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Dihydroxyacetone phosphate, DHAP, in the crystalline state: monomeric and dimeric forms

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ABSTRACT

It was shown that dihydroxyacetone phosphate may exist in both monomeric DHAP ($C_3H_7O_6P$) and dimeric DHAP-dimer ($C_6H_{14}O_{12}P_2$) form. Monomeric DHAP was obtained in the form of four crystalline salts: $CaCl(DHAP)\cdot 2.9H_2O$ (**7a**), $Ca_2Cl_3(DHAP)\cdot 5H_2O$ (**7b**), $CaCl(DHAP)\cdot 2H_2O$ (**7c**), and $CaBr(DHAP)\cdot 5H_2O$ (**7d**) by crystallization from aqueous solutions containing DHAP acid and $CaCl_2$ or $CaBr_2$, or by direct crystallization from a solution containing DHAP precursor and $CaCl_2$. At least one of the salts is stable and may be stored in the crystalline state at room temperature for several months. The dimeric form was obtained by slow saturation of free DHAP syrup with ammonia at −18 °C and isolated in the form of its hydrated diammonium salt $(NH_4)_2(DHAP\text{-dimer})\cdot 4H_2O$ (**8**). The synthesis of the compounds, their crystallization, and crystal structures determined by X-ray crystallography are described. In all **7a–d** monomeric DHAP exists in the monoanionic form in an extended (in-plane) cisoid conformation, with both hydroxyl and ester oxygen atoms being synperiplanar to the carbonyl O atom. The crucial structural feature is the coordination manner, in which the terminal phosphate oxygen atoms act as chelating as well as bridging atoms for the calcium cations. Additionally, the DHAP monoanions chelate another Ca^{2+} by the α-hydroxycarbonyl moiety, in a manner observed previously in dihydroxyacetone (DHA) calcium chloride complexes. In dimeric **8** the anion is a trans isomer with the dioxane ring in a chair conformation with the hydroxyl groups in axial positions and the phosphomethyl group in an equatorial position.

1. Introduction

The synthesis of dihydroxyacetone phosphate (DHAP, **7**) and its isolation in the form of a stable, easy-to-store compound, as well as the structural investigations of DHAP in monomeric (**7**) as well as in dimeric form (**8**), was the main aim of the project dealing with the chemical conversion of the simplest ketose, dihydroxyacetone (DHA, **2**), into its phosphate ester, DHAP (**7**). It is also a final part of the series of our previous papers on the structural characterization of the intermediates on this chemical pathway (Scheme 1).[1–5]

In living cells sugars are primed for metabolic transformations by phosphorylation. In case of DHAP, there are multiple pathways to it, mainly DHA phosphorylation and L-glycerol 3-phosphate (G-3P) oxidation in the mitochondrial glycerophosphate shuttle. DHAP is also produced in the glycolytic pathway by the cleavage of D-fructose 1,6-bisphosphate (Fru-1,6-BP) (Scheme 2).[6,7]

DHAP (**7**) is one of the most important biochemical intermediates of high importance for all living cells. As a phosphate ester of a ketotriose (DHA), it plays a crucial role in energy-producing processes and in many biosyntheses. It acts as a substrate for at least 18 different enzymes, including triosephosphate isomerase, glycerol-3-phosphate dehydrogenase, dihydroxyacetone phosphate acyltransferase, and several types of aldolases. DHAP is involved in 25 different enzymatic reactions (most of which are shown in Scheme 2) and 9 different metabolic pathways, mainly in the area of carbohydrate metabolism, but also that of cofactors and vitamin metabolism.[6,7] Thus, dihydroxyacetone phosphate plays a crucial role in, for example, gluconeogenesis, fructose and mannose metabolism, glycerol and fatty acid metabolism, pentose phosphate pathway, the Calvin cycle, biosynthesis of triacylglycerols and (ether)phospholipids, glycerophosphate shuttle, and finally, in glycolysis. What is interesting is the fact that only three from eleven glycolytic intermediates have not been yet crystallized. Two of them are just DHAP and its isomer (glyceraldehyde 3-phosphate) GAP.[8]

Reversible reactions involving DHAP-dependent aldolases have been commonly used in the synthesis of synthetic sugars and related chiral compounds on a preparative scale. They accept a broad spectrum of aldehydes, but all of them are very specific to DHAP (for review of the application of DHAP-dependent aldolases in the asymmetric organic synthesis see Refs. 9–14). The use of aldolases (EC 4.1.2) as catalysts for the aldol condensation helps to avoid difficulties in controlling the stereo- and regioselectivity of

