

Accumulated Bending Energy Elicits Neutral Sphingomyelinase Activity in Human Red Blood Cells

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SUPPLEMENTARY MATERIAL

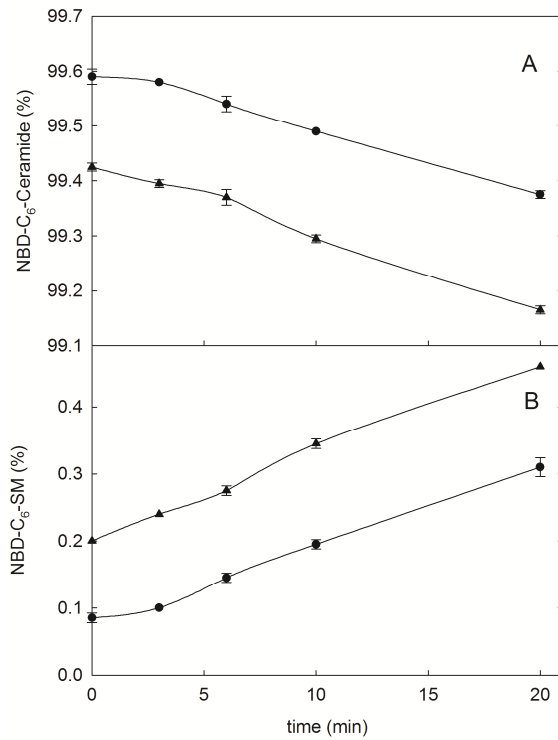


Figure S1. Spingomyelin synthase activity in human RBC labeled with NBD-C₆-Ceramide. Cells were labeled with NBD-C₆-Ceramide, incubated in hypotonic (●) or isotonic (▲) buffer and analysed after elution in a HPLC column. (A) variations of NBD-C₆-Ceramide and (B) variations of NBD-C₆-SM. Average values \pm SD (n = 3)

Table S1. Characterization of sphingomyelinase activity under hypotonic and hypertonic conditions. Average values \pm SEM (n=3-12)

pH [̄]	Cations	Other additions	hypotonic		hypertonic	
			Initial rate, nmoles SM/min x mg prot	% activity	Initial rate, nmoles SM/min x mg prot	% activity
Neutral	1 mM Mg ²⁺ , 1 mM Ca ²⁺	none	31.83 \pm 3.53	100	16.8 \pm 1.36	100
Neutral	1 mM Mg ²⁺	none	6.28 \pm 6.1	20	7.45 \pm 3.43	44
Neutral	1 mM Ca ²⁺	none	3.92 \pm 14.12	12	11.27 \pm 3.23	67
Neutral	1 mM Mg ²⁺ , 1 mM Ca ²⁺	20 μ M GW4869	7.79 \pm 10.02	24	3.52 \pm 1.56	21
Neutral	1 mM Mg ²⁺ , 1 mM Ca ²⁺	10 μ M spiroepoxide	0	0	10 \pm 2.94	59
Neutral	1 mM Mg ²⁺ , 1 mM Ca ²⁺	1 mg/ml trypsin	14.14 \pm 4.71	44	<i>nd</i>	<i>nd</i>
Neutral	1 mM Mg ²⁺ , 1 mM Ca ²⁺	1 mg/ml α -chymotrypsin	0	0	<i>nd</i>	<i>nd</i>
Acidic	1 mM Mg ²⁺ , 1 mM Ca ²⁺	none	0	0	0	0
Acidic	1 mM Mg ²⁺ , 1 mM Ca ²⁺ , 0.1 Zn ²⁺	none	0	0	0	0

[̄]Neutral conditions were assayed in a buffer containing 32 mM HEPES, 5mM glucose, pH 7.4; acidic conditions were assayed in 32 mM sodium acetate, 5mM glucose, pH 5.0. *nd* indicates not determined

Table S2. Variations in the amount of different sphingomyelin species present in the human erythrocyte membrane. Average values \pm SD (n=4)

SM species	0 min, moles	% species at 0 min	6min, moles	% decrease for each species
14:0	5.87E-10 \pm 5.47E-11	3.1	5.56E-10 \pm 5.60E-11	5.2
16:1	3.98E-10 \pm 2.26E-11	2.1	3.35E-10 \pm 4.34E-11	15.6
16:0	8.27E-09 \pm 2.95E-10	43.8	6.83E-09 \pm 7.31E-10	17.4
d16:0 ⁼	2.71E-10 \pm 9.98E-12	1.4	2.19E-10 \pm 1.73E-11	19.3
18:1	4.75E-10 \pm 4.17E-11	2.5	4.63E-10 \pm 3.20E-11	2.7
18:0	9.31E-10 \pm 5.33E-11	4.9	8.57E-10 \pm 4.97E-11	7.9
20:1	1.59E-10 \pm 1.57E-11	0.8	1.54E-10 \pm 1.55E-11	3.0
20:0	3.56E-10 \pm 3.25E-11	1.9	3.30E-10 \pm 2.68E-11	7.4
22:1	3.98E-10 \pm 3.13E-11	2.1	3.40E-10 \pm 2.30E-11	14.6
22:0	1.38E-09 \pm 1.01E-10	7.3	1.25E-09 \pm 9.88E-11	9.5
24:2	7.01E-10 \pm 3.45E-11	3.7	6.23E-10 \pm 1.57E-11	11.1
24:1	2.52E-09 \pm 1.16E-10	13.3	2.21E-09 \pm 6.18E-11	12.4
24:0	1.88E-09 \pm 1.23E-10	9.9	1.64E-09 \pm 8.62E-11	12.7
d24:0	1.80E-10 \pm 1.31E-11	1.0	1.56E-10 \pm 4.03E-12	13.5
26:1	1.88E-10 \pm 1.57E-11	1.0	1.75E-10 \pm 1.47E-11	6.8
26:0	2.00E-10 \pm 1.94E-11	1.1	1.88E-10 \pm 1.67E-11	6.3

⁼d indicates "dihydroxy base"