Mechanistic Pathway on Human α-Glucosidase Maltase-Glucoamylase Unveiled by QM/MM Calculations

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Supporting Information

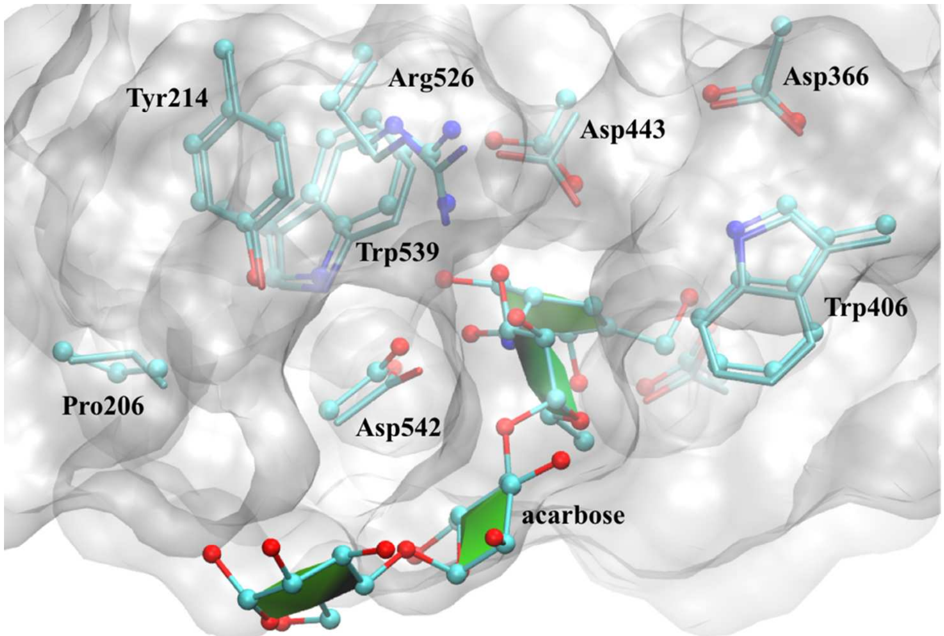

Figure S1. Superimposition of some active site residues of the NtMGAM (depicted in sticks) and NtMGAM:acarbose (shown as ball-and-sticks) crystallographic structures.

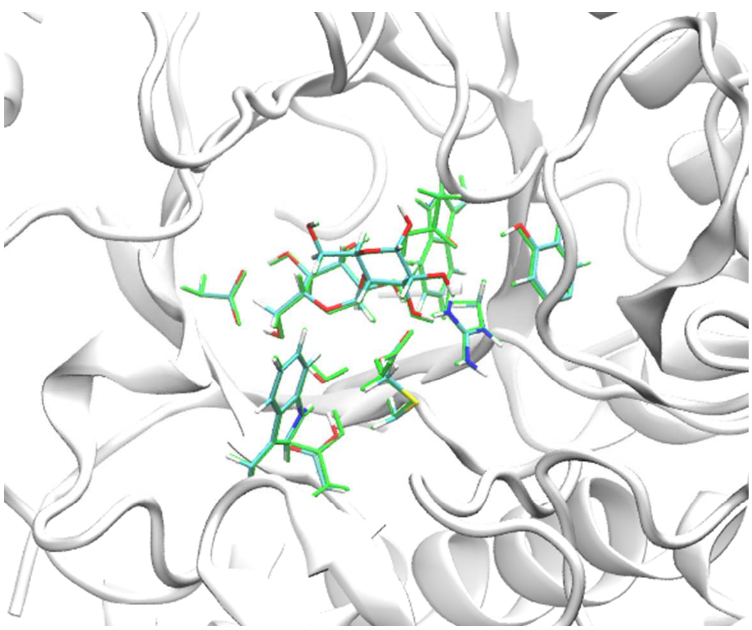

Figure S2. Superimposition of the reactant state geometries optimized with B3LYP with and without the D3 dispersion correction. The QM region optimized with B3LYP and B3LYP-D3 approaches are colored at element type and green, respectively.

