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Proposed Mechanism of Bacterial Hydroxyapatite Biosynthesis and Comparative Molecular Docking Analysis of Hydroxyapatite and Osteogenic Factors with Alkaline Phosphatase

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Abstract

Purpose and aim: Hydroxyapatite ss a biocompatible calcium phosphate mineral used in bone tissue engineering and regenerative medicine could be synthesized by Bacillus species. Molecular mechanisms of bacterial hydroxyapatite biomineralization as well as comparative molecular interactions of hydroxyapatite and conventional osteogenic supplements with osteogenic marker proteins remain poorly understood. This paper aimed to investigate a putative molecular mechanism of bacterial hydroxyapatite biosynthesis and predicted interaction of hydroxyapatite with alkaline phosphatase using an integrated bioinformatics and molecular docking approach.

Material and Methods: Molecular docking was performed using Molegro Virtual Docker, and protein–protein interactions were analyzed using the STRING database.

Results and Discussion: Tricalcium phosphate showed favorable interact-tions with the *Bacillus* membrane proteins YtaF and alkaline phosphatase, with binding energies of −105.096 and −110.086 kcal/mol, respectively. STRING analysis suggested a functional association between YtaF and proteins involved in alkaline phosphatase regulation, supporting a putative biomineralization pathway. Docking analysis of osteogenic compounds revealed that hydroxyapatite exhibited the strongest predicted interaction with alkaline phosphatase (−147.45 kcal/mol), followed by dexamethasone, β-glycerophosphate, and ascorbic acid.

Conclusion: These findings provide a hypothetical framework for bacterial hydroxyapatite formation and suggest potential molecular interactions between hydroxyapatite and osteogenic path-ways. Experimental validation is required to confirm these predictions.

What is "already known":	• Several *Bacillus* species are capable of synthesizing hydroxyapatite through biomineralization processes. • Hydroxyapatite is widely used in bone tissue engineering because of its excellent biocompatibility and osteoconductive properties. • Alkaline phosphatase is a key biomarker of osteogenic differentiation and plays an essential role in mineralization.

What this article adds:	• This study proposes a putative molecular mechanism for bacterial hydroxyapatite biosynthesis involving YtaF, PhoD, and their predicted protein–protein interactions. • It provides the first comparative molecular docking analysis of hydroxyapatite and conventional osteogenic supplements against alkaline phosphatase. • The findings generate computational hypotheses that may guide future experimental studies on bacterial biomineralization and hydroxyapatite-mediated osteogenesis.

1. Introduction

Hydroxyapatite is one of the most important bioceramics used in medicine, and it has received a lot of attention in recent years. It possesses a chemical composition and structure that is strikingly similar to the minerals found in bone and teeth. This bioactive and biocompatible substance interacts effectively with bone tissue and develops a direct bond with it after being implanted in the body [1, 2]. Hydroxyapatite attaches to, grows in, and encourages the creation of new bone tissue, as well as stimulating bone development into its porous region and resulting in bone cell differentiation and proliferation. It has a bone inclination, does not absorb, does not degrade, and eventually achieves strong adhesion to bone tissue [3]. In general, bone tissue can self-repair; however, the capacity of bone to self-repair is lost in cases such as severe bone tissue injury and aging. So researchers introduced hydroxyapatite for replacement in bone tissue. Therefore, hydroxyapatite has made great advances in orthopedics and implant and bone graft implantation. It is also utilized in oral and maxillofacial implants to raise deteriorated gums that are unable to hold dentures or implant grafts [4, 5]. Numerous chemical and biological methods are used to HA synthesize which are divided into chemical synthesis (solid-state, mechano-chemical, acid-base, sol-gel, ultrasonic-chemical, microwave, multiple emulsion, chemical deposition, conversion hydrothermal and soluble ignition) and biological synthesis. Sources of biological HA include mammalian bones such as livestock, poultry and fishes bone, coral, shrimp shells, snakeskin, hedgehog thorns, and some plants and algae [2]. The most important advantages of biological HA over chemical HA is similarities in the physical structure and chemical composition of natural hydroxyapatite with human bone tissue. The presence of trace element such as magnesium, sodium, potassium, iron, zinc even in small amounts in biological HA is other advantag of this material that have an important role in the process of bone repair and regeneration. Bioloical synthesis of HA not only cost effective but also ease to accomplish [4]. One of the newest method in biological synthesis of nanoHA is biomineralization method via bacteria including *Serratia marcescens, Enterobacteria, Staphylococcus,* and *Alkanindiges illinoisensis* [4, 6]. In osteogenesis procecc in human body, biomineralisation is the procecc by wich HA particls settels down in exteracellular matrix. Then Tissue Nonspecific Alkaline Phosphatase enzyme (TNAP) hydrolyzis pyrophosphate and provides inorganic phosphate to simulate biomineralisation. Alkaline phpsphatase is also present in some bacteria and mentioned process is posible in bacteria as previosly observed some alkaline phosphatase possetive bacteia can synthesis HA patricle [2, 7]. As mentioned befor hydroxyapatite can differentiate stem cells.

