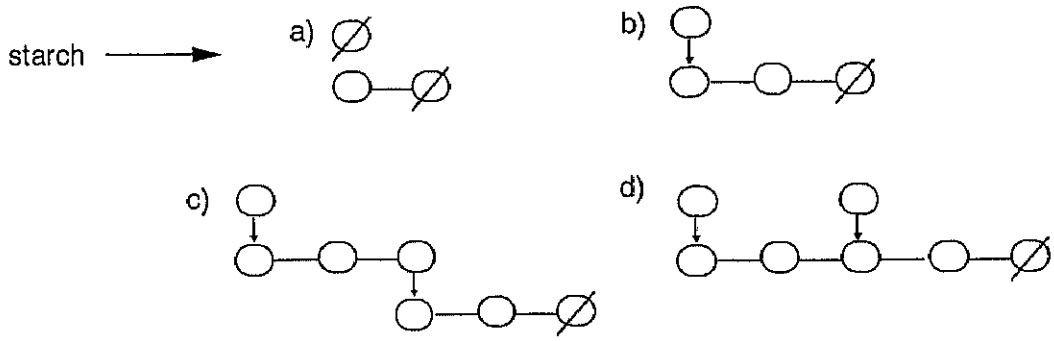
APPENDIX

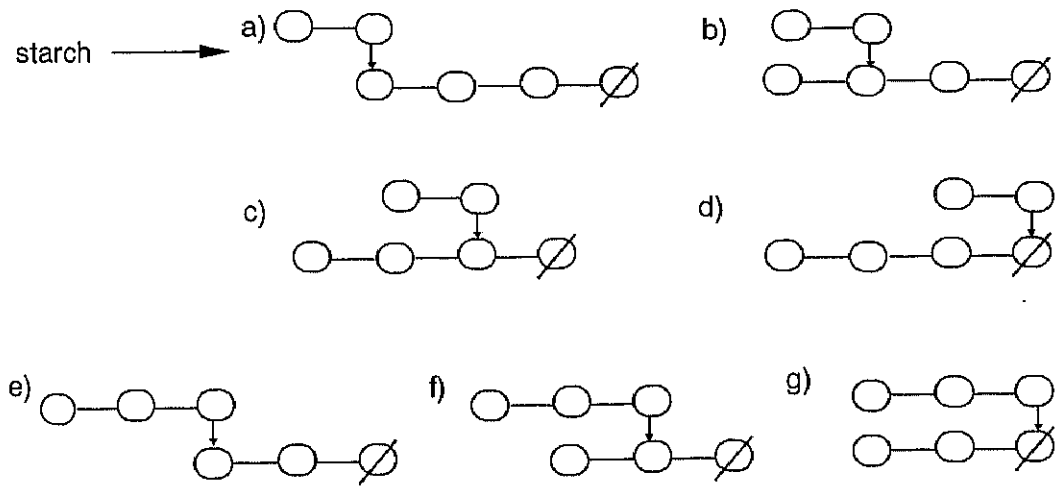
Schema 1: Starch degradation pattern of amylases:

α-Amylases:

a) Saccharifying type:

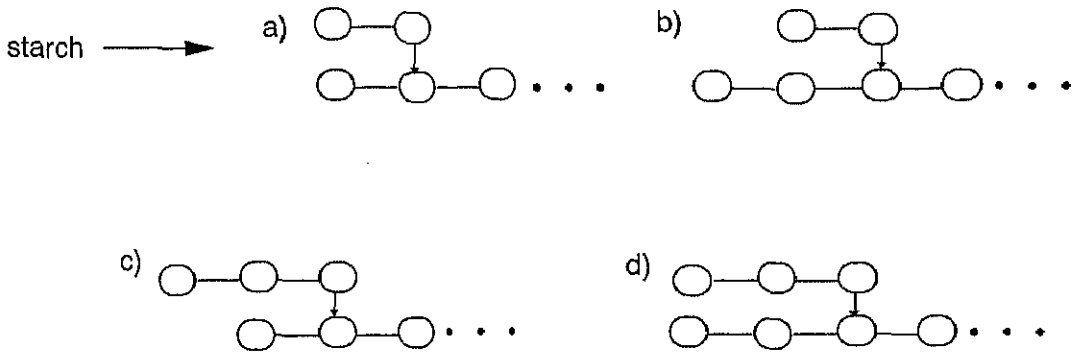


b) Liquefying type:

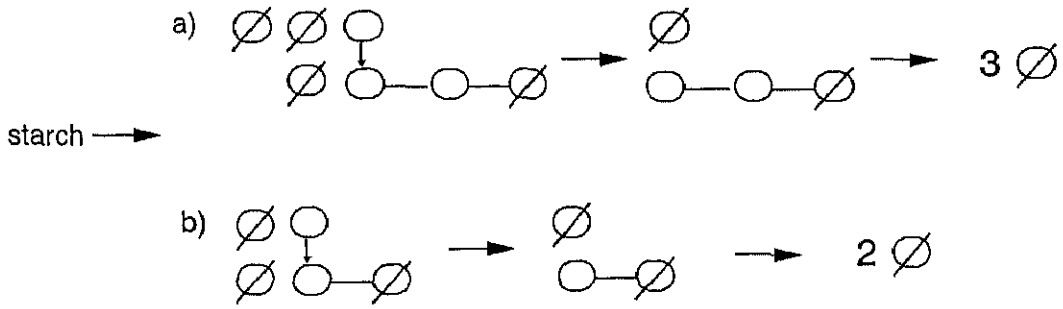


Schema 1 (continue)

β-Amylases:



Glucoamylases:



Schema 1. Starch degradation pattern of amylases.

Schema 1 was generated from the book "Handbook of Amylases and Related Enzymes", 1988. ⬭: glucose residue, ∅: reducing end glucose residue or free glucose, — : α-1.4-glucosidic linkage, ↓: α-1.6-glucosidic linkage.

TABLES

Table 1-1. A list of reservoir conditions in Crystal Screen I

#	BUFFER	SALT	PRECIPIPANT
1.	0.1 M Na Acetate pH 4.6	0.02 M $CaCl_2(2H_20)$	30% v/v MPD
2.	-	-	0.4 M K, Na Tartrate ($4H_20$)
3.	-	-	0.4 M Ammonium dihydrogen phosphate
4.	0.1 M Tris HCl pH 8.5	-	2.0 M $(NH_4)_2SO_4$
5.	0.1 M Na Hepes pH7.5	0.2 M Na_3 citrate ($2H_20$)	30% v/v MPD
6.	0.1 M Tris HCl pH 8.5	0.2 M $MgCl_2(6H_20)$	30% w/v PEG 4000
7.	0.1 M Na Cacodylate pH6.5	-	1.4 M Na acetate ($3H_20$)
8.	0.1 M Na Cacodylate pH6.5	0.2 M Na_3 citrate ($2H_20$)	30% v/v isopropanol
9.	0.1 M Na Citrate pH 5.6	0.2 M CH_3COONH_4	30% w/v PEG 400
10.	0.1 M Na Acetate pH 4.6	0.2 M CH_3COONH_4	30% w/v PEG 400
11.	0.1 M Na Citrate pH 5.6	-	1.0 M Ammonium dihydrogen phosphate
12.	0.1 M Na Hepes pH 7.5	-	0.2 M $MgCl_2(6H_20)$
13.	0.1 M Tris HCl pH 8.5	0.2 M Na_3 citrate ($2H_20$)	30% w/v PEG 400
14.	0.1 M Na Hepes pH7.5	0.2 M $CaCl_2(2H_20)$	28% w/v PEG 400
15.	0.1 M Na Cacodylate pH6.5	0.2 M $(NH_4)_2SO_4$	30% w/v PEG 8000
16.	0.1 M Na Hepes pH7.5	-	1.5 M $Li2SO_4(H_20)$
17.	0.1 M Tris HCl pH 8.5	0.2 M $Li2SO_4(H_20)$	30% w/v PEG 4000
18.	0.1 M Na Cacodylate pH6.5	0.2 M Mg acetate ($4H_20$)	20% w/v PEG 8000
19.	0.1 M Tris HCl pH 8.5	0.2 M CH_3COONH_4	30% v/v isopropanol
20.	0.1 M Na Acetate pH 4.6	0.2 M $(NH_4)_2SO_4$	25% w/v PEG 4000
21.	0.1 M Na Cacodylate pH6.5	0.2 M Mg acetate ($4H_20$)	30% v/v MPD
22.	0.1 M Tris HCl pH 8.5	0.2 M Na acetate ($3H_20$)	30% w/v PEG 4000
23.	0.1 M Na Hepes pH7.5	0.2 M $MgCl_2(6H_20)$	30% w/v PEG 400
24.	0.1 M Na Acetate pH 4.6	0.2 M $CaCl_2(2H_20)$	20% v/v isopropanol

(continued)

25.	0.1 M Imidazole pH6.5	-	1.0 M Na acetate ($3H_20$)
26.	0.1 M Na Citrate pH 5.6	0.2 M CH_3COONH_4	30% v/v MPD
27.	0.1 M Na Hepes pH7.5	0.2 M Na_3 citrate ($2H_20$)	20% v/v isopropanol
28.	0.1 M Na Cacodylate pH6.5	0.2 M Na acetate ($3H_20$)	30% w/v PEG 8000
29.	0.1 M Na Hepes pH7.5	-	0.8 M K, Na Tartrate ($4H_20$)
30.	-	0.2 M $(NH_4)_2SO_4$	30% w/v PEG 8000
31.	-	0.2 M $(NH_4)_2SO_4$	30% w/v PEG 4000
32.	-	-	2.0M $(NH_4)_2SO_4$
33.	-	-	4.0 M Na formate
34.	0.1 M Na Acetate pH 4.6	-	2.0 M Na formate
35.	0.1 M Na Hepes pH7.5	-	0.8 M KH_2PO_4 & 0.8 M NaH_2PO_4
36.	0.1 M Tris HCl pH 8.5	-	8% w/v PEG 8000
37.	0.1 M Na Acetate pH 4.6	-	8% w/v PEG 8000
38.	0.1 M Na Hepes pH7.5	-	1.4 M Na acetate ($2H_20$)
39.	0.1 M Na Hepes pH7.5	-	2% v/v PEG 400 & 2.0M $(NH_4)_2SO_4$
40.	0.1 M Na Citrate pH 5.6	-	20% v/v isopropanol & 20% w/v PEG 4000
41.	0.1 M Na Hepes pH7.5	-	10% v/v isopropanol & 20% w/v PEG 4000
42.	-	0.05 M KH_2PO_4	20% w/v PEG 8000
43.	-	-	30% PEG 1500
44.	-	-	0.2 M Mg formate
45.	0.1 M Na Cacodylate pH6.5	0.2 M Zn acetate ($2H_20$)	18% w/v PEG 8000
46.	0.1 M Na Cacodylate pH6.5	0.2 M Ca acetate	18% w/v PEG 8000
47.	0.1 M Na Acetate pH 4.6	-	2.0M $(NH_4)_2SO_4$
48.	0.1 M Tris HCl pH 8.5	-	1.0 M Ammonium dihydrogen phosphate
49.	-	1.0 M $Li_2SO_4(H_20)$	2% w/v PEG 8000
50.	-	0.5 M $Li_2SO_4(H_20)$	15% w/v PEG 8000

Table 1-2. Crystal parameters and Data collection statistics

Crystal system	Monoclinic	
Space group	$P2_1$	
Unit-cell parameters (Å, °)	a= 53.5 b=92.8 c=53.1, β=109.4	
Number of total reflections	183694	
Number of unique reflections	29849	
Resolution range (outermost, Å)	20-2.0	(2.09-2.00)[a]
$I/\sigma(I)>3$(%)	81.4	(54.1)
Completeness (%)	89	(73)
Rmerge (%)[b]	7.4	(22.1)
Multiplicity	6.2	

[a]Values in parentheses are for the outermost shell.

[b]$R_{merge} = \sum h \sum i |I(h)i - \langle I(h) \rangle| / \sum h \sum i I(h)i$, where $\langle I(h) \rangle$ is the average intensity of the i observations of reflection h.

Table 2-1. Structure refinement statistics

Resolution range (Å)	8.0-2.0
σ cut-off	2
Reflections used	28367
R-factor	0.156
R_{free}-factor	0.197
No. of protein atoms	3909
No. of metal atoms	4
No. of water molecules	320
Ramachandran plot in the most favored/additional region (%)	87.7/11.8
Average *B*-factor (Å^2)	9.6
Protein atoms (Å^2)	9.1
Metal ions (Å^2)	5.7
Solvent atoms (Å^2)	18.6
RMS deviations	
from ideal bond lengths (Å^2)	0.008
from ideal bond angles (°)	1.4
from ideal dihedral angles (°)	24.2

†*R*-factor is defined as $R = \Sigma | | F_{obs} | - | F_{calc} | | / \Sigma | F_{obs} |$. R_{free}-factor was calculated using 10% of the unique reflections.

