Electronic Supplementary Material (ESI) for RSC Advances

Mechanistic comparison of saccharide depolymerization catalyzed by dicarboxylic acids and glycosidases

Henning Kayser[a], Francisco Rodríguez-Ropero[b], Walter Leitner[a,c], Marco Fioroni[d]*,
Pablo Domínguez de María[a]*

SUPPLEMENTARY INFORMATION

Scheme S1. Acid-catalyzed mechanisms for the depolymerization of polysaccharides to release oligomers and sugars.

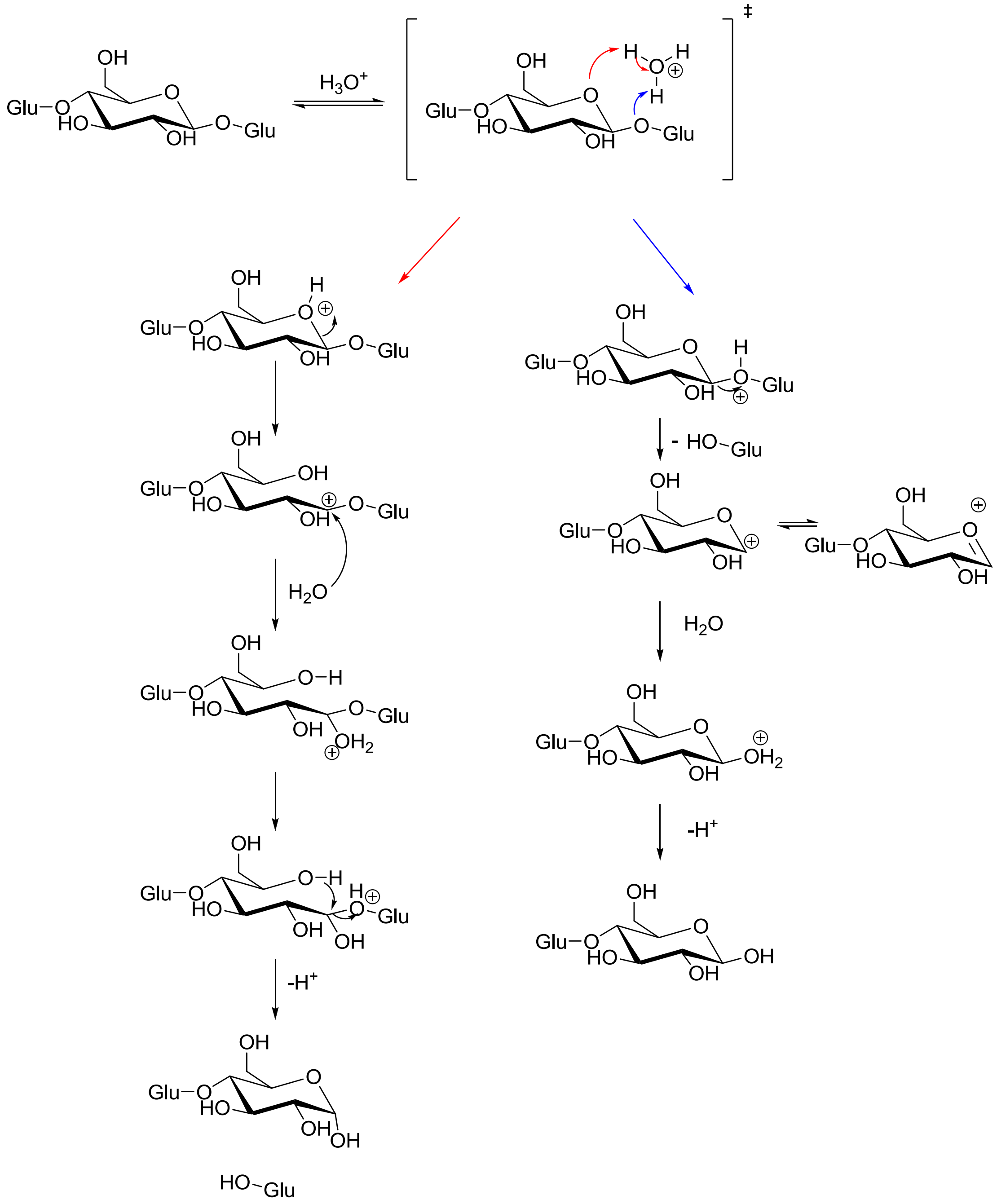


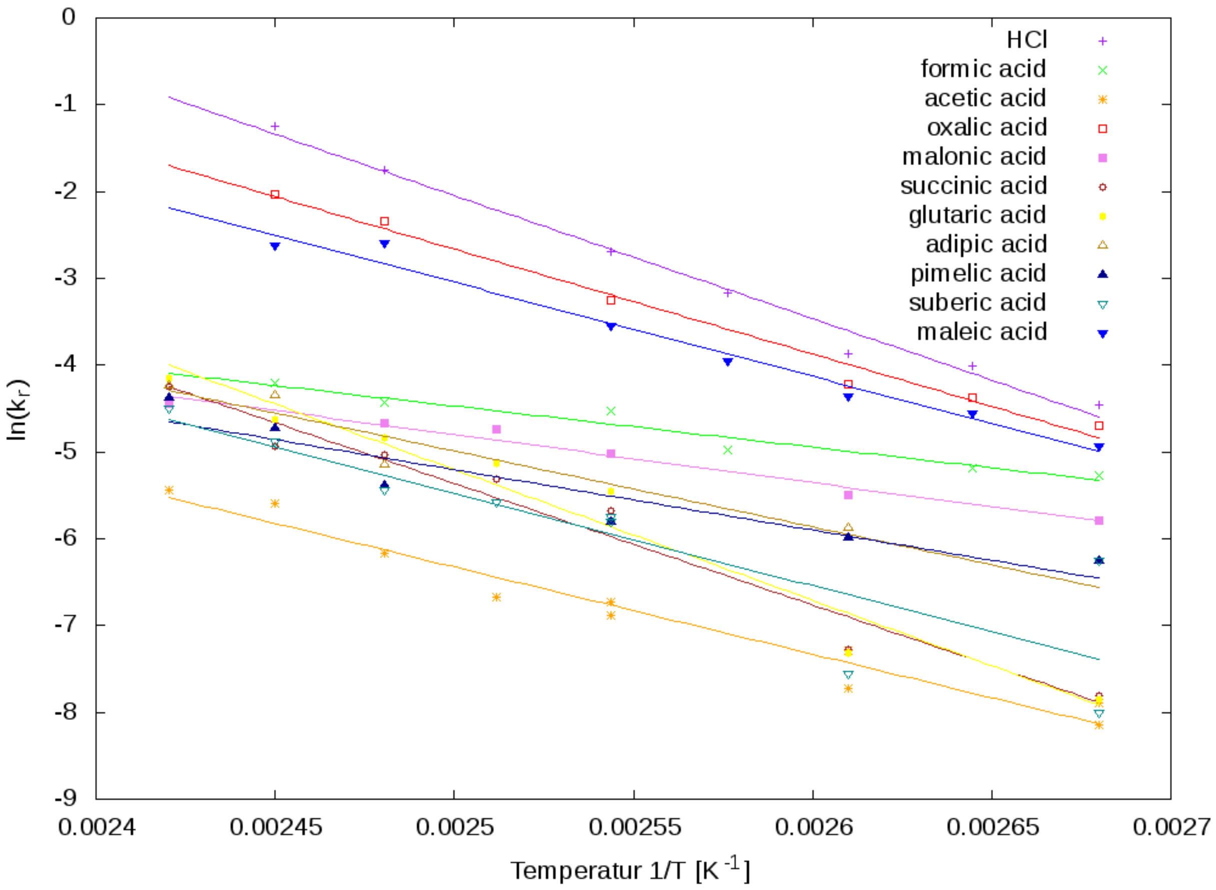
Table S1. Assessment of the acid-mediated degradation of glucose to 5-hydroxymethylfurfural (HMF). Conditions: Acids at different pH; Cellobiose 50 gL^{-1}; 125 °C. Analytics: HPLC. Jasco HPLC equipped with a SugarSH1011 (8mm * 300 mm) column (0.01% aq. acetic acid as eluent) and an UV/Vis- as well as a RI-detector.