Undifferentiated cells such stem cells from human exfoliated deciduous teeth (SHED) which is a type of mesenchymal stem cell, multiply fast in their early stages to attain a suitable cell density before beginning the differentiation process in response to certain stimuli. This stem cells have the ability to differentiate into odontoblasts, osteoblast, chondrocytes, neuronal cells, hepatocytes, adipocytes. When cells acquire differentiation signals, they become committed and progressively cease multiplying while beginning to exhibit early markers like alkaline phosphatase and osterix. Osteoblast differentiation and bone production from stem cells require Osterix and alkalin phosphatase. Osterix (Osx) is a zinc-finger containing protein and osteoblast-specific transcription factor. Osteopontin, osteonectin, and osteocalcin are all expressed by cells during the differentiation process. The development of SHED into osteoblast cells is caused by this progressive upregulation. In in vitro research, osteogenic factors are used to differentiate stem cells. These factors include dexamethasone, ascorbic acid, and β-glycerophosphate [8-10].

Biologically synthesized hydroxyapatite has attracted increasing attention as a biomaterial for bone regeneration because of its structural similarity to natural bone mineral. Therefore, understanding both the biosynthesis of bacterial hydroxyapatite and its potential interactions with osteogenic markers is important for evaluating its biomedical applications [10-12]. In this study, we first investigated a putative mechanism for bacterial hydroxyapatite biosynthesis using protein interaction and docking analyses. Subsequently, we evaluated the predicted interaction of hydroxyapatite with alkaline phosphatase and compared it with commonly used osteogenic supplements employed for SHED differentiation. The present study aimed to compare the predicted interactions of hydroxyapatite and commonly used osteogenic supplements (dexamethasone, ascorbic acid, and β-glycerophosphate) with alkaline phosphatase, a key marker of osteogenic differentiation, using molecular docking analysis. According to research, hydroxyapatite toothpaste can also be used to partially rebuild dental enamel cavities.

2. Method

2.1. Study Design

This study employed a bioinformatics and molecular docking approach to investigate the potential osteogenic activity of hydroxyapatite (HA) compared with conventional osteogenic differentiation factors. In addition, a protein–protein interaction analysis was performed to propose a possible mechanism for bacterial hydroxyapatite biosynthesis in *Bacillus* species.

2.2. Protein Selection and Structure Retrieval

Proteins associated with osteogenic differentiation of stem cells from human exfoliated deciduous teeth (SHED) and bacterial hydroxyapatite production were selected based on literature reports. Human alkaline phosphatase, an early marker of osteogenic differentiation, was used as the target protein for docking studies. The crystal structure of alkaline phosphatase (PDB ID: 1ZED) was obtained from the Protein Data Bank (RCSB PDB). Alkaline phosphatase was selected as the target protein because it is an early and well-established marker of osteogenic differentiation and plays a direct role in extracellular matrix mineralization through the generation of inorganic phosphate required for hydroxyapatite formation.

