Metal –ion promoted cleavage of nucleoside diphosphosugars: A model for reactions of phosphodiester bonds in carbohydrates

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Abstract. Cleavage of five different nucleoside diphosphosugars has been studied in the presence of Cu^{2+} and Zn^{2+} complexes. The results show that metal ion catalysts promote the cleavage *via* intramolecular transesterification whenever a neighbouring HO-group can adopt a *cis* orientation with respect to the phosphate. The HO-group attacks the phosphate and two monophosphate products are formed. If such nucleophile is not available, Cu^{2+} complexes are able to promote a nucleophilic attack of an external nucleophile e.g. a water molecule or metal ion coordinated HO-ligand, on phosphate. With the Zn^{2+} complex studied, this was not observed.

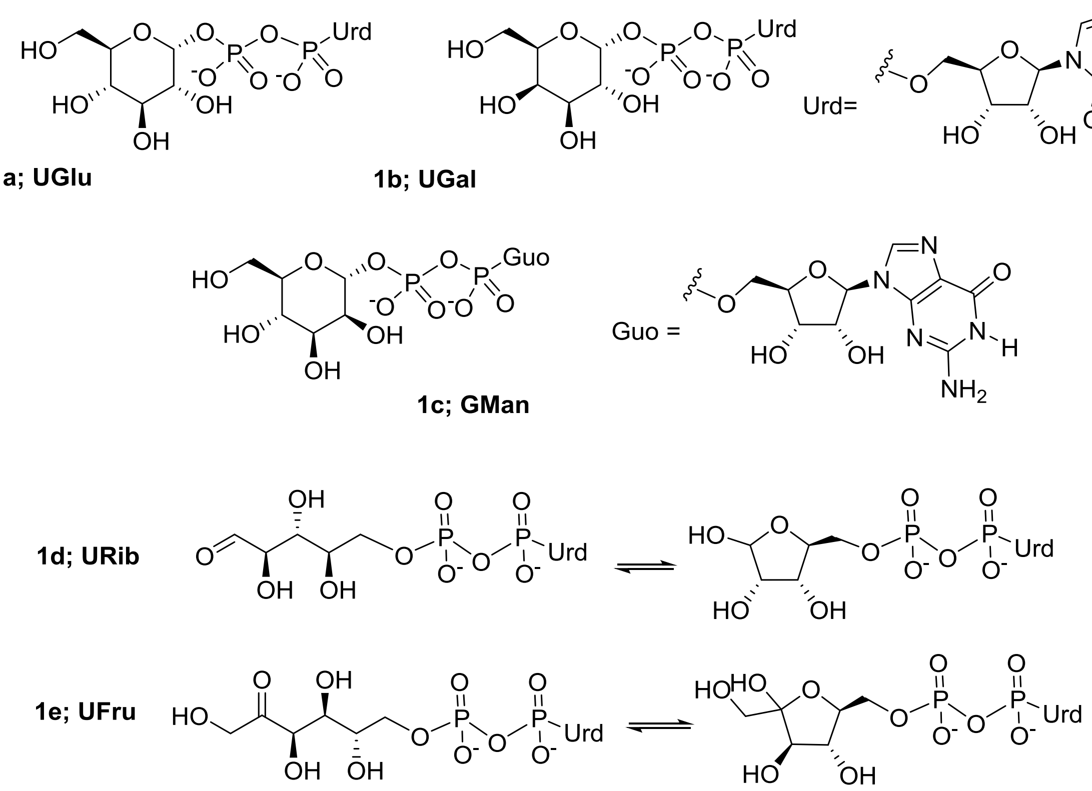
Keywords: Carbohydrates, phosphodiesters, cleavage, mechanisms, metal ion catalysts

Introduction

Carbohydrate-phosphate –linkages are ubiquitous in biological systems. Phosphodiester bonds of DNA and RNA are the best known example, but phosphodiester bonds are found in other biomolecules as well. For example, cell-surface carbohydrates and lipopolysaccharides contain repeating oligosaccharide sequences linked together through phosphodiester bonds (1). While the reactions of phosphodiester bonds in RNA and DNA have been extensively studied (2), less is known about the reactivity of phosphodiester bonds in other biomolecules (3). As the molecular environment around a phosphodiester bond in carbohydrates, for example, is more versatile than in RNA and DNA (1) the reactivities vary significantly, and different reaction routes are available as is shown by the examples collected in *Ref.3.*

The present work studies metal ion promoted cleavage of nucleoside diphosphate sugars. In chemical research they can be regarded as simple and convenient model compounds for studies on reactions of carbohydrate phosphodiester linkages. At the first stage of the reactivity studies, the use of nucleoside diphospho sugars simplifies the reactions system, since diphosphate and monophosphate moieties are good leaving groups. Furthermore, a nucleic acid base allows a convenient detection with a UV-detector. On the other hand, nucleoside diphospho sugars form a series of substrates which can be used to obtain further information on catalytic strategies employed by metal ion catalysts in reactions of biological phosphates.

