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Studies on the Reactivity of Bisglycoaldehyde Phosphodiester in Alkaline Solution

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Abstract: The behaviour of bisglycoaldehyde phosphodiester in alkaline solution has previously been investigated by reducing, dephosphorylating and acetylating the products. The detection of threitol and erythreitol tetraacetates by GC coupled with kinetic arguments suggested that bisglycoaldehyde phosphodiester undergoes rapid intramolecular aldolisation to give a mixture of erythrose and threose-2,4-cyclophosphates (Pitsch, S.; Pombo-Villar, E.; Eschenmoser, A. *Helv. Chim. Acta* **1994**, *77*, 2251). In this paper, electrospray mass spectroscopy, deuteration studies and comparison with synthetic materials are used to confirm and augment these earlier findings.

Introduction

In a recent publication from this laboratory a novel aldolisation of achiral monomers **1** to RNA was suggested[1]. The proposed polymerisation mechanism requires that the phosphoketonic enolate of one monomer reacts with the aldehyde group of another, Fig. 1.

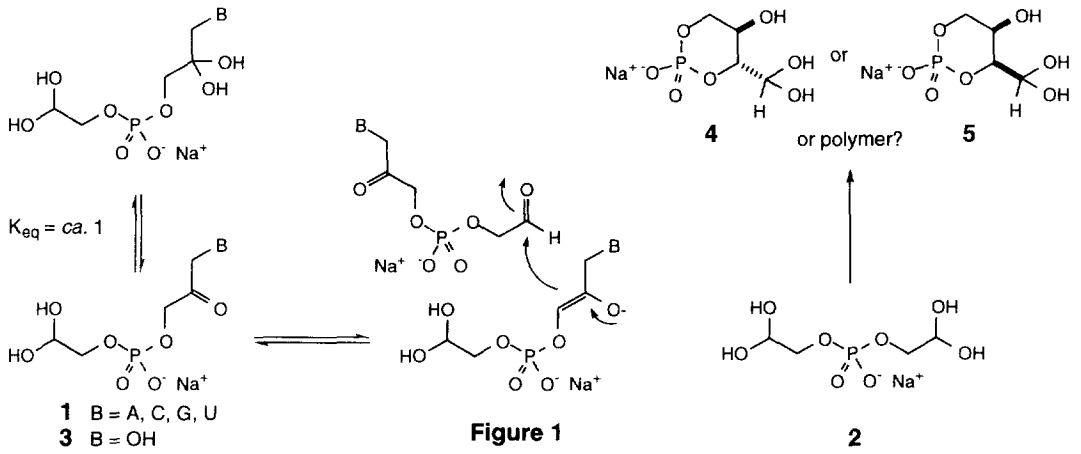

Figure 1

The solution structures of the simpler compounds, bisglycoaldehyde phosphodiester **2** and dihydroxyacetonephosphoglycoaldehyde **3** suggested that preferential aldehyde *cf.* ketone hydration might predispose the crossed-aldol selectivity we invoked[2,3]. A key point in relation to polymerisation is the requirement for predominantly *intermolecular* aldolisation of biscarbonylphosphodiesters. This point has been raised by Eschenmoser in his pioneering studies directed at the chemical aetiology of the natural nucleic acids. Preliminary analysis of the reactivity of **2** in alkaline solution has been reported by the Zürich group who concluded on kinetic grounds that *intramolecular* aldolisation giving **4** and **5** was predominant[4]. The importance of this issue prompted us to investigate this behaviour further by using electrospray mass spectroscopy (ESMS) and product authentification by synthesis.