For investigation of the bacterial hydroxyapatite synthesis mechanism, the calcium-binding protein homologous to YtaF (PDB ID: 3LI6) and alkaline phosphatase PhoD (PDB ID: 2YEQ) were selected as representative membrane-associated proteins of *Bacillus* spp. YtaF and PhoD were selected as representative proteins because they are associated with two fundamental processes involved in bacterial hydroxyapatite biomineralization. YtaF is a predicted membrane-associated calcium-binding protein that may participate in calcium uptake, whereas PhoD is a well characterized alkaline phosphatase responsible for releasing inorganic phosphate through phosphate hydrolysis. Together, these proteins were used to model the coordinated roles of calcium acquisition and phosphate metabolism during hydroxyapatite formation.

2.3. Ligand Preparation

Hydroxyapatite, dexamethasone, ascorbic acid, β-glycerophosphate, and tricalcium phosphate were selected as ligands. Three-dimensional structures of the ligands were retrieved from the PubChem database and converted into appropriate formats for docking analysis. Prior to docking, ligand structures were energy-minimized using the default settings of the docking software.

2.4. Representation of Hydroxyapatite for Molecular Docking

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is a crystalline biomineral and cannot be represented computationally as an entire crystal lattice during conventional molecular docking simulations. Therefore, a molecular fragment representing the fundamental chemical unit of hydroxyapatite was constructed and used as the ligand. The three-dimensional structure was obtained from the PubChem database and geometry optimization was performed using the default parameters of Molegro Virtual Docker prior to docking. The objective of this approach was not to reproduce the complete crystalline structure of hydroxyapatite but rather to evaluate the potential interactions between hydroxyapatite-associated calcium and phosphate groups and amino acid residues within the target protein. Similar

reductionist approaches have been used in computational studies of mineral–protein interactions where representative structural units are employed to estimate binding tendencies. Molecular docking was therefore used as a comparative screening tool to assess the relative interaction propensity of hydroxyapatite and osteogenic compounds toward alkaline phosphatase rather than to model the complete biomineralization process.

2.5. Molecular Docking Analysis

Molecular docking was performed using Molegro Virtual Docker (MVD) version 2013 v6.0.1. Docking simulations were conducted using the MolDock SE algorithm with an energy threshold of 100. Protein structures were prepared by removing water molecules and adding hydrogen atoms where necessary.

For osteogenic differentiation analysis, alkaline phosphatase was docked against hydroxyapatite, dexamethasone, ascorbic acid, and β-glycerophosphate. Binding energies and interacting amino acid residues were recorded for each protein–ligand complex.

To investigate bacterial biomineralization, tricalcium phosphate was docked against the calcium-binding protein (YtaF homolog) and alkaline phosphatase (PhoD). The docking pose with the lowest binding energy was selected as the most favorable interaction. Two-dimensional interaction maps and three-dimensional complex structures were generated using Molegro Virtual Docker. For each protein–ligand pair, the docking pose with the lowest predicted binding energy was selected for analysis. Multiple independent docking runs and statistical analyses were not performed because the study was designed as a preliminary comparative screening using identical computational parameters for all ligands.

2.6. Protein–Protein Interaction Network Analysis

Protein–protein interaction analysis was performed using the STRING database. Proteins associated with calcium uptake, sporulation, phosphate metabolism, and alkaline phosphatase regulation in *Bacillus* spp. were analyzed to identify functional relationships potentially involved in hydroxyapatite biosynthesis. Interaction networks were visualized, and proteins with direct or indirect associations with YtaF and PhoD were used to propose a putative biomineralization pathway. Protein–protein interaction analysis was performed using the STRING database to explore functional associations among proteins involved in calcium uptake, sporulation, phosphate metabolism, and alkaline phosphatase regulation in *Bacillus* spp. Proteins were selected based on published evidence regarding their potential roles in bacterial biomineralization. The resulting interaction network was used to propose a hypothetical molecular pathway and was interpreted qualitatively.

2.7. Data Analysis

Binding affinity was evaluated based on docking scores (kcal/mol), where more negative values indicated stronger predicted interactions. Amino acid residues participating in protein–ligand interactions were identified and compared among ligands. Docking results were interpreted to estimate the relative affinity of hydroxyapatite and conventional osteogenic factors toward osteogenic marker proteins and to infer possible molecular mechanisms underlying bacterial hydroxyapatite formation. The present study was designed as an exploratory computational analysis. So, molecular dynamics simulations and experimental validation were not included, as the primary objective was to generate hypotheses regarding protein ligand interactions for subsequent investigation.

