

ESRF

Experiment title:

x-ray structure of acceptor stem of
E.coli tRNA^{ALA}-bromine derivative
MAD- EXPERIMENT

Experiment

number:

ls-639

Beamline:

BW14

Date of Experiment:

from: 24.01.1997 to: 25.01.1997

Date of Report:

10.04.1997

Shifts: 3

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Received at ESRF:

15 AVR. 1997

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Report:

We tried to perform an MAD experiment with an System containing chemically bonded Br Atoms within the RNA molecule. Therefore we could crystallize the RNA and we got small crystals with dimensions of 150x150x150 μm . The EXAFS experiment (Fig.1) shows a small signal from the bromine anomalous scatterer at the expected energy range. We performed several MAD experiments for 4 wavelength with 3 crystals. The crystal diffracted up to 2.5 \AA .Data reduction and scaling gave reasonable statistics (Fig2.). Unfortunately we could not determine the coordinates of the bromine sites. We used Patterson and direct methods, but failed every time. An MALDI experiment of the RNA -solution which was used for crystallization showed only the signal due to the debrominated molecules. Recently we could solve the structure with an SIRAS approach coming from an iodine derivative .The difference fourier to the bromine system gave also no solution. These evidences showed that the experiment failed due to the determination of the bromine sites.

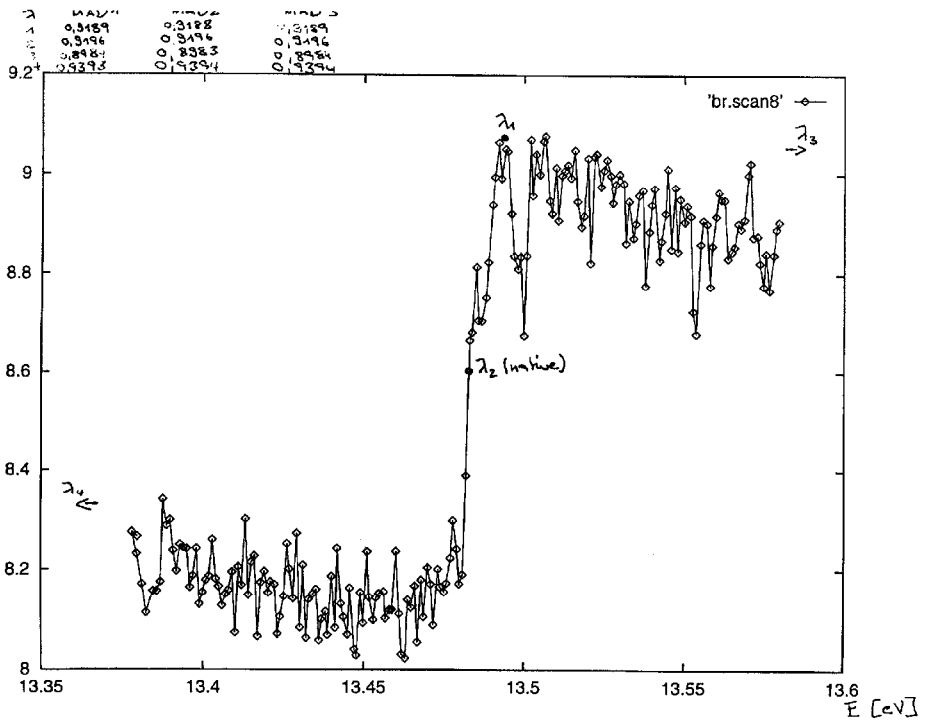


Fig.1 EXAFS scan for the brominated RNA crystal

By 4SINTH/LASQ bins (all statistics use <I>,<I>-etc)

STABLE: Analysis against resolution:
 SGRAPHS: Rfactor v Resolution: N:2,4,6
 : Average I, sd and Sigma : A:2,8,9,11: I:/sigma: N:2,10: \$S

