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Metal induced structural changes observed in hexameric insulin

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ABSTRACT

The metal ions in insulin hexamer play a crucial role in the T to R conformational transitions. We have determined the crystal structures of $2Mn^{2+}$, $1Rb^{1+}$ and $4Ni^{2+}$ human arg-insulin and compared them with the $2Zn^{2+}$ structure. The first two structures exist in the $T3R3^f$ state like the native $2Zn^{2+}$ arg-insulin, while the $4Ni^{2+}$ adopts a T6 conformation. The metal coordination is found to be tetrahedral in all the structures except that of nickel where a dual octahedral and tetrahedral coordination is found at one site. Rubidium occupies only one of the high affinity metal binding sites. The metal induced structural changes observed, have been explained.

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1. Introduction

Metal ions have important implications in defining the function and stability of a protein through coordination to specific groups of a protein molecule. The role of metal ions on insulin storage, release and conformation is a well-known phenomenon [1–3]. Insulin, a protein hormone produced by the beta cells in the Islets of Langerhans of the pancreas, is stored in the form of hexamers with the help of Zn^{2+} ions. An increase in the sugar level stimulates the beta cells to produce and release insulin more than normally required. Even though the insulin monomer is believed to bind a receptor, the metal zinc plays a key role in its storage as a hexamer in the beta cells before being released. A number of divalent metal ions such as Co^{2+}, Cu^{2+}, Ni^{2+}, Fe^{2+} and Mn^{2+} have been shown to replace the zinc ion [4]. In addition, monovalent metal ions like Tl^{1+}, Li^{1+} and Rb^{1+} were also shown to bind insulin [5]. Thus the role of metal ions on insulin storage and release has been an area of research interest over a long period of time.

The hexameric insulin has been shown to exist as an allosteric complex, from various crystallographic [7–9] and spectroscopic studies [10–12]. In the presence of ions such as chloride or thiocyanate [8,13] the first eight residues of the B-chain undergo a conformational transition from extended to α-helical [14–18]. Each monomer is denoted as T (Tensed) or R (Relaxed) according to whether the first eight residues of the B-chain adopt an extended (T) or α-helical (R) conformation [10]. Thus the nomenclature was designated as T6 or R6 if all six monomers in the hexamer are in T or R conformation respectively and T3R3 if three monomers are in T conformation and the remaining three in R conformation. The T3R3 conformation was later denoted as $T3R3^f$ since it was observed that in the R3 trimer the first three residues of the B-chain are in an extended conformation. Hereafter, we will use the T3R3 notation instead of $T3R3^f$ for the sake of simplicity.

The 2Zn native insulin in the crystalline state, adopts a T6 conformation in the absence of chloride ions or phenol derivatives in the crystallization medium with zinc taking up octahedral coordination with three symmetry-related B10/D10 (C and D refers to the A and B chains of molecule 2 of the dimer, respectively) histidine imidazole nitrogen atoms and three water molecules. However, the 4Zn native insulin adopts a T3R3 conformation with a complex zinc coordination compared to that of 2Zn insulin [18,19]. In this structure, apart from the usual zinc ions at the B10/D10 site, the other zinc ions occupy sites at the surface, tetrahedrally coordinated by the histidine imidazole groups of B5 and B10, and two water molecules. These structures clearly demonstrate that the insulin conformation is also controlled by the number of metal ions binding to it. In addition, insulin crystallizes in the cubic space group in a zinc free form in T state bringing out the flexible nature of the insulin [5,6] and also indicating that the T conformation is better for release of metal atoms.

The conformational state of the hexamer is reflected in the type of metal coordination as well. The T conformation in the insulin hexamer is linked with a site of octahedral metal coordination on the three-fold axis coordinated with three B10/D10 histidines and

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Table 1
Crystallization conditions of Human arg-insulin with different metals. The protein concentration was 10 mg/ml in ammonia and the pH was 6.5–7.0.

Arg-insulin with	Well solution	Drop composition
Zinc[a]	15% acetone, 0.06 M zinc sulphate, 0.05 M sodium citrate	4 μl protein, 2 μl well solution
Nickel	0.2 M sodium citrate, 0.12 M nickel chloride, 1 M ammonium sulphate	4 μl protein, 3 μl well solution
Manganese	0.1 M sodium citrate, 1 M ammonium sulphate	4 μl protein, 2 μl well solution, 2 μl 0.1 M manganese chloride
Rubidium	0.2 M sodium citrate, 16% acetone	4 μl protein, 2 μl well solution, 2 μl 0.1 M rubidium chloride

[a] The zinc crystallization condition is provided for comparison.

three water molecules generated from symmetry. On the other hand, in the R conformation, it is always tetrahedral coordination, whether on or off axis, because of steric hindrance. It has been shown that the R6 conformer is assumed in the presence of phenol [8,12,19,20] which interacts with cysteines 6 and 11 of the A-chain.

