Bioorganic & Medicinal Chemistry Letters, Vol 2, No 12, pp. 1513-1518, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

MECHANISTIC STUDIES OF BIOLOGICAL GLYCOSYLATION. DETERMINATION OF THE GLUCOSYLATING REACTIVITY OF URIDINE-5'-DIPHOSPHO-α-D-GLUCOSE (UDPG) AND ASSESSMENT OF THE CATALYTIC POWER OF THE GLYCOSYLTRANSFERASES.

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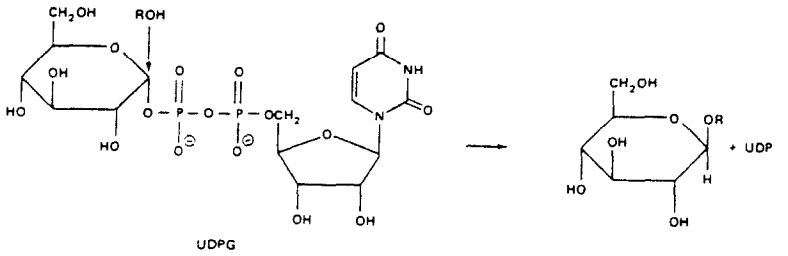
(*Received* 19 *August* 1992)

Abstract: Enzymic rate enhancements of the glycosyltransferases are estimated to be in the order of 10^{10}, as revealed by a determination for the first time of the magnitude of the spontaneous glucosylating reactivity (which prevails only below pH 3) of the prototypical 'activated' co-substrate of biological glycosylation, UDPG.

Biological glycosylation is largely, perhaps wholly, accomplished by enzymes that utilise as 'activated' co-substrates the glycosyl derivatives of either nucleoside pyrophosphates or polyprenol pyrophosphates.[2] The former class of co-substrates form the largest group, for one of several nucleosides (e.g. uridine, cytidine, adenine) may be linked to one of a whole range of glycosyl groups (e.g. glucosyl, galactosyl, mannosyl, glucuronyl, ribosyl). The prototype of the series, uridine-5'-diphospho-α-D-glucose (UDPG), is the co-substrate employed in the biosynthesis of glycogen, cellulose, and a whole host of other polysaccharides, glycolipids, and glycoproteins (many of the latter, of course, possessing more than one type of sugar and requiring other 'activated' glycosylating co-substrates). At a much less complex level, UDPG is also utilised as a co-substrate in the biotransformation in insects and plants of natural, and xenobiotic, alcohols and phenols into 'glucose conjugates'. These UDPG-dependent processes effect, in the main, either O-glucosylation or N-glucosylation of a substrate. In all cases, whether the substrate be a large or small biomolecule, the sites (e.g. hydroxyl or amino groups) of glucosylation are, of course, nucleophilic groupings. Since O- and N-glucosylation are mechanistically congeneric, the prototype of all of the biological glycosylations that deploy glycosyl derivatives of nucleoside pyrophosphates as co-substrates can be formulated as the glucosyltransferase-mediated glucosylation of an alcohol, ROH, by UDPG. Since, moreover, all of the nucleoside co-substrates are α-XDP-glycosides (none are β-), the prototypical formation from an alcohol (ROH) of its β-glucoside can, as Axelrod and co-workers[3] (in their work on mammalian steroidal β-glucuronides) recognised more than 30 years ago, be viewed as a glucosyltransferase-mediated nucleophilic displacement at C-1 of α-UDPG with

expulsion of uridine-5′-diphosphate (UDP) (Scheme 1). (Clearly, when α-glucosides are formed, a double displacement reaction of α-UDPG is required, presumably involving the intermediacy of a glucosylated enzyme. That type of process will not concern us here.)