Table 2-2. Metal ion binding sites in BSTA and BLA

	Ligand		Distance (Å)	
Metal ion	BSTA	BLA	BSTA	BLA
CaI	Asp 105 OD1	Asn 104 OD1	2.4	2.4
	Asp 197 O	Aasp194 O	2.3	2.4
	Asp 197 OD1	Asp 194 OD1	2.5	2.5
	Asp 203 OD1	Asp 200 OD1	2.4	2.4
		Asp 200 OD2		3.0
	His 238 O	His235 O	2.3	2.4
	Wat	Wat	2.4	2.6
Na	Asp 162 OD1	Asp 161 OD1	2.5	2.4
	Asp 186 OD2	Asp 183 OD2	2.5	2.7
	Asp 197 OD1	Asp 194 OD1	2.9	3.1
	Asp 197 OD2	Asp 194 OD2	2.5	2.5
	Asp 203 OD2	Asp 200 OD2	2.4	2.4
	Leu 204 O	Leu 201 O	2.3	2.5
CaII	Asp 162 OD1	Asp 161 OD1	2.6	2.6
	Asp 162 OD2	Asp 161 OD2	2.6	2.6
	Ala 184 O	Ala 181 O	2.3	2.4
	Asp 186 OD1	Asp 183 OD1	2.5	2.4
	Asp 205 OD1	Asp 202 OD1	2.5	2.5
	Wat	Asp 204 OD1	2.5	2.6
	Wat	Wat	2.4	2.5
CaIII	Gly 303 O	Gly 300 O	2.3	2.6
	Phe 305 O	Tyr 302 O	2.3	2.3
	Ser 406 O	His 406 O	2.4	2.6
	Asp 407 OD2	Asp 407 OD2	2.5	2.3
	Asp 430 OD1	Asp 430 OD1	2.6	2.6
	Asp 430 OD2	Asp 430 OD2	2.6	2.6
	Wat	Wat	2.4	2.9

Table 2-3. Molecular volumes and void's volumes in BSTA and BLA

	BSTA	BLA
Standard volume of molecule x10^4 (Å^3)	8.8	8.79
	Cavity volumes (Å^3)	
Catity #	BSTA	BLA
1	4.3	9.5
2	-	0.9
3	4	-
4	0.5	-
5	7	5.4
6	2	-
7	1	0.6
8	0.6	-
9	0.8	-
10	26	1.4
Total volumes of voids	46	18

The standard van der Waals volumes and volumes of the voids were calculated with VOIDOO (Kleywegt & Jones, 1994). Cavities were detected using probe radius 1.5Å and atom fattening factor 1.09.

Table 2-4. Accessible surfaces, voids and volumes

	BSTA	BLA
Total solvent accessible surface area (ASA; $Å^2$)[a]	17,479	17,388
Total (% of total) ASA of polar residues	5133 (29)	5081 (29)
Total (% of total) ASA of hydrophobic residues	2214 (12.7)	2141 (12)
Total (% of total) ASA of charged residues	2645 (15)	4899 (28)
Volumes and voids ($Å^3$):		
Volume of molecules	88470	87930
Total void's volumes[b]	46	18
Molecular packing densities	1.17	1.19

Hydrophobic residues	Ala, Ile, Leu, Met, Phe, Pro, Trp, Val
Polar residues	Ser, Gln, Asn, Gly, Thr, Cys, Tyr
Charged residues	Asp, Arg, Glu, His, Lys

[a]Accessible surfaces were calculated with GRASP (Anthony *et al.*, 1991)

[b]The standard van der Waals volumes and volumes of the voids were calculated with VOIDOO (Kleywegt & Jones, 1994). Voids were detected using probe radius 1.5Å and atom fattening factor 1.09.

Table 2-5. Summary of main-chain side-chain and side-chain side-chain atoms hydrogen-bond interactions in BSTA

	DONOR				ACCEPTOR					
RES	ATOM1		ATOM2		ATOM3		ATOM4		RES	Dist23
Domain A										
ASN	4	C	5	N	396	OH	396	CZ	TYR(C)	2.88
ASN	5	CG	5	ND2	391	O	391	C	ARG(C)	2.84
ASN	5	CG	5	ND2	395	O	395	C	ALA	2.88
THR	6	C	7	N	39	OG1	39	CB	THR	2.90
TYR	11	CZ	11	OH	370	OH	370	CZ	TYR	2.78
TRP	13	C	14	N	333	OE2	333	CD	GLU	2.90
TYR	14	C	15	N	13	OE1	13	CD	GLU	2.95
TYR	15	CZ	15	OH	334	O	334	C	PRO	2.75
THR	21	CB	21	OG1	19	OD1	19	CG	ASP	2.69
LYS	25	CE	25	NZ	11	OH	11	CZ	TYR	2.90
ASN	32	CG	32	ND2	29	OE1	29	CD	GLU	3.05
SER	34	CB	34	OG	94	O	94	C	ALA	2.64
SER	35	CB	35	OG	32	O	32	C	ASN	2.66
THR	39	CB	39	OG1	7	O	7	C	THR	3.27
TRP	42	CE2	42	NE1	101	OD2	101	CG	ASP	2.98
TYR	47	CZ	47	OH	101	O	101	C	ASP	2.67
LYS	48	CE	48	NZ	64	OD2	64	CG	ASP	2.89*
LYS	48	CE	48	NZ	68	O	68	C	PHE	2.97
LYS	48	CE	48	NZ	70	O	70	C	GLN	3.23
LYS	48	CE	48	NZ	73	O	73	C	ALA	2.95
THR	49	C	50	N	54	OD2	54	CG	ASP	3.19
THR	50	CB	50	OG1	54	OD2	54	CG	ASP	2.74
ARG	52	CD	52	NE	18	OD2	18	CG	ASP	2.89*
ARG	52	CZ	52	NH2	18	OD2	18	CG	ASP	3.11*
SER	52	C	53	N	51	OG	51	CB	SER	3.29
SER	53	CB	53	OG	18	OD2	18	CG	ASP	2.90
GLY	55	C	56	N	54	OD1	54	CG	ASP	2.90
TYR	57	CZ	57	OH	101	OD2	101	CG	ASP	2.72
GLY	57	C	58	N	54	OD1	54	CG	ASP	3.26
TYR	60	CZ	60	OH	111	OD1	111	CG	ASP(B)	2.97
TYR	60	CZ	60	OH	111	OD2	111	CG	ASP(B)	2.80
TYR	62	C	63	N	61	OD1	61	CG	ASP	3.25
TYR	63	CZ	63	OH	214	OE2	214	CD	GLU	2.77
GLY	65	C	66	N	64	OD1	64	CG	ASP	3.15
GLU	66	C	67	N	64	OD1	64	CG	ASP	2.90
PHE	67	C	68	N	64	OD1	64	CG	ASP	3.14
GLN	70	CD	70	NE2	61	OD2	61	CG	ASP	2.96
GLN	70	CD	70	NE2	64	OD2	64	CG	ASP	3.03
LYS	71	CE	71	NZ	109	O	109	C	GLY(B)	2.63
ARG	75	CZ	75	NH1	18	OD1	18	CG	ASP	2.90*
ARG	75	CZ	75	NH1	76	O	76	C	THR	2.78
ARG	75	CZ	75	NH2	18	O	18	C	ASP	3.30
THR	76	CB	76	OG1	79	O	79	C	GLY	3.18
TYR	77	C	78	N	76	OG1	76	CB	THR	3.26
TYR	78	CZ	78	OH	55	O	55	C	VAL	2.66
THR	79	C	80	N	83	OE1	83	CD	GLN	2.99

(continued)

LYS	81	CE	81	NZ	67	OE2	67	CD	GLU	2.85*
TYR	84	CZ	84	OH	222	OH	222	CZ	TYR	2.82
VAL	97	C	98	N	229	OD2	229	CG	ASP	2.84
TYR	99	CZ	99	OH	361	OH	361	CZ	TYR	2.83
VAL	102	C	103	N	47	OH	47	CZ	TYR	2.90

Domain B

HIS	106	CG	106	ND1	105	OD2	105	CG	ASP	2.86*
THR	113	CB	113	OG1	137	OE1	137	CD	GLN	3.20
GLU	119	C	120	N	131	OG	131	CB	SER	3.12
ASN	122	CG	122	ND2	128	OE1	128	CD	GLN	2.74
SER	123	C	124	N	122	OD1	122	CG	ASN	3.08
ARG	126	CD	126	NE	123	O	123	C	PRO	2.96
ARG	126	CZ	126	NH1	195	O	195	C	ASN	3.20
ARG	126	CZ	126	NH1	198	O	198	C	TYR	2.92
ASN	126	C	127	N	125	OD1	125	CG	ASP	3.24
SER	131	CB	131	OG	132	O	132	C	GLY	2.81
TYR	134	CZ	134	OH	120	OE2	120	CD	GLU	2.66
TYR	134	CZ	134	OH	176	OH	176	CZ	TYR	2.87
GLN	136	C	137	N	167	OD1	167	CG	ASP	2.94
TRP	139	CE2	139	NE1	168	OE1	168	CD	GLU	3.04
THR	140	CB	140	OG1	114	OE1	114	CD	GLU	2.83
LYS	141	CE	141	NZ	143	OD2	143	CG	ASP	2.70*
ASP	142	C	143	N	111	OD2	111	CG	ASP	2.95
GLY	145	C	146	N	70	OE1	70	CD	GLN(A)	2.93
ARG	146	C	147	N	70	OE1	70	CD	GLN(A)	3.21
ARG	147	CD	147	NE	61	OD2	61	CG	ASP(A)	2.89*
ARG	147	CZ	147	NH1	150	O	150	C	THR	2.81
ARG	147	CZ	147	NH1	152	O	152	C	SER	2.83
ARG	147	CZ	147	NH2	61	OD1	61	CG	ASP(A)	3.03*
THR	150	CB	150	OG1	147	O	147	C	ARG	3.14
TYR	151	CZ	151	OH	67	OE1	67	CD	GLU(A)	2.65
SER	152	CB	152	OG	214	OE2	214	CD	GLU(A)	3.18
LYS	155	CE	155	NZ	143	O	143	C	ASP	2.77*
ARG	157	CZ	157	NH1	142	O	142	C	PHE	3.30
ARG	157	CZ	157	NH1	156	O	156	C	TRP	2.82
ARG	157	CZ	157	NH2	143	OD1	143	CG	ASP	2.88
TRP	157	C	158	N	114	OE1	114	CD	GLU	2.82
ASP	164	C	165	N	198	OH	198	CZ	TYR	3.15
TRP	166	CE2	166	NE1	168	OE1	168	CD	GLU	2.80
SER	168	C	169	N	167	OD1	167	CG	ASP	2.95
SER	169	CB	169	OG	167	OD1	167	CG	ASP	2.64
ARG	170	CD	170	NE	167	OD2	167	CG	ASP	2.81*
ARG	170	CZ	170	NH2	135	O	135	C	GLN	2.57
ARG	170	CZ	170	NH2	167	OD2	167	CG	ASP	3.00*
LYS	171	CE	171	NZ	168	OE2	168	CD	GLU	3.16*
ARG	174	CZ	174	NH1	173	O	173	C	SER	3.16
LYS	177	CE	177	NZ	129	OE2	129	CD	GLU	2.96*
ARG	179	CZ	179	NH2	129	OE2	129	CD	GLU	2.75*
LYS	183	CE	183	NZ	159	O	159	C	TYR	2.67
LYS	183	CE	183	NZ	161	O	161	C	PHE	2.83
LYS	183	CE	183	NZ	205	OD2	205	CG	ASP	2.81*
TRP	185	CE2	185	NE1	126	O	126	C	ARG	3.02
ASP	185	C	186	N	197	OD2	197	CG	ASP	2.84

(continued)

GLU	191	C	192	N	190	OD2	190	CG	ASP	3.03
ASN	195	CG	195	ND2	186	O	186	C	ASP	3.04
ASP	196	C	197	N	195	OD1	195	CG	ASN	2.95
TYR	198	CZ	198	OH	201	O	201	C	TYR	2.66
MET	205	C	206	N	186	OD2	186	CG	ASP	3.00
Domain A										
ASP	206	C	207	N	205	OD1	205	CG	ASP	3.02
THR	213	CB	213	OG1	209	O	209	C	PRO	3.23
LYS	216	CE	216	NZ	246	OD1	246	CG	ASP	2.94*
SER	217	CB	217	OG	213	O	213	C	THR	3.07
TRP	218	CE2	218	NE1	62	O	62	C	LEU	2.80
LYS	220	CE	220	NZ	254	OE1	254	CD	GLN	2.87
THR	225	CB	225	OG1	221	O	221	C	TRP	2.84
THR	226	CB	226	OG1	222	O	222	C	TYR	2.69
ARG	232	CZ	232	NH1	101	OD1	101	CG	ASP	2.97*
ARG	232	CZ	232	NH1	234	OD1	234	CG	ASP	3.04*
ALA	234	C	235	N	264	OE1	264	CD	GLU	2.91
LYS	237	CE	237	NZ	266	O	266	C	TRP	2.78
HIS	238	CG	238	ND1	196	O	196	C	TYR	2.74
LYS	240	CE	240	NZ	187	O	187	C	TRP	2.87
TRP	247	CE2	247	NE1	215	O	215	C	LEU	2.96
SER	249	CB	249	OG	245	O	245	C	PRO	2.96
SER	249	CB	249	OG	246	O	246	C	ASP	2.95
ARG	252	CD	252	NE	258	O	258	C	PRO	2.87
ARG	252	CZ	252	NH1	283	OG1	283	CB	THR	3.05
ARG	252	CZ	252	NH2	259	O	259	C	LEU	3.00
ARG	252	CZ	252	NH2	283	O	283	C	THR	3.21
SER	253	CB	253	OG	249	O	249	C	SER	2.90
SER	253	CB	253	OG	250	O	250	C	TYR	3.09
THR	255	CB	255	OG1	251	O	251	C	VAL	2.76
LYS	257	CE	257	NZ	227	OD1	227	CG	ASN	2.98
LYS	257	CE	257	NZ	228	O	228	C	ILE	2.75
LYS	257	CE	257	NZ	229	OD1	229	CG	ASP	3.30*
THR	261	CB	261	OG1	259	O	259	C	LEU	3.08
TYR	265	CZ	265	OH	190	OD1	190	CG	ASP(B)	2.63
ASP	268	C	269	N	267	OG	267	CB	SER	3.19
ASN	270	C	271	N	269	OD1	269	CG	ASP	3.16
LYS	272	CE	272	NZ	190	OD1	190	CG	ASP(B)	3.12*
LYS	272	CE	272	NZ	190	OD2	190	CG	ASP(B)	3.11*
ASN	275	CG	275	ND2	188	OE2	188	CD	GLU(B)	3.11
LYS	279	CE	279	NZ	188	OE1	188	CD	GLU(B)	2.80*
THR	280	CB	280	OG1	276	O	276	C	TYR	2.78
LEU	290	C	291	N	288	OD1	288	CG	ASP	3.23
HIS	292	CG	292	ND1	327	O	327	C	VAL	2.69
LYS	294	CE	294	NZ	309	O	309	C	THR	3.14
THR	297	CB	297	OG1	293	O	293	C	ASN	2.92
SER	299	CB	299	OG	296	O	296	C	TYR	2.75
LYS	300	CE	300	NZ	343	OD2	343	CG	ASP	3.09*
SER	301	CB	301	OG	297	O	297	C	THR	2.80
THR	304	CB	304	OG1	301	O	301	C	SER	2.82
ARG	307	C	308	N	306	OD1	306	CG	ASP	3.09