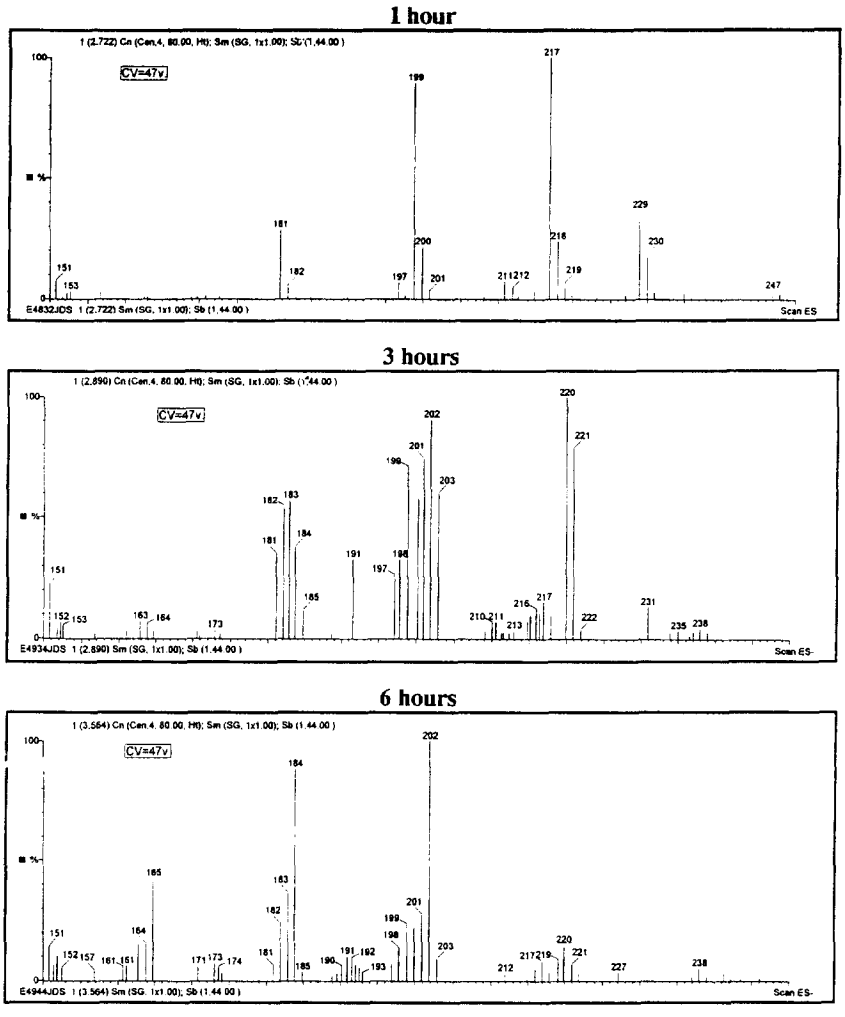

Figure 2

Aliquots were withdrawn from the alkaline reaction at the times indicated and were quenched by addition of a solution of DCl in D_2O (prepared by addition of oxalyl chloride to D_2O) to an apparent pD = 7. Prior to mass spectroscopy, the samples were diluted in H_2O/CH_3CN. The samples were ionised by electrospray with a cone-voltage of 47V and spectra recorded in negative ion mode. Higher cone-voltages resulted in spectra corresponding to more extensive dehydration.

Results and Discussion

In their investigation of the aldolisation of **2**, the Zürich group reduced product mixtures and subjected the resultant compounds to dephosphorylation and acetylation before analysis by GC[5]. The detection of the two tetrol-tetraacetates was consistent with inter or intramolecular aldolisation of **2** giving **4** and **5** or some polymer but dependence of reaction rate on the concentration of **2** *suggested* the latter. We have found electrospray mass spectroscopy (ESMS) to be a powerful technique for the analysis of sugar-phosphates, in particular because the soft nature of the ionisation method allows different hydration states to be detected[3,6]. ESMS analysis of the products formed on treatment of **2** at pH 9.5 confirmed the findings of the Eschenmoser group, species with masses of 199 and 181 being detected in addition to a peak with a mass of 163 corresponding to dehydration of either tetrose-2,4-cyclophosphate. To investigate the relative rates of enolisation and intramolecular aldolisation a series of experiments was carried out in alkaline D_2O ([**2**] = 70 mM, apparent pD = 9.5 (NaOD)). The spectra corresponding to 1, 3 and 6 hours of reaction are shown in Fig. 2. Little deuteration or cyclisation has occurred after 1 hour. After 3 hours the residual starting material is predominantly tri- and tetradeuterated whereas cyclisation products are mainly undeuterated, mono- and dideuterated, Fig. 3. This demonstrates that enolate deuteration and cyclisation are competitive and that a primary kinetic isotope effect is operative. After 6 hours cyclisation has proceeded further and the products are predominantly trideuterated (except for the dehydration product which is dideuterated with molecular mass 165) suggesting reversibility of the aldol reaction or exchange of the product. At the end of the reaction, the products are detected in three hydration states. The relative intensities of the peaks corresponding to these three hydration states vary with applied cone-voltage, high cone-voltages causing dehydration. We tentatively assign the peak at molecular mass 165 to the α,β-unsaturated aldehyde **6**, Fig. 3.