The type of metal that binds to insulin governs the storage, release, stability, conformation and coordination geometry. A correlation between the conformation and the metal releasing ability, based on the metal releasing efficiency, is ordered as T6 > T3R3 > R6. Further, one could also follow the same order in terms of its function, which is indirectly controlled by the metal releasing efficiency.

It was proposed by Engels et al. [17] that in a R to T transition the A-chain N-terminal helix (A_N) compresses into the space abandoned by the pre-existing B1–B8 helix and in the T to R transition the B-chain N-terminal region first rotates while remaining largely stretched and then winds up to join the extending B9–B19 helix. The crystal structure of Co^{2+} native insulin hexamer [21] reported earlier has been described as one possessing a conformation that could be a possible intermediate in the T to R transition pathway. But for this crystal structure, there is no other crystallographic study relating to metal induced T to R transition of insulin, even though its implications are widely mentioned and accepted in literature, well supported by spectroscopic and dynamics studies [12,17,22]. Despite having a fold similar to that of 2Zn insulin, i.e., T6 conformation, the electron densities for residues B1–B4 and D1–D4 of the cobalt structure were reported to be poorly defined, indicating (the authors surmised) "about three or more different conformations for this region". This led us to believe that the use of metals other than cobalt and zinc may help us to pin down further intermediates in the T–R pathway. For the present studies, a modified form of insulin, namely the human arg-insulin (an arginine residue attached to the N terminal of the A-chain of human insulin) with reduced activity has been used. 2Zn insulin molecule in this structure (PDB ID 2QIU) adopts a T3R3 conformation [23] instead of a T6 observed in the 2Zn native insulin, which has been induced by the N-terminal substitution made at the A-chain. This indicated that the alterations in the A-chain are pivotal in the conformational transitions in insulin [23], which is further supported by the T6 to R6 transition caused by binding of phenol to the A-chain [9,13,21]. Apart from conformational transition, dehydration also induces structural changes in the A_N helix [24]. To explore the structural changes induced by other metal ions further, metals such as nickel(II), manganese(II) and rubidium(I) were complexed with human arg-insulin and their three-dimensional structures were established. Since arg-insulin adopted a T3R3 (intermediate) state in the 2Zn form, it was felt that complexing different metals with arg-insulin could help us capture the structural changes more precisely than using the native insulin.

2. Materials and methods

2.1. Crystallization, structure solution and refinement

Human arg-insulin expressed in yeast, obtained from Sigma chemicals, was used for crystallization. Crystals were grown using the hanging drop method using the conditions given in Table 1. Intensity data for $2Zn^{2+}$, $2Mn^{2+}$ and $4Ni^{2+}$ complexes were measured using *mar345* Image Plate system on a Rigaku rotating anode

Table 2
The crystallographic data and structure refinement statistics of human arg-insulin, with different metal ions. Values for the highest resolution shell are given in the parentheses.

	Zinc[a]	Manganese	Rubidium	Nickel
Space group	H3	H3	H3	H3
Unit cell parameters				
$a = b$ (Å)	80.49	80.68	80.52	83.95
c (Å)	37.64	37.51	38.32	40.22
Resolution (Å)	40.26–2.0 (2.05–2.00)	40.36–2.25 (2.31–2.25)	25.79–2.08 (2.13–2.08)	35.2–2.0 (2.05–2.00)
No. of unique reflections	6115	4331	4169	6805
Average redundancy	5.0(4.8)	4.9(4.9)	1.3(1.2)	2.6(2.3)
Completeness (%)	100(100)	96.3(95.3)	70.3(52.0)	96.8(83.0)
Overall R_{merge}	0.13(0.44)	0.15(0.59)	0.24(0.65)	0.08(0.46)
Mean ($I/\sigma I$)	3.4(1.2)	3.3(1.0)	2.2(0.8)	5.0(1.0)
AMoRe model (PDB ID)	1ZNI	1TRZ	1TRZ	1OS4[b]
Refinement statistics				
R value (%)	19.6	18.5	23.7	22.1
R_{free} value (%)	24.9	26.3	29.7	25.5
R.M.S. deviations from ideal				
Bond lengths (Å)	0.08	0.04	0.11	0.04
Bond angles (°)	2.1	2.6	7.7	3.0
Dihedral angles (°)	15.5	17.4	24.2	11.5
No. of water atoms	57	41	22	36
Zinc/manganese/rubidium/nickel atoms	2	2	1	4

[a] The zinc data is provided for comparison.
[b] Only a dimer unit was taken for the molecular replacement search.

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