The cleavage of five different nucleoside diphosphosugars uridine 5'-diphospho-1-α-D-glucose (**1a**, UGlu), uridine 5'-diphospho-1-α-D-galactose (**1b**, UGal), guanosine 5'-diphospho-1-α-D-mannose (**1c**, GMan), uridine 5'-diphospho-5-D-ribose (**1d**, URib), and uridine 5'-diphospho-6-D-fructose (**1e**, UFru) under neutral conditions in the presence of metal ion catalysts. These substrates can be divided into two groups depending on the position of the diphosphate moiety. **1a-c** are glycosylic compounds, where the phosphate group is attached to an anomeric carbon and under acidic conditions these compounds exhibit chemical properties similar to those of glycosides (4). Substrates **1d** and **1e** can be called reductive nucleoside diphospho sugars (5). In these substrates the anomeric HO-group is unsubstituted and they exist in solution as a mixture of cyclic and acyclic forms and their reactivity is therefore different from that of glycosylic substrates.



1a; UGlu

1b; UGal

1c; GMan

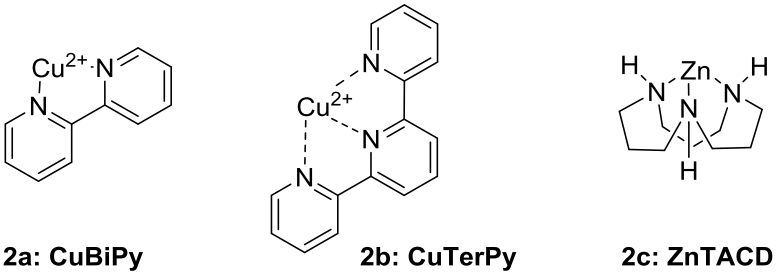
1d; URib

1e; UFru

Chart 1. Structures of nucleoside diphosphosugars studied

In biological systems compounds **1a-c** serve as glycosyl donors where the diphosphate moiety is an easily replaceable leaving group. Glycosylation reactions are catalyzed by glycosyl transferase enzymes and they involve a nucleophilic substitution at C1 (6). Another reaction in biological systems is the hydrolysis of excess or hazardous nucleoside diphosphosugars that is catalysed by nudix hydrolases (7). Hydrolysis usually involves a nucleophilic attack on a phosphate and two monophosphates are formed as products (7). Mannose derivatives make an exception, and they are cleaved by a nucleophilic attack on C1, which results in a release of a nucleoside diphosphate. Both transferases and hydrolases usually utilize metal ions, most typically Mg^{2+} or Mn^{2+}, as cofactors, although metal ion –independent variants are also known (6,7). Metal ion cofactors are usually involved in interactions with the phosphate group.

The cleavage of substrates **1a-e** was studied in the presence of three different catalysts, Cu^{2+}-bipyridine (**2a**, CuBiPy), Cu^{2+}-terpyridine (**2b**, CuTerPy) and Zn^{2+}-triazacyclododecane (**2c**, ZnTACD). These complexes are known to enhance the cleavage of different types of biological phosphates, such as phosphodiester bonds of RNA, cyclic monophosphates and RNA model compounds (8), as well as dinucleoside oligophosphates including the mRNA cap-structure (9). The results obtained were discussed in comparison to those hose obtained with other types of phosphate containing substrates.



2a: CuBiPy **2b: CuTerPy** **2c: ZnTACD**

Chart 2. Structures of metal complexes employed as catalysts

Experimental

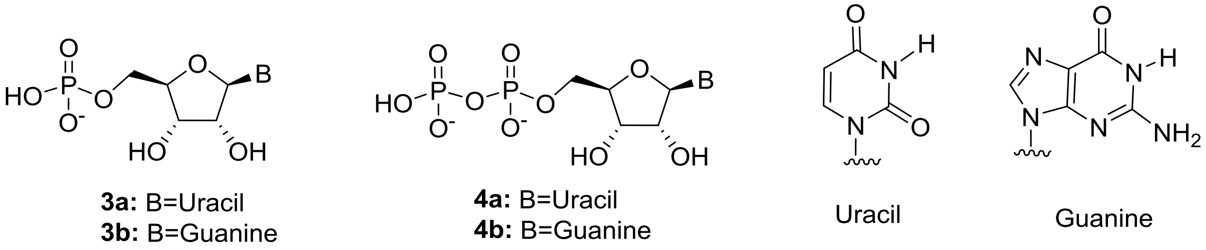
Nucleoside diphosphosugars **1a-c** were commercially available from SigmaAldrich and they were used as received. Substrates **1d** and **1e** were prepared as explained in detail in *Supplementary material*. Metal complexes **2a-c** were prepared by mixing appropriate amounts of the metal chloride salt and the ligand in aqueous solution. All reagents used in reaction solutions and background electrolyte (BGE) solutions were of analytical grade and solutions were prepared in purified water. The pH of reaction solutions was adjusted with HCl, NaOH or 50 mM MOPSO (3-morpholino-2-hydroxypropanesulphonic) buffer, pH 7.0. Ionic strength of reaction solutions was adjusted with NaCl to 0.1 M.