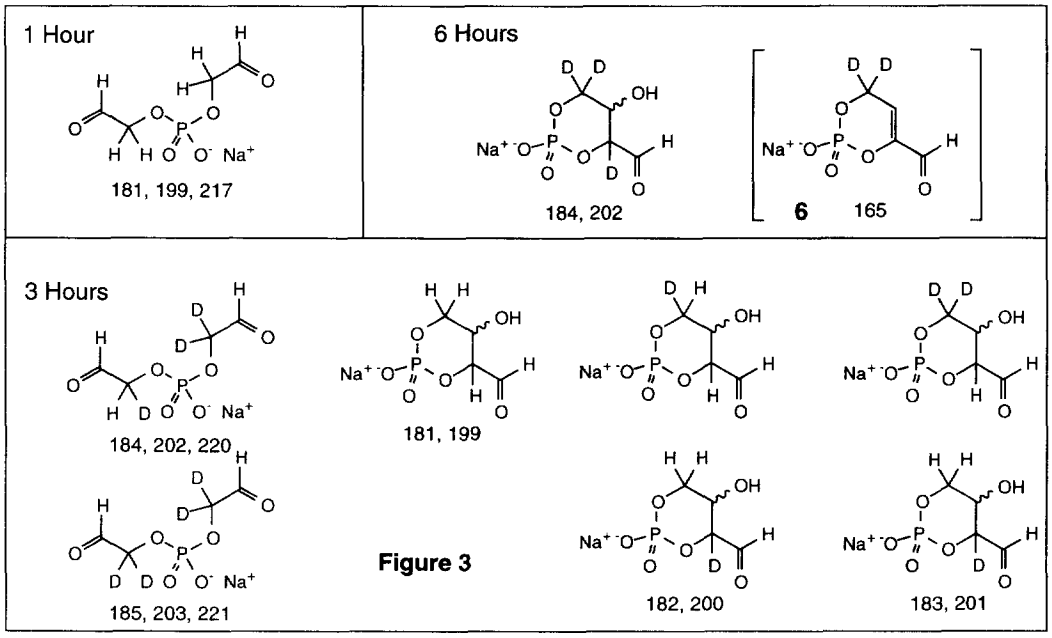

Figure 3

To confirm that the peak at molecular mass 163 (165 when di-deuterated) is associated with dehydration of the threose-2,4-cyclophosphates and is not an alternate reaction product we synthesised the threose derivative **5**, Fig. 4.

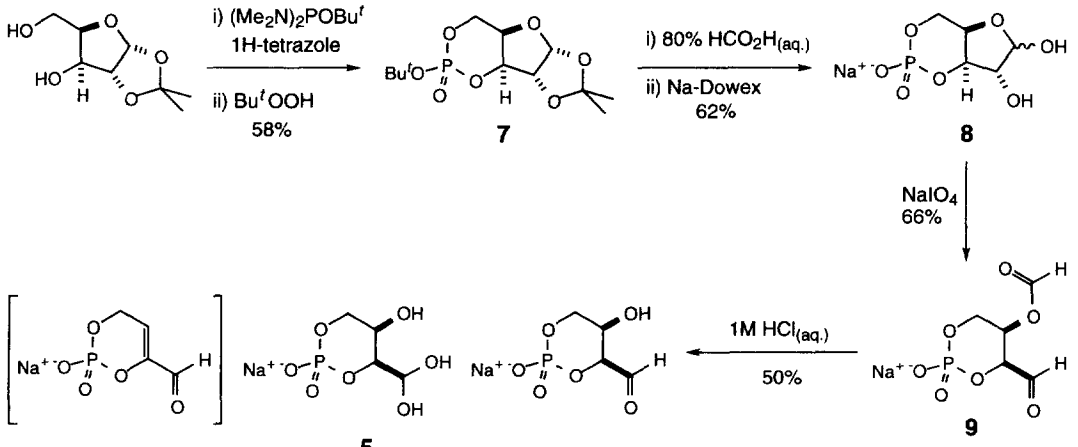

Figure 4

Treatment of 1,2-*O*-isopropylidene-D-xylofuranose with *tert*-butoxy-*bis*-(dimethylamino)-phosphine[2] and 1H-tetrazole followed by oxidation with *tert*-butylhydroperoxide gave the cyclophosphate **7** as a single diastereoisomer after crystallisation from ether. Acid deprotection gave D-xylofuranose-3,5-cyclophosphate **8** which was oxidised with sodium periodate to give 3-formyl-D-threose-2,4-cyclophosphate **9**. Deformylation by treatment with acid gave D-threose-2,4-cyclophosphate **5** which gave peaks at masses of 199, 181 and 163 by ESMS. It thus appears likely that the peak at molecular mass 163 (165 in the D_2O experiments) in the spectrum of the bisglycoaldehyde phosphodiester reaction products does correspond to the α,β-unsaturated aldehyde **6**. The accessibility of **6** in solution would allow the further deuteration of the first formed aldol cyclisation products and provide a simple mechanism for the thermodynamic equilibration of **4** and **5** observed in the work of the Eschenmoser group.

The intramolecular aldolisation of bisglycoaldehyde phosphodiester **2** indicates that the phosphoglycoaldehyde carbonyl group of a phosphodiester is a good aldol acceptor despite its high degree of hydration. Examination of models representing likely transition-states for the intramolecular aldolisation of **2** suggests that subsitution giving **3** or the monomers **1** will lead to severe steric encumbrance to intramolecular reaction. This, coupled with our demonstration that the phosphoketonic enolate of dihydroxyacetonephosphoglycoaldehyde is kinetically preferred, suggests that alternate reactions will ensue with these compounds. Intermolecular aldolisation thus remains a viable reaction mode for the monomers **1** but aldolisation routes to simple poly sugar-phosphates such as polythreose-2,4-phosphates must now be considered less likely.

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