Acid	**pH**	**[Cellobiose] gL^{-1}**	**Reaction time (h)**	**HMF (%)**
HCl	1.56	50	0.5	0.03
HCl	1.56	50	2	0.60
HCl	1.44	50	1	0.40
HCl	2.01	50	2	0.12
Maleic	1.88	50	2	0.25
Maleic	1.88	50	22	3.70
Fumaric	2.63	50	3	0.60
Oxalic	1.30	50	3	1.40
Oxalic	1.70	50	1	0.27
Formic	2.67	50	1	0.16

Table S2. Activation energies of cellobiose hydrolysis with different carboxylic acids, based on exponential Arrhenius plot, $k_1 (T) = A\ e^{-E‡/RT}$. Pre-exponential factor (A) was fixed at $A = 1\cdot 10^{11}$ considering the $\Delta S^{‡}$ to be analogous in such homogeneous systems.

Acid	**$E^{‡}$ (kJ mol^{-1})**
HCl	99 ± 3
Formic	98 ± 14
Acetic	100 ± 8
Oxalic	99 ± 6
Malonic	103 ± 12
Succinic	99 ± 15
Glutaric	97 ± 9
Adipic	97 ± 37
Pimelic	97 ± 20
Suberic	98 ± 12
Maleic	99 ± 16

Figure S1.


0
-1
-2
-3
-4
-5
-6
-7
-8
-9
ln(k_r)
0.0024
0.00245
0.0025
0.00255
0.0026
0.00265
0.0027
Temperatur 1/T [K^-1]
HCl
formic acid
acetic acid
oxalic acid
malonic acid
succinic acid
glutaric acid
adipic acid
pimelic acid
suberic acid
maleic acid

DETERMINATION OF pK_a AT DIFFFERENT DIELECTRIC CONSTANTS.

To estimate the Glu pK_a shift (ΔpK_a) at different dielectric constants (ε), a simple acetic acid molecule was used (acetic acid: $pK_a = 4.74$, Glu: $pK_a = 4.25$). The expression reported by Shields *et al.*[1] allows the determination of pK_a of a BH acid from a known pK_a acid of an AH acid:

[illegible]

Where G_{gas} and G_{sol} are respectively the free energy values in the gas and solvated phases, R is the constant for ideal gases and T is the temperature. If AH is acetic acid in water ($\varepsilon = 78.39$), with a known pK_a (4.74), and BH is the same acetic acid but at different dielectric medium, since both AH and BH are the same species (acetic acid), terms related to the free energy in the gas phase may be suppressed ($G_{gas}(B^-) = G_{gas}(A^-)$ and $G_{gas}(BH) = G_{gas}(AH)$). Consequently the pK_a of acetic acid at different dielectric media (pK_a (BH) can be obtained from:

$$pK_a\,(BH) = pK_a\,(AH) + [\Delta G_{sol}(B^-) - \Delta G_{sol}(A^-) - \Delta G_{sol}(BH) + \Delta G_{sol}(AH)] / 2.303\ RT$$

To assess the effect of the polarity of the local aqueous environment more quantitatively, acetic acid was used as model for Quantum Mechanical calculations of solvation energies. The variation of the pK_a resulting from the change in the dielectric constant of the medium was estimated using Eq. 1, in analogy to the method of Shields *et al.*[1] for the prediction of pK_a values. The expression provides the pK_a

calculation of a given acid as a function of media, using tabulated pK_a values in water. (Eq. 1).

$$pK_a^{\varepsilon} = pK_a^{water} + [\Delta G_{sol}^{\varepsilon}\,(\text{carboxylate}) - \Delta G_{sol}^{water}(\text{carboxylate}) - \Delta G_{sol}^{\varepsilon}\,(\text{acid}) + \Delta G_{sol}^{water}\,(\text{acid})] / 2.303\,RT \qquad \textbf{Eq. 1}$$

The main advantage of Eq. 1 with respect to other previously proposed methodologies for pK_a estimation of weak acids, is that the value of the proton free energy is no longer required. Thus inaccuracies in values of such proton-related magnitudes – which must be estimated from experimental data, and not from QM calculations –, do not lead to any influence in the final result. Figure S2 depicts the increase of pK_a (starting from pK_a value of acetic acid in aqueous solution, 4.74). As observed, for acetic acid the difference ΔpK_a significantly increases when ε decreases.

