Supporting Information

for

Diabetes-Associated Mutations in Human Insulin: Crystal Structure and Photo-Cross-Linking Studies of A-Chain Variant Insulin *Wakayama*

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Purpose of Supplement

The purpose of the Supplement is to provide five figures and two sets of tables. Figure S1 provides an overview of the crystal structure of Leu^{A3}-insulin as ribbon model with alignment of molecules 1 and 2. Figures S2 and S3 illustrate additional regions of the electron-density map respectively depicting bound phenol molecules and one of the zinc coordination sites. Figure S4 provides CD spectra of photo-stable Pmp insulin analogs. Figure S5 provides a Ramachandran map of Leu^{A3}-insulin (molecules 1 and 2). The first set of supplemental tables provides quantitative comparisons between Leu^{A3}-insulin and wild-type insulin (Tables S1-A, B, and C). The second set of supplemental tables provides distances between the A3 side chain in structures of Leu^{A3}-insulin and wild-type insulin (Tables S2-A and S2-B, respectively).

Figure S1. Stereoview showing superposition of crystal structure of Leu^{A3} insulin of molecule 1 and molecule 2. (A) Ribbon representation of crystal structure. The A-chain of molecule 1 is shown in red and the B-chain in green. Ribbon model of molecule 2 is shown in black. Disulfide bridges between residues Cys^{A6} and Cys^{A11}, Cys^{A7} and Cys^{B7}, and Cys^{A20} and Cys^{B19} are shown in yellow. (B) Stereo packing schemes of Leu^{A3}-insulin (red) in the hydrophobic core of molecule 1. Residues Ile^{A2}, Leu^{A16}, and Tyr^{A19} are shown in gray; residues Leu^{B11}, Leu^{B15}, Phe^{B24} and Tyr^{B26} in green and residues Cys^{A7} and Cys^{B7} in yellow. Molecule 2 is shown in black.

Fig. S1

Figure S2. *2Fo-Fc* electron density map contoured at a level of $1.0\ \sigma$. (A) Atomic positions of residues A1-A4 of Leu^{A3}-insulin (molecule 1). (B) Atomic positions of residues A1-A4 of Leu^{A3} insulin (molecule 2). (C) Phenol molecule in Leu^{A3}-insulin of molecule 1. The phenol molecule is capable of forming hydrogen bonds to the carbonyl O atom of Leu^{A6} (dashed line; a contact of 2.87 Å) and the N atom of Cys^{A11} (dashed line, a contact of 2.87 Å). (D) Phenol molecule in the cubic structure of molecule 2. It is capable of forming hydrogen bonds to the carbonyl O atom of Leu^{A6} (dashed line; contact of 2.81 Å) and the N atom of Cys^{A11} (dashed line; contact of 3.12 Å).

A **B** **C**

molecule 1

D

molecule 2

Fig. S2

Figure S3. *2Fo-Fc* electron density map contoured at a level of $1.0\ \sigma$. (A) Zinc ion in the R_3 trimer of the Leu^{A3}-insulin hexamer of molecule 1, shown parallel to the crystallographic 3-fold axis. Coordination geometry is tetrahedral. (B) R_3 trimer of the Leu^{A3}-insulin hexamer of molecule 2.

A

molecule 1

B

molecule 2

Fig. S3

Figure S4. CD studies of Pmp derivatives of insulin. Far-UV CD spectra of analogues containing *para*-amino-phenylalanine (Pmp) at A3 and A14 (triangles and open circles, respectively) relative to spectrum of human insulin (filled circles). Pmp^{A3}- and Pmp^{A14} derivatives also contain a biotin tag at A1 as described in text. Spectra were observed at pH 7.4 and 4 °C. The spectrum of the Pmp^{A3} analogue suggests a perturbation in secondary structure or dynamics; however, a contribution of an ordered Pmp ring in this spectral region cannot be excluded.

Fig. S4

Figure S5. Ramachandran map of Leu^{A3}-insulin (molecules 1 and 2). Map resembles that of R-state protomers of wild-type insulin (PDB entries 1BEN, 1EV3, 1EV6, 1G7A, 1G7B, 1MPJ, 1TRZ, 1TYL, 1TYM, 1ZNJ, and 3MTH).

Fig. S5

Table S1-A. RMSD of Leu^{A3}-insulin (molecule 1) relative to wild-type R-state protomers^{a,b}