Kinetic reactions were carried out in Eppendorf tubes in a water bath, the temperature of which was adjusted to 50.0±0.1 °C. Reactions were followed by withdrawing 50 µl aliquots from reactions solutions (ideally, ten during two half-lives). Fast acid or base catalyzed reactions were quenched by adding concentrated BGE solution to bring the pH of samples to a nearly neutral value. With metal catalyzed reaction EDTA solution was used for quenching, if necessary. Samples were stored in an ice bath until analysis.

Samples were analysed at ambient temperature by capillary zone electrophoresis with UV-detection at 254 nM. Fused silica capillary of 57 cm effective length and 75 µm i.d. was employed in the analysis. Background electrolyte was 50 mM phosphate buffer, pH 7.0, and voltage of 30 kV was applied. Under these conditions migration times of the substrates were approximately ten minutes and those of products ten to fifteen minutes. Mole fraction of the starting material remaining was observed as a function of reaction time, and rate constants were calculated by applying the integrated first-order rate law.

Results and discussion

Metal complex promoted cleavage of **1a-e** was studied at pH 7.0. Cleavage in the absence of metal ion catalysts was studied under acidic, basic and neutral conditions in order to obtain information on the possible reaction routes and reactivity of different types of sugar nucleotides. Progress of the reactions were followed by observing the disappearance of starting materials and formation of UV active products, nucleoside monophosphates uridine monophosphate (**3a**; UMP) and guanosine monophosphate (**3b;** GMP) and corresponding diphosphates uridine diphosphate (**4a**, UDP) and guanosine diphosphate (**4b;** GDP). Rate constants for the disappearance of the starting material are collected in table 1.



3a: B=Uracil
3b: B=Guanine

4a: B=Uracil
4b: B=Guanine

Uracil

Guanine

Chart 3. Structures of nucleoside monophosphates and diphosphates formed as UV-active products in reactions of **1a-e**

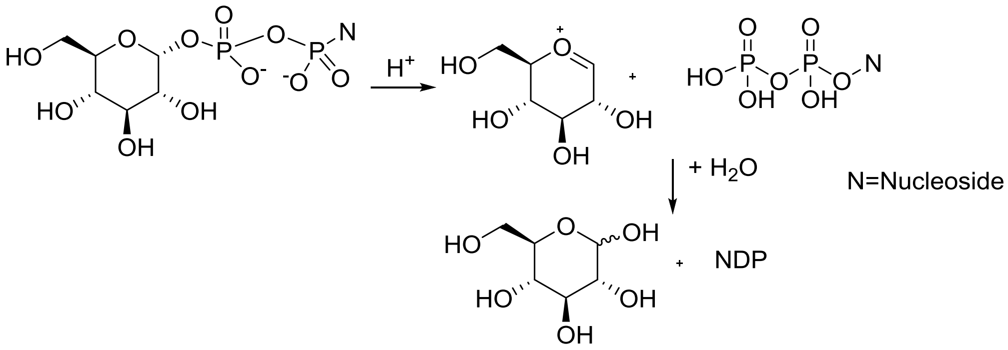
Table 1. Rate constants of the decomposition of nucleoside diphosphosugars at 50 °C in the presence and in the absence of metal ion catalysts. Metal ion catalyzed reactions have been studied in 50 mM MOPSO buffer at pH 7.0. UV-active products of the reactions are shown in brackets; major product is mentioned first.

Catalyst /Conditions	***k*(1a) $/10^{-5}s^{-1}$**	***k*(1b) $/10^{-5}s^{-1}$**	***k*(1c) $/10^{-5}s^{-1}$**	***k*(1d) $/10^{-5}s^{-1}$**	***k*(1e) $/10^{-5}s^{-1}$**	***k*(4b) $/10^{-5}s^{-1}$**
10 mM HCl	3.89±0.07 (UDP)	15.8±0.6 (UDP)	2.48±0.07 (GDP)	0.43±0.02[a] (UMP)	1.67±0.04[a] (UMP)	
10 mM NaOH	17.8±0.3 (UMP)	103±3 (UMP)	5.87±0.05 (GDP)	8.2±0.9 (UMP, UDP)	10±1 (UMP, UDP)	
pH 7.0 50 mM MOPSO	0.011±0.001 (UMP)	0.019±0.001 (UMP)	nd[b]	nd[b]	0.0029±0.0001 (UMP; UDP)	0.044±0.001
2 mM CuTerPy	2.4±0.2 (UMP)	4.2±0.2 (UMP)	0.047±0.002 (GMP,GDP)	0.83±0.04 (UMP)	2.08±0.05 (UMP)	
5 mM CuTerPy	12.5±0.2 (UMP)	23.9±0,5 (UMP)	0.130±0.004 (GMP,GDP)	4.3±0.1 (UMP)	10.4±0.2 (UMP)	0.024±0.004
2 mM CuBiPy	43±2 (UMP)	89±1 (UMP)	1.60±0.04 (GMP,GDP)	6.3±0.4 (UMP)	8.1±0.2 (UMP)	
5 mM CuBiPy	131±3 (UMP)	273±3 (UMP)	3.4±0.2 (GMP,GDP)	12.7±0.4 (UMP)	22±1 (UMP)	0.84±0.03
5 mM ZnTACD	11.3±0.2 (UMP)	20.8±0.6 (UMP)	0.040±0.002 (GDP, GMP)	0.57±0.02 (UMP)	0.79±0.02 (UMP)	0.030±0.002