(continued)

ARG	308	CD	308	NE	306	OD1	306	CG	ASP	2.92*
ARG	308	CZ	308	NH2	306	OD2	306	CG	ASP	3.15*
THR	309	CB	309	OG1	306	O	306	C	ASP	2.84
THR	314	CB	314	OG1	311	O	311	C	MET	2.72
LEU	314	C	315	N	288	OD1	288	CG	ASP	3.10
MET	315	C	316	N	288	OD2	288	CG	ASP	2.82
LYS	316	C	317	N	314	OG1	314	CB	THR	3.29
ASN	328	C	329	N	332	OG1	332	CB	THR	2.90
HIS	330	CG	330	ND1	10	OE1	10	CD	GLN	2.85
ASP	330	C	331	N	329	OD1	329	CG	ASN	2.82
THR	331	C	332	N	329	OD1	329	CG	ASN	3.05
THR	332	CB	332	OG1	328	OD1	328	CG	ASP	2.71
THR	332	CB	332	OG1	329	O	329	C	ASN	3.21
GLY	334	C	335	N	339	OE1	339	CD	GLN	2.93
GLN	336	CD	336	NE2	15	OH	15	CZ	TYR	3.01
SER	340	CB	340	OG	292	NE2	292	CE1	HIS	2.76
SER	340	CB	340	OG	338	O	338	C	LEU	2.81
VAL	341	C	342	N	328	OD2	328	CG	ASP	3.02
ASP	342	C	343	N	299	OG	299	CB	SER	3.30
TRP	345	CE2	345	NE1	299	O	299	C	SER	2.81
LYS	347	CE	347	NZ	328	OD2	328	CG	ASP	2.71*
LYS	347	CE	347	NZ	368	OD1	368	CG	ASP	2.84*
TYR	351	CZ	351	OH	368	OD2	368	CG	ASP	2.66
THR	356	CB	356	OG1	352	O	352	C	ALA	2.76
ARG	357	CZ	357	NH1	401	OD1	401	CG	ASP(C)	2.80*
ARG	357	CZ	357	NH2	401	OD2	401	CG	ASP(C)	2.79*
GLN	357	C	358	N	399	OE1	399	CD	GLN(C)	2.78
GLU	358	C	359	N	359	OE1	359	CD	GLU	2.88
PHE	364	C	365	N	351	OH	351	CZ	TYR	3.21
TYR	369	CZ	369	OH	385	OD1	385	CG	ASP	2.74
SER	379	CB	379	OG	369	O	369	C	TYR	2.70
LYS	381	CE	381	NZ	369	OH	369	CZ	TYR	2.79
LYS	381	CE	381	NZ	385	OD2	385	CG	ASP	2.91*
ARG	391	CD	391	NE	6	O	6	C	GLY	3.05
ARG	391	CZ	391	NH1	355	O	355	C	LEU	2.98
ARG	391	CZ	391	NH2	6	O	6	C	GLY	2.87
ARG	392	CD	392	NE	35	O	35	C	SER	3.21
ARG	392	CZ	392	NH1	393	OD2	393	CG	ASP	2.91*
ARG	392	CZ	392	NH2	35	O	35	C	SER	2.93

Domain C

TYR	396	CZ	396	OH	392	O	392	C	ARG(A)	2.83
GLN	399	CD	399	NE2	359	OE1	359	CD	GLU(A)	3.20
GLN	399	CD	399	NE2	359	OE2	359	CD	GLU(A)	3.27
HIS	400	CG	400	ND1	412	OG1	412	CB	THR	3.24
TRP	411	CE2	411	NE1	356	OG1	356	CB	THR(A)	2.86
THR	412	CB	412	OG1	439	O	439	C	TYR	2.70
ARG	413	CD	413	NE	397	O	397	C	GLY	3.07
ARG	413	CZ	413	NH1	356	O	356	C	THR(A)	2.85
VAL	415	C	416	N	422	OG	422	CB	SER	2.91
THR	417	CB	417	OG1	418	OE1	418	CD	GLU	3.15
GLU	417	C	418	N	418	OE1	418	CD	GLU	2.76
LYS	419	CE	419	NZ	3	O	3	C	PRO(A)	2.88

(continued)

SER	422	CB	422	OG	419	O	419	C	LYS	2.56
THR	429	CB	429	OG1	432	O	432	C	PRO	3.09
GLY	430	C	431	N	429	OG1	429	CB	THR	3.19
SER	435	CB	435	OG	469	OE2	469	CD	GLU	2.87
LYS	436	CE	436	NZ	404	OD1	404	CG	ASP	2.77*
LYS	436	CE	436	NZ	408	O	408	C	ILE	2.81
TYR	438	C	439	N	402	OH	402	CZ	TYR	2.91
LYS	441	C	442	N	439	OH	439	CZ	TYR	3.18
GLN	442	C	443	N	443	OE1	443	CD	GLN	2.74
GLN	443	CD	443	NE2	414	OE1	414	CD	GLU	3.04
THR	452	C	453	N	451	OD1	451	CG	ASP	2.96
THR	453	CB	453	OG1	451	OD1	451	CG	ASP	2.66
ASN	454	C	455	N	453	OG1	453	CB	THR	3.19
ASN	455	CG	455	ND2	476	OG	476	CB	SER	3.29
ASN	455	CG	455	ND2	477	O	477	C	VAL	2.81
ARG	455	C	456	N	451	OD2	451	CG	ASP	2.96
ARG	456	CD	456	NE	458	OD1	458	CG	ASP	2.94*
ARG	456	CZ	456	NH1	473	OD1	473	CG	ASN	2.82
ARG	456	CZ	456	NH2	458	OD2	458	CG	ASP	2.92*
ASP	457	C	458	N	458	OD1	458	CG	ASP	2.67
ASP	464	C	465	N	463	OD1	463	CG	ASN	2.76
GLY	465	C	466	N	463	OD1	463	CG	ASN	2.94
TRP	466	C	467	N	463	OD1	463	CG	ASN	2.96
LYS	471	CE	471	NZ	469	O	469	C	GLU	3.06
ASN	473	CG	473	ND2	455	O	455	C	ASN	3.08
SER	478	CB	478	OG	451	OD2	451	CG	ASP	2.92
VAL	478	C	479	N	451	OD1	451	CG	ASP	3.10
TRP	480	CE2	480	NE1	471	O	471	C	LYS	2.95
ARG	482	C	483	N	483	OT			ARG	2.73

(A)-residue of domain A
(B)-residue of domain B
(C)-residue of domain C
* - salt bridges

Table 2-6. Summary of main-chain side-chain and side-chain side-chain atoms hydrogen-bond interactions in BLA

	DONOR				ACCEPTOR					
RES	ATOM1		ATOM2		ATOM3		ATOM4		RES	Dist23
Domain A										
ASN	3	C	4	N	396	OH	396	CZ	TYR(C)	3.15
ASN	4	CG	4	ND2	391	O	391	C	ARG	2.78
ASN	4	CG	4	ND2	395	O	395	C	ALA	2.78
THR	5	C	6	N	38	OG1	38	CB	THR	2.88
TYR	10	CZ	10	OH	28	OD2	28	CG	ASP	2.57
TRP	12	C	13	N	12	OE2	12	CD	GLU	3.14
TRP	12	C	13	N	330	OE1	330	CD	GLN	2.90
TYR	13	C	14	N	12	OE2	12	CD	GLU	2.63
TYR	14	CZ	14	OH	333	OE1	333	CD	GLN	2.94
ASN	17	CG	17	ND2	51	OE1	51	CD	GLN	2.90
GLN	19	C	20	N	18	OD1	18	CG	ASP	2.97
LYS	23	CE	23	NZ	20	OE1	20	CD	GLN	2.40
LYS	23	CE	23	NZ	82	OE2	82	CD	GLU	2.99*
ARG	24	CZ	24	NH1	18	OD1	18	CG	ASP	2.95*
ARG	24	CZ	24	NH2	18	OD2	18	CG	ASP	2.39*
SER	29	CB	29	OG	89	O	89	C	SER	3.10
TYR	31	CZ	31	OH	385	OE2	385	CD	GLU	2.36
TRP	41	CE2	41	NE1	100	OD2	100	CG	ASP	2.99
TYR	46	CZ	46	OH	100	O	100	C	ASP	2.68
LYS	47	CE	47	NZ	63	OD2	63	CG	ASP	2.79*
LYS	47	CE	47	NZ	67	O	67	C	PHE	2.85
LYS	47	CE	47	NZ	72	O	72	C	THR	3.15
THR	48	C	49	N	53	OD2	53	CG	ASP	2.96
THR	49	CB	49	OG1	53	OD2	53	CG	ASP	2.96
GLN	51	CD	51	NE2	72	OG1	72	CB	THR	2.86
GLY	54	C	55	N	53	OD1	53	CG	ASP	2.64
TYR	56	CZ	56	OH	100	OD2	100	CG	ASP	2.65
GLY	56	C	57	N	53	OD1	53	CG	ASP	3.17
TYR	59	CZ	59	OH	110	OD1	110	CG	ASP(B)	2.71
TYR	59	CZ	59	OH	110	OD2	110	CG	ASP(B)	2.89
TYR	61	C	62	N	60	OD1	60	CG	ASP	3.06
TYR	62	CZ	62	OH	211	OE2	211	CD	GLU	2.67
GLY	64	C	65	N	63	OD1	63	CG	ASP	3.18
GLU	65	C	66	N	63	OD1	63	CG	ASP	2.94
PHE	66	C	67	N	63	OD1	63	CG	ASP	3.00
GLN	69	CD	69	NE2	60	OD2	60	CG	ASP	2.94
GLN	69	CD	69	NE2	63	OD2	63	CG	ASP	2.94
THR	72	CB	72	OG1	74	O	74	C	ARG	2.69
ARG	74	CZ	74	NH1	17	OD1	17	CG	ASN	2.69
ARG	74	CZ	74	NH1	75	O	75	C	THR	2.98
ARG	74	CZ	74	NH1	76	O	76	C	LYS	3.22
ARG	74	CZ	74	NH2	51	OE1	51	CD	GLN	3.24
THR	75	CB	75	OG1	78	O	78	C	GLY	3.18
LYS	76	CE	76	NZ	15	O	15	C	MET	2.70
TYR	76	C	77	N	75	OG1	75	CB	THR	3.18
TYR	77	CZ	77	OH	54	O	54	C	VAL	2.61
THR	78	C	79	N	82	OE1	82	CD	GLU	2.87

(continued)

LYS	80	CE	80	NZ	66	OE1	66	CD	GLU	3.01*
LYS	80	CE	80	NZ	150	OH	150	CZ	TYR(B)	3.23
LYS	80	CE	80	NZ	222	OE2	222	CD	GLU	2.59*
GLN	84	CD	84	NE2	222	O	222	C	GLU	3.12
SER	89	CB	89	OG	85	O	85	C	SER	3.02
SER	92	CB	92	OG	89	O	89	C	SER	2.72
ARG	93	CZ	93	NH1	29	OG	29	CB	SER	3.17
VAL	96	C	97	N	226	OD2	226	CG	ASP	2.78
TYR	98	CZ	98	OH	358	OH	358	CZ	TYR	2.85
VAL	101	C	102	N	46	OH	46	CZ	TYR	3.01

Domain B

LYS	106	CE	106	NZ	59	O	59	C	TYR	2.95
GLU	118	C	119	N	130	OG	130	CB	SER	3.09
ALA	122	C	123	N	121	OD1	121	CG	ASP	2.92
ARG	125	CD	125	NE	122	O	122	C	PRO	2.95
ARG	125	CZ	125	NH1	195	O	195	C	TYR	2.82
ASN	125	C	126	N	124	OD1	124	CG	ASP	3.14
ARG	127	CZ	127	NH1	121	OD2	121	CG	ASP	2.81*
HIS	133	CG	133	ND1	117	O	117	C	ALA	3.29
HIS	133	CG	133	ND1	131	O	131	C	GLY	3.21
LYS	135	C	136	N	166	OD1	166	CG	ASP	3.04
LYS	136	CE	136	NZ	114	OD1	114	CG	ASP	2.70*
TRP	138	CE2	138	NE1	167	OE1	167	CD	GLU	2.93
THR	139	CB	139	OG1	113	OE1	113	CD	GLU	2.53
HIS	141	C	142	N	110	OD2	110	CG	ASP	2.65
PHE	142	C	143	N	110	OD2	110	CG	ASP	3.04
GLY	144	C	145	N	69	OE1	69	CD	GLN(A)	2.96
ARG	145	C	146	N	69	OE1	69	CD	GLN(A)	3.01
ARG	146	CD	146	NE	60	OD2	60	CG	ASP(A)	2.77*
ARG	146	CZ	146	NH1	149	O	149	C	THR	2.82
ARG	146	CZ	146	NH1	151	O	151	C	SER	2.92
ARG	146	CZ	146	NH2	60	OD1	60	CG	ASP(A)	3.09*
TYR	150	CZ	150	OH	66	OE2	66	CD	GLU(A)	2.75
ASP	151	C	152	N	152	OD1	152	CG	ASP	2.58
PHE	152	C	153	N	151	OG	151	CB	SER	3.28
LYS	154	CE	154	NZ	142	O	142	C	HIS	2.94
HIS	156	CG	156	ND1	113	OE2	113	CD	GLU	2.76
TRP	156	C	157	N	113	OE1	113	CD	GLU	2.77
ASP	163	C	164	N	195	OH	195	CZ	TYR	3.12
TRP	165	CE2	165	NE1	167	OE1	167	CD	GLU	2.94
SER	167	C	168	N	166	OD1	166	CG	ASP	3.02
SER	168	CB	168	OG	166	OD1	166	CG	ASP	2.90
ARG	169	CD	169	NE	166	OD2	166	CG	ASP	2.64*
ARG	169	CZ	169	NH2	134	O	134	C	LEU	2.52
LYS	170	CE	170	NZ	167	OE2	167	CD	GLU	2.77*
ASN	172	CG	172	ND2	164	O	164	C	ASP	2.48
LYS	176	CE	176	NZ	180	O	180	C	LYS	3.24
LYS	180	CE	180	NZ	158	O	158	C	TYR	2.71
LYS	180	CE	180	NZ	202	OD2	202	CG	ASP	2.52*
TRP	182	CE2	182	NE1	125	O	125	C	ARG	2.98
ASP	182	C	183	N	194	OD2	194	CG	ASP	2.74
SER	187	CB	187	OG	189	OE1	189	CD	GLU	2.73
GLU	188	C	189	N	187	OG	187	CB	SER	3.22
ASN	192	CG	192	ND2	183	O	183	C	ASP	3.05