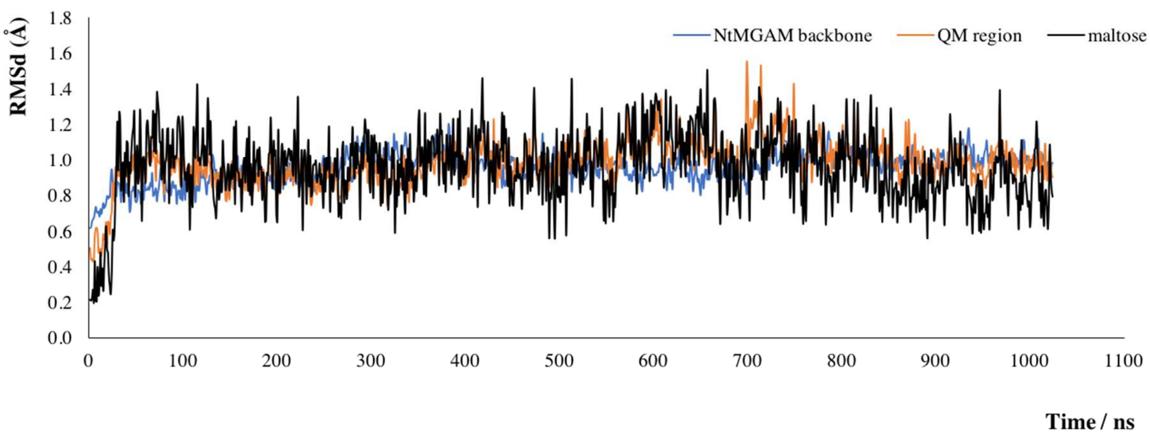

Figure S3. Representation of the RMSd values obtained for the enzyme backbone atoms (blue), the QM region (orange) and the maltose molecule (black) during the MD simulation of NtMGAM:maltose complex.

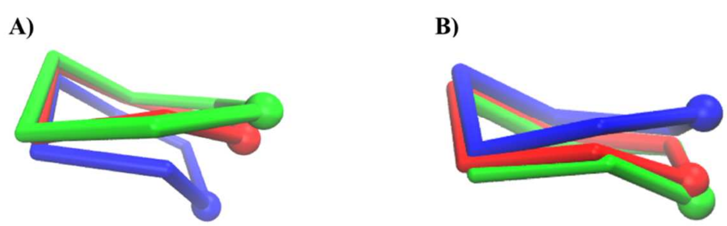

Figure S4. Superimposition of the gluc-1 ring conformations in the three stationary points of the first (a) and second (b) mechanistic step of the human NtMGAM reaction. The reactants, TS and products are colored at blue, red, and green, respectively. The C1 atom is shown as a sphere.