[a] 0.1 M HCl, [b] No significant cleavage was observed in two weeks at 50 °C.

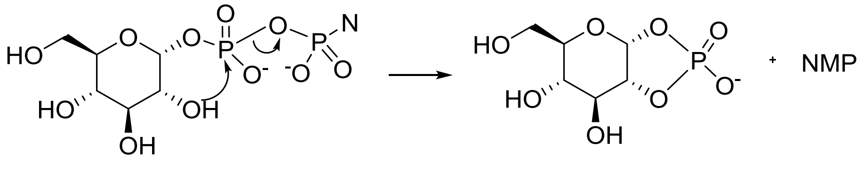
Reaction routes in the absence of metal ion catalysts. Substrates studied fall into two categories: UGlu, UGal and GMan are glycosylic substrates with the diphosphate moiety attached to

C1 of the pyranose ring. Under acidic conditions these substrates react similarly to glycosides: the aglycon is released and a water molecule attacks the oxocarbenium ion formed (*Scheme 1a*). Consistent with this, a nucleoside diphosphate is formed as the UV active product in 10 mM HCl.



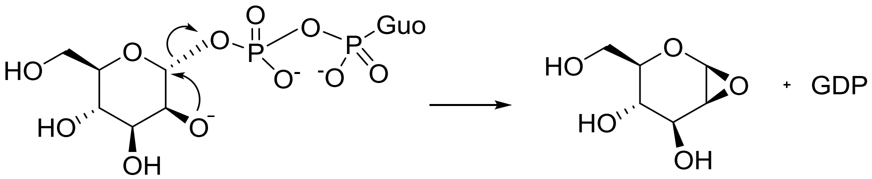
Scheme 1a: Hydrolysis of glycosylic substrates under acidic conditions. A nucleoside diphosphate NDP is formed as the UV-active product.

As the acid concentration decreases, transesterification, where a neighbouring HO -group attacks on the α-phosphorous (*Scheme 1b*), starts to compete with acid-catalysed hydrolysis, whenever a *cis*-orientation of nucleophile and phosphate is possible. This is the case with UGlu and UGal, and consistent with this, a nucleoside monophosphate product, UMP (**3a**) is observed as the sole UV active product under neutral and basic conditions. Under acidic conditions UMP could be formed also from UDP (**4a**) as a result of hydrolysis, but this is not a feasible reaction route under neutral conditions since the diphosphate hydrolysis is very slow (10).



Scheme 1b: Cleavage by intramolecular transesterification results in a formation of a 5-membered cyclic phosphate. A nucleoside monophosphate NMP is formed as the UV-active product

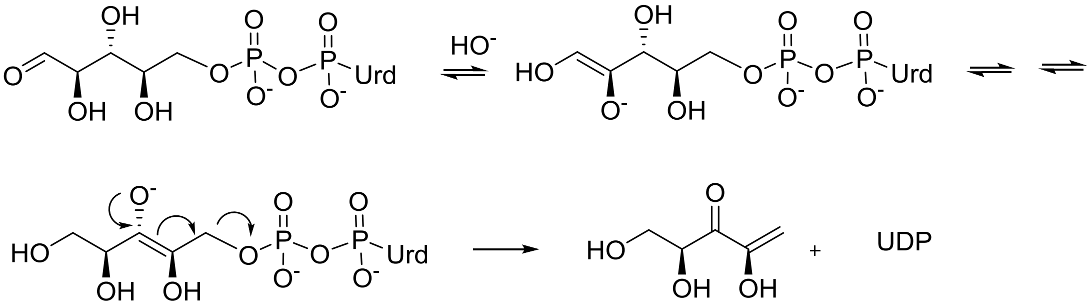
With GMan (**1c**) the situation is different, since the neighbouring HO-group is in *trans* – orientation. Acidic hydrolysis proceeds as shown in *Scheme 1a* and guanosine diphosphate (**4b**) is formed as the UV-active product. Transesterification is not, however, possible and no significant cleavage was observed under neutral conditions. Under alkaline conditions GMan is cleaved, but at a slower rate than UGlu or UGal. Nucleoside diphosphate **4b** is formed as the UV-active product. This reaction most probably proceeds through a nucleophilic substitution of the diphosphate moiety by the neighbouring HO-group at C1 (*Scheme 1c*). This reaction route has been previously reported with other types of mannose-1-phosphates and it involves a deprotonation of the nucleophilic HO-group (11). At pH 7 the proportion of nucleophilic 2-oxyanion is very low and no cleavage was observed in two weeks at 50 °C.