(continued)

ASP	193	C	194	N	192	OD1	192	CG	ASN	2.91
TYR	195	CZ	195	OH	198	O	198	C	TYR	2.69
TYR	202	C	203	N	183	OD2	183	CG	ASP	2.85
TYR	203	CZ	203	OH	103	O	103	C	ILE	2.62
ASP	203	C	204	N	202	OD1	202	CG	ASP	2.88
Domain A										
ARG	214	CZ	214	NH1	62	OH	62	CZ	TYR	2.59
ARG	214	CZ	214	NH1	150	O	150	C	TYR(B)	2.84
ARG	214	CZ	214	NH2	150	O	150	C	TYR(B)	2.89
TRP	215	CE2	215	NE1	61	O	61	C	LEU	2.97
THR	217	CB	217	OG1	213	O	213	C	LYS	3.23
TYR	219	CZ	219	OH	100	O	100	C	ASP	2.79
ARG	229	CZ	229	NH1	100	OD1	100	CG	ASP	2.85*
ARG	229	CZ	229	NH1	231	OD1	231	CG	ASP	3.01*
LYS	234	CE	234	NZ	263	O	263	C	TRP	2.78
HIS	235	CG	235	ND1	193	O	193	C	TYR(B)	2.62
LYS	237	CE	237	NZ	204	OD1	204	CG	ASP(B)	2.77*
ARG	242	CZ	242	NH1	243	OD1	243	CG	ASP	2.61*
TRP	244	CE2	244	NE1	212	O	212	C	ILE	3.02
ARG	249	CD	249	NE	255	O	255	C	GLU	2.78
ARG	249	CZ	249	NH1	280	O	280	C	ASN	2.83
ARG	249	CZ	249	NH2	256	O	256	C	MET	3.01
LYS	251	CE	251	NZ	250	OE2	250	CD	GLU	2.72*
THR	252	CB	252	OG1	248	O	248	C	VAL	2.61
LYS	254	CE	254	NZ	225	O	225	C	LEU	2.72
THR	258	CB	258	OG1	256	O	256	C	MET	3.15
TRP	263	CE2	263	NE1	290	OH	290	CZ	TYR	3.27
SER	264	CB	264	OG	266	O	266	C	ASP	3.18
ASP	265	C	266	N	264	OG	264	CB	SER	3.12
GLY	267	C	268	N	266	OD1	266	CG	ASP	3.14
ALA	268	C	269	N	266	OD2	266	CG	ASP	3.01
ASN	272	CG	272	ND2	185	OE2	185	CD	GLU(B)	2.92
TYR	273	CZ	273	OH	281	NE2	281	CE1	HIS	2.75
LYS	276	CE	276	NZ	185	OE1	185	CD	GLU(B)	2.69*
LYS	276	CE	276	NZ	272	OD1	272	CG	ASN	2.95
THR	277	CB	277	OG1	273	O	273	C	TYR	2.79
ASN	280	CG	280	ND2	242	O	242	C	ARG	3.05
ASN	280	CG	280	ND2	246	OD1	246	CG	ASN	3.00
HIS	281	CG	281	ND1	280	OD1	280	CG	ASN	2.65
HIS	289	CG	289	ND1	324	O	324	C	VAL	2.64
GLN	291	CD	291	NE2	306	O	306	C	LYS	3.03
GLN	291	CD	291	NE2	309	O	309	C	ASN	2.85
SER	296	CB	296	OG	293	O	293	C	HIS	2.91
THR	297	CB	297	OG1	293	O	293	C	HIS	2.90
THR	297	CB	297	OG1	294	O	294	C	ALA	3.24
TYR	302	CZ	302	OH	291	OE1	291	CD	GLN	2.46
ARG	304	C	305	N	303	OD1	303	CG	ASP	3.18
ARG	305	CD	305	NE	303	OD1	303	CG	ASP	2.92*
ARG	305	CZ	305	NH2	303	OD2	303	CG	ASP	2.93*
VAL	311	C	312	N	285	OD1	285	CG	ASP	2.96
VAL	312	C	313	N	285	OD2	285	CG	ASP	2.73
SER	313	C	314	N	311	OG1	311	CB	THR	3.06

(continued)

SER	314	CB	314	OG	311	O	311	C	THR	2.76
LYS	315	CE	315	NZ	271	OE1	271	CD	GLU	2.93*
LYS	315	CE	315	NZ	271	OE2	271	CD	GLU	3.15*
LYS	319	CE	319	NZ	279	O	279	C	PHE	2.83
SER	320	CB	320	OG	285	OD2	285	CG	ASP	3.15
ASN	325	C	326	N	329	OG1	329	CB	THR	3.06
ASN	326	CG	326	ND2	328	OD2	328	CG	ASP	3.22
HIS	327	CG	327	ND1	9	OE1	9	CD	GLN	2.88
ASP	327	C	328	N	326	OD1	326	CG	ASN	2.92
THR	328	C	329	N	326	OD1	326	CG	ASN	3.13
THR	329	CB	329	OG1	325	OD1	325	CG	ASP	2.62
GLN	330	CD	330	NE2	12	OE2	12	CD	GLU	2.75
GLY	331	C	332	N	336	OE1	336	CD	GLU	2.80
GLN	333	CD	333	NE2	327	O	327	C	HIS	3.17
SER	337	CB	337	OG	289	NE2	289	CE1	HIS	2.76
SER	337	CB	337	OG	335	O	335	C	LEU	3.05
THR	338	CB	338	OG1	329	O	329	C	THR	2.81
VAL	338	C	339	N	325	OD2	325	CG	ASP	2.91
GLN	339	C	340	N	296	OG	296	CB	SER	2.95
TRP	342	CE2	342	NE1	296	O	296	C	SER	2.96
TRP	342	CE2	342	NE1	340	OE1	340	CD	GLN	3.03
LYS	344	CE	344	NZ	325	OD2	325	CG	ASP	2.71*
LYS	344	CE	344	NZ	365	OD1	365	CG	ASP	2.62*
TYR	348	CZ	348	OH	365	OD2	365	CG	ASP	2.43
THR	353	CB	353	OG1	349	O	349	C	ALA	2.76
ARG	354	CZ	354	NH1	401	OD1	401	CG	ASP(C)	2.84*
ARG	354	CZ	354	NH2	401	OD2	401	CG	ASP(C)	3.01*
GLU	354	C	355	N	399	OE1	399	CD	GLN(C)	2.83
PHE	361	C	362	N	348	OH	348	CZ	TYR	3.20
TYR	367	CZ	367	OH	28	OD2	28	CG	ASP	2.61
THR	369	CB	369	OG1	376	OE1	376	CD	GLU	2.62
LYS	369	C	370	N	12	OE1	12	CD	GLU	2.86
GLY	370	C	371	N	376	OE1	376	CD	GLU	2.81
SER	372	C	373	N	376	OE2	376	CD	GLU	3.16
SER	373	CB	373	OG	376	OE2	376	CD	GLU	2.82
ARG	374	C	375	N	373	OG	373	CB	SER	2.92
ARG	375	CD	375	NE	336	OE2	336	CD	GLU	2.85*
ARG	375	CZ	375	NH2	336	OE1	336	CD	GLU	2.99*
ILE	376	C	377	N	369	OG1	369	CB	THR	2.93
ARG	391	CD	391	NE	5	O	5	C	GLY	3.26
ARG	391	CZ	391	NH1	352	O	352	C	LEU	2.76
ARG	391	CZ	391	NH2	5	O	5	C	GLY	2.85

Domain C

TYR	396	CZ	396	OH	392	O	392	C	LYS(A)	2.80
GLN	399	CD	399	NE2	401	OD1	401	CG	ASP	3.01
HIS	406	CG	406	ND1	407	OD1	407	CG	ASP	3.12*
TRP	411	CE2	411	NE1	353	OG1	353	CB	THR(A)	2.88
THR	412	CB	412	OG1	439	O	439	C	TYR	2.58
ARG	413	CD	413	NE	397	O	397	C	GLY	2.93
ARG	413	CZ	413	NH1	353	O	353	C	THR(A)	3.18
ARG	413	CZ	413	NH2	397	O	397	C	GLY	3.15
ASP	415	C	416	N	422	OG	422	CB	SER	2.75
SER	417	C	418	N	416	OD1	416	CG	ASP	2.68
SER	418	CB	418	OG	416	OD1	416	CG	ASP	2.48

(continued)

SER	422	CB	422	OG	416	O	416	C	ASP	3.09
SER	422	CB	422	OG	419	O	419	C	VAL	2.87
THR	429	CB	429	OG1	432	O	432	C	PRO	3.12
GLY	430	C	431	N	429	OG1	429	CB	THR	2.99
LYS	436	CE	436	NZ	404	OD1	404	CG	ASP	2.66*
LYS	436	CE	436	NZ	408	O	408	C	ILE	3.02
TYR	438	C	439	N	402	OH	402	CZ	TYR	2.99
ARG	441	C	442	N	439	OH	439	CZ	TYR	3.25
GLN	442	C	443	N	443	OE1	443	CD	GLN	2.56
GLN	443	CD	443	NE2	414	OE1	414	CD	GLU	3.16
ASN	444	CG	444	ND2	414	O	414	C	GLU	2.95
ASN	444	CG	444	ND2	440	O	440	C	VAL	2.72
TRP	449	CE2	449	NE1	447	OE1	447	CD	GLU	2.71
HIS	450	CG	450	ND1	481	O	481	C	VAL	3.20
THR	452	C	453	N	451	OD1	451	CG	ASP	3.00
THR	453	CB	453	OG1	451	OD1	451	CG	ASP	2.67
ASN	454	C	455	N	451	OD2	451	CG	ASP	3.15
ASN	454	C	455	N	453	OG1	453	CB	THR	3.16
ASN	455	CG	455	ND2	453	OG1	453	CB	THR	3.19
ASN	455	CG	455	ND2	477	O	477	C	VAL	2.72
ARG	455	C	456	N	451	OD2	451	CG	ASP	2.97
ARG	456	CD	456	NE	473	OD1	473	CG	ASN	3.11
ARG	456	CZ	456	NH1	458	OE1	458	CD	GLU	2.57*
SER	456	C	457	N	458	OE2	458	CD	GLU	2.84
SER	457	CB	457	OG	458	OE2	458	CD	GLU	3.09
ALA	464	C	465	N	463	OD1	463	CG	ASN	2.92
GLY	465	C	466	N	463	OD1	463	CG	ASN	3.17
TRP	466	C	467	N	463	OD1	463	CG	ASN	3.00
ASN	473	CG	473	ND2	455	O	455	C	ASN	3.20
SER	478	CB	478	OG	451	OD2	451	CG	ASP	2.90
ILE	478	C	479	N	451	OD1	451	CG	ASP	2.88
TYR	480	CZ	480	OH	471	O	471	C	HIS	2.71
GLN	482	CD	482	NE2	421	OD1	421	CG	ASN	3.09

(A)-residue of domain A
(B)-residue of domain B
(C)-residue of domain C
* - salt bridges

Table 2-7. The number of hydrogen bonding interactions in BLA and BSTA, excluding main-chain and main-chain hydrogen bonds

hydrogen bonds	main-chain and side-chain atoms		side-chain and side-chain atoms				Total	
	BSTA	BLA	BSTA		BLA		BSTA	BLA
Domain A	81	80	40	(18)[a]	51	(18)	121	131
Domain B	29	30	17	(9)	10	(5)	46	40
Domain C	26	30	14	(3)	14	(3)	40	44
Domain A/B[b]	4	5	10	(5)	9	(4)	14	14
Domain A/C[c]	4	4	2	(0)	3	(2)	6	7
Total	144	149	83	(35)	87	(32)	227	236

[a]( ): the number of salt bridges

[b]Inter-domain between A and B

[c]Inter-domain between A and C

Table 2-8. Comparison of BSTA, BLA and BAA for some criteria determining thermostability of proteins

criteria	effect[a]	BSTA	BLA	BAA
Helices-content/protein-content ratio of β-branched residues[b, c]	(-)	0.22	0.18	0.19
Number of prolines[d]	(+)	21	15	18
Number of Asn and Gln[e]	(-)	39	43	46
Arg/Arg+Lys ratio[f]	(+)	0.38	0.43	0.40

[a] (+) : factors which positively affect the thermostability of proteins
(-) : factors which negatively affect the thermostability of proteins
[b] β-branched residues: Val, Thr, Ile
[c] Vogt *et al.*, 1997
[d] Matthews *et al.*, 1987
[e] Klibanov *et al.*, 1985
[f] Mrabet *et al.*, 1992

FIGURES

REGION I

```
                Ca1
BSTA   99:DVVFDH 104
BLA   100:DVVINH 105
BAA    97:DVVLNH 102
TAKA  116:DVVANH 121
BSUA   96:DAVINH 101
PPA    96:DAVINH 101
Bar    93:DIVINH  98
          * *  *
```

REGION II

```
                A  Ca1
BSTA  200:GFRLDAVKHIKFS--------FFPD 246
BLA   197:GFRLDAVKHIKFS--------FLRD 243
BAA   197:GFRIDAAKHIKFS--------FLRD 243
TAKA  202:GLRIDTVKHVQKD--------FWPG 218
BSUA  172:GFRFDAAKHIELPDDGSYGSQFWPN 196
PPA   194:GFRLDASKH------------MWPG 205
Bar   181:AWRLDFARG------------YSPE 193
                 *   *
```

REGION III

```
                A
BSTA  262:VGEYWS-- 267
BLA   259:VAEYWQ-- 264
BAA   259:VAEYWQ-- 264
TAKA  228:IGEVLD-- 233
BSUA  206:YGEILQ-- 211
PPA   231:FQEVIDLG 238
Bar   208:VAEVWD-- 213
             *
```

REGION IY

```
                A
326:FVDNHD 331
323:FVDNHD 328
323:FVENHD 328
291:FVENHD 297
263:WVESHD 269
293:FVDNHD 299
287:AATFVD 293
           *
```

BSTA - α-amylase from *Bacillus stearothermophilus*;
BLA - α-amylase from *Bacillus licheniformis*;
BAA - α-amylase from *Bacillus amyloliquiefacines*;
TAKA -α-amylase from *Aspergillus oryzae*;
BSUA -α-amylase from *Bacillus subtilis*;
PPA - pig pancrease α-amylase;
Bar - barley α-amylase, type II (amy2);

Ca1 - calcium ion binding sites ;
A - amino acids at active sites;
▭ - amino acids nearby the active sites;
*- conserved amino acids;

Figure 1. Sequence alingnment of four highly conserved regions of some α-amylases, corresponding to the active and conserved calcium ion binding site. Figure reproduced from Nakajima *et al*., 1996.