2.8. Docking Protocol Considerations

Since the objective of this study was to compare the relative binding tendencies of different ligands under identical computational conditions, all docking simulations were performed using the same protein preparation procedure, scoring function, and docking parameters. A separate validation procedure, such as redocking of a co-crystallized ligand or benchmarking against experimental binding data, was not performed. Therefore, the docking scores were interpreted as comparative predictions rather than absolute binding affinities.

3. Results and Discussion

Molegro Virtual Docker created the modeled structure of interactions with the highest energy and a two-dimensional diagram with different residues, as shown in Figure 1 and Table 1. The docking analysis was conducted between tricalcium phosphate as a ligand and calmodulin-like protein and alkaline phosphatase involved in the *Bacillus* sp. membran

Table 1. The result of docking analysis between Ca_2-binding protein and alkaline phosphatase as a membrane protein of *Bacillus sp.* and tricalcium phosphate as a ligand

Bacteria	protein	Ortholog protein	BDP ID code	Binding energy (kcal/mol)	Possible amino acids involved in the interaction
Bacillus sp.	YtaF	EhCaBP1	3LI6	-105.096	Lys- Ser
Bacillus sp.	Alkaline phosphatase D	PhoD	2YEQ	-110.086	Lys- Lys

The docking analysis showed that tricalcium phosphate interacted with both YtaF and PhoD proteins. Binding energies of -105.096 kcal/mol and -110.086 kcal/mol were obtained for YtaF and PhoD, respectively. YtaF is a *Bacillus* sp. membrane protein with at least four transmembrane domains involved in sporulation. YtaF shares structural similarities with several calmodulin-like Ca^{2+}-binding proteins, most notably EhCaBP1 from Entamoeba histolytica (PDB ID: 3LI6). EhCaBP1 possesses four canonical EF-hand Ca^{2+}-binding motifs, and these motifs are aligned with a stretch of 12 amino acid residues in YtaF. Therefore, YtaF is predicted to function as a Ca^{2+}-binding protein [13, 14]. Based on the docking results, YtaF exhibited a favorable predicted interaction with tricalcium phosphate. This computational finding suggests that YtaF may participate in calcium acquisition during bacterial biomineralization; however, this proposed role remains hypothetical and requires experimental confirmation [15, 16].

3.1. Protein–Protein Interaction with Calcium

Based on the STRING analysis network, YtaF, a Ca^{2+}-binding protein involved in sporulation, was connected to CoaE (dephospho-CoA kinase), which catalyzes the phosphorylation of the 3'-hydroxyl group of dephosphocoenzyme A to form coenzyme A. The network also included PhoR and PhoD, proteins involved in alkaline phosphatase regulation and synthesis (Figure 2).

The STRING interaction network predicted functional associations between YtaF and proteins involved in phosphate metabolism and alkaline phosphatase regulation. These predicted interactions provide a basis for proposing a hypothetical mechanism of bacterial hydroxyapatite biosynthesis but do not constitute experimental evidence of protein function or regulation. Based on the docking and protein–protein interaction analyses, a hypothetical mechanism of bacterial hydroxyapatite biosynthesis is proposed as follows: YtaF, a calcium-binding protein, absorbs tricalcium phosphate and provides calcium ions required for hydroxyapatite crystal formation. Following the protein–protein interactions identified in Figure 2, YtaF may contribute to the activation of alkaline phosphatase, which can release phosphate from insoluble phosphate-containing compounds. Consequently, alkaline phosphatase could contribute to phosphate availability required for hydroxyapatite formation, although this proposed mechanism remains to be experimentally validated.