Scheme 1

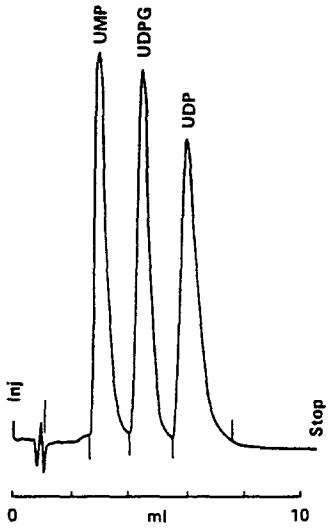


Biological glycosylation can be grouped with the two other major biological alkylation processes, methylation and prenylation. S-Adenosylmethionine (SAM) and dimethylallyl pyrophosphate (DMAPP) are the 'activated' co-substrates, respectively, used by many methyltransferases and prenyltransferases. Although Schowen and Coward and coworkers[4] have determined the spontaneous methylating reactivity of SAM and estimated the catalytic power of the methyltransferases to be in the order of 10^{16}, and Tidd's determination of the spontaneous alkylating reactivity of DMAPP and related compounds[5] provides the wherewithal to do likewise for the prenyltransferases, there have been, to our knowledge, no studies of the measurement of the spontaneous glycosylating reactivity of any glycosyl derivative of a nucleoside pyrophosphate which would yield similar data pertaining to the magnitude of the catalytic role of the glycosyltransferases. To this end we have determined the rates of hydrolysis of α-UDPG in the pH range 1-10, and therefrom we have identified the magnitude of its spontaneous glucosylating reactivity (towards water) and the pH range at which this reactivity prevails.

As the intention was to follow the course of the reaction by quantitative HPLC determination of the declining concentration of α-UDPG using spectrophotometric detection of its uridine chromophore, a system capable of resolving α-UDPG from its likely uridine-containing hydrolysis products, UDP and uridine-5'-phosphate (UMP), was developed (Fig 1). From the results of pilot experiments, a temperature of 60 °C was chosen for the determination of the pH rate profile of α-UDPG. The rate data that were obtained (standard conditions for rate measurements were used[6]) are shown in Fig 2.

Clearly, among the several possible C-O and P-O hydrolytic cleavage reactions of α-UDPG, there was

a need to identify and quantify the hydrolytic process yielding uridine-5'-diphosphate (UDP) by C-O fission (Scheme 1, HOH for ROH). The quantitative HPLC method that was used for the assay of α-UDPG yielded,



Conditions: 10 μL from a solution of about 1 mg/mL injected by valve onto a 200 X 4.5 mm column packed with Partisil ODS 2. The mobile phase was 0.00225 M tetrabutylammonium hydroxide, 0.00225 M tetraethyl-ammonium hydroxide, 0.04 M ammonium dihydrogen orthophosphate, adjusted to pH 6 with KOH in aqueous 10% methanol. The eluant was monitored at λ= 262 nm.

Fig 1. HPLC chromatogram of a mixture of α-UDPG, UDP, and UMP.

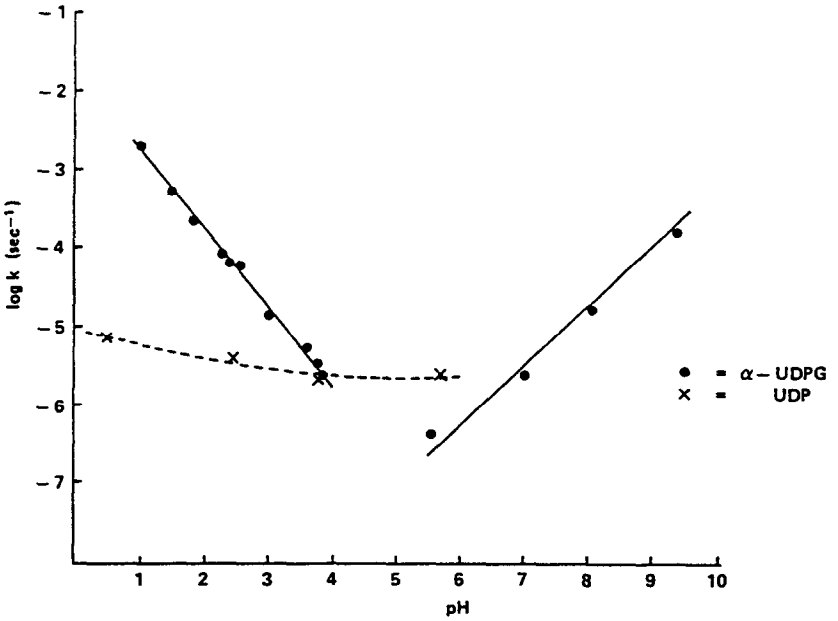


Fig 2. The pH-rate profiles at 60 °C of the hydrolysis of α-UDPG and UDP.

simultaneously, an assay of both UMP and UDP (see Fig 1), and this permitted an estimate to be made of the amounts of each of these reaction products formed during each run at a given pH value.