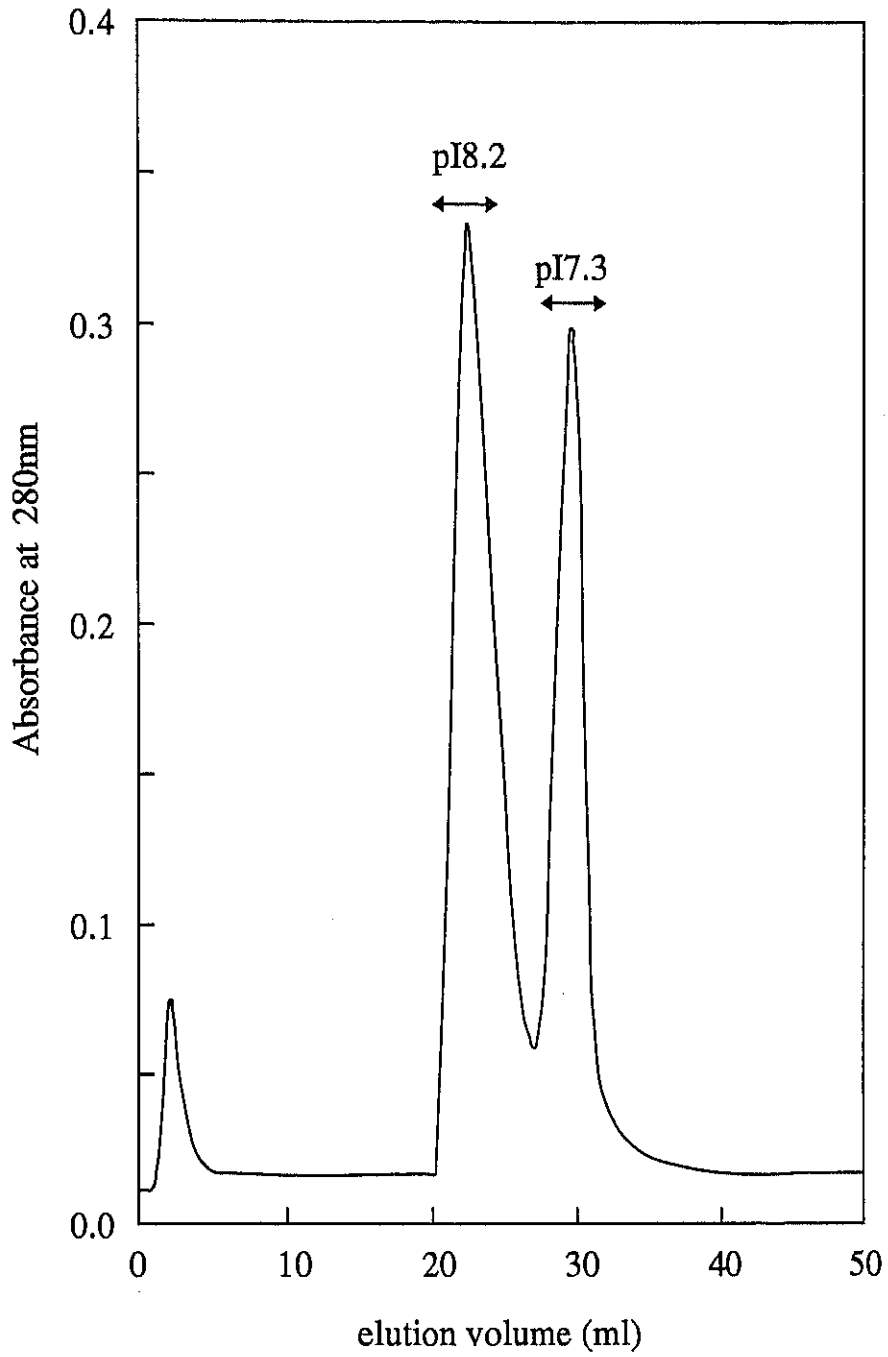


Figure 1-1. Chromatofocusing of BSTA. Conditions: column, Mono P HR 5/20; start buffer, 25mM diethanolamine-HCl diluted 1 to 20 (pH 6.0); flow rate, 0.3 ml/min. The pooled fractions are indicated by arrows.

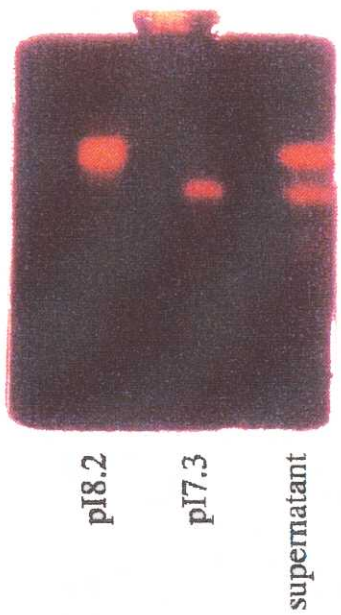


Figure 1-2. Isoelectricfocusing of culture supernatant and of protein fractions with pI 7.3 and pI 8.2 after chromatofocusing. A PhastGel IEF3-9 (Pharmacia) was used. The gel was incubated with 1% starch solution and stained with iodine for activity. The pI values were estimated using the Broad pI Calibration Kit (Pharmacia) on a CBB- stained gel (data not shown).

pTUB617

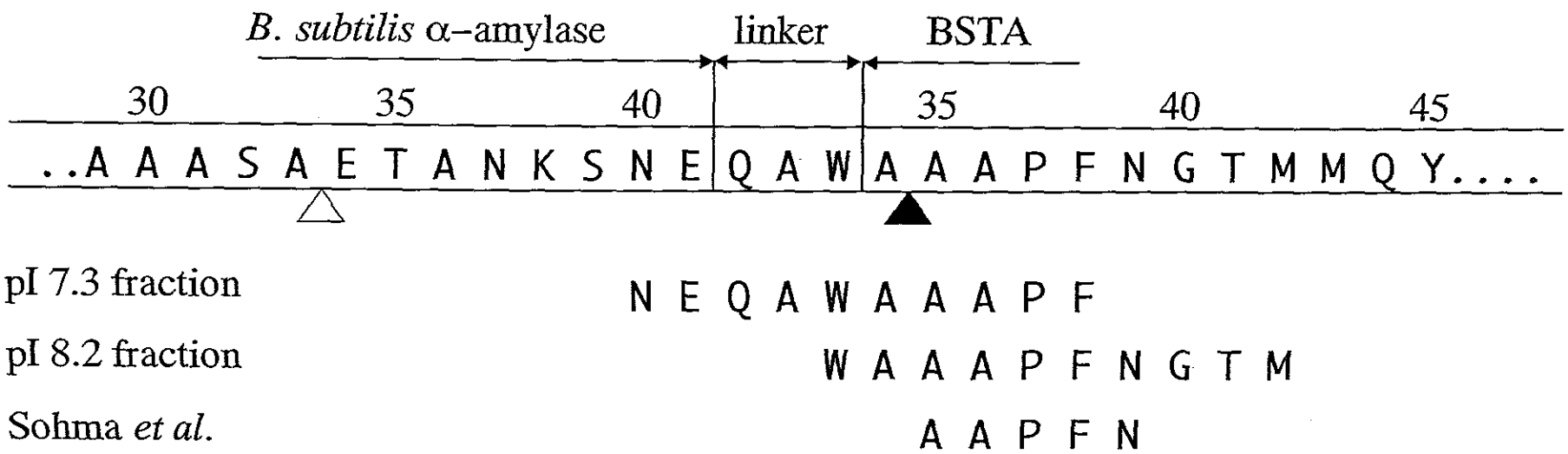


Figure 1-3. N-terminal amino acid sequence of BSTA fused with *Bacillus Subtilis* α-amylase sequence in pTUB617 and of the fractions with pI7.3 and pI8.2 purified in this study and reported by Sohma *et al.*, (1987). Amino acid are numbered from the initiator Met of each protein. Open triangle shows signal peptide cleavage site of *Bacillus subtilis* α-amylase (Takase *et al.*, (1988)); filled triangle signal peptide cleavage site of BSTA (Sohma *et al.*, (1987)). Ala35 of the precursor protein of BSTA corresponds to the N-terminal residue (Ala1) of mature BSTA.

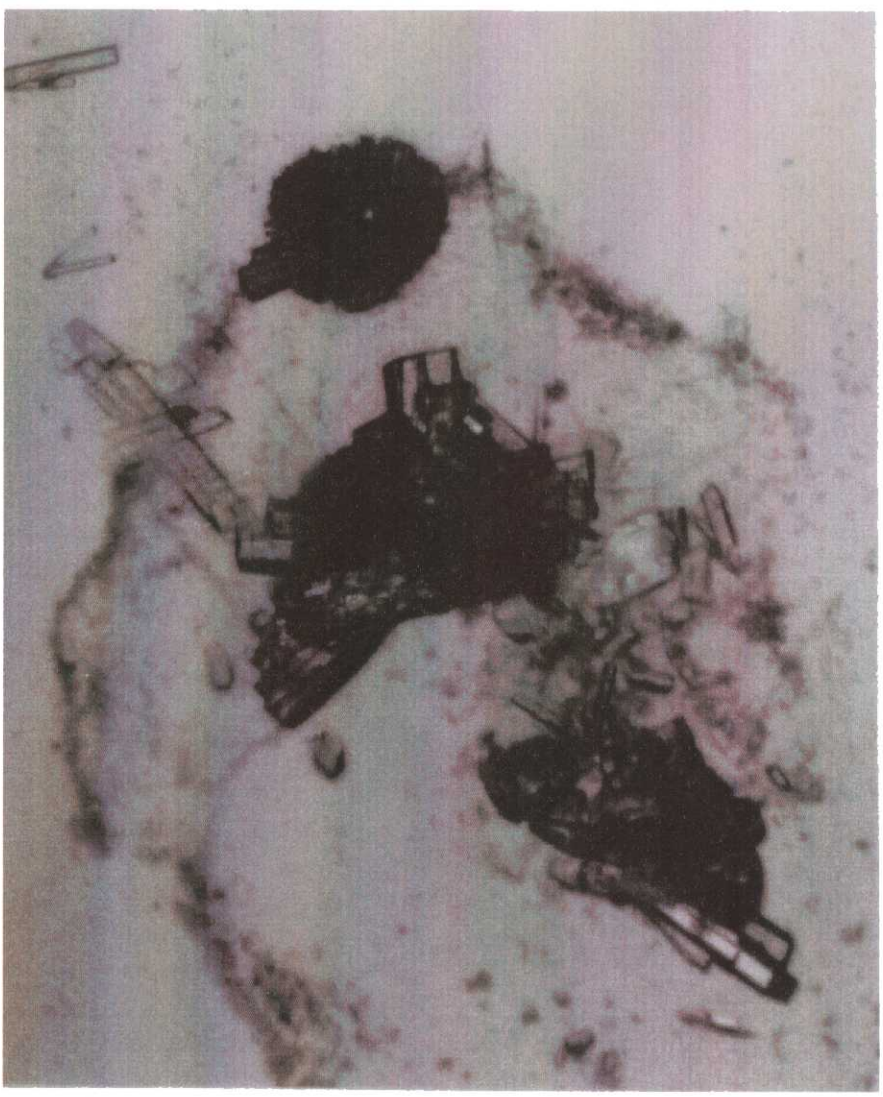


Figure 1-4. Cluster of BSTA crystals. A piece of about 0.1x0.05x0.02 mm cut out of the cluster was used for data collection.

Crystallization condition: 0.035M Na-acetate (pH 4.6), 0.035M CaCl2, 6.25% (v/v) 2-propanol, in the presence of 1.23% (w/v) acarbose.