The proposed mechanism is consistent with previous studies on microbial biomineralization. It has been reported that Bacillus species produce alkaline phosphatase enzymes capable of hydrolyzing phosphate-containing compounds and releasing inorganic phosphate ions, which subsequently combine with calcium ions to form hydroxyapatite crystals. Therefore, the predicted involvement of YtaF in calcium acquisition together

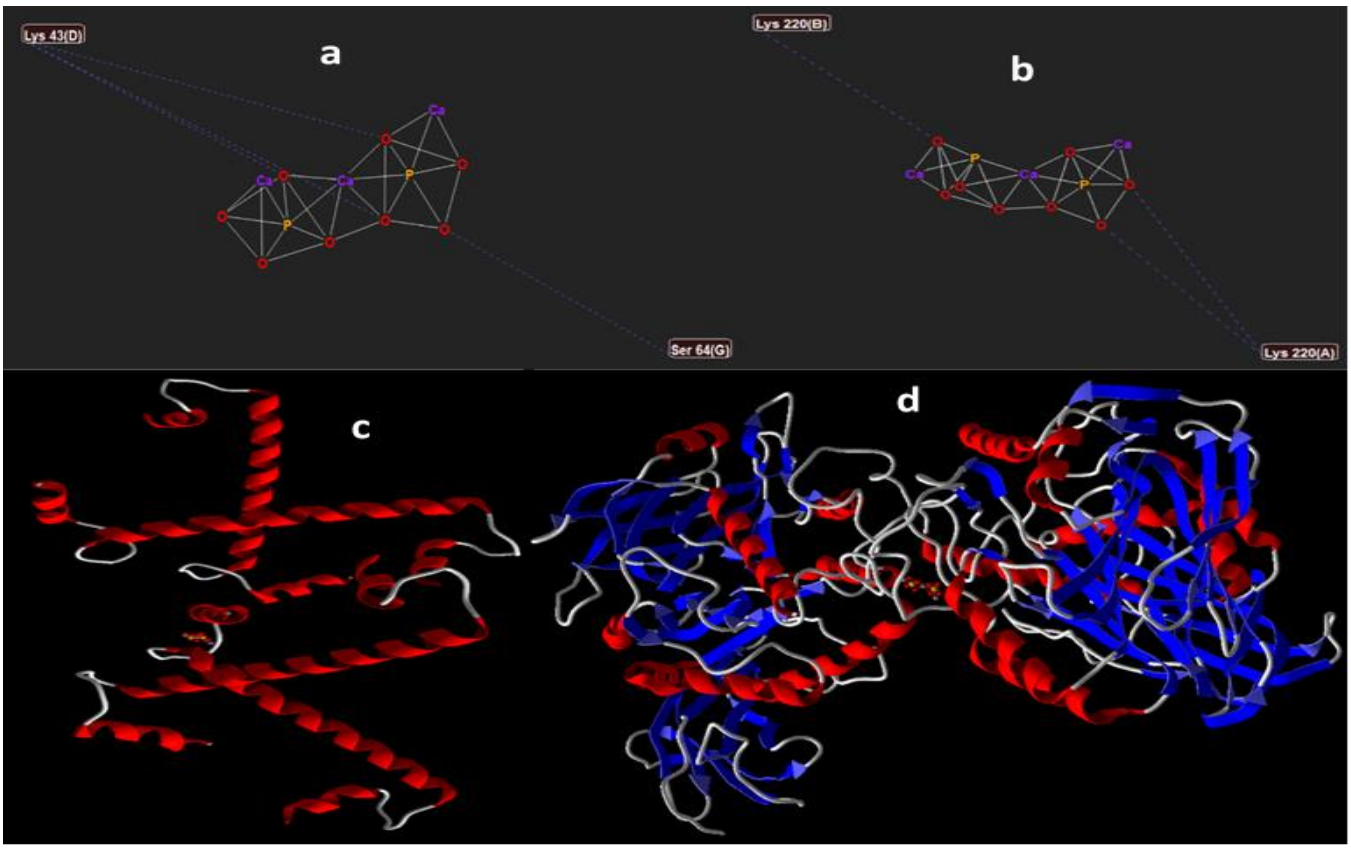

Figure 1. Top; Two-dimensional diagram of interactions with residues in docking results between (**a)** Ca-binding protein from *Bacillus sp*. and tricalcium phosphate as a ligand (**b)** alkaline phosphatase of *Bacillus sp*. and tricalcium phosphate as a ligand. Down; Modeled structure of the interaction between (**c)** Ca-binding protein from *Bacillus sp*. and tricalcium phosphate as a ligand (**d)** alkaline phosphatase of *Bacillus sp*. and tricalcium phosphate as a ligand (ligands are shown by the blue arrow). They were designed by Molegro Virtual Docker.