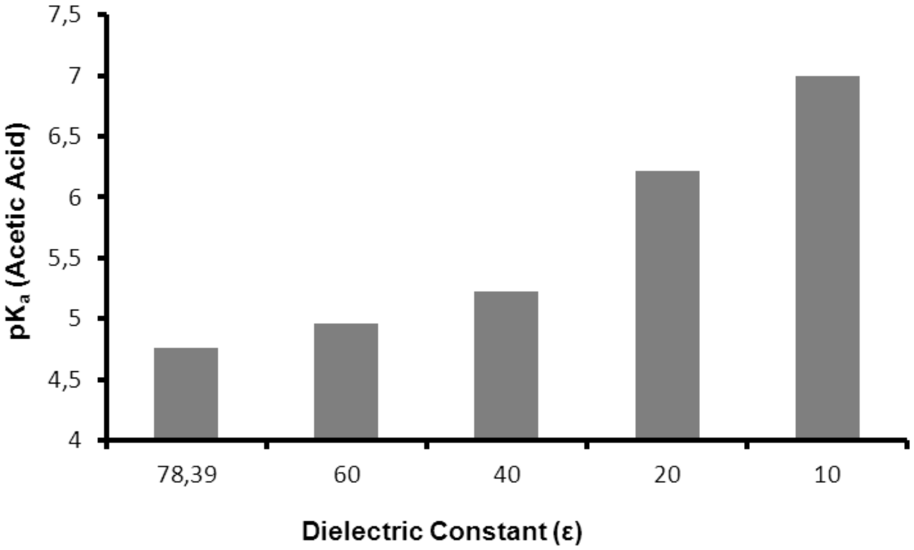


Figure S2. pK_a values for acetic acid at different dielectric constants (ε), retrieved by means of Quantum Mechanical (QM) calculations and Equation 1.

OTHER ASSESSMENTS

Geometry of each trasition state (TS) is depicted in Figure S3. As observed, for the concerted mechanism the distance between the proton in Glu162 and the glycosidic oxygen is 2.14 Å, different from the observed in the acidic catalysis (1.36 Å).

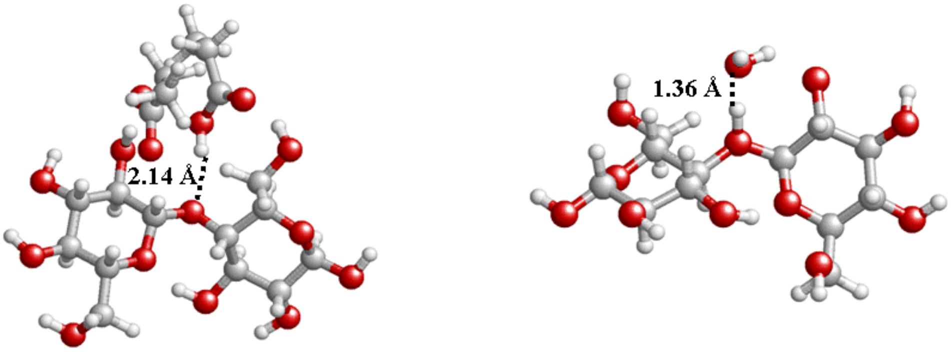


Figure S3. Transition state (TS) geometries for the cooperative mechanism (left) and acidic attack (right).

