



	Experiment title: Structural studies on BrefeldinA ADP-Ribosylated Substrate (BARS)	Experiment number: LS1803, LS1933
	Beamline: ID14-3 ID14-1 ID14-1 ID14-3 ID14-2	Date of experiment: from 07-09-2000 to 09-09-2000 from 23-11-2000 to 25-11-2000 from 09-02-2001 to 12-02-2001 from 28-04-2001 to 30-04-2001 from 09-06-2001 to 10-06-2001
Shifts to BAG: 9 9 9 12 6	Local contact(s): Steffi ARZT Hassan BELRHALL Hassan BELRHALL Edward MITCHELL Stéfanie MONACO	<i>Received at ESRF:</i>
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Brefeldin A-ADP Ribosylated Substrate protein (BARS) is a newly discovered enzyme associated to the Golgi membrane, where it exerts a control on the chemical and electrostatic properties of phosphatidic acid. Acting putatively as an acyl-transferase, BARS is held responsible of the control of macroscopic Golgi events such as fission and/or vesiculation, thus controlling eventually the delivery of protein products.

BARS has a high sequence similarity to the G-terminal Binding Protein (CtBP) family of transcription regulators. Thus BARS might be a multifunctional protein and have an additional role in transcription.

A truncated form of rat brain BARS has been crystallized in two different crystal forms by vapour diffusion method at room temperature. Crystals of bipyramidal shape (crystal form 1) grow within few days. They belong to the space group $P6_222/P6_422$ with cell parameters $a=b=88.7 \text{ \AA}$, $c=163.2 \text{ \AA}$, $\alpha=\beta=90^\circ$, $\gamma=120^\circ$, and one molecule per asymmetric unit. They diffract up to $1.95 \text{ \AA}$, using synchrotron radiation. The other crystal form (crystal form 2) is characterized by a hexagonal prism morphology, and these crystals grow within few weeks. They belong to space group $P3_121/P3_221$ with cell parameters $a=b=145.0 \text{ \AA}$, $c=262.0 \text{ \AA}$, $\alpha=\beta=90^\circ$, $\gamma=120^\circ$, and six molecules per asymmetric unit. They diffract up to $4.5 \text{ \AA}$, using a rotating anode X-ray source.

No significant sequence similarity has been detected between BARS and proteins of known 3D structures. Therefore, isomorphous replacement method is required for phasing the X-ray data.

During the experiments carried out at ESRF we collected many possible heavy atom derivatives both for crystal forms 1 and 2. Moreover for crystal form 2 we also collected a native data set and for crystal form 1 a complex with NAD (see tables below). Inspection of Patterson maps didn't reveal the presence of any heavy atom bound to the protein. At the moment we are crystallizing a Se-Met substituted BARS, and we plan a MAD experiment at the Se edge on ID29 during the next round of BAG beam time.

References

- Weigert, R, Silletta, M.G., Spanò, S., Turacchio, G., Pericola, C., Colanzi, A., Senatore, S., Mancini, R., Polishchuk, E.V., Salmons, M., Facchiano, F., Burger, K.N.J., Mironov, A., Luini, A. & Corda, D.

Nature (1999) **402**, 429-433

- Spanò, S., Silletta, M.G., Colanzi, A., Alberti, S., Ciucci, G., Valente, C., Fusella, A., Salmons, M., Mironov, A., Luini, A. & Corda, D.

J. Biol. Chem. (2000) **274**, 17705-17710

Table: Summary of BARS data collection statistics (ID14-3, from 07-09-2000 to 09-09-2000)