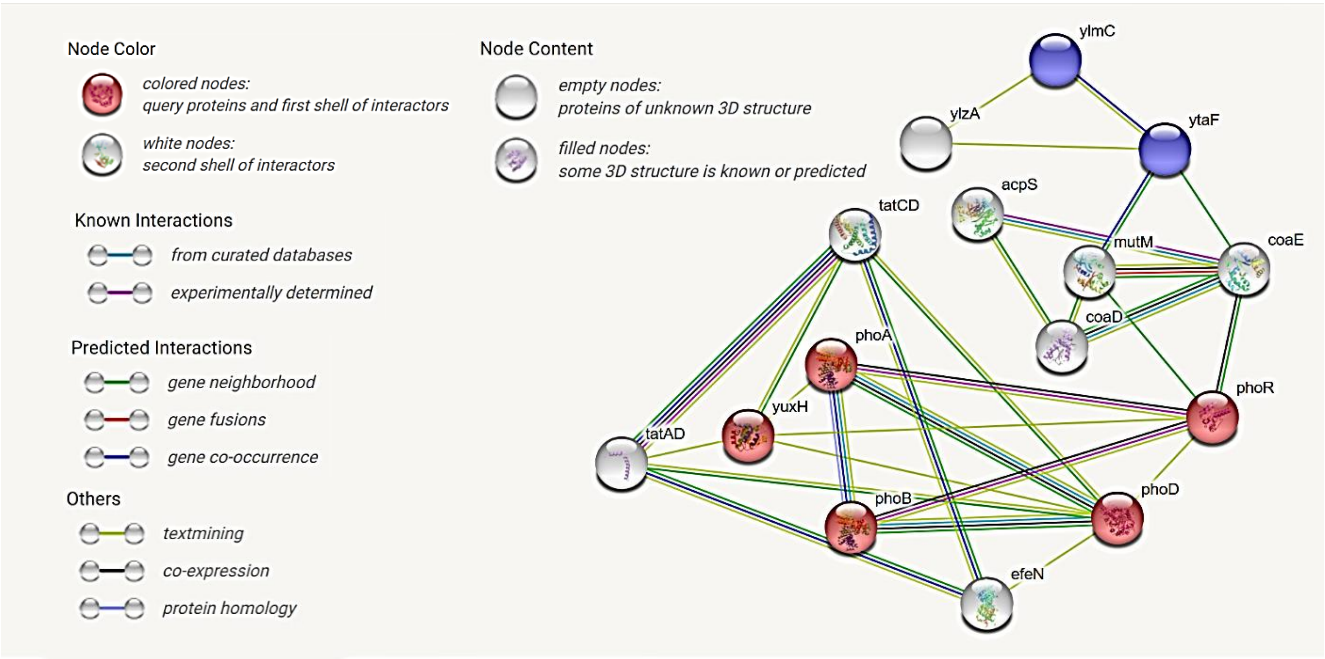

Figure 2. Probable protein-protein interaction network of *bacillus* membrane to the synthesis of bacterial nano-HA. Blue nodes are sporulation-assisted proteins, red nodes are proteins that are involved in alkaline phosphatase synthesis, and the edges show the interaction between proteins. Other nodes represent membrane proteins involved in the process.

with PhoD-mediated phosphate release may provide favorable conditions for hydroxyapatite biomineralization in *Bacillus* spp [7, 17]. Urease is also considered a key enzyme in the process of hydroxyapatite formation because it facilitates the association of calcium and phosphate ions within the hydroxyapatite crystal structure. Eventually, hydroxyapatite may be synthesized on the bacterial membrane surface. Subsequently, the synthesized crystals may bind to negatively charged teichoic acids. Because teichoic acids possess an indented serrated structure, hydroxyapatite crystals may aggregate into woven or braided morphologies [4, 18].

