Appendices

Appendix A. Parvalbumin metal ion-binding parameters.

Table A-1. CA^{2+}- AND MG^{2+}- BINDING PARAMETERS FOR PARVALBUMINS

Column	PVWT	F102W	PVEF	PVEF-E101D
Maximal binding sites (mol/mol)	2.02	2.18	1.31	1.07
Association constant $K_{Ca}(M^{-1})$[a]	2.89×10^{8}	2.66×10^{8}	1.21×10^{8}	1.54×10^{6}
Association constant K_{Mg} (M^{-1})[b]	2.83×10^{4}	2.50×10^{4}	3.88×10^{4} [c]5.13×10^{4}	[c]2.82×10^{5}
Dissociation rate K_{Ca} (S^{-1})[d]	3.6, 0.6	4.0, 0.5	2.0	630.0
Dissociation rate K_{Mg} (S^{-1})[d]		0.9	0.95	0.8

Data are from Li *et al*., 2000.
All data represent the average of at least 3 determinations.
PVWT = wild type parvalbumin. PVEF = D51A/F102W mutant.
PVEF-E101D = D51A/E101D/F102W mutant.
[a]Constants were determined by equilibrium dialysis.
[b]Constants were calculated from a competition experiment in the presence of 2 mM Mg^{2+}.
[c]Constant was determined by fluorescence titration.
[d]Rates were determined by stopped flow.

Appendix B. Mulliken population analyses of the parvalbumin binding sites.

The Mulliken population analysis is a scheme for assigning charges by dividing orbital overlap evenly between the atoms involved. This method is somewhat arbitrary, as are all methods of assigning charge distribution, because atomic charges are not quantum mechanical observables. Therefore, charge distribution is not explicitly predictable from first principles (Foresman and Frisch, 1995). The binding loops were modeled with just the sidechains of the coordinating residues, terminated at the alpha carbon, and the coordinating carbonyl groups in each binding site were represented from the alpha carbon of the amino acid containing the coordinating carbonyl to the alpha carbon of the subsequent residue. Hydrogens were generated to replace the bonds from the mainchain to the alpha carbons by placing them on the vector between the alpha carbon and the mainchain atom, at the appropriate distance for a C-H bond. The coordinates for the hydrogens were derived using the equations listed below.

$$x(t) = x_0 + t\,(x_1 - x_0),$$

$$y(t) = y_0 + t\,(y_1 - y_0), \text{ and}$$

$$z(t) = z_0 + t\,(z_1 - z_0),$$

where t = (length of a C-H bond/length of the mainchain atom-Cα bond),

x_0 = x coordinate of the Cα, and x_1 = x coordinate of the mainchain atom.

The results of the Mulliken population analyses for the two parvalbumin Ca^{2+}-binding sites are given in Tables B-1 and B-2.

Table B-1. MULLIKEN POPULATION ANALYSIS FOR THE PARVALBUMIN CD SITE

ASP 51	**PHE 57**	**GLU 62**
1 Cα -0.242927	30 Hα 0.178209	55 Cα -0.233764
2 Hα 0.125288	31 Cα -0.251932	56 Hα 0.061021
3 Hα 0.018027	32 Hα 0.052321	57 Hα 0.076880
4 Hα 0.090159	33 Hα 0.124063	58 Hα 0.056153
5 Cβ -0.241985	34 C 0.508096	59 Cβ -0.184990
6 Hβ 0.061232	35 O -0.561090	60 Hβ 0.067142
7 Hβ 0.058734	36 N -0.513870	61 Hβ 0.121612
8 Cγ 0.554857	37 H_N 0.213273	62 Cγ -0.225480
9 Oδ -0.656250	38 Cα -0.130230	63 Hγ 0.101753
10 Oδ -0.672508	39 Hα 0.231770	64 Hγ 0.085329
ASP 53	40 Hα 0.058924	65 Cδ 0.568834
11 Cα -0.219037	41 Hα 0.087125	66 Oε -0.697822
12 Hα 0.095880	**GLU 59**	67 Oε -0.626053
13 Hα 0.119640	42 Cα -0.266663	**CAL 109**
14 Hα 0.009164	43 Hα 0.042213	68 Ca 1.461887
15 Cβ -0.235583	44 Hα 0.154506	
16 Hβ 0.087743	45 Hα 0.056601	
17 Hβ 0.054471	46 Cβ -0.181166	
18 Cγ 0.598582	47 Hβ 0.033881	
19 Oδ -0.713666	48 Hβ 0.139263	
20 Oδ -0.650703	49 Cγ -0.226278	
SER 55	50 Hγ 0.069048	
21 Cα -0.258579	51 Hγ 0.089586	
22 Hα 0.023508	52 Cδ 0.563695	
23 Hα 0.155473	53 Oε -0.659446	
24 Hα 0.065513	54 Oε -0.654578	
25 Cβ 0.082361		
26 Hβ 0.118323		
27 Hβ 0.021902		
28 Oγ -0.567767		
29 Hγ 0.328322	Sum of Mulliken charges = -2.00000	

Calculations were performed in Gaussian (Frisch et al., 1995).

Table B-2. MULLIKEN POPULATION ANALYSIS FOR THE PARVALBUMIN EF SITE

ASP 90	**MET 96**
1 Cα -0.211333	31 Hα 0.197131
2 Hα 0.112768	32 Cα -0.250180
3 Hα 0.015540	33 Hα 0.067943
4 Hα 0.082379	34 Hα 0.115393
5 Cβ -0.250140	35 C 0.480969
6 Hβ 0.054279	36 O -0.572768
7 Hβ 0.059234	37 N -0.509073
8 Cγ 0.558734	38 H 0.218970
9 Oδ -0.681104	39 Cα -0.116808
10 Oδ -0.640302	40 Hα 0.214847
ASP 92	41 Hα 0.070643
11 Cα -0.216653	42 Hα 0.099177
12 Hα 0.007159	**GLU 101**
13 Hα 0.118436	43 Cα -0.229988
14 Hα 0.095255	44 Hα 0.065653
15 Cβ -0.228973	45 Hα 0.074841
16 Hβ 0.068358	46 Hα 0.057428
17 Hβ 0.054425	47 Cβ -0.206162
18 Cγ 0.585829	48 Hβ 0.071538
19 Oδ -0.684168	49 Hβ 0.112889
20 Oδ -0.677984	50 Cγ -0.210222
ASP 94	51 Hγ 0.101220
21 Cα -0.192851	52 Hγ 0.095963
22 Hα 0.006399	53 Cδ 0.563511
23 Hα 0.154888	54 Oε -0.697204
24 Hα 0.070311	55 Oε -0.632088
25 Cβ -0.248076	**CAL 110**
26 Hβ 0.102872	56 Ca 1.422261
27 Hβ 0.049704	**H_2O 111**
28 Cγ 0.577378	57 O -0.536229
29 Oδ -0.719242	58 H 0.216717
30 Oδ -0.645450	59 H 0.335954

Sum of Mulliken charges = -2.00000

Calculations were performed in Gaussian (Frisch et al., 1995).