The role of the dielectric constant (ε) in the TS was studied, providing insights on which of the possible reaction paths would be followed by the reactants. The ΔΔG of the two TS were calculated at different dielectric constant values (ε = 80 to 1, with ε = 80 as reference). In both cases energies increased at decreasing ε values (see Table S2). At ε = 20 the ΔΔG for the concerted mechanism increased 2.70 kcal mol^{-1} whereas the acidic attack did 4.56 kcal mol^{-1}.

Table S3. ΔΔG calculated considering the reference TS in water (ε=80) for each of the acidic and concerted mechanism, comparing the trend for both the acidic and concerted TS, energies dramatically increase at low ε values.

ε	**ΔΔG (TS) (kcal mol^{-1}) Acid Catalysis**	**ΔΔG (TS) (kcal mol^{-1}) Concerted mechanism**
80	0.00	0.00
60	0.68	0.31
40	1.53	0.90
20	4.56	2.70
6	17.93	10.91
1	85.86	36.12

CALCULATED DISTANCES BETWEEN CARBOXYLIC ACIDS

- H-H distance is the distance between the two H atoms in the carboxylic groups C-OO-H ··· H-OO-C within the diacid
- C-C distance is the distance between the two C atoms in the carboxylic groups H-OO-C ··· C-OO-H
- H-O distance is the shortest distance between one H atom in one carboxylic group and the non-protonated O atom in the other C-OO-H ··· O=C
- O-O distance is the distance between the two non-protonated O atoms C=O ··· O=C
- The energy is included to indicate which one of the two possible minima is the most stable.
- All calculations were done at the B3LYP/6-31G(d) level of calculation.

Table S4. Diprotonated diacids.

Compound	H-H (Å)	C-C (Å)	H-O (Å)	O-O (Å)	ΔE (kcal/mol)
Oxalic (“cis”)	4.51	1.53	4.24	2.81	4.21
Oxalic (“trans”)	3.84	1.54	2.09	3.47	0.00
Malonic (“cis”)	6.08	2.50	4.43	3.24	1.78
Malonic (“trans”)	3.94	2.60	1.78	4.16	0.00
Succinic (“cis”)	6.89	3.87	5.80	5.22	0.00
Succinic (“trans”)	6.70	3.86	4.81	5.02	4.35
Glutaric (“cis”)	7.18	5.08	7.00	7.03	0.00
Glutaric (“trans”)	7.61	5.13	5.95	6.51	5.69
Adipic (“cis”)	8.36	6.40	8.09	7.54	0.00
Adipic (“trans”)	7.60	6.46	7.30	8.33	1.99
Pimelic (“cis”)	9.50	7.69	9.43	9.64	0.00
Pimelic (“trans”)	10.04	7.63	8.68	8.60	4.43
Suberic (“cis”)	10.92	8.95	10.48	10.00	0.00
Suberic (“trans”)	10.12	8.97	9.39	10.75	1.22
Maleic (cis)	2.52	3.45	3.77	5.79	5.69
Maleic (trans)	3.90	3.30	1.67	4.46	0.00
Fumaric (cis)	7.44	3.82	5.96	5.12	0.00
Fumaric (trans)	6.82	3.84	5.96	5.11	0.62

Table S5. Monoprotonated diacids.

Compound	C-C (Å)	H-O (Å)	O-O (Å)
Oxalic	1.58	1.65	2.92
Malonic	2.59	3.68	3.36
Succinic	3.37	1.36	4.34
Glutaric	5.14	3.88	6.28
Adipic	6.51	6.26	7.54
Pimelic	7.75	6.62	8.83
Suberic	9.01	10.25	9.92
Maleic	3.21	5.51	3.59
Fumaric	3.89	5.96	5.10
E162-E282	5.01	4.20	4.62

Table S6. Deprotonated diacids.

Compound	C-C (Å)	O-O (Å)
Oxalic	1.62	2.78
Malonic	2.70	3.84
Succinic	4.05	4.82
Glutaric	5.35	5.33
Adipic	6.64	7.24
Pimelic	7.87	7.94
Suberic	9.17	9.55
Maleic	2.97	1.53
Fumaric	4.02	5.12

Reference

1 M.D. Liptak, G.C. Shields, *Int. J. Quatum Chem*. 2001, **85**, 727-741.