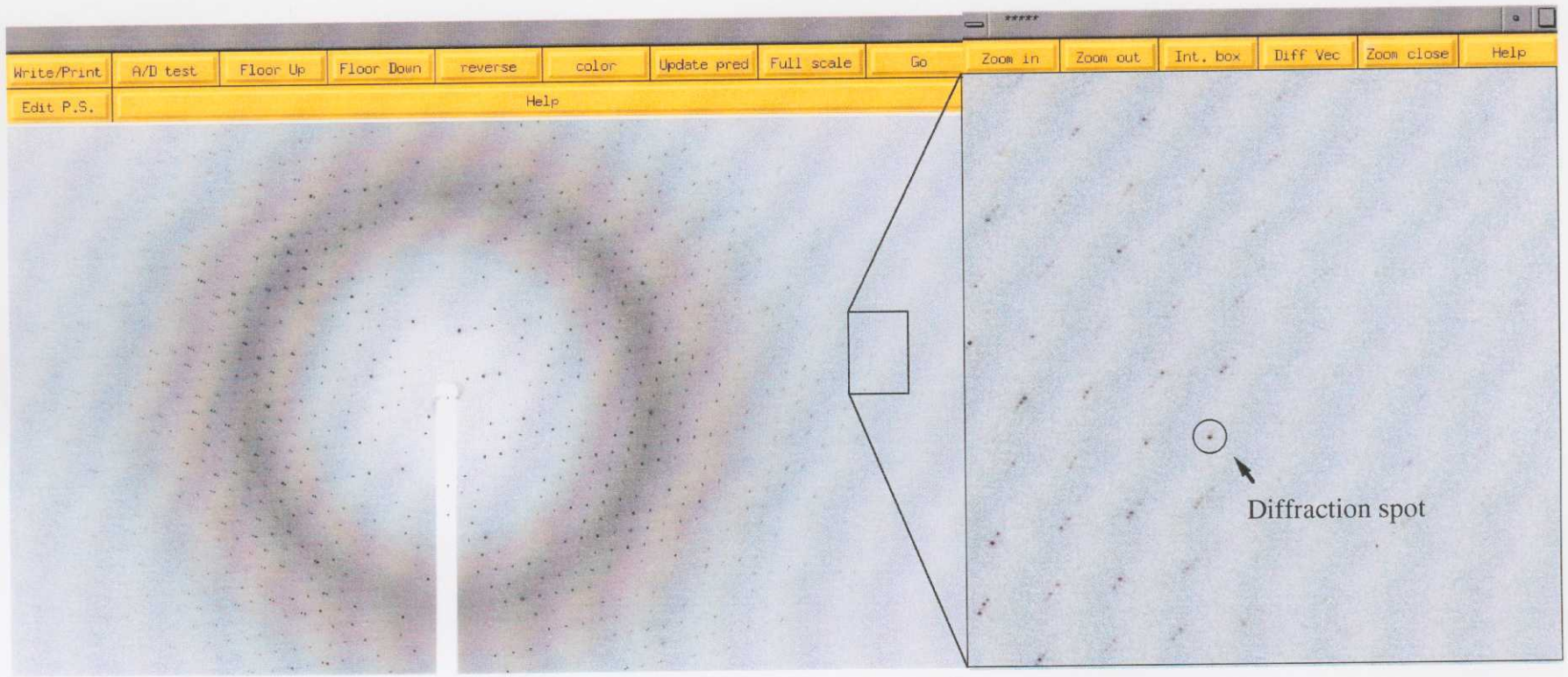


Figure 1-5. A 5.0 ° oscillation photograph for the crystals of BSTA. The image was taken on Weissenberg camera detector system at the beamline BLA6A at the Photon Factory. A close-up view corresponds to a resolution limit of 1.9 Å.

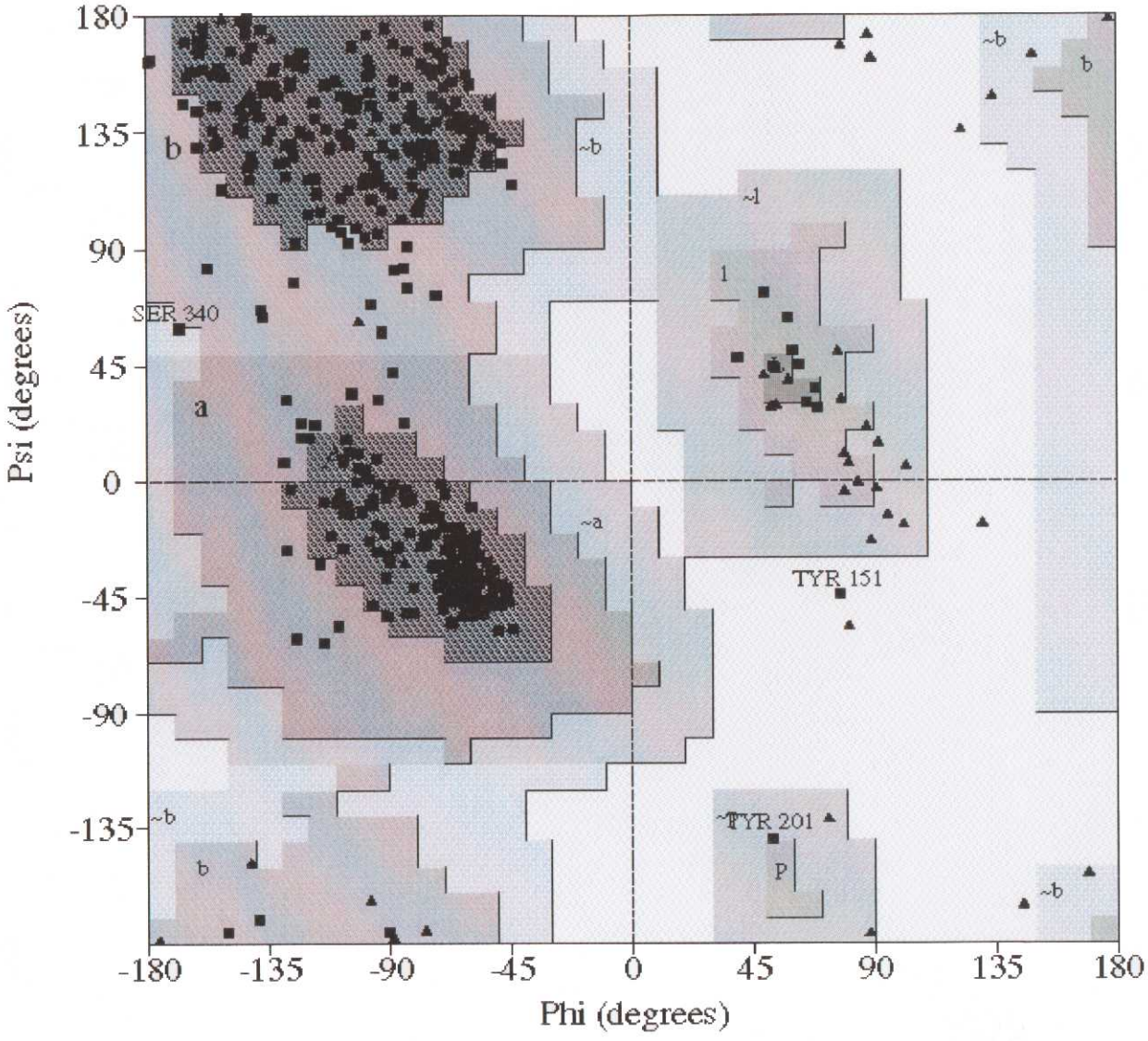


Figure 2-1. Ramachandran plot (Ramakrishnan & Ramachandran, 1965) of the atomic coordinates from the final molecular model of α-amylase from *Bacillus stearothermophilus*. There are only one outlier (Tyr151) in the plot. This figure was generated using the program PROCHECK (Laskowski, 1993).

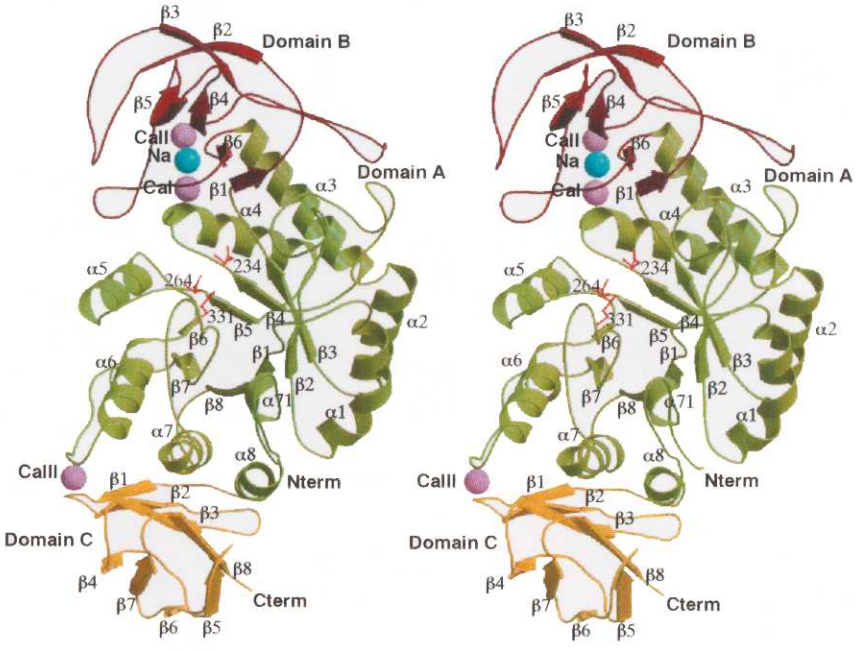


Figure 2-2. Ribbon model of BSTA generated with the program MOLSCRIPT (Kraulis, 1991); Metal ions are shown in spheres. Active site residues are numbered and are shown in liquorice. Domains are shown in different colours.

Figure 2-4. Superimposed Cα-models of BSTA (red) and (BLA) gray. BSTA is numbered at every 20 residues.

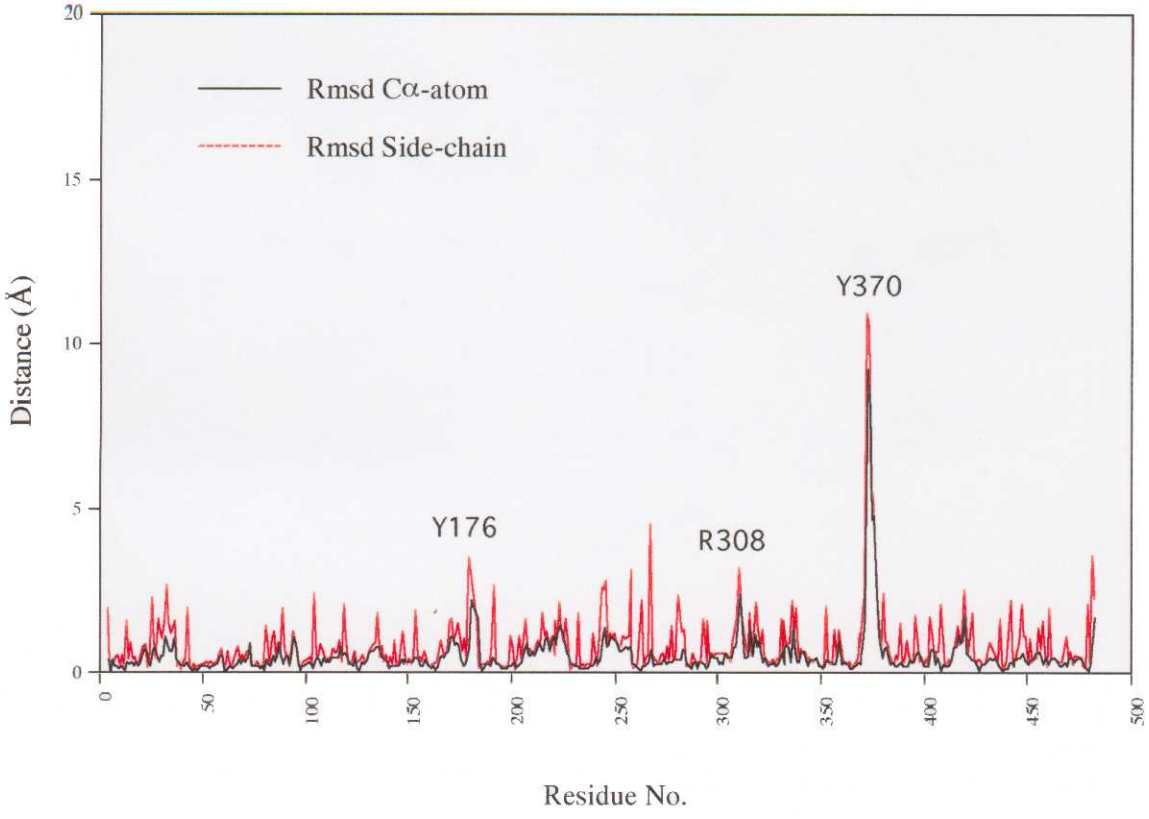


Figure 2-5. RMSD Plot of Cα and side chain atoms between BSTA and BLA.

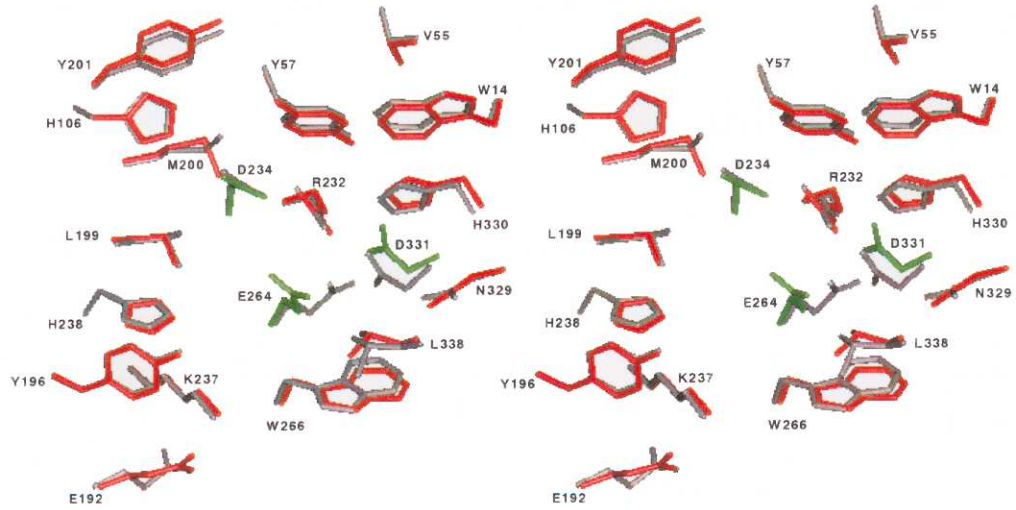


Figure 2-6. Stereoview of a superposition of the residues involved in active sites of BSTA (red) and BLA (gray). Catalytic residues are shown in green. Only BSTA residues are labeled.