structures	main-chain (Å)	side-chain (Å)
1BEN R (4ZN T3R3)	0.78	1.31
1TRZ R (4ZN T3R3)	0.92	1.36
1TYL R (4ZN T3R3)	0.49	1.04
1TYM R (4ZN T3R3)	0.57	1.11
1ZNI R (4ZN T3R3)	0.78	1.58
1MPJ R (4ZN T3R3)	0.63	1.49
3MTH R (4ZN T3R3)	0.78	1.68
6INS R(mini-proinsulin)	0.65	1.46
1G7A R1 (rhomb T3R3)	0.79	1.40
R2	0.65	1.22
1LPH R (KP T3R3)	0.93	1.61
1EV3 R1 (rhomb R2)	0.58	1.29
R2	0.57	0.10
1ZEG R1 (rhomb R2)	0.63	1.15
R2	0.54	1.34
1ZEH R1 (rhomb R2)	0.78	1.40
R2	0.63	1.58
1ZNJ R1 (monoclinic)	0.55	1.20
R2	0.50	1.27
R3	0.38	1.03
R4	0.50	1.06
R5	0.49	1.02
R6	0.54	0.95
1EV6 R1 (monoclinic)	0.52	1.15
R2	0.56	1.12
R3	0.58	1.37
R4	0.63	1.16
R5	0.52	1.14
R6	0.55	1.04
average	0.62 (±0.13)	1.26 (±0.20)

^aStructures were aligned pairwise according to the main-chain atoms of residues B3-B28 and A2-A20. The baseline RMSDs between molecules 1 and 2 in the crystal structure of Leu^{A3}-insulin are 0.47 Å (main chain) and 1.25 Å (side chain). ^bT₃R₃^f structures contain R^f promoters, which differ from canonical R state in R₆ zinc-phenol hexamers due to fraying of terminal residues B1 and B2 in B-chain α -helix.

Table S1-B. RMSD of Leu^{A3}-insulin (molecule 2) relative to wild-type R-state protomers^{a,b}

structures	main-chain (Å)	side-chain (Å)
A3-Leu (cubic R1)	0.47	1.25
1BEN R (4ZN T3R3)	0.82	1.57
1TRZ R (4ZN T3R3)	0.86	1.50
1TYL R (4ZN T3R3)	0.49	1.24
1TYM R (4ZN T3R3)	0.54	1.31
1ZNI R (4ZN T3R3)	0.80	1.72
1MPJ R (4ZN T3R3)	0.68	1.59
3MTH R (4ZN T3R3)	0.79	1.72
6INS R(mini-proinsulin)	0.68	1.66
1G7A R1 (rhomb T3R3)	0.72	1.45
R2	0.72	1.34
1LPH R (KP T3R3)	0.92	1.69
1EV3 R1 (rhomb R2)	0.42	1.00
R2	0.54	1.17
1ZEG R1 (rhomb R2)	0.54	1.34
R2	0.44	0.91
1ZEH R1 (rhomb R2)	0.74	1.58
R2	0.54	1.18
1ZNJ R1 (monoclinic)	0.49	1.09
R2	0.46	1.14
R3	0.36	1.00
R4	0.47	1.31
R5	0.35	1.14
R6	0.43	1.08
1EV6 R1 (monoclinic)	0.40	1.11
R2	0.53	1.26
R3	0.60	1.36
R4	0.54	1.23
R5	0.37	1.04
R6	0.50	1.07
average	0.57 (±0.16)	1.30 (±0.24)

^aStructures were aligned pairwise according to the main-chain atoms of residues B3-B28 and A2-A20. The baseline RMSDs between molecules 1 and 2 in the crystal structure of Leu^{A3}-insulin are 0.47 Å (main chain) and 1.25 Å (side chain). ^bT₃R₃ structures contain R^f promoters, which differ from canonical R state in R₆ zinc-phenol hexamers due to fraying of terminal residues B1 and B2 in B-chain α-helix

Table S1-C. Baseline variability among wild-type crystal structures: RMSD between a prototypical wild-type insulin R state (1TRZ) and other wild-type crystal structures^a

structures	Main-chain (Å)	side-chain (Å)
1BEN R (4ZN T3R3)	0.17	0.47
1TYL R (4ZN T3R3)	0.23	0.49
1TYM R (4ZN T3R3)	0.16	0.46
1ZNI R (4ZN T3R3)	0.21	0.57
1MPJ R (4ZN T3R3)	0.34	0.72
3MTH R (4ZN T3R3)	0.31	0.80
6INS R(mini-proinsulin)	0.96	1.34
1G7A R1 (rhom T3R3)	0.53	1.03
R2	0.78	1.25
1LPH R (KP T3R3)	0.53	1.00
1EV3 R1 (rhom R2)	0.91	1.37
R2	0.85	1.03
1ZEG R1 (rhom R2)	0.93	1.44
R2	0.90	1.55
1ZEH R1 (rhom R2)	1.03	1.45
R2	0.92	1.53
1EV6 R1(monoclinic)	0.85	1.35
R2	0.73	1.17
R3	0.99	1.57
R4	0.76	1.20
R5	0.75	1.41
R6	0.73	1.24
1ZNJ R1 (monoclinic)	0.78	1.23
R2	0.69	1.42
R3	0.78	1.39
R4	0.71	1.03
R5	0.76	1.23
R6	0.75	1.19
average	0.68 (±0.26)	1.14 (±0.34)

^aStructures were pairwise aligned according to the main-chain atoms of residues B3-B28 and A2-A20.