Scheme 1c: Base-catalysed cleavage of GMan produces a nucleoside diphosphate as the UV-active product

The other two substrates URib (**1d**) and UFru (**1e**) are reducing sugar compounds with a free hemiacetal group. Since the anomeric HO is unsubstituted, glycoside hydrolysis does not take place, but the substrates react through the transesterification route similar to that in *Scheme 1b* under acidic and neutral conditions. The reaction most probably proceeds through the acyclic form, where the 4-OH in ribose and 5-OH in fructose can adopt the required *cis*-orientation and their attack on α-phosphate produces a five-membered cyclic phosphate that is favoured as a product over six-membered rings in phosphoester reactions (2a). Transesterification is, however, slower than that of UGlu and UGal, which most probably results from an entropy penalty for a reaction involving a more flexible structure.

Under alkaline conditions the cleavage of URib and UFru produces UMP and UDP in approximately 3:1 ratio. We have previously speculated that a formation of a nucleoside diphosphate is to be attributed to keto-enol equilibria followed by β-elimination that results in the release of nucleoside diphosphate leaving group (*Scheme 1d*) (5). This kind of reaction sequence can be proposed also for UFru, although the reaction may be even more complicated in this case. The carbon backbone of fructose-6-phosphate has been proposed to be cleaved into two three carbon fragments under alkaline conditions prior to the phosphate elimination (12). The present information does not, however, allow any speculation about the reaction route which results in the elimination of the nucleoside diphosphate from UFru.



Scheme 1d: mechanism proposed previously (5) for the base-catalysed cleavage of URib. UDP is formed as the UV-active product

Metal ion promoted reactions. The results collected in Table 1 show that metal ion promoted cleavage produces a monophosphate as the sole UV-active product whenever a nucleophile in *cis*-orientation is available. This is consistent with a report by Nunez and Barker, who have observed that UGlu and UGal were cleaved by several metal aquo ions, whereas GMan was not (13). As hydrolysis of a corresponding nucleoside diphosphate is much slower than the cleavage of a nucleoside diphosphosugar, it can be concluded that monophosphate is formed from the intact substrate.

In case of GMan both a monophosphate **3b** and a diphosphate **4b** are observed as UV-active products. With CuBiPy and CuTerPy the formation of GMP predominates, whereas the slow cleavage in the presence of ZnTACD seems to favour **4b** as a product. Rate constants of the cleavage of the corresponding nucleoside diphosphate show that some of the monophosphate may be formed by the hydrolysis of a diphosphate. With Cu^{2+} complexes this is a minor pathway, but in the case of ZnTACD the rates of GMan cleavage and GDP hydrolysis are comparable. It would seem hence that metal ion complexes promote nucleophilic substitution both at the phosphate group and at C1, with Cu^{2+} complexes favouring the former reaction and ZnTACD promoting preferably the latter.

Catalytic activity of metal complexes varies depending on the substrate. CuBiPy is the most efficient of catalysts: an approximately 10000 –fold rate-enhancement is observed with UGlu, UGal and UFru. The uncatalysed cleavage of GMan is so slow that no significant cleavage was observed in two weeks. Assuming a detection limit of 2 % of substrate cleaved, it can be estimated that the rate constant of the uncatalysed reaction is less than $2\cdot10^{-8}$ s^{-1}. The rate constant of $3.4\cdot10^{-5}$ s^{-1} obtained in the presence of 5 mM CuBiPy represents, hence, a significant rate-enhancement. CuBiPy differs from the other two complexes in that that it promotes the hydrolysis of a nucleoside diphosphate, even though the rate-enhancement is modest.

CuTerPy is less efficient as catalyst than CuBiPy and the catalytic activity seems to depend more clearly on the structure of the substrate. 5 mM CuBiPy promotes the cleavage of GMan 26 times more efficiently than 5 mM CuTerPy. With Uglu and UGal the difference is approximately 10 fold, and with URib and UFru only two to three –fold. Another difference between CuBiPy and CuTerPy is the concentration dependence: rate constants obtained in the presence of CuBiPy show a first-order on dependence on the catalyst concentration, whereas a second-order dependence is observed with CuTerPy. The difference between the catalysts has been previously attributed to dimerization of the complexes (9a). Dimer is the catalytically active species, and while CuBiPy is fully dimerised under the experimental conditions, dimerization of CuTerPy is still incomplete.