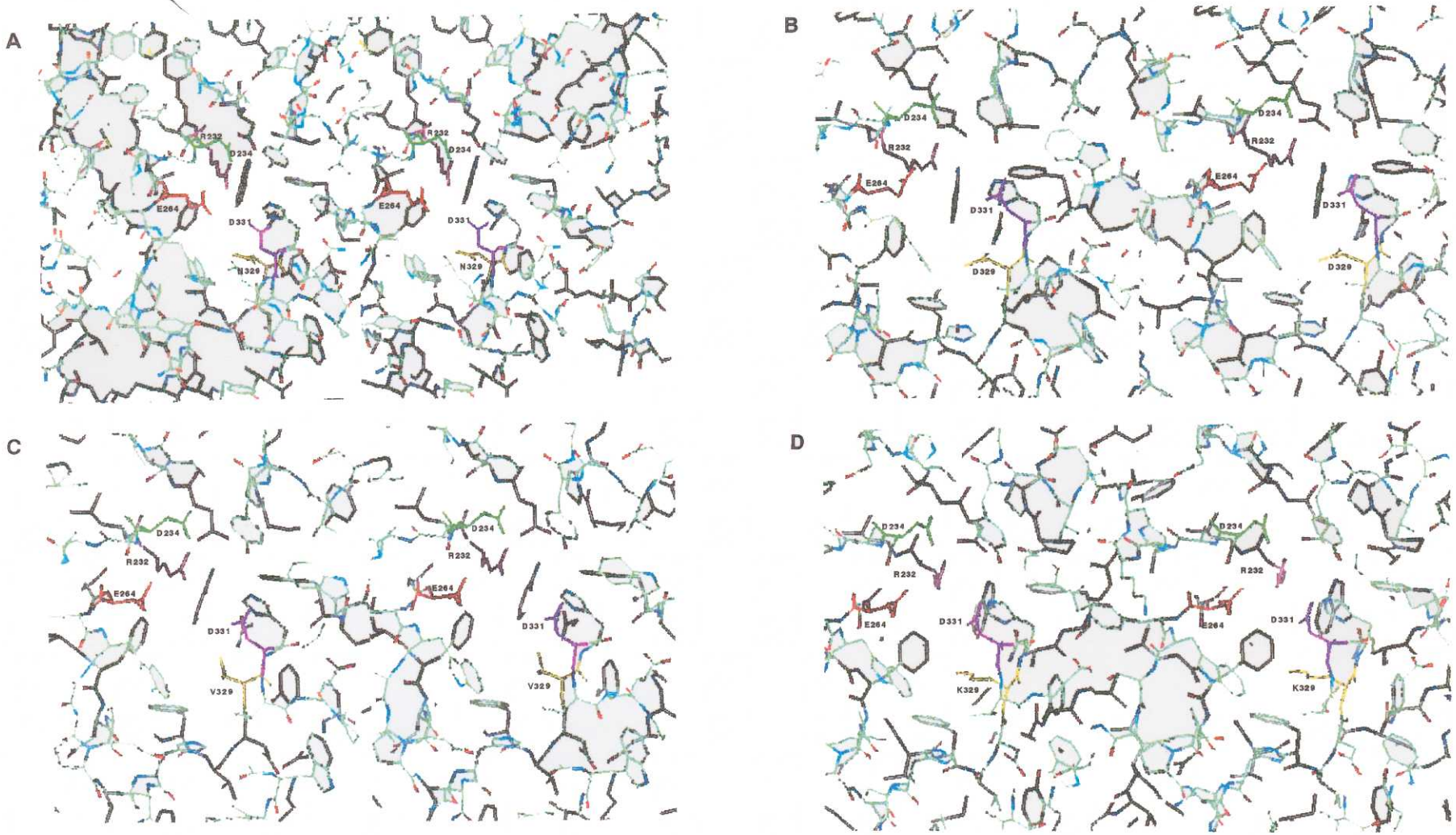


Figure 2-7. Stereoview of molecular dynamics simulation of the mutants at the Asn329 located near the active site. Mutated residues are drawn in yellow and are shown separately in a. Wild type b. N329D c. N329V d. N329K. The catalytic-site amino acids are colored and are numbered.

A

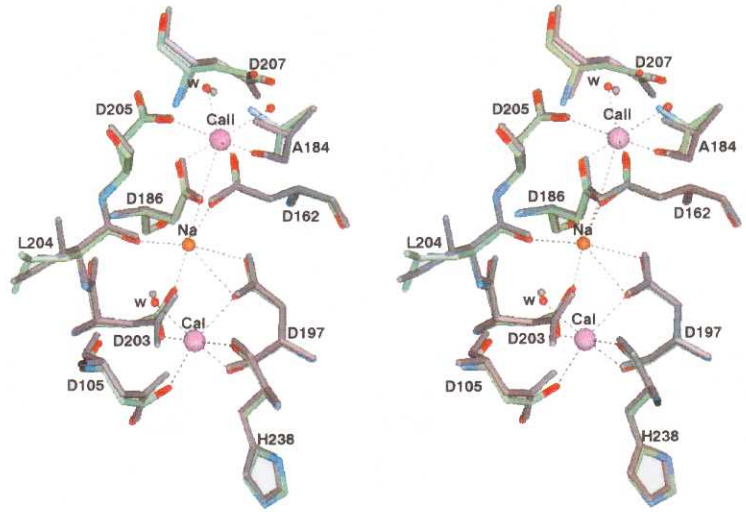
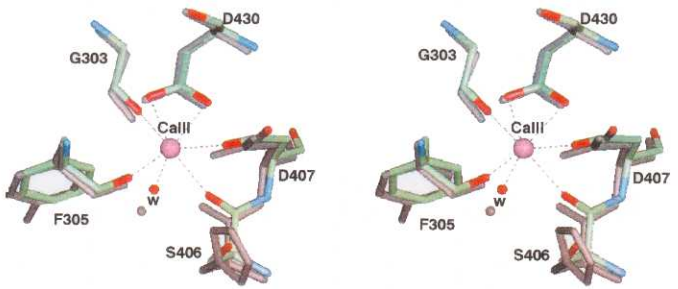


Figure 2-8. Stereoview of the superposition of the amino acid residues involved in metal-binding in BSTA (shown by element; atoms are colored in: C-green, N-blue and O-red) and in BLA (gray). Water molecules (w) are shown in small spheres. A water molecule involved in CaII-binding in BSTA is marked with an asterisk: (A) Ca^{2+}-Na^{+}-Ca^{2+} triad; (B) CaIII.

B


D430
G303
CaIII
D407
W
F305
S406
D430
G303
CaIII
D407
W
F305
S406

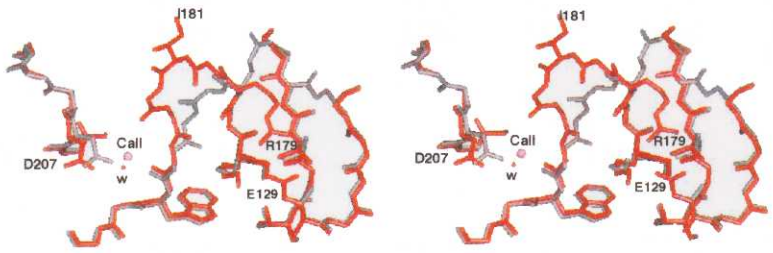


Figure 2-9. Superposition of the site of two amino acid residue insertion Ile181 - Gly182 in BSTA. BSTA is drawn in red and BLA in gray. Calcium ion is drawn as a pink ball. Residue are numbered according to BSTA.

```
BSTA  174:RIYKFRGIGKAWDW 187
BLA   173:RIYKFQG--KAWDW 184
BAA   171:RIFKFRGEGKAWDW 184
LAMY  176:KIYKFRGTGKAWDW 189
          ** ** *  *****
```

Figure 2_10. Sequence alignment of insertion site at the residues 180I-181G in BSTA, BLA, BAA and LAMY. Conserved residues are marked with asterisk.

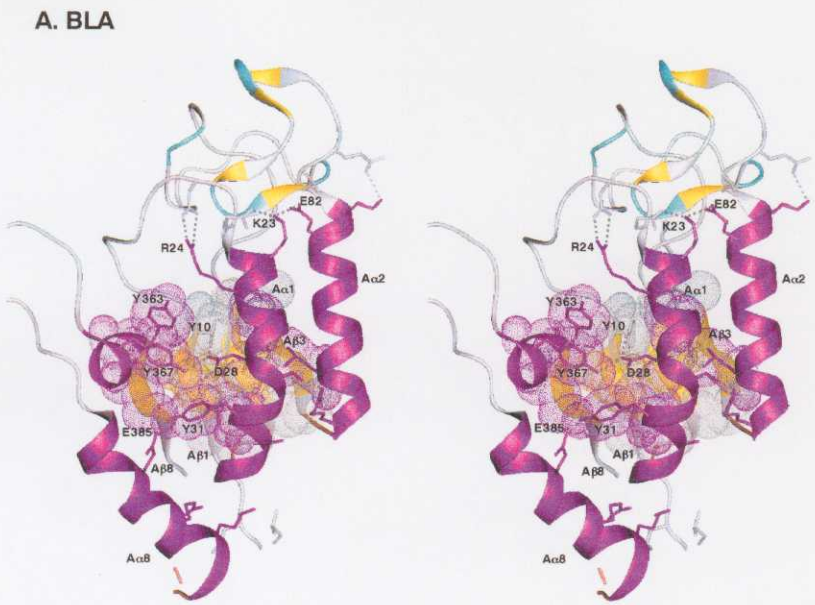
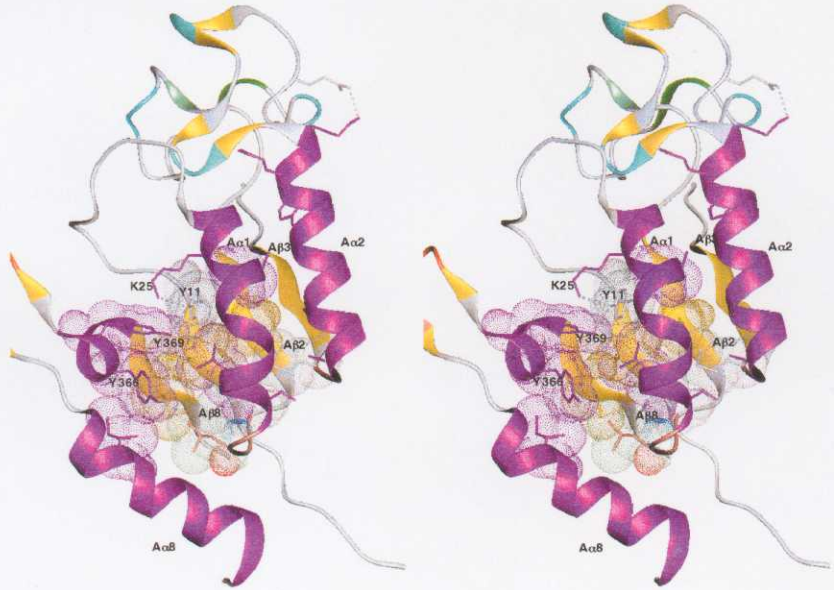
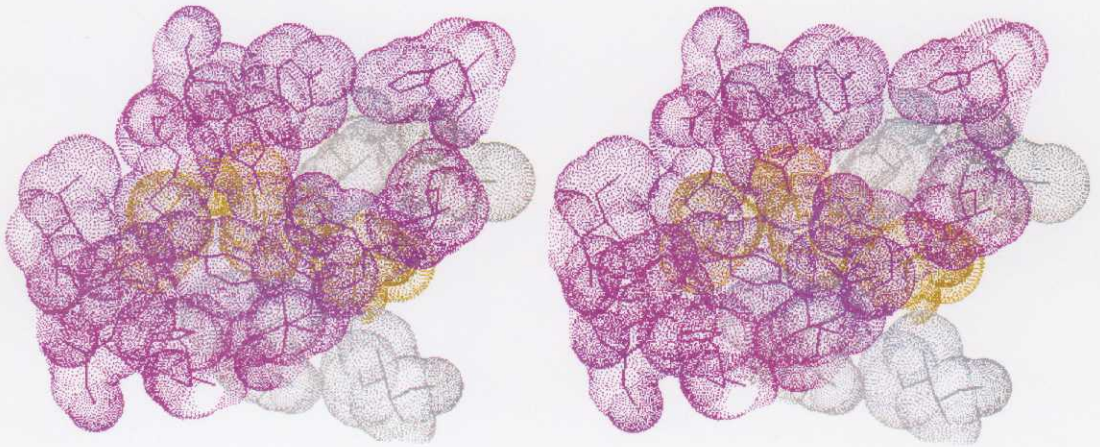


Figure 2-11. Hydrogen bonding and hydrophobic stacking interactions around Aα1, Aα2, Aα8, Aβ2 and Aβ3 a. BLA b. BSTA; Hydrophobic interactions are given in close-up view: c. BLA d. BSTA. Hydrogen bonding interactions are drawn in dash lines: hydrophobic stacking interactions are drawn as dot surfaces. Residues and dot surfaces are colored by secondary structure. Voids in BSTA are labeled with arrows.

B. BSTA

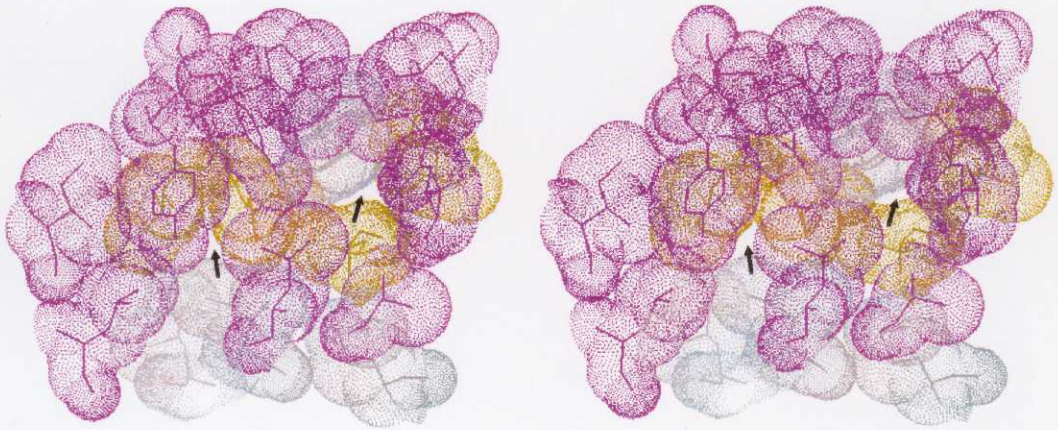

Aα1
Aβ3
Aα2
K25
Y11
Y369
Aβ2
Y366
Aβ8
Aα8
Aα1
Aβ3
Aα2
K25
Y11
Y369
Aβ2
Y366
Aβ8
Aα8

C. BLA



Hydrophobic interactions are given in close-up view: c. BLA d. BSTA. Hydrophobic stacking interactions are drawn as dot surfaces. Residues and dot surfaces are colored by secondary structure. Voids in BSTA are labeled with arrows.