Table S2-A: Contacts of A3 side chain in Leu^{A3}-insulin^a

						molecule 1	molecule 2
A3	-LEU	-CD1	---	A2	-ILE-CG1	5.7	7.5
A3	-LEU	-CD1	---	A2	-ILE-CG2	4.3	5.5
A3	-LEU	-CD1	---	A2	-ILE-CD1	6.5	8.3
A3	-LEU	-CD2	---	A2	-ILE-CG2	5.7	4.9
A3	-LEU	-CD1	---	A4	-GLU-OE1	6.5	5.7
A3	-LEU	-CD1	---	A4	-GLU-OE2	6.6	5.0
A3	-LEU	-CD2	---	A4	-GLU-OE1	5.8	6.9
A3	-LEU	-CD2	---	A4	-GLU-OE2	5.3	6.4
A3	-LEU	-CD1	---	A6	-CYS-CB	6.9	9.0
A3	-LEU	-CD1	---	A7	-CYS-SG	4.2	5.7
A3	-LEU	-CD2	---	A7	-CYS-SG	5.9	3.6
A3	-LEU	-CD2	----	B4	-GLN -NE2	3.8	5.4
A3	-LEU	-CD2	----	B4	-GLN -OE1	4.7	6.3
A3	-LEU	-CD1	---	B4	-GLN -NE2	5.4	3.9
A3	-LEU	-CD1	---	B4	-GLN -OE1	5.8	5.1
A3	-LEU	-CD1	----	B7	-CYS -SG	5.6	7.5
A3	-LEU	-CD2	----	B7	-CYS -SG	7.5	5.2
A3	-LEU	-CD1	---	B11	-LEU -CD1	3.9	6.1
A3	-LEU	-CD2	---	B11	-LEU -CD1	6.4	3.8
A3	-LEU	-CD1	----	B26	-TYR -OH	3.4	3.9
A3	-LEU	-CD2	----	B26	-TYR -OH	3.8	3.7
A3	-LEU	-CD1	----	B28	-PRO -CB	4.9	3.6
A3	-LEU	-CD1	----	B28	-PRO -CG	6.2	4.9
A3	-LEU	-CD2	----	B28	-PRO -CB	3.6	5.1
A3	-LEU	-CD2	----	B28	-PRO -CG	4.9	6.3

^aThe Leu^{A3} side chains in molecules 1 and 2 each project from the N-terminal A-chain α -helix to contact the C-terminal β -strand within a corresponding crevice lined.

Table S2-B: Contacts of residue A3 side chain in native insulin^a

						T state		R state	
						molecule 1	molecule 2	molecule 1	molecule 2
A3	-VAL	-CG1	---	A2	-ILE-CG2			4.0	5.9
A3	-VAL	-CG2	---	A2	-ILE-CG1	6.2	5.8	6.9	5.6
A3	-VAL	-CG2	---	A2	-ILE-CG2	4.1	4.0	5.7	3.6
A3	-VAL	-CG1	---	A4	-GLU-OE1	4.2	4.8	5.1	5.2
A3	-VAL	-CG1	---	A4	-GLU-OE2	5.3	3.8	5.3	7.2
A3	-VAL	-CG2	---	A4	-GLU-OE1	5.0	5.0	3.7	5.2
A3	-VAL	-CG2	---	A4	-GLU-OE2	5.4	4.8	3.4	6.0
A3	-VAL	-CG1	---	A6	-CYS-CB	7.4	6.8	7.2	7.5
A3	-VAL	-GG1	---	A6	-CYS-SG	8.6	7.4	8.3	8.7
A3	-VAL	-CG1	---	A7	-CYS-CB	4.1	6.2	6.1	4.6
A3	-VAL	-CG1	---	A7	-CYS-SG	4.6	7.2	5.1	4.2
A3	-VAL	-CG1	---	B11	-LEU -CD1	7.8	6.7	4.5	5.9
A3	-VAL	-CG1	---	B11	-LEU -CD2	5.7	4.2	6.8	8.2
A3	-VAL	-CG2	---	B11	-LEU -CD1	6.9	6.0	6.3	4.6
A3	-VAL	-CG2	---	B11	-LEU -CD2	4.6	4.1	8.3	6.9
A3	-VAL	-CG1	----	B26	-TYR -OH	5.9	3.7	3.4	5.6
A3	-VAL	-CG2	----	B26	-TYR -OH	4.2	3.7	5.8	3.6
A3	-VAL	-CG1	---	B28	-PRO -CB	5.9	3.9	4.3	6.2
A3	-VAL	-CG1	---	B28	-PRO -CG	7.1	5.0	5.6	7.6
A3	-VAL	-CG2	---	B28	-PRO -CB	4.0	4.2	5.7	4.8
A3	-VAL	-CG2	---	B28	-PRO -CG	5.0	4.9	7.2	6.1

^aThe Val^{A3} side chains in T-state protomers in each case project from the N-terminal A-chain α -helix to contact the C-terminal β -strand within a corresponding crevice lined. PDB entries 4INS in T state, and 1ZNJ in R-state.