Are the Majority of a_2-Ions Cyclic?

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Supporting Information

Cell culture and prep. PC12 cells (European Collection of Cell Cultures) were cultured on poly-ornithine-coated culture cover slips in DMEM supplemented with 10% heat-inactivated horse serum and 5% fetal bovine serum. To promote cell differentiation, the medium was changed to DMEM supplemented with 5% heat-inactivated horse serum, 2.5% fetal bovine serum, and 100 ng/ml mouse submaxillary NGF (Alomone Labs Ltd., Israel) with 100 μM ATP (Roche, Indianapolis, USA). The cells were kept under differentiating conditions for 2–3 days, and the differentiation medium was replaced every 24 h.

PC12 cells were lysed using 0.1% Rapigest (Waters, Sweden), heating at 40C for 10 min. The extracted proteins were reduced with 5mM DTT (Sigma) during 10 min at 95C and alkylated with 10 mM iodoacetamide (Sigma) for 30 min at room temperature. Subsequently, cells were digested overnight using trypsin in a 1:50 trypsin:protein ratio. The tryptic peptides were filtered using a 10 kDa cut off filter (Pall Life Science) and dried in a speed vac.

LC-MS/MS analysis. 500 ng aliquots of peptides were re-dissolved in 0.1% formic acid prior to LC-MS/MS analysis. Analyses were performed in an online fashion using a Proxeon HPLC (Proxeon Biosystems, Odense, Denmark) coupled to a Velos-Orbitrap (ThermoFinnigan, Bremen, Germany) mass spectrometer, and employing a nanoelectrospray ion source (Proxeon Biosystems, Odense, Denmark). A 15-cm fused silica emitter (75-μm inner diameter, 375-μm outer diameter; Proxeon Biosystems) was used as the analytical column. The emitter was packed in-house with a methanol slurry of reversed-phase, fully end-capped Reprosil-Pur C18-AQ 3-μm resin (Dr. Maisch GmbH, Ammerbuch. Entringen, Germany) using a pressurized "packing bomb" operated at 50-60 bar (Proxeon Biosystems). Mobile phase consisted of 0.1% formic acid in water (v/v) (buffer A) and 0.1% formic acid and in ACN (v/v) (buffer B). Ten microliters of prepared peptide mixture was automatically loaded onto the column and rinsed with 5% buffer B at a flow rate of 700 nL/min followed by a 98-min gradient from 4 to 30% buffer B at a constant flow rate of 300 nL/min. Data was acquired in a data-dependent mode, with MS^1 performed in the Orbitrap at 60000 resolving power, and the 5 most intense peaks subjected to MS^2 analysis. Higher-collisional energy dissociation (HCD) was employed for MS^2 analysis, using a normalized collisional energy of 40, 0.1 ms of activation time, and a 2.0 Dalton isolation width. Resulting fragment ions were analyzed in the Orbitrap at 7500 resolution.

AA	G	A	V	L	I	F	Y	H
G	0	0.5789	0.8909	0.9579	0.9529	0.9583	1	0.6667
A	0.459	0.7419	0.9496	0.9609	0.9296	0.8471	0.8955	0.875
V	0.8362	0.9297	0.959	0.9856	0.9857	0.8614	0.9623	1
L	0.7912	0.9007	0.9805	0.9858	0.9897	0.994	0.9365	0.7742
I	0.7961	0.9686	0.9514	0.9738	0.9674	0.9649	0.9787	0.8571
F	0.2024	0	0.2336	0.142	0.2879	0.04	0.1333	0.3103
Y	0.381	0.1333	0.0294	0.1346	0.2949	0.1111	0.1875	0.5
H	0.125	0	0.3333	0.1977	0.6286	0	0	0