The computational findings presented in this study may have implications for the future development of bacterial-derived hydroxyapatite in biomedical applications. Previous studies have demonstrated the potential of biologically synthesized hydroxyapatite for bone tissue engineering, dental biomaterials, and enamel remineralization. However, the present study did not directly investigate these applications. Therefore, these potential uses should be regarded as future perspectives rather than conclusions derived from the current computational analyses. Therefore, the predicted bacterial capacity for hydroxyapatite production, together with the reported probiotic and antimicrobial properties of *Bacillus coagulans* and *Bacillus subtilis*, suggests their potential for future investigation in oral healthcare formulations. However, this potential should be verified through experimental studies evaluating hydroxyapatite production, stability, and clinical efficacy [19, 20]

3.2. Docking Analysis of Hydroxyapatite and Osteogenic Compounds

Using Molegro Virtual Docker 2013 v6.0.1 with the MolDock SE algorithm and an energy threshold of 100, docking of alkaline phosphatase (PDB ID: 1ZED) against hydroxyapatite and osteogenic factors (dexamethasone, ascorbic acid, and β-glycerophosphate) was performed. Crystal structures were obtained from the RCSB PDB and PubChem databases.

Table 2 illustrate the modeled structures with the highest-energy interactions and the corresponding two-dimensional interaction maps. The docking analysis was conducted between alkaline phosphatase as the protein target and osteogenic compounds as ligands.

The reported docking energies provide a comparative measure of the predicted binding affinity between each ligand and alkaline phosphatase. More negative binding energies indicate thermodynamically more favorable interactions under the computational conditions employed. Since alkaline phosphatase is directly involved in phosphate metabolism and mineralization, the stronger predicted interaction of hydroxyapatite with this enzyme may reflect a greater potential for molecular association during biomineralization. However, docking scores alone cannot establish biological function, enzymatic activity, or osteogenic efficacy. Therefore, these computational findings should be interpreted as hypothesis generating observations that require validation through molecular dynamics simulations and experimental investigations.

Among the tested compounds, hydroxyapatite exhibited the lowest docking energy (-147.45 kcal/mol), followed by dexamethasone (-108.3 kcal/mol), β-glycerophosphate (-97.4 kcal/mol), and ascorbic acid (-86.80 kcal/mol). The docking study demonstrated that hydroxyapatite exhibited stronger predicted binding to alkaline phosphatase than the investigated osteogenic compounds. However, molecular docking represents only a computational estimation of protein–ligand interactions. Osteogenic differentiation is a complex biological process involving numerous transcription factors, signaling molecules, and extracellular matrix proteins, including Osterix, Runx2, osteocalcin, and osteopontin. These findings are consistent with previous studies [21, 22], which emphasize that osteogenic differentiation is a highly regulated and multifactorial process involving key transcription factors such as Runx2 and Osterix as well as extracellular matrix proteins including osteocalcin and osteopontin. In addition, the limitations of molecular docking in fully capturing biological complexity have been widely acknowledged in the literature.

Table 2. Docking analysis between alkaline phosphatase and hydroxyapatite or osteogenic compounds.

protein	Ligand	Binding energy (kcal/mol)	Possible amino acids involved in the interaction
Alkaline phosphatase	Hydroxyapatite	-147.45	Ala154(A), Ser155(A), Gly93(A), Arg166(A), His432(A), Glu429(A), Asp91(A), Asp357(A), Thr95(A), Ala96(A)
	Dexamethasone	-108.3	Gln27(A), Glu347(A), His447(A), Gly443(A), Tyr246(A), Lys87(A)
	Ascorbic acid	-86.80	Glu347(A), Gln27(A), Gly443(A), His447(A), Gln445(A)
	β-glycerophosphate	-97.4	Arg166(A), Asp91(A), Thr95(A), Ser155(A), Glu429(A), His432(A)

Although Runx2, Osterix, osteocalcin, and osteopontin are essential regulators of osteogenic differentiation, they were not included in the present computational analysis because the primary objective was to investigate molecular interactions with a protein directly involved in mineralization. Future studies should extend the analysis to additional osteogenic markers to provide a more comprehensive understanding of the molecular mechanisms underlying hydroxyapatite-induced osteogenesis [23, 24].