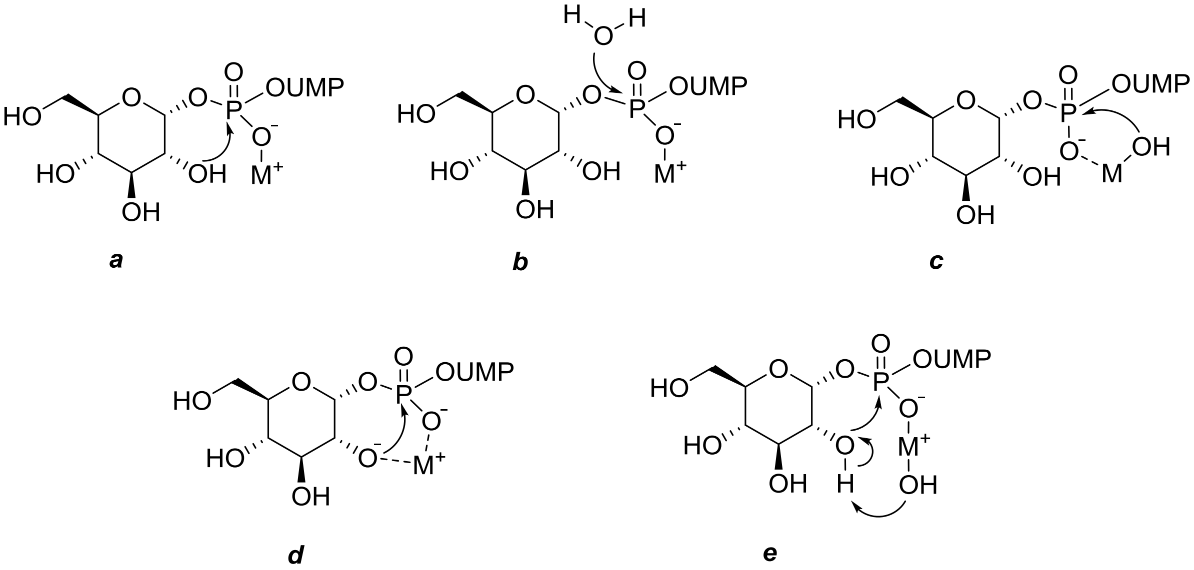
A mechanistically interesting observation is that while the cleavage of all other substrates is second-order in CuTerPy, a first-order dependence is observed with GMan as a substrate. This behavior was unexpected and the result was confirmed by determining the rate constant at four different concentrations (2.0, 5.0, 7.0 and 10.0 mM). The plot log *k* vs. log *c* gave a slope of 1.16±0.04 consistent with first-order dependence on CuTerPy. One might suggest that the first-order dependence is only apparent and results from significant contribution from the uncatalysed reaction. This is not, however, the case, but the catalytic activity even at the lowest concentration of 2 mM CuTerPy is at least 20 –fold.

Catalysis by ZnTACD shows the clearest dependence on the substrate structure. With UGlu and UGal 5 mM CuBiPy is approximately ten times more efficient as a catalyst than ZnTACD is. With GMan the difference is 85 –fold and with URib and UFru 22- and 28 –fold, respectively.

***Mechanism of the metal ion promoted reactions*.** Metal ion complexes can promote the cleavage of phosphoesters in a number of ways (8). A phosphate bound metal complex may act as an electrophile assisting the nucleophilic attack by an internal (*Scheme 2a*) or external nucleophile (*Scheme 2b*). Phosphate-bound metal ion catalyst can also provide nucleophilic catalysis by activating the attacking nucleophile by direct coordination. In the present case there are two possibilities: the nucleophile may be a metal-bound hydroxo or water ligand (*Scheme 2c*), or a sugar HO-group (*Scheme 2d*). Metal-bound hydroxo ligand may also act as a general base that deprotonates the attacking nucleophile (*Scheme 2e*). General acid catalysis for the departure of the leaving group by an aquo ligand is also possible, but in this case it is improbable, because nucleoside mono- and diphosphates are good leaving groups and protonation is not necessary.

Earlier studies with RNA models have shown that with Zn^{2+} complexes the catalytic activity correlates with a pK_a –value of a metal-bound aquo ligand, and general acid-base –catalysis has been proposed as the most probable catalytic strategy employed (8). The correlation is not extended to Cu^{2+} complexes, but CuTerPy is more active a catalyst than could be expected on the basis of its acidity. With RNA models CuTerPy is nearly ten times more efficient a catalyst than CuBiPy is. The difference has been attributed to dimerization of CuBiPy; a dimer is inefficient as a catalyst for the cleavage of RNA model compounds.

