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## *In silico* analysis of heme binding effect on the formation of mouse arginyl-tRNA-protein transferase 1 and protein LIAT1 complex

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*Ab initio* prediction of mouse arginyl-tRNA-protein transferase 1 (R-transferase, EC 2.3.2.8) and protein LIAT1 structures was performed by I-Tasser server. Molecular docking studies of the protein models revealed that potential heme binding sites didn't coincide with the sites of interaction with protein partner in both R-transferase and LIAT1 protein. Heme docking to the complex of two proteins showed LIAT1 protein to provide more preferable sites for heme binding than R-transferase. Heme attachment to the cavities in the core of R-transferase involved in catalysis and the alterations of LIAT1 protein conformation could be the additional mechanisms of heme inhibiting action on protein arginylation.

**Key words:** *heme binding, arginyl-tRNA-protein transferase, LIAT1 protein, 3D-modelling, docking.*

## *In silico* аналіз ефекту зв'язування гему на утворення комплексу аргініл-тРНК-протеїнтрансферази 1 і протеїну LIAT1 миші

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*Ab initio* передбачення структури аргініл-тРНК-протеїнтрансферази 1 (R-трансфераза, КФ 2.3.2.8) і протеїну LIAT1 миші було проведено за допомогою серверу I-Tasser. Молекулярний докінг протеїнових моделей виявив, що потенційні сайти зв'язування гему не співпадають з сайтами взаємодії з протеїнами-партнерами як у R-трансферази, так і у LIAT1 протеїну. Докінг гему до комплексу двох протеїнів показав, що протеїн LIAT1 надає сайти зв'язування, яким гем віддає перевагу у порівнянні з R-трансферазою. Приєднання гему до порожнин в корі R-трансферази, які задіяні у каталізі, та зміни конформації протеїну LIAT1 можуть явитись додатковими механізмами інгібуючої дії гему на аргінілювання білків.

**Ключові слова:** *зв'язування гему, аргініл-тРНК-протеїнтрансфераза, LIAT1 протеїн, 3D-моделювання, докінг.*

## *In silico* анализ эффекта связывания гема на образование комплекса аргинил-тРНК-протеинтрансферазы 1 и белка LIAT1 мыши

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*Ab initio* предсказание структуры аргинил-тРНК-протеинтрансферазы 1 (R-трансфераза, КФ 2.3.2.8) и белка LIAT1 мыши было проведено с помощью сервера I-Tasser. Молекулярный докинг белковых моделей выявил, что потенциальные сайты связывания гема не совпадают с сайтами взаимодействия с белками-партнерами как у R-трансферазы, так и у белка LIAT1. Докинг гема к комплексу двух белков показал, что белок LIAT1 предоставляет более предпочтительные сайты связывания гема в сравнении с R-трансферазой. Присоединение гема к полостям в коре R-трансферазы, задействованным в катализе, и изменения конформации белка LIAT1 могут явиться дополнительными механизмами ингибирующего действия гема на аргинилирование белков.

**Ключевые слова:** *связывание гема, аргинил-тРНК-протеинтрансфераза, белок LIAT1, 3D-моделирование, докинг.*

### Introduction

Arginyl-tRNA-protein transferase 1 (ATE1) or R-transferase (EC 2.3.2.8) is one of the executor enzymes of the N-end rule pathway of protein degradation (Varshavsky, 2011). It attaches arginine as one of the primary destabilizing residues to N-terminals of proteins and is regulated by a number of different factors,

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including oxidative stress (Hu et al., 2005). ATE1-deficient mice have lethal defects in cardiac and vascular development (An et al., 2006; Lee et al., 2012).

ATE1 sequence contains three Cys-Pro and one Pro-Cys patterns, which are putative heme regulatory motifs (HRM) and are common to various heme-binding proteins (Zhang, Guarente, 1995). Two of four HRM-like motifs from mouse ATE1 – $C^{71}C^{72}P$ и $HSC^{411}P$ – are conservative for many species. According to experimental data hemin induces disulfide bond formation between Cys71-Cys72 in $C^{71}C^{72}P$ motif and can bind at several sites including $C^{411}P$ thus inhibiting R-transferase activity in mouse under micromolar concentrations (Hu et al., 2008). The character of heme binding and the affinity of other sites of this protein for heme have not been discussed yet.

N-terminal arginylation *in vitro* by R-transferase was shown to be stimulated by protein LIAT1 (Brower et al., 2014). The ability of LIAT1 protein to bind heme hasn't been investigated. Neither full models of these proteins nor homologous solved structures are available for today. Mechanism of their interaction is also unknown. So, we performed *in silico* modelling of R-transferase and LIAT1 protein structures, prediction of heme-binding sites in the unbound proteins and their complex and molecular docking of protein LIAT1 with R-transferase with or without bound heme.

## Materials and methods

The amino acid sequences and protein annotations were loaded from UniProt DB: *Mus musculus* arginyl-tRNA-protein transferase 1 (canonical isoform, 516 aa; http://www.uniprot.org/uniprot/Q9Z2A5) and protein LIAT1 (228 aa; http://www.uniprot.org/uniprot/Q810M6). Protein domains were analyzed by the help of InterPro (http://www.ebi.ac.uk/interpro/) and Pfam (http://pfam.xfam.org/) servers.

*Ab initio* protein structure prediction was performed using I-TASSER server (Yang et al., 2015) without or with template (http://zhanglab.ccmb.med.umich.edu/I-TASSER/).

