptimierung der glycerin-3-phosphat Synthese, PhD. Universität Bielefeld, Bielefeld

Hettwer J, Oldenburg H, Flaschel E (2002) Enzymic routes to dihydroxyacetone phosphate or immediate precursors. J Mol Catal B Enzym 19:215–222

Hirschbein BL, Mazenod FP, Whitesides GM (1982) Synthesis of phosphoenolpyruvate and its use in adenosine triphosphate cofactor regeneration. J Org Chem 47:3765–3766

Itoh N, Tujibata Y, Liu JQ (1999) Cloning and overexpression in *Escherichia coli* of the gene encoding dihydroxyacetone kinase isoenzyme I from *Schizosaccharomyces pombe*, and its application to dihydroxyacetone phosphate production. Appl Microbiol Biotechnol 51:193–200

Jung SH, Jeong JH, Miller P, Wong CH (1994) An efficient multigram-scale preparation of dihydroxyacetone phosphate. J Org Chem 59:7182–7184

Kroemer M, Merkel I, Schulz GE (2003) Structure and catalytic mechanism of L-rhamnulose-1-phosphate aldolase. Biochemistry 42:10560–10568

Liu J-Q, Odani M, Yasuoka T, Dairi T, Itoh N, Kataoka M, Shimizu S, Yamada H (2000) Gene cloning and overproduction of low-specificity D-threonine aldolase from *Alcaligenes xylosoxidans* and its application for production of a key intermediate for parkinsonism drug. Appl Microbiol Biotechnol 54:44–51

Mahmoudian M, Noble D, Drake CS, Middleton RF, Montgomery DS, Piercey JE, Ramlakhan D, Todd M, Dawson MJ (1997) An efficient process for the production of *N*-acetylneuraminic acid using *N*-acetylneuraminic aldolase. Enzyme Microb Technol 20:393–400

Meyer O, Rohmer M, Grosdemange-Billiard C (2004) Short and efficient synthesis of a stock material of dihydroxyacetone phosphate from glycidol. Tetrahedron Lett 45:7921–7923

Meyer O, Ponaire S, Rohmer M, Grosdemange-Billiard C (2006) Lewis acid mediated regioselective ring opening of benzylglycidol with dibenzyl phosphate: short and attractive synthesis of dihydroxyacetone phosphate. Org Lett 8:4347–4350

Nagano N, Orengo CA, Thornton JM (2002) One fold with many functions: the evolutionary relationships between TIM barrel families based on their sequences, structures and functions. J Mol Biol 321:741–765

Nobelmann B, Lengeler JW (1996) Molecular analysis of the *gat* genes from *Escherichia coli* and of their roles in galactitol transport and metabolism. J Bacteriol 178:6790–6795

Noguchi T, Shiba T (1998) Use of *Escherichia coli* polyphosphate kinase for oligosaccharide synthesis. Biosci Biotechnol Biochem 62:1594–1596

Oldenburg H (1998) Kinetische Untersuchungen und Prozessoptimierung der Produktion von Dihydroxyacetonphosphat mit Hilfe der glycerinphosphat-oxidase, PhD. Universität Bielefeld, Bielefeld

Operdoes FR (1987) Compartmentation of carbohydrate metabolism in trypanosomes. Annu Rev Microbiol 41:127–151

Ozaki A, Toone EJ, von der osten CH, Sinskey AJ, Whitesides GM (1990) Overproduction and substrate specificity of a bacterial fuculose-1-phosphate aldolase: a new enzymatic catalyst for stereocontrolled aldol condensation. J Am Chem Soc 112:4970–4971

Panke S, Wubbolts MG (2005) Advances in biocatalytic synthesis of pharmaceutical intermediates. Curr Opin Chem Biol 9:188–194

Pederson RL, Esker J, Wong CH (1991) An improved synthesis of dihydroxyacetone phosphate. Tetrahedron 47:2643–2648

Periana RA, Motiu-DeGrood R, Chaing Y, Hupe DJ (1980) Does substrate rather than protein provide the catalyst for α-proton abstraction in aldolase? J Am Chem Soc 102:3923–3927

Phillips SA, Thornalley PJ (1993) The formation of methylglyoxal from triose phosphates. Eur J Biochem 212:101–105

Pradines A, Klaebe A, Perie J, Paul F, Monsan P (1988) Enzymatic synthesis of phosphoric monoesters with alkaline phosphatase in reverse hydrolysis conditions. Tetrahedron 44:6373–6386

Resnick SM, Zehnder AJB (2000) In vitro ATP regeneration from polyphosphate and AMP by polyphosphate: AMP phosphotransferase and adenylate kinase from *Acinetobacter johnsonii* 210A. Appl Environ Microbiol 66:2045–2051

Richard JP (1991) Kinetic parameters for the elimination reaction catalyzed by triosephosphate isomerase and an estimation of the reaction's physiological significance. Biochemistry 30:4581–4585

Rowan AS, Hamilton CJ (2006) Recent developments in preparative enzymatic synthesis of carbohydrates. Nat Prod Rep 23:412–443

Sanchez-Moreno I, Garcia-Garcia JF, Bastida A, Garcia-Junceda E (2004) Multienzyme system for dihydroxyacetone phosphate-dependent aldolase catalyzed C–C bond formation from dihydroxyacetone. Chem Commun 14:1634–1635