At pH 1-3, the major hydrolysis product was found to be UDP (ca.95%), with minor amounts of UMP. Moreover the rate of formation of UDP in this pH range was virtually identical to the rate of disappearance of α-UDPG, once allowance had been made for the rate of hydrolysis of UDP (to UMP), which we also measured. The rate profile at 60 °C for UDP in the pH range 1-6 is, for ease of comparison, superimposed on the α-UDPG rate profile in Fig 2, and was very similar to that of adenosine-5'-phosphate (ADP) determined by Miller and Westheimer[7] at 95 °C.

At pH 3-4, the major hydrolysis product was found to be UMP, with minor amounts of UDP. As is evident from Fig 2, the rate of hydrolysis of α-UDPG to UDP is about equal to that of the hydrolysis of UDP to UMP at pH 4, and this means that at this pH it would be hydrolysed as fast as it was formed. Clearly, although all, or a part of, the UMP could have been formed directly from α-UDPG, it seems more likely that all of it is formed via the hydrolysis of initially-formed UDP.

UDP, which is stable under mild alkaline conditions (like ADP[7]) and would have been expected to have persisted if it had been formed, was not a reaction product in the pH range 7-10. Consequently, only a few measurements in this pH range were made. The rate profile for α-UDPG in the pH range 7-10 is probably best explained by hydroxide ion-catalysed formation of uridine-5'-monophosphate (UMP) and glucose-1,2-cyclic monophosphate, as observed by Leloir and co-workers[8] during their structural studies.

The shape of the rate profile of α-UDPG at pH 1-3 was very similar to that of α-D-glucose-1-phosphate (α-GP), determined by Bunton and co-workers.[9a] In isotope experiments using $H_2{}^{18}O$, Bunton and co-workers[9a] found that in this pH region the hydrolysis of α-GP occurred wholly via C-O fission. For comparison, our data and those for α-D-glucose-1-phosphate,[9a] methyl phosphate,[9b] dimethylallyl phosphate,[5] and dimethylallyl pyrophosphate[5] in the pH range 1-8 (all extrapolated to 60 °C) appear in Figure 3. Although UDP could clearly have derived from α-UDPG via a P-O or a C-O fission process, we have not as yet conducted any experiments with $H_2{}^{18}O$ to check this. However, the near-identical slopes of the profiles for α-UDPG and α-GP, together with the closely similar slopes for dimethylallyl phosphate and dimethylallyl pyrophosphate in the pH range 1-7 (which Tidd has shown[5] to be due to C-O fission of each) point to a similar C-O fission for α-UDPG at pH 1-3. It is noteworthy that for glucosyl and dimethylallyl esters, monophosphate appears to be about as good a leaving group as pyrophosphate. As is well known, C-O fission of methyl phosphate is negligible at pH 1-3.

The mechanism of the hydrolysis of α-UDPG at pH 1-3 would be expected to be analogous to that proposed[9a] for α-GP and therefore probably involves the slow ionisation via an $S_N1(C)$ process of the neutral molecule (Scheme 2), or less likely, of the monoanion.