Therefore, the present findings should be considered preliminary and require confirmation through cellular differentiation assays and gene expression analyses. Nevertheless, the stronger predicted interaction between hydroxyapatite and alkaline phosphatase indicates a potentially favorable molecular association under the computational conditions employed. These results should not be interpreted as direct evidence of enhanced osteogenic differentiation or biological activity but rather as preliminary computational observations that warrant further investigation using molecular dynamics simulations, biochemical assays, and cell-based osteogenic differentiation studies. These findings are in agreement with recent studies demonstrating that nano-hydroxyapatite can enhance osteoblastic activity and upregulate alkaline phosphatase expression, thereby promoting osteogenic differentiation. Moreover, alkaline phosphatase has been shown to directly mediate calcium phosphate and hydroxyapatite formation in biomimetic systems, supporting its central role in bone mineralization and osteomimetic processes [1, 25].

Bio-nano-hydroxyapatite has broad potential in bone tissue engineering, dental biomaterials, bone cements, and enamel remineralization because of its similarity to natural bone mineral. However, these applications were not investigated in the present study and should be considered future perspectives rather than direct outcomes of the current computational findings [26].

3.3. Limitations

The present study has several limitations that should be considered when interpreting the findings. First, the proposed mechanisms are based exclusively on computational analyses, including molecular docking and protein–protein interaction network prediction, and therefore should be regarded as hypothesis-generating rather than definitive evidence of biological function. Experimental validation using biochemical assays, gene expression analysis, protein interaction studies, and cellular osteogenic differentiation models is required to confirm the proposed molecular mechanisms.

Second, the molecular docking protocol was not validated by redocking co-crystallized ligands or benchmarking against experimental binding data, and the docking results were not complemented by molecular dynamics simulations or MM/-PBSA/MM/GBSA binding free energy calculations. Consequently, the reported docking scores should be interpreted as comparative predictions rather than absolute measures of binding affinity or complex stability.

Third, hydroxyapatite is a crystalline inorganic biomaterial rather than a conventional small-molecule ligand. Therefore, docking simulations were

performed using a representative molecular structure instead of a complete crystal lattice, which may not fully capture the physicochemical complexity of hydroxyapatite–protein interactions.

Finally, the computational analysis was limited to selected proteins associated with bacterial biomineralization and osteogenesis, including YtaF, PhoD, and alkaline phosphatase. Other important regulators of osteogenic differentiation, such as Runx2, Osterix, osteocalcin, and osteopontin, were not investigated. Moreover, the proposed role of YtaF in bacterial hydroxyapatite biosynthesis represents a computational hypothesis inferred from docking and STRING analyses and requires further experimental confirmation. Future studies integrating validated computational approaches with molecular, biochemical, and in vitro/in vivo experiments will be essential to verify and extend the findings of the present study. Consequently, docking simulations were performed using a representative molecular structure instead of a complete crystal lattice. Therefore, the calculated docking scores should be interpreted as indicators of potential local interactions between hydroxyapatite functional groups and protein residues rather than absolute measures of biological activity. Nevertheless, molecular docking remains a useful preliminary tool for comparing relative binding tendencies among compounds and generating hypotheses that can be tested experimentally.

10. Conclusion

This study provides a computational framework for understanding bacterial hydroxyapatite biomineralization by proposing a putative role of YtaF and its association with alkaline phosphatase during hydroxyapatite formation. In addition, the predicted strong interaction between hydroxyapatite and alkaline phosphatase suggests a possible molecular basis for its osteogenic potential. These findings generate testable hypotheses for future studies; however, experimental validation is required to confirm the proposed biomineralization mechanism and the predicted protein–ligand interactions.

11. Declarations

11.1. Acknowledgements

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11. 2. Conflict of Interest

The authors declare no conflict of interest.

11.3. Authors Contributions

Formal analysis, graphic investigation and writing-original draft preparation Sabereh Nouri. writing-review and editing, Giti Emtiazi, Oguzhan Gunduz, Songul Ulag. supervision, Giti Emtiazi, project administration, personal.

11.4. Using Artificial Intelligent chatbots

AI chatbots used for graphic abstract.

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