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Langmuir-Blodgett Technology for Drugs Production and Delivery: Insights and Implications from an *In Silico* Study

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Abstract

Insulin dynameomics here reported as a database pertaining to a series of Molecular Dynamics (MD) ran for Protein Data Bank (PDB) entries consisting in bioinformatics and molecular dynamics simulation of Langmuir-Blodgett and classical insulin with and without insulin receptor, appears to have profound implications for drug design and endocrinology. The slight differences in conformation and dynamics here reported may indeed explain why LB-insulin is more stable when binds to its receptor (lower free energy) and this could be useful when designing new drugs and pharmaceuticals.

Keywords

Molecular dynamics, Protein data bank, Free interface diffusion, Counter-ion diffusion, Langmuir-blodgett, RMDS

Introduction

Nanobiotechnologies are powerful tools emerging from the convergence of nanotechnology, molecular biology and post-genomics medicine. They, indeed, play a major role in the field of molecular medicine and, in particular, of molecular oncology.

Crystallography is an important tool in the biomedical field. Different crystallization techniques have been proposed over the past decades, such as the classical vapor hanging drop method, its variant the sitting drop method, dialysis, cryotemperature, gel, batch, and the innovative microgravity (space) techniques like free interface diffusion (FID) and counter-ion diffusion (CID). Langmuir-Blodgett (LB)–based crystallization approach [1-3] has been shown to represent a valid alternative for fast crystallizing proteins which are difficult to obtain with other techniques [4-6].

A new multi-scale and multi-dimensional approach termed as "dynameomics" [7], arising from the coupling of protein crystallography and molecular dynamics, has been coined by Daggett.

The present investigation is aimed at systematically exploring structural differences among insulin protein structures, obtained with HD (Hanging Drop proteins crystals) and LB (Langmuir-Blodgett based protein crystal) approaches (Figure 1).

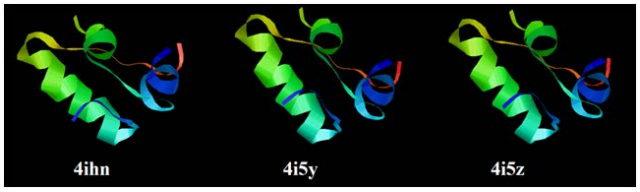

Figure 1: 4ihn (LB-insulin), 4i5y and 4i5z (HD-insulins).

Material and Methods

Protein crystallization

Human insulin (from *Homo sapiens*) was crystallized by classical and LB nanotemplate method with the structure solved using X-ray crystallography, as previously described [5].

In silico analysis

Protein-peptide/receptor superimposition was carried out using the MultiProt (Figure 2) simultaneous alignment software [8-13], which represent a fully automated, highly efficient computational tool enabling a fast structural comparison of different biomolecules/macromolecular complexes. Root-mean-square deviation (RMSD) for C-α atoms was used as the similarity measure for all structures.

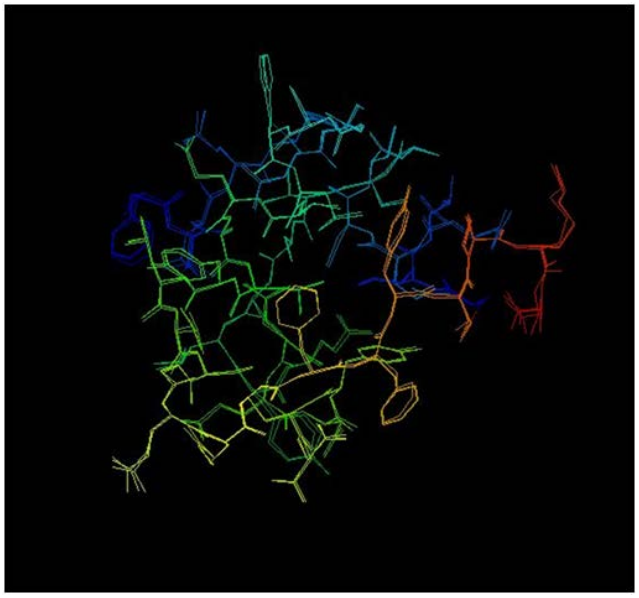

Figure 2: 4ihn, 4i5y and 4i5z superimposed with MultiProt.