D. BSTA



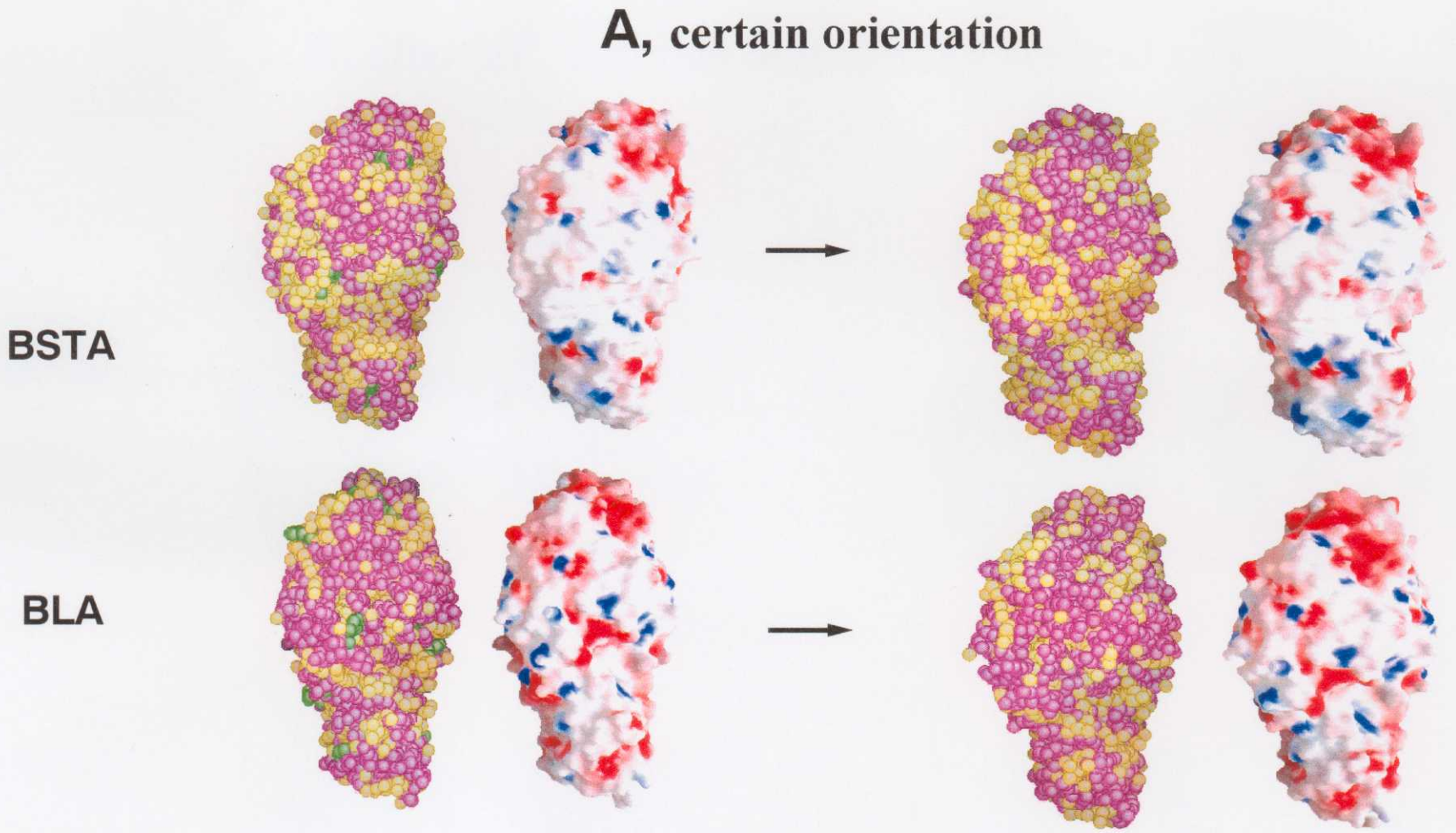
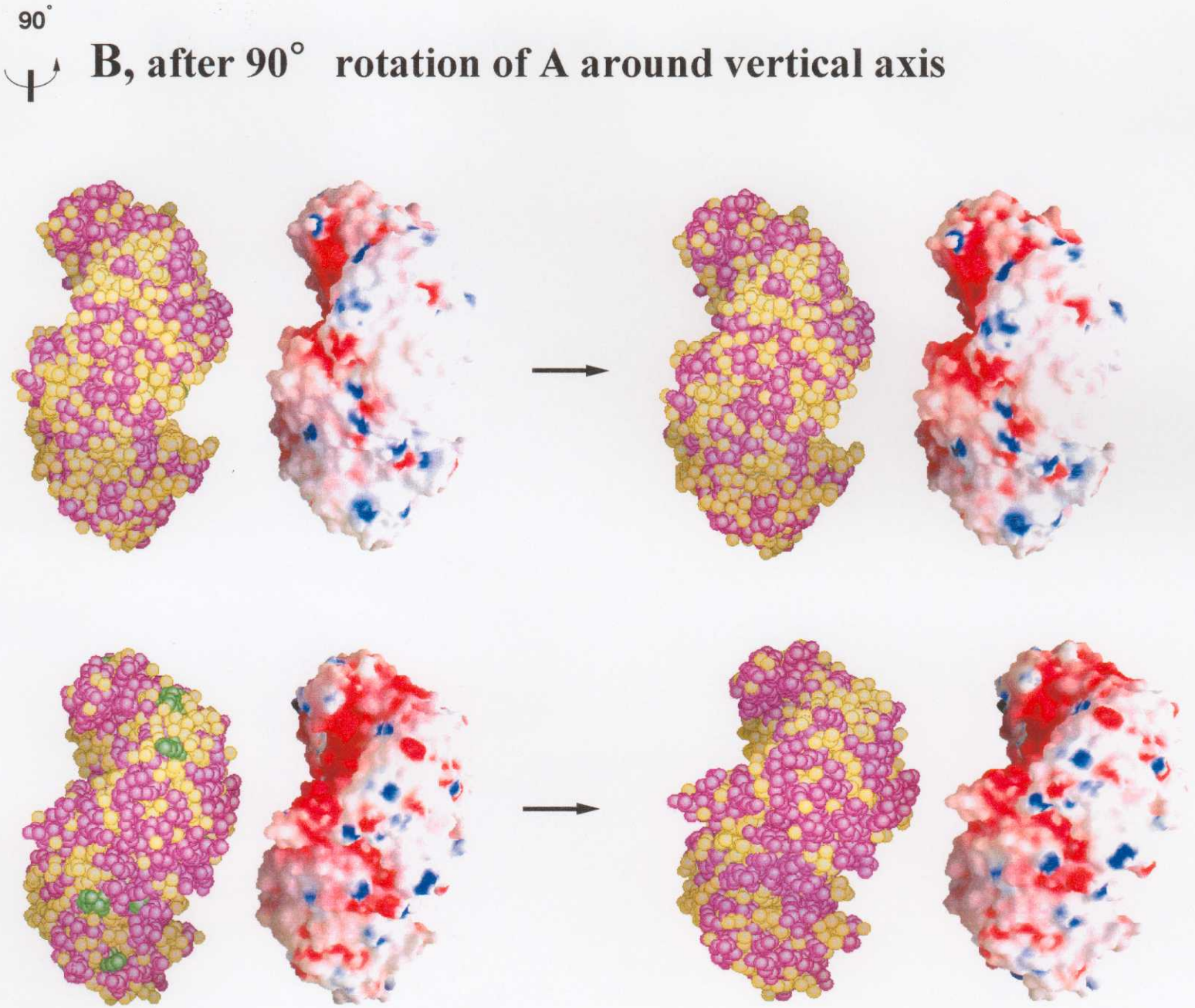


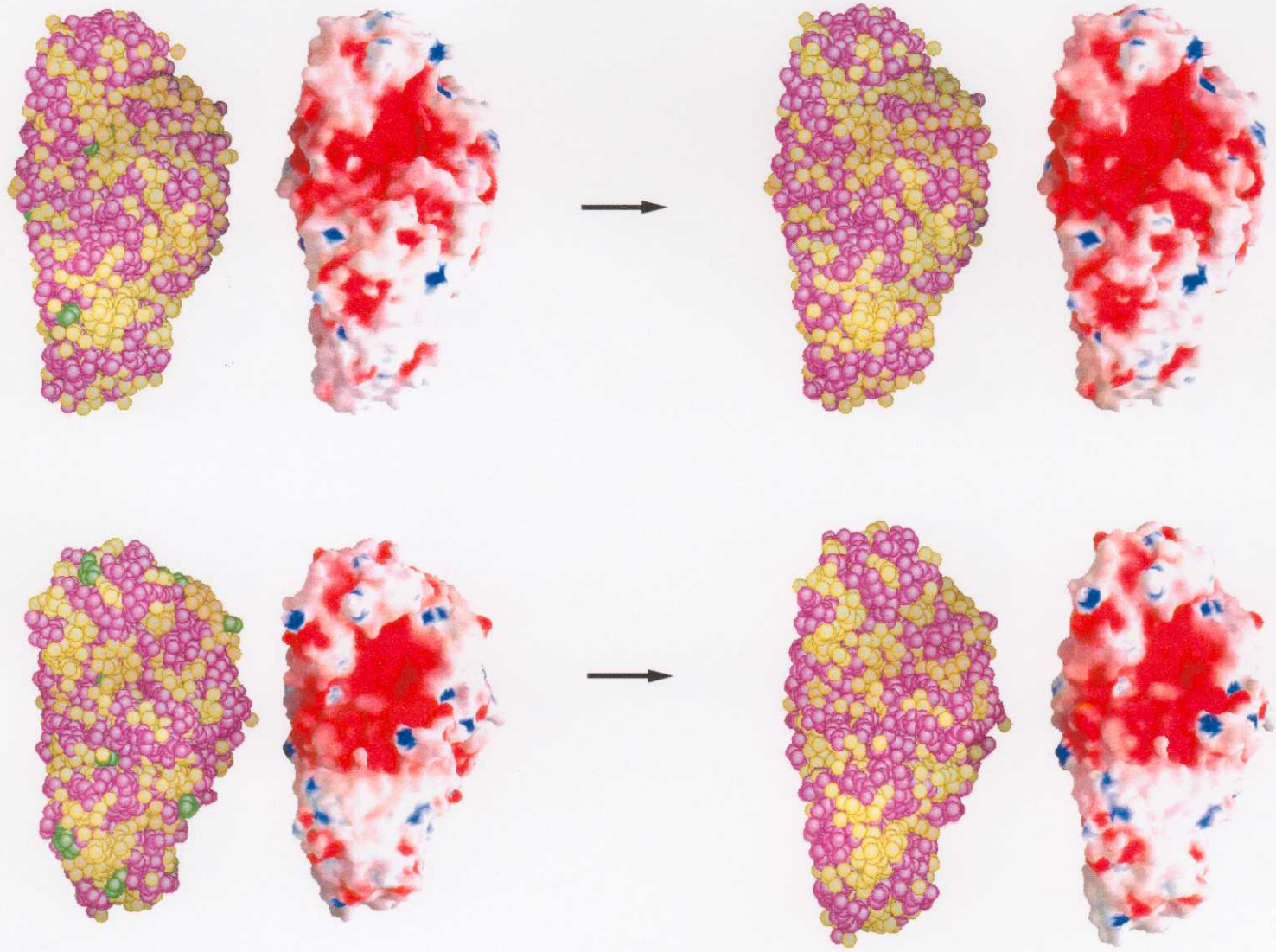
Figure 2-12. Distribution and contribution of His residues to the polar and electrostatic surfaces of BSTA and BLA. Atoms of His residues are colored in green, of polar and charged residues in magenta, of nonpolar residues in yellow. The electrostatic surfaces were calculated using probe radius of 1.4 Å. Positively and negatively charged electric potencials are indicated in blue and red. In calculation of surfaces, a charge of (-0.4) is assigned for ND1 atom of histidine. The model is viewed from four different orientations. Surfaces are generated using program GRASP (Nicholls, 1991).


90°
B, after 90° rotation of A around vertical axis
BSTA
BLA

C, after180° rotation of A around vertical axis

BSTA

BLA



D, after 270° rotation of A around vertical axis

BSTA

BLA

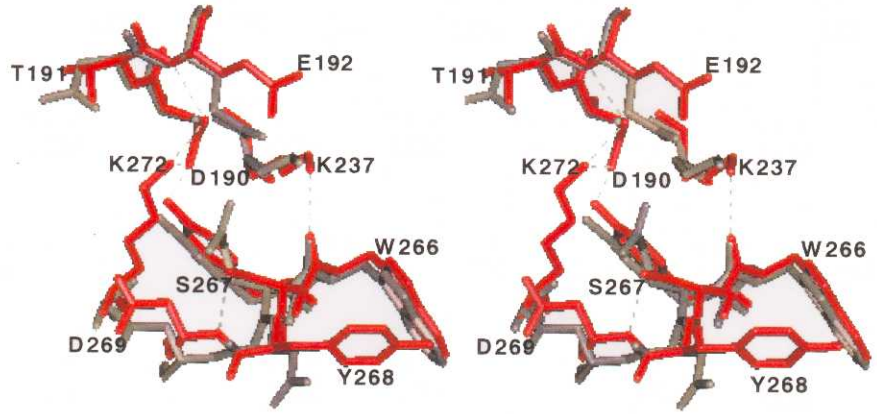


Figure 2-13. Stereoview of superposition of the area of Lys over BLA. BSTA is drawn in red and BLA in gray. Residues are labeled according to BSTA.