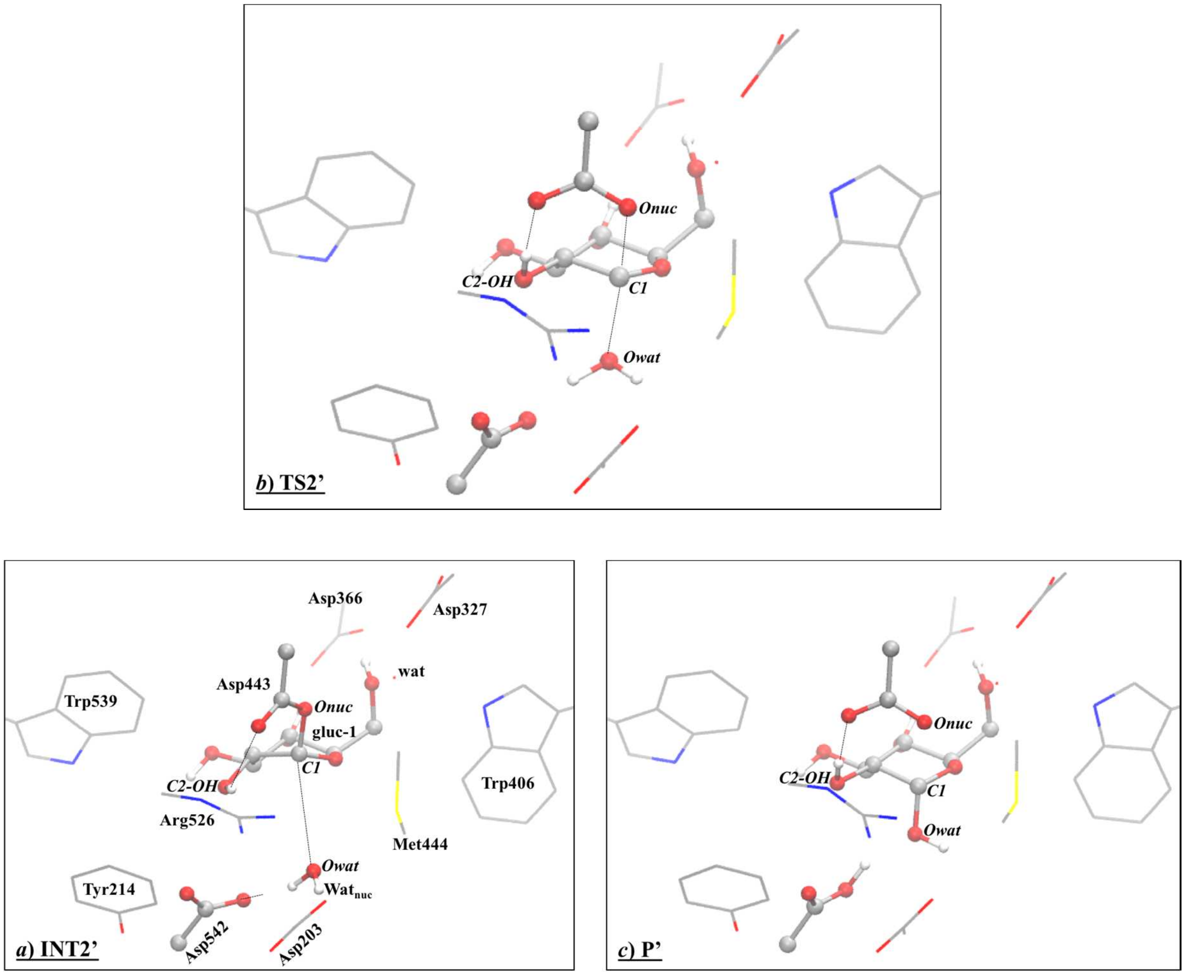

Figure S5. Representation of the reactants (INT2'), transition state (TS2') and products (P') structures of the alternative deglycosylation step, emphasizing the main interactions established. All atoms are depicted in balls-and-sticks.

Table S1. List of the atoms included in the high-layer of the QM/MM calculations.

atom number	element	atom type	residue	atom number	element	atom type	residue
1	C	CG	Tyr214	72	C	CD1	Trp539
2	C	CD1	Tyr214	73	C	CD2	Trp539
3	C	CD2	Tyr214	74	C	CE2	Trp539
4	C	CE1	Tyr214	75	C	CE3	Trp539
5	C	CE2	Tyr214	76	N	NE1	Trp539

6	C	CZ	Tyr214	77	C	CZ2	Trp539
7	O	OH	Tyr214	78	C	CZ3	Trp539
8	H	HD1	Tyr214	79	C	CH2	Trp539
9	H	HD2	Tyr214	80	H	HB2	Trp539
10	H	HE1	Tyr214	81	H	HB3	Trp539
11	H	HE2	Tyr214	82	H	HD1	Trp539
12	H	HH	Tyr214	83	H	HE1	Trp539
13	C	CB	Asp327	84	H	HE3	Trp539
14	C	CG	Asp327	85	H	HZ2	Trp539
15	O	OD1	Asp327	86	H	HZ3	Trp539
16	O	OD2	Asp327	87	H	HH2	Trp539
17	H	HB2	Asp327	88	C	CB	Asp542
18	H	HB3	Asp327	89	C	CG	Asp542
19	C	CB	Asp327	90	O	OD1	Asp542
20	C	CG	Asp327	91	O	OD2	Asp542
21	O	OD1	Asp366	92	H	HB2	Asp542
22	O	OD2	Asp366	93	H	HB3	Asp542
23	H	HB2	Asp366	94	H	HD2	Asp542
24	H	HB3	Asp366	95	O	O1	Maltose (gluc-2)
25	H	HD2	Asp366	96	H	HO1	Maltose (gluc-2)
26	C	CB	Trp406	97	C	C1	Maltose (gluc-2)
27	C	CG	Trp406	98	C	C2	Maltose (gluc-2)
28	C	CD1	Trp406	99	O	O2	Maltose (gluc-2)
29	C	CD2	Trp406	100	C	C3	Maltose (gluc-2)
30	C	CE2	Trp406	101	O	O3	Maltose (gluc-2)
31	C	CE3	Trp406	102	C	C4	Maltose (gluc-2)
32	N	NE1	Trp406	103	O	O4	Maltose (gluc-2)
33	C	CZ2	Trp406	104	C	C5	Maltose (gluc-2)
34	C	CZ3	Trp406	105	O	O5	Maltose (gluc-2)
35	C	CH2	Trp406	106	C	C6	Maltose (gluc-2)
36	H	HB2	Trp406	107	O	O6	Maltose (gluc-2)
37	H	HB3	Trp406	108	H	H1	Maltose (gluc-2)
38	H	HD1	Trp406	109	H	H2	Maltose (gluc-2)
39	H	HE1	Trp406	110	H	H3	Maltose (gluc-2)
40	H	HE3	Trp406	111	H	H4	Maltose (gluc-2)
41	H	HZ2	Trp406	112	H	H5	Maltose (gluc-2)
42	H	HZ3	Trp406	113	H	H2O	Maltose (gluc-2)
43	H	HH2	Trp406	114	H	H3O	Maltose (gluc-2)
44	C	CB	Asp443	115	H	H61	Maltose (gluc-2)