	Hg derivative (1)	Ho derivative (1)		
Space Group	<i>P</i> 6 ₂ 22/ <i>P</i> 6 ₄ 22	<i>P</i> 6 ₂ 22/ <i>P</i> 6 ₄ 22		
Unit cell (Å)	<i>a</i> = <i>b</i> =85.6, <i>c</i> =162.7	<i>a</i> = <i>b</i> =89.4, <i>c</i> =160.6		
Resolution (Å)	35.0-2.7	35.0-3.2		
N° measurements	100201	58884		
N° unique reflections	15280	6807		
Completeness (%)	83.9	99.7		
Rsym (%)	9.2	6.5		
Mosaicity (°)	0.9	0.5		
	Lu derivative (1)	Sm derivative (1)	Yt derivative (1)	
Space Group	<i>P</i> 6 ₂ 22/ <i>P</i> 6 ₄ 22	<i>P</i> 6 ₂ 22/ <i>P</i> 6 ₄ 22	<i>P</i> 6 ₂ 22/ <i>P</i> 6 ₄ 22	
Unit cell (Å)	<i>a</i> = <i>b</i> =89.5, <i>c</i> =160.4	<i>a</i> = <i>b</i> =85.6, <i>c</i> =162.6	<i>a</i> = <i>b</i> =89.3, <i>c</i> =160.3	
Resolution (Å)	35.0-3.35	35.0-3.1	35.0-3.4	
N° measurements	58232	57519	35017	
N° unique reflections	5953	7402	5928	
Completeness (%)	100.0	99.5	99.9	
Rsym (%)	7.5	7.0	6.5	
Mosaicity (°)	0.5	0.5	0.5	

Table: Summary of BARS data collection statistics (ID14-3, from 23-11-2000 to 25-11-2000)

	Hg2 derivative (1)
Space Group	$P6_222/P6_422$
Unit cell (Å)	$a=b=85.6, c=162.6$
Resolution (Å)	35.0-2.7
N° measurements	184821
N° unique reflections	18430
Completeness (%)	99.7
Rsym (%)	10.9
Mosaicity (°)	0.9

Table: Summary of BARS data collection statistics (ID14-1, from 09-02-2001 to 12-02-2001)

	NAD-complex (1)	native (2)
Space Group	$P6_222/P6_422$	$P3_121/P3_221$
Unit cell (Å)	$a=b=88.7, c=163.0$	$a=b=145.2, c=263.7$
Resolution (Å)	40.0-2.3	40.0-3.55
N° measurements	188264	141318
N° unique reflections	17299	38605
Completeness (%)	99.3	98.7
Rsym (%)	5.9	17.1
Mosaicity (°)	0.7	0.7

Table: Summary of BARS data collection statistics (ID14-3, from 28-04-2001 to 30-04-2001)

	Au derivative (2)	Os derivative (2)	Pt derivative (2)
Space Group	$P3_121/P3_221$	$P3_121/P3_221$	$P3_121/P3_221$
Unit cell (Å)	$a=b=143.5, c=262.8$	$a=b=143.5, c=263.9$	$a=b=143.6, c=263.9$
Resolution (Å)	30.0-3.8	30.0-3.5	30.0-3.55
N° measurements	97792	161427	161942
N° unique reflections	31932	40752	40886
Completeness (%)	99.1	99.1	98.1
Rsym (%)	15.1	12.8	11.1
Mosaicity (°)	0.7	0.5	10.1

Table: Summary of BARS data collection statistics (ID14-2, from 09-06-2001 to 10-06-2001)

	Gd derivative (2)	Eu derivative (2)
Space Group	$P3_121/P3_221$	$P3_121/P3_221$
Unit cell (Å)	$a=b=143.7, c=263.5$	$a=b=144.0, c=264.5$
Resolution (Å)	40.0-3.3	30.0-3.35
N° measurements	310990	144263
N° unique reflections	48785	42101
Completeness (%)	99.2	91.0
Rsym (%)	8.9	14.3
Mosaicity (°)	0.4	0.9

	I derivative (2)	La derivative (2)
Space Group	$P3_121/P3_221$	$P3_121/P3_221$
Unit cell (Å)	$a=b=144.6, c=262.6$	$a=b=144.0, c=264.5$
Resolution (Å)	35.0-4.0	40.0-4.0

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N° measurements	162576	142000
N° unique reflections	26011	27095
Completeness (%)	92.2	99.7
Rsym (%)	13.0	17.8
Mosaicity (°)	0.9	0.9

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