In summary, these results reveal that α-UDPG is a glucosylating agent only at pH 1-3 with a reactivity only marginally greater than that of α-D-glucose-1-phosphate. Extrapolation to pH 7.4 and to 37°C of the rate data gives a value of 2 x 10^{-11} sec^{-1} for the glucosylating reactivity (towards water) of α-UDPG. Though data

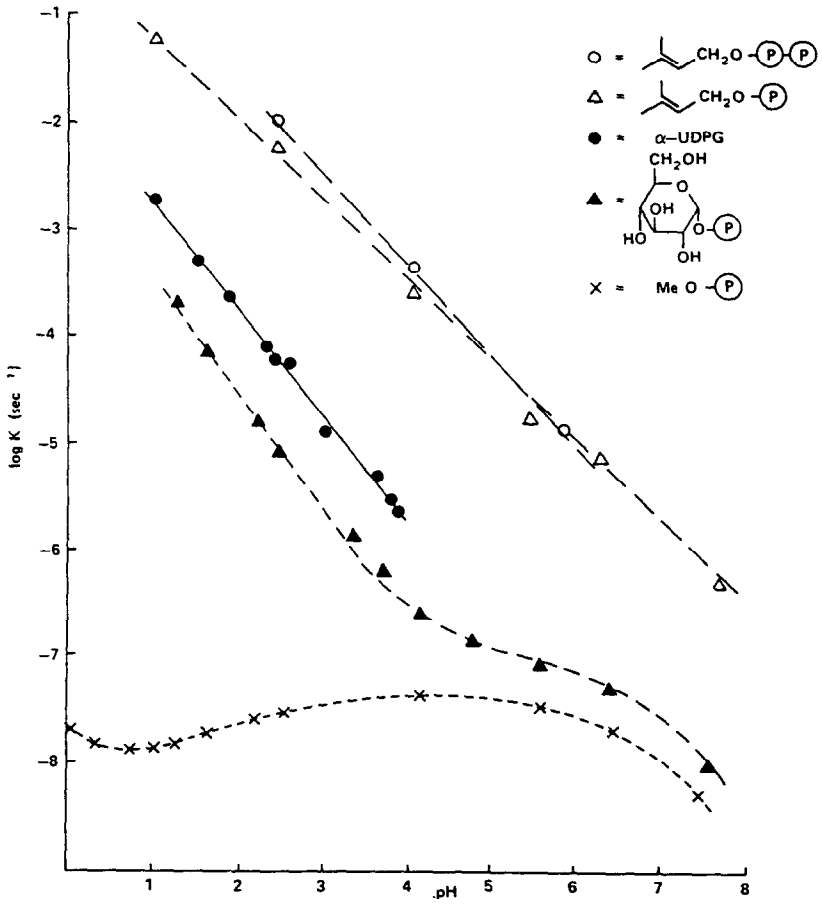
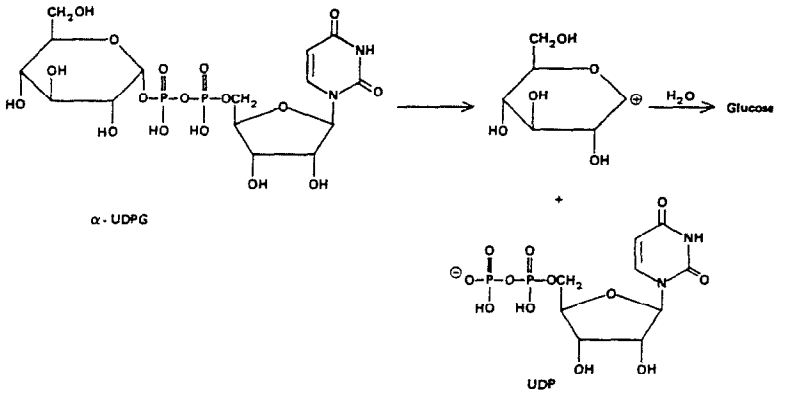


Fig 3. The pH-rate profiles at 60 °C of the hydrolysis of methyl phosphate, dimethylallyl phosphate, dimethylallyl pyrophosphate, α-D-glucose-1-phosphate and α-UDP.

Scheme 2



for enzymic rates of glycosyltransferases are sparse,[10] the indications are that enzymic rate enhancements are of the order of 10^{10}.

References and Notes

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6. The reactions were carried out in an appropriate buffer, in duplicate, at 60°C. The ionic strength of all solutions was kept at 1.0 by addition of suitable quantities of KCl. First-order rate constants were calculated from data acquired over at least four half-lives.(No corrections of the rate data for buffer catalysis were necessary, since representative checks for buffer catalysis at pH 3.8 (formate) and pH 8.0 (Tris) showed there to be none.

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