45	C	CG	Asp443	116	H	H62	Maltose (gluc-2)
46	O	OD1	Asp443	117	H	H6O	Maltose (gluc-2)
47	O	OD2	Asp443	118	C	C1	Maltose (gluc-1)
48	H	HB2	Asp443	119	C	C2	Maltose (gluc-1)
49	H	HB3	Asp443	120	O	O2	Maltose (gluc-1)
50	C	CG	Met444	121	C	C3	Maltose (gluc-1)
51	S	SD	Met444	122	O	O3	Maltose (gluc-1)
52	C	CE	Met444	123	C	C4	Maltose (gluc-1)
53	H	HG2	Met444	124	O	O4	Maltose (gluc-1)
54	H	HG3	Met444	125	C	C5	Maltose (gluc-1)
55	H	HE1	Met444	126	O	O5	Maltose (gluc-1)
56	H	HE2	Met444	127	C	C6	Maltose (gluc-1)
57	H	HE3	Met444	128	O	O6	Maltose (gluc-1)
58	C	CD	Met444	129	H	H1	Maltose (gluc-1)
59	N	NE	Met444	130	H	H2	Maltose (gluc-1)
60	C	CZ	Arg526	131	H	H3	Maltose (gluc-1)
61	N	NH1	Arg526	132	H	H4	Maltose (gluc-1)
62	N	NH2	Arg526	133	H	H5	Maltose (gluc-1)
63	H	HD2	Arg526	134	H	H2O	Maltose (gluc-1)
64	H	HD3	Arg526	135	H	H3O	Maltose (gluc-1)
65	H	HE	Arg526	136	H	H61	Maltose (gluc-1)
66	H	HH11	Arg526	137	H	H62	Maltose (gluc-1)
67	H	HH12	Arg526	138	H	H4O	Maltose (gluc-1)
68	H	HH21	Arg526	139	H	H6O	Maltose (gluc-1)
69	H	HH22	Arg526	140	O	O	Water
70	C	CB	Trp539	141	H	H1	Water
71	C	CG	Trp539	142	H	H2	Water

Table S2. Hydrogen bonds between the NtMGAM and the substrate during the MD simulation.

NtMGAM residue	maltose	% of occurrence	average distance / Å
Asp542	gluc-1 / C2-OH	90.4	2.73
Asp203	gluc-2 / C2-OH	61.6	2.74
Arg526	gluc-2 / C3-OH	58.2	2.84
Asp327	gluc-1 / C4-OH	50.7	2.72
Asp327	gluc-1 / C6-OH	47.5	2.69

Asp443	gluc-1 / C2-OH	46.7	2.77
Asp203	gluc-2 / C2-OH	43.4	2.77
His600	gluc-1 / C3-OH	42.6	2.88
Asp203	gluc-2 / C3-OH	39.4	2.76
His600	gluc-1 / C4-OH	16.5	2.89
Asp542	gluc-2 / C3-OH	8.9	2.81
Asp542	gluc-2 / C4-OH	2.4	2.83
Arg526	gluc-1 / C2-OH	2.4	2.91
Asp443	gluc-1 / C6-OH	1.9	2.86
Gln603	gluc-2 / C6-OH	1.0	2.72
Tyr299	gluc-2 / C6-OH	0.5	2.74

Table S3. Activation and reaction free energies attained with six different density functionals, obtained with the combined free-rotor/harmonic oscillator approximation for a threshold vibrational temperature of 120 K (~100 cm^{-1}, as proposed by Grimme et al.) for the NtMGAM catalytic mechanism. All values are in $kcal.mol^{-1}$.

density functional	**TS1**	**INT**	**TS2**	**P**
B1B95	18.0	-7.2	14.7	-2.2
MPW1B95	19.2	-6.5	14.9	-2.4
CAM-B3LYP	19.3	-4.9	13.9	-1.7
PBE1PBE	18.5	-5.9	14.0	-0.9
wB97X-D	21.1	-3.5	15.4	-1.8
BB1K	20.9	-6.6	15.8	-2.9
corrections				
Thermal correction to Enthalpy	-2.3	-1.6	-2.6	-0.6
Entropy	1.0	0.3	-0.6	-2.8