N <=>	Dmin(A)	Rfac	Rcum	Ranom	Nanom	AV_I	SIGMA I/sigma	sd	Nmeas	Nref	Ncent	FRCBIAS	Nbias	P5	
1	0.0183	7.40	0.043	0.043	0.037	42	25721.	1729.7	14.9	3805.66	329	51	10	-0.0327	60
2	0.0340	5.42	0.053	0.049	0.044	75	25668.	2012.4	12.8	3895.55	575	81	9	-0.0352	121
3	0.0498	4.48	0.052	0.051	0.030	98	32251.	2849.7	11.3	4820.87	746	109	12	-0.0382	163
4	0.0655	3.91	0.057	0.053	0.027	116	36327.	3091.8	11.7	5477.65	877	127	11	-0.0110	194
5	0.0813	3.51	0.052	0.053	0.029	125	41490.	3536.1	11.7	6411.64	919	133	9	-0.0173	199
6	0.0970	3.21	0.059	0.054	0.026	137	42280.	4327.2	9.8	6595.66	1038	146	10	-0.0249	228
7	0.1128	2.98	0.064	0.056	0.038	159	34177.	4016.0	8.5	5603.55	1027	165	12	-0.0089	218
8	0.1285	2.79	0.071	0.057	0.067	166	34329.	3503.7	9.5	2640.02	653	166	8	-0.0236	133
9	0.1443	2.63	0.093	0.058	0.064	166	11849.	3546.8	7.7	2032.23	595	165	12	-0.0197	119
10	0.1600	2.50	0.111	0.059	0.098	157	8379.	3358.8	6.2	1594.01	364	113	6	-0.0164	40

BY INTENSITY ranges (all statistics use <I>,<I>-etc)

STABLE: Analysis against intensity:
 SGRAPHS: Rfactor v Intensity: N:1,2,3: \$S

\$S	Imax	Kfac	Ranom	Nanom	AV_I	SIGMA I/Sigma	sd	Nmeas	Nref	Ncent	FRCBIAS	Nbias	Nindep	Abs.BIAS	P5
125.	-1.571	-2.022	27	-421.	1042.0	-0.4	478.4	84.	23	4	-24.6262	10	7	947.8	
250.	1.470	3.651	8	558.	1204.2	0.5	420.8	32.	7	1	0.4390	8	3	270.6	
375.	1.214	1.036	5	576.	1016.7	0.6	348.5	32.	6	1	0.0925	4	3	-65.1	
500.	1.624	0.705	9	442.	1008.0	0.4	448.1	41.	9	1	0.4850	9	4	-124.0	
625.	0.970	0.390	7	788.	1068.4	0.7	365.5	44.	7	0	0.3985	11	6	471.0	
750.	1.415	0.498	8	687.	1189.1	0.5	367.8	44.	7	0	-1.4157	7	3	1770.9	
875.	0.958	0.317	12	867.	1367.5	0.6	434.8	57.	11	1	-0.3142	17	7	-295.2	
1000.	0.754	0.443	12	1077.	1150.7	0.9	430.5	60.	12	1	0.2680	15	7	274.5	
1125.	0.464	0.267	12	1367.	962.1	1.4	408.4	53.	11	1	0.0958	19	5	131.1	
1250.	0.589	0.354	11	1555.	1376.6	1.1	507.2	69.	12	2	-0.8328	9	4	-860.5	
1375.	0.467	0.304	11	1479.	910.6	1.6	500.1	55.	12	1	0.0943	11	5	152.9	
1500.	0.654	0.319	10	1855.	1622.4	1.1	585.1	64.	12	2	-0.0317	12	7	-65.6	
695991.	0.057	0.036	1109	33159.	3234.8	10.3	5160.7	6488.	1129	84	-0.0192	1390	638	-705.5	

TOTALS 0.059 0.038 1241 30284. 3108.3 9.7 4741.0 7123. 1258 99 -0.0187 1515 699 -632.4

Completeness and multiplicity, including reflections measured only once

STABLE: Completeness & multiplicity v. resolution:
 SGRAPHS: Completeness v Resolution :N:2,5: \$S
 : Multiplicity v Resolution :N:2,7: \$S

N <=>	Dmin	Nmeas	Nref	Npos	Multiplicity	\$S
1	0.018	7.40	332	52	93.9	6.4
2	0.034	5.42	577	84	99.7	6.9
3	0.050	4.48	750	110	103.0	6.8
4	0.066	3.91	878	127	102.3	6.9
5	0.081	3.51	923	135	97.9	6.8
6	0.097	3.21	1041	147	96.3	7.1
7	0.113	2.98	1048	171	103.6	6.1
8	0.129	2.79	684	175	99.7	3.9
9	0.144	2.63	646	181	96.0	3.5
10	0.160	2.50	511	171	87.2	3.0

Total 7390 1353 97.4 5.5

Fig.2 Data reduction statistics (Agrovata output) for best Crystal at $\lambda=0.9188 \text{ \AA}$

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