Sawada H, Takagi Y (1964) The metabolism of L-rhamnose in *Escherichia coli*, 3. L-Rhamnulose-phosphate aldolase. Biochim Biophys Acta 92:26–32

Schoevaart R, van Rantwijk F, Sheldon RA (1999) Carbohydrates from glycerol: an enzymatic four-step, one-pot synthesis. Chem Commun 24:2465–2466

Schoevaart R, van Rantwijk F, Sheldon RA (2000a) A four-step enzymatic cascade for the one-pot synthesis of non-natural carbohydrates from glycerol. J Org Chem 65:6940–6943

Schoevaart R, van Rantwijk F, Sheldon RA (2000b) Stereochemistry of nonnatural aldol reactions catalyzed by DHAP aldolases. Biotechnol Bioeng 70:349–352

Schultz PG, Yin J, Lerner RA (2002) The chemistry of the antibody molecule. Angew Chem Int Ed 41:4427–+

Schürmann M, Sprenger GA (2001) Fructose-6-phosphate aldolase is a novel class I aldolase from *Escherichia coli* and is related to a novel group of bacterial transaldolases. J Biol Chem 276:11055–11061

Schürmann M, Schürmann M, Sprenger GA (2002) Fructose 6-phosphate aldolase and 1-deoxy-D-xylulose 5-phosphate synthase from *Escherichia coli* as tools in enzymatic synthesis of 1-deoxysugars. J Mol Catal B Enzym 19:247–252

Shiba T, Tsutsumi K, Ishige K, Noguchi T (2000) Inorganic phosphate and polyphosphate kinase: their novel biological functions and applications. Biochemistry (Moscow) 65:315–323

Silvestri MG, deSantis G, Mitchell M, Wong C-H (2003) Asymmetric aldol reactions using aldolases, In: Denmark SE (ed) Topics in stereochemistry. Wiley, pp 267–342

Suau T, Alvaro G, Benaiges MD, Lopez-Santin J (2006) Influence of secondary reactions on the synthetic efficiency of DHAP-aldolases. Biotechnol Bioeng 93:48–55

Sun J, van den Heuvel J, Soucaille P, Qu Y, Zeng A-P (2003) Comparative genomic analysis of *dha* regulon and related genes for anaerobic glycerol metabolism in bacteria. Biotechnol Prog 19:263–272

Takami M, Suzuki Y (1994) Synthesis of novel phosphatidyldihydroxyacetone *via* transphosphatidylation reaction by phospholipase D. Biosci Biotechnol Biochem 58:2136–2139

Valentin ML, Bolte J (1995) A convenient synthesis of DHAP from acetone. Bull Soc Chim Fr 132:1167–1171

van Herk T, Hartog AF, Schoemaker HE, Wever R (2006) Simple enzymatic in situ generation of dihydroxyacetone phosphate and its use in a cascade reaction for the production of carbohydrates: Increased efficiency by phosphate cycling. J Org Chem 71:6244–6247

Vidal L, Durany O, Suau T, Ferrer P, Benaiges MD, Caminal G (2003) High-level production of recombinant His-tagged rhamnulose 1-phosphate aldolase in *Escherichia coli*. J Chem Technol Biotechnol 78:1171–1179

von der Osten CH, Sinskey AJ, Barbas CF, Pederson RL, Wang YF, Wong CH (1989) Use of a recombinant bacterial fructose-1,6-diphosphate aldolase in aldol reactions-preparative syntheses of 1-deoxynojirimycin, 1-deoxymannojirimycin, 1,4-dideoxy-1,4-imino-D-arabinitol, and fagomine. J Am Chem Soc 111:3924–3927

Whalen LJ, Wong CH (2006) Enzymes in organic synthesis: aldolase-mediated synthesis of iminocyclitols and novel heterocycles. Aldrichimica Acta 39:63–71

Wong CH, Whitesides GM (1983) Synthesis of sugars by aldolase-catalyzed condensation reactions. J Org Chem 48:3199–3205

Wong CH, Whitesides GM (1994) Enzymes in synthetic organic chemistry. Pergamon, Tarrytown, NY, USA

Wong C-H, Halcomb RL, Ichikawa Y, Kajimoto T (1995) Enzymes in organic synthesis: application to the problems of carbohydrate recognition (part 1). Angew Chem Int Ed 34:412–432

Yanase H, Okuda M, Kita K, Shibata K, Sakai Y, Kato Y (1995) Enzymatic preparation of [1,3-C-13] dihydroxyacetone phosphate from [C-13] methanol and hydroxypyruvate using the methanol-assimilating system of methylotrophic yeasts. Appl Microbiol Biotechnol 43:228–234

Zelic B, Gostovic S, Vuorilehto K, Vasic-Racki D, Takors R (2004) Process strategies to enhance pyruvate production with recombinant *Escherichia coli*: from repetitive fed-batch to in situ product recovery with fully integrated electrodialysis. Biotechnol Bioeng 85:638–646

Zhao H, van der Donk WA (2003) Regeneration of cofactors for use in biocatalysis. Curr Opin Biotechnol 14:583–589