Clustering was performed with the Protein (Structure) Comparison, Knowledge, Similarity and Information (PROCKSI) server (Figure 3), which enables a fast simultaneous alignment and structural comparison of multiple proteins. The files for building tree diagrams were prepared according to the Newick format (file extension .newick), which exploits a standard that makes use of the correspondence between trees and nested parentheses. The trees were visualized using a tree editor, Dendroscope. The clustering algorithm was used, selecting the Ward distance both as all-against-all and as all-against-target options.

Protein-peptide molecular docking was performed utilizing the CABS-dock server (Figure 4) for flexible protein-peptide docking, in that this server (accessible at http://biocomp.chem.uw.edu.pl/CABSdock/) enables a highly efficient modeling of full peptide flexibility and significant flexibility of a protein receptor.

Protein-receptor interaction and binding energy were predicted, modelled and computed using the PRISM server.

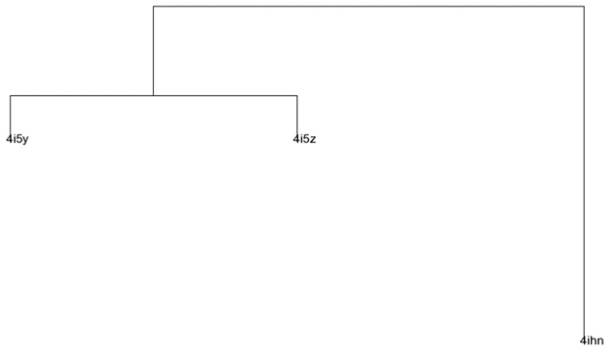

Figure 3: 4ihn, 4i5y and 4i5z clustered with PROCKSI.

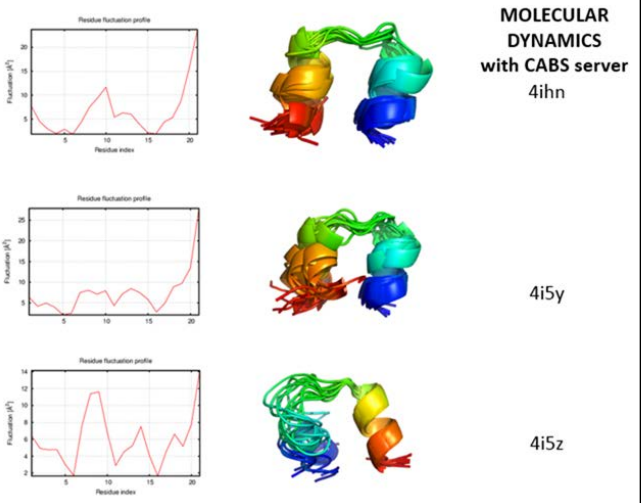

Figure 4: Molecular dynamics with CABS server.

Results

4ihn, 4i5y and 4i5z are the same protein insulins but exhibit some slight structural differences above all for the side-chain dynamics. 4i5y and 4i5z are very similar (RMSD 0.04), whereas 4ihn exhibits some differences (overall RMSD 0.11, with 4i5z 0.14, with 4i5y 0.15). This difference in RMSD becomes more important (overall RMSD 0.14, with 4i5z 0.15, with 4i5y 0.14) when aligning insulins and insulin receptor (Table 1). These slight differences in conformation and dynamics may explain why LB-insulin is more stable when binds to its receptor (lower free energy).

Discussion

In the present article, we have presented extensive bioinformatics and fast-MD simulations of three insulin proteins that we previously solved (namely, 4ihn, 4i5y and

Table 1: Binding energy computed for different macromolecular complexes. *In silico* study of insulins alone and binded to the receptor 3loh either LB crystallized (4ihn) or classical HD crystallized (4i5y, 4i5z).