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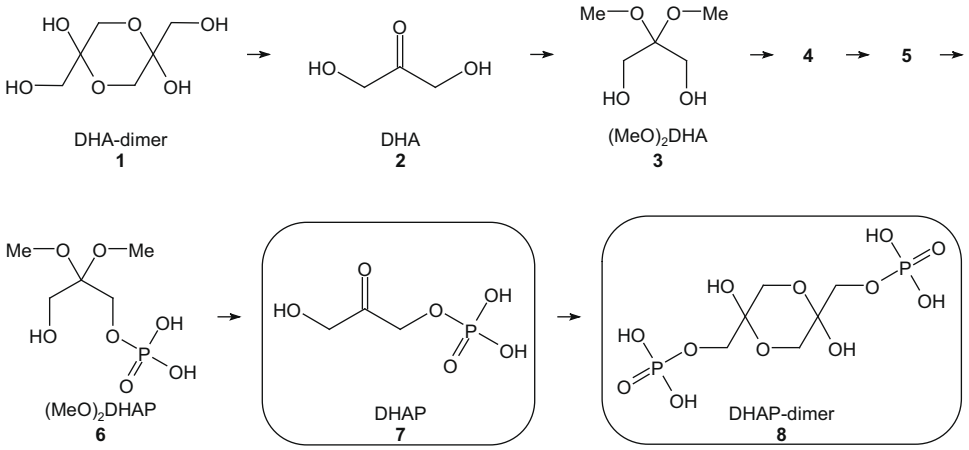
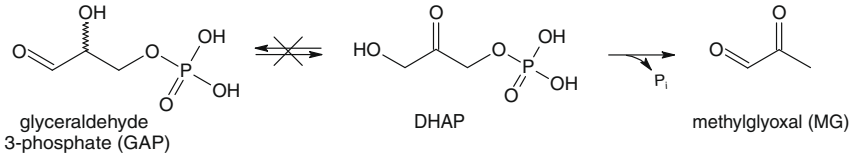


Scheme 1. Chemical pathway leading from DHA to DHAP.

the reaction by numerous blocking/unblocking steps, the use of chemical catalysts activating the acceptor, donor or both, the use of non-aqueous solvents and other difficulties. Instead, the aldolase-catalyzed reactions may be carried out in aqueous solutions at room temperature, and they lead to the enantiomerically pure products from achiral or racemic substrates.

It is known that DHAP, similar to DHA, exists in water as a mixture of two monomeric forms: the free carbonyl, ketone (K), and the *gem*-diol (hydrate, H).[15–18] The ketone/hydrate ratio was found to be both temperature and pH dependent. The amount of the keto form is increased at higher temperatures,[15,16,18] and its proportion in neutral aqueous solution was established by Gray and Barker as 55% at 25 °C and 63% at 37 °C[18] and by Reynolds et al., as 15% at 4 °C, 55% at 20 °C, and even 83% at 37 °C.[16] Additionally, Reynolds et al.[16] stated that DHAP aqueous solution at 20 °C and neutral pH (fully ionized DHAP anion) consists of free keto (K), hydrated keto (H, *gem*-diol), and enol forms in proportions 55:44:1. One percent of the enolic species in DHAP water solution was also observed at 30 °C, and it is rather huge proportion in comparison with 0.02% for dihydroxyacetone (DHA), 0.04% for monohydroxyacetone, and 0.17% for monohydroxyacetone phosphate under the same conditions.[16] The overall pH dependence of ketone/hydrate ratio reveals that the amount of the keto form is increased with pH increase at temperatures below 23 °C, and it is increased with pH decrease at temperatures above 23 °C.[16] All in all, it was also shown that the keto form of DHAP is the form of the substrate handled by the enzyme (α-glycerophosphate dehydrogenase, aldolase, and triosephosphate isomerase).[15,16] It is to be mentioned that the existence of the dimeric form of dihydroxyacetone phosphate, DHAP-dimer, has been postulated but probably never observed experimentally.[16,18] Moreover, its possible role in the biochemical processes has not been even taken into consideration. For comparison, it is known that commercial, solid dihydroxyacetone, which is 100% in the dimeric form, DHA-dimer, dissociates in water solution into a mixture of two monomeric forms: the free ketone and the hydrate in a ratio 4:1.[19] It has also been shown that the crystalline DHA monomer kept at room temperature undergoes dimerization, which is complete in 25–30 days, and then polymerization within several months.[20] Additionally, it has been shown that the DHA-dimer to DHA interconversion process in aqueous solutions is reversible and temperature dependent; increasing the temperature of dihydroxyacetone aqueous solutions favors the formation of monomeric DHA from DHA-dimer.[21] Taking this into consideration, a similar process should not be excluded in the case of DHAP.

It has been shown that DHA in water may undergo enolization and interconversion into DL-glyceraldehyde,[21,22] even though the enolization reactions are known to require the acid–base catalysis. Probably because of enolization, DHAP is unstable in the whole pH range, and its stability is the lowest under alkaline conditions. For example, 0.1 M NaOH solution at room temperature was shown to be the conditions that degrade DHAP with a halftime of about 2 min.[23] It was also shown that in 1 N hydroxide solution at room temperature DHAP fully hydrolyzes just in 20 min.[24] However, DHAP enolization does not lead to the respective aldehyde. Instead, the hydrolysis of DHAP occurs, resulting in inorganic phosphate and 2-oxo-propanal (also called pyruvaldehyde or methylglyoxal, MG), instead of DHA, as shown in the scheme below:



DHAP enolization is the first step of its nonenzymatic elimination (hydrolysis) and possible isomerization.[23] In an aqueous solution, the endiolate intermediate undergoes an immediate elimination to produce MG. (The elimination reaction is ~100 times faster than the isomerization.) This cannot take place in vivo, because DHAP must be converted into GAP for glycolysis, and the synthesis of toxic MG should be under control. Therefore, in living organisms these two processes (reversible isomerization and irreversible elimination) are controlled by two different enzymes: triosephosphate isomerase, TIM, EC 5.3.1.1, and methylglyoxal synthase, MGS, EC 4.2.3.3 (Scheme 2). It is known that the first step of the reactions catalyzed by both TIM and MGS is the forma-