More information on the catalysts can be obtained by studying the results obtained with diadenosine oligonucleotides. With diadenosine triphosphate ApppA (**6a**) as a substrate the situation is quite the opposite: 2 mM CuTerPy and CuBiPy are 20 and 300 times more efficient catalysts than ZnTACD at pH 7.0 and at 60 °C (9a). As no intramolecular nucleophile is available, metal ion catalysts enhance either a nucleohilic attack of an external nucleophile as in *Scheme 2b* or provide the attacking nucleophile as in *Scheme 2c*. Factors that influence on the catalytic activity are the strength of binding and Lewis acidity, which are more pronounced if the complex is dimeric, and a coordination geometry that would allow the nucleophilic catalysis depicted in *Scheme 2c.*



Scheme 2: Possible catalysis mechanisms for metal ion catalysis in reactions of biological phosphates. **a**. Electrophilic catalysis of an intramolecular nucleophilic attack; **b.** Electrophilic catalysis of an external nucleophilic attack; **c.** Electrophilic and nucleophilic catalysis on a nucleophilic attack by external nucleophile; **d**.Electrophilic and nucleophilic catalysis on a nucleophilic attack by internal nucleophile; **e.** Electrophilic and general base catalysis on a nucleophilic attack by internal nucleophile

The results obtained in the present work show that both Zn^{2+} and Cu^{2+} complexes possess properties that facilitate the cleavage of nucleoside diphosphosugars. The importance of binding/coordination properties Cu^{2+} complexes is shown by that, that similarly to the situation with dinucleoside oligophosphates, CuBiPy is a better catalyst than CuTerPy and ZnTACD. Comparison between the present results and those obtained previously (9a) with dinucleoside oligophosphates shows, however, that UGlu and UGal are more reactive in the presence of metal ion catalysts than **6a** is**,** indicating that the intramolecular HO group is involved in the reaction. This shows that the metal ion catalyst acts either as a mere electrophile as in *Scheme 2a*, or as a bifunctional catalyst providing either nucleophilic (*Scheme 2d*) or general base catalysis (*Scheme 2e*)

The importance of properties typical for Zn complexes is shown by a rough estimation of catalytic activities (*k*catalysed/*k*uncatalysed) (9a,14) which shows that only the catalytic activity of ZnTACD significantly increases when an intramolecular nucleophile is introduced in the reaction system. With CuTerPy, and particularly with CuBiPy, the increase is more modest. This suggests that while an interaction between the catalyst and the attacking HO-group is essential for the catalysis by ZnTACD, it is less significant with CuTerPy and CuBiPy. We have previously (8) proposed that ZnTACD can act as a general base deprotonating the attacking nucleophile as in *Scheme 2e*, and similar catalysis mechanism can be proposed here, as well.

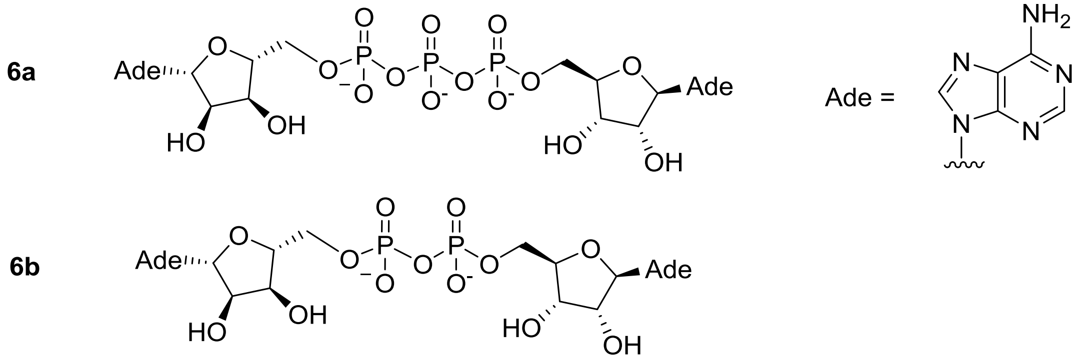


Chart 4. Structures of diadenosine oligophosphates studied previously (13)

Results obtained with GMan are mechanistically interesting. The formation of GMP suggests that a nucleophilic attack on the phosphate takes place. The nucleophile in this case is either a metal bound aquo/hydroxo ligand or a water molecule as in *Scheme 2b* or *2c*. Formation of GDP shows that also the nucleophilic substitution at C1 is enhanced.

Another interesting observation is that the reaction of GMan is only first-order in CuTerPy while a second-order dependence is observed with other substrates. A corresponding phenomenon has been reported before with diadenosine oligophosphates as substrates (9a), where the reaction order depends on the size of the substrate. While the cleavage of a triphosphate bridge of triphosphate **6a** shows a clear second-order dependence on CuTerPy and CuTACD, the cleavage of diadenosine diphosphate **6b** is less strongly dependent on catalyst concentration. These observations suggest that the reaction order reflects how the substrate interacts with the dimeric catalyst. While the triphosphate bridge can interact with two metal centers, with a diphosphate substrate only one interaction is possible.