Molecular Complex	Binding Energy
4ihn and 3loh	-21.99 kcal/mol
4i5y and 3loh	-18.09 kcal/mol
4i5z and 3loh	-19.28 kcal/mol

4i5z). We have shown that these proteins, even though being structurally identical, exhibit some slight differences, above all for the side-chain and fluctuation dynamics that become more important and evident when insulin binds to its receptor (Figure 5 and 6). Indeed from what is well known for insulin in the literature and even in Wikipedia apparently, preparing insulin with Langmuir-Blodgett approach could be useful for a more effective treatment of metabolic impairments. Could also contribute the apparently poorly resolved domains in Hanging Drop proteins crystals to turn into more unstable secondary domains in *in silico* simulations.

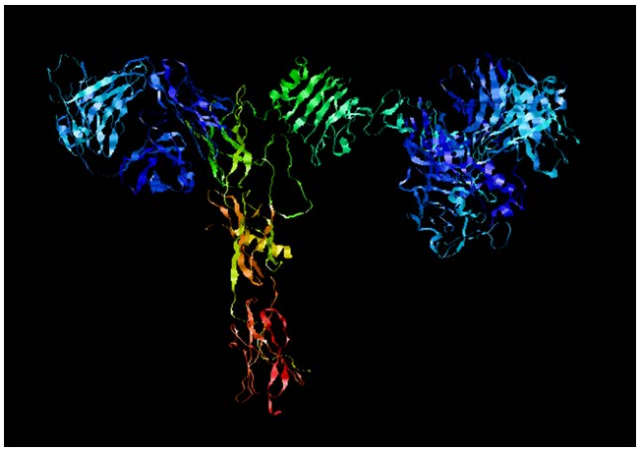

Figure 5: Interaction between 4ihn and 3loh.

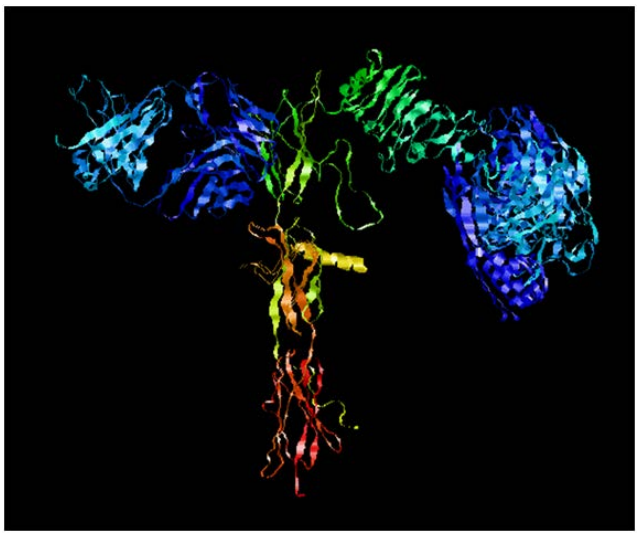

Figure 6: Insulin receptor and insulins superimposed.

The role of Langmuir-Blodgett technology for drugs production and delivery [14-20], for biocatalysis [21-26] and in general [27-29] was known since long time, mostly in experimental terms but also *in silico* [22]. Details can be found in the quoted papers and relevant to this communication we must remember that over forty five 3D Enzymatic Structures were deposited in RCSB Protein Data Bank by the Fondazione ELBA-Nicolini and Nanoworld Institute over the past several years.

Conclusions

This *in silico* study has shown that proteins obtained with the LB crystallization approach (4ihn) are more stable both alone and when binding to the receptor than the proteins obtained with classical HD crystallization technique (4i5y, 4i5z). This investigation could have practical implications for the field of molecular endocrinology and drug design (work in progress). The manuscript is carrying indeed the clear idea of stability of insulin crystallized by Langmuir-Blodgett techniques, which is interesting and promising result which could have practical use in pharmaceutics. Limits and significance of this and previous *in silico* studies emerge from the recent most comprehensive publication in all aspects of LB nanotechnology [30].

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