With nucleoside diphospho sugars this could mean that the CuTerPy promoted cleavage of GMan producing GMP involves an electrophilic catalysis by one Cu^{2+} center. The catalyst may even be a dimer, but if the second Cu^{2+} is not involved in the interactions that facilitate the cleavage, only a first-order dependence on CuTerPy is observed. In contrast to GMan, a second-order dependence on CuTerPy is observed with other substrates suggesting two interactions between the catalyst and substrate. As the other interaction favours the reaction, it is logical to assume that the attacking nucleophile is either directly or indirectly in contact with the catalyst as in *Scheme 2d* or *2e*.

In the presence of ZnTACD the cleavage of GMan produces GDP as the predominant product showing that TACD preferably enhances the substitution at C1 rather than the intermolecular nucleophilic attack on the phosphate. As mentioned above, we have previously proposed that ZnTACD can act as a general base that deprotonates the attacking OH nucleophile, and this mechanism is possible here, as well. While nucleophilic catalysis is kinetically indistinguishable, it is clear that electrophilic catalysis only or nucleophilic attack by the Zn –coordinated aquo ligand are not feasible.

Comparison to biological reactions of nucleoside diphosphosugars. The results discussed above show clearly that the role of metal ion catalysts in chemical systems is different from that in enzymatic reactions of nucleoside diphosphosugars. As discussed in the introduction, glycosyl transfer catalyzed by glycosyl transferases involves a nucleophilic substitution at C1, and the role of a metal ion cofactor, most commonly Mn^{2+} or Mg^{2+}, is to stabilize the departing diphosphate (6). Interestingly, the results by Nunez and Barker show that in a chemical system these metal ions act quite differently: while Mn^{2+} aquo ion promotes the cleavage of UGal, Mg^{2+} is inefficient as a catalyst (13). Furthermore, Mn^{2+} and other metal aquo ions studied enhance the cleavage by transesterification, *i.e.* a nucleophilic attack on phosphate, not on C1. Different reaction routes and different roles of metal ion catalysts are most likely to be attributed to the efficiency of nucleophilic attack in enzymatic reactions. Because of optimal position of a suitable nucleophile, the reaction is fast, and in the absence of leaving group stabilization, its departure would become the rate-determining step of the reaction. In chemical reactions the nucleophilic attack is less efficient, and therefore stabilization of the leaving group is not necessary, but metal ion catalysts that can enhance the nucleophilic attack are the most efficient catalysts.

Hydrolysis by nudix hydrolases is closer to the reactions studied in the present work, and it is interesting to note that the regiospesifity is similar, even though metal ions involved, and most likely also their roles, are different. As mentioned in the Introduction, Nudix hydrolases typically promote a nucleophilic attack on the phosphate, even with glycosylic substrates, and two monophosphate products are formed (7). The exception of the rule is GMan (**1c**) that is hydrolysed through a nucleophilic attack on C1. The same difference was observed in the present work in the presence of Zn^{2+} and Cu^{2+} complexes, as well as under alkaline conditions. As the demand for a *cis*-oriented neighbouring HO-nucleophile is the same, this suggests that the interactions to this group are important also in the hydrolase –catalysed reaction.

Conclusions. Even though different reaction routes are available, as shown by the reactions under acidic and basic conditions, metal ion catalysts studied in the present work promote the cleavage of nucleoside diphosphosugars through the transesterification route whenever the neighbouring HO-group can adopt a *cis* orientation with respect the phosphate group. Intramolecular nucleophilic (*Scheme 2d*) or general base catalysis (*Scheme 2e*) by the metal ion catalysts are the most probable catalysis mechanisms, but different complexes may prefer different mechanisms. The structure of the substrate and the catalyst apparently affect the efficiency of the catalyzed reaction and different mechanisms may be employed by different catalysts. In case *cis* –oriented HO-group is not available, Cu complexes electrophilically promote the cleavage at the phosphate. Nucleophile in this case is either a water molecule (*Scheme 2b*) or an aquo ligand attached to the metal ion (*Scheme 2c*). All metal ion catalysts also enhance the nucleophilic substitution at C1 of mannose in GDP, but this reaction is slow. Catalysis, in this case, most probably enhances the deprotonation of 2-OH group.

When the results obtained in the present work are contrasted to the known specificity of enzymatic reactions of nucleoside diphosphosugars, it is seen that results obtained with chemical models can only be extended to biological systems with caution. The role of the metal ions, and hence also the required properties, can be completely different, because enzymes contain a number of other functional groups that can be involved in the catalysis. However, the main aim of the present work was to study the cleavage of nucleoside diphosphosugars as a model for chemical cleavage of phosphodiester bonds in carbohydrates, and considering this, the results are promising. It would seem that metal ion catalysts may offer a method to efficiently cleave phosphodiester bonds in carbohydrates under mild conditions. Even then, one has to keep in mind that the reactions of phosphodiester bonds under neutral conditions involve almost always a poor leaving group, whereas nucleoside monophosphates and nucleoside diphosphates in the present work are good leaving groups. Reactivity and selectivity may hence be different in carbohydrate phosphodiesters.

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