The choice of prediction server was based on its high citation and high rank in CASP7-10 (http://predictioncenter.org/casp10/groups_analysis.cgi). The template choice for R-transferase was guided by its similarity to N-acyltransferases superfamily (IPR016181) revealed by sequence analysis (Rai et al., 2006). So two known structures were chosen as additional templates for prediction: 2Q4V:A (human acetyltransferase SAT2) and 1P4N:A (bacterial peptidoglycan synthase FemX). LIAT1 protein modelling was performed without template. For each variant of prediction I-TASSER server provided five models selected by a confidence score (C-score). Checking of models was carried out on-line by Verify (http://services.mbi.ucla.edu/Verify_3D/) and Errat servers (http://services.mbi.ucla.edu/ERRAT/). Structural alignment of protein structures obtained was performed by TM-align server (http://zhanglab.ccmb.med.umich.edu/TM-align).

Docking of proteins was performed by ZDOCK (Pierce et al., 2014), version ZD3.0.2F, without stating of interacting residues (http://zdock.umassmed.edu/). Structure file (*.pdb) for heme molecule was loaded from PubeChem (http://www.ebi.ac.uk/pdbe-srv/pdbechem/chemicalCompound/show/HEM). Docking of proteins with heme as ligand was carried out by on-line tool PatchDock, Beta 1.3 Version (Schneidman-Duhovny et al., 2005; http://bioinfo3d.cs.tau.ac.il/PatchDock/). Docking variants with heme ring not crossed or touched by amino acid side chains were selected for further analysis and the predictions with best scores are presented in results section.

Visualization and analysis of structures (including the selection of residues within certain distance to ligand or other protein chain) were carried out by the help of PyMOL (The PyMOL Molecular Graphics System, Version 1.3, Schrödinger, LLC) and SwissProt viewer 4.1.0 (http://spdbv.vital-it.ch).

## Results and discussion

The structural models of R-transferase obtained by I-Tasser revealed great differences in the secondary structure from mostly β-organized first model to almost no β-sheets in model 4 (template-free prediction). Model 2 (template-free) containing disulfide bond between Cys71-Cys23; and models 3 and 4 of protein LIAT1 almost fully organized into β-structures and rod-like, were excluded from further analysis. $HSC^{411}P$ sequence was proposed (Hu et al., 2008) to be proximal in the folded enzyme to the disulfide-forming region $C^{71}C^{72}P$ and the thioredoxin-like motif $C^{23}GYC^{26}$. It was assumed that $HSC^{411}P$ and $C^{71}C^{72}P$ are located adjacent to each other and play role in the ligand binding. Among models obtained for R-transferase no one had all three sites in close proximity to each other. In the model 4 (template-free) $C^{71}C^{72}P$ is located close to the Cys411 but not to Cys23; in models 3 and 5 (template-free) $C^{71}C^{72}P$ is located close to Cys23 but not to Cys411. So R-transferase models 3-5 (template-free) and LIAT1 protein models 1, 2 and

5 were selected for docking analysis. In models 3 and 4 of R-transferase $C^{71}C^{72}P$ motif was placed on the surface, but in model 5 this motif is in the cavity so disulfide $C^{71}C^{72}$ bond formation could significantly change the conformation of the core protein region (Fig. 1, A).

Amino acids in neighbourhood of heme ring in LIAT1 protein were represented mostly by histidines and motifs of charged amino acids but not by cysteines (Table 1).

**Table 1.**

**Analysis of PatchDock docking results (heme as ligand and selected protein model structures of R-transferase or LIAT1 protein as targets)**

| Protein model | Contact area | Total Score | Number of AA | Amino acids in neighborhood to heme in the models (predicted to be within 6,5 Å to heme iron) |
|---|---|---|---|---|
| R-transferase | | | | |
| model 3 | 892,6 | 6346 | 7 AA | S34; Y35; W55; C79; H80; P81; P441 |
| model 4 | 816,1 | 6236 | 8 AA | E13; K209; K215; E216; S251; S259; V351; Y403 |
| model 5 | 870,5 | 6334 | 7 AA | H171; Y298; Q312; F313; V364; Y365; Y367; F406 |
| LIAT1 protein | | | | |
| model 1 | 845,8 | 6514 | 14 AA | E19; E20; E22; E23; A48; K55; A100; P102; R103; D104; K105; E107; L130; Q133 |
| model 2 | 942,7 | 7166 | 11 AA | H87; L90; H96; G97; L98; E101; L159; N160; K163; R164; R166 |
| model 5 | 870,5 | 6726 | 12 AA | S85; F86; H87; I89; N108; Q110; S115; D141; G142; I143; L144; T145 |

Amino acids residues within 6,5 Å from the heme iron in R-transferase models included K215 involved in posttranslational modification (by UniProtKB); aromatic amino acids, Cys79 but not Cys71-72 or Cys411. Such predictions may be linked with mostly geometric approach of the PatchDock tool that doesn't take into account metal coordination and oxidative effects of heme iron. Heme binding at additional sites except HRM-like is in agreement with the experimental analyses of R-transferase mutants (Hu et al., 2008).

Heme docking to selected protein model structures revealed heme placement in the cavities in the central parts of the molecules (Fig. 1, A-C). Heme binding in deep cavity found in the core of the LIAT1 protein model 2 (Fig. 1, D) could greatly affect protein conformation and interaction with R-transferase.

R-transferase works as monomer but the interaction with protein LIAT1 increases its catalytic activity (Brower et al., 2014) so we have modelled the complex of these proteins using ZDOCK tool. An internal conserved 53-residue segment of LIAT1 (113–165) known to be sufficient for binding to R-transferase (Brower et al., 2014) is mostly exposed in model 5 of LIAT1 protein. Higher Z-scores and number of interacting amino acids were shown for complexes of R-transferase model 4 with any selected model of LIAT1 protein (Table 2) comparing to model 5 of R-transferase. In five of six docking variants (Table 2) the amino acid region 113–122 of LIAT1 protein, containing first of three PC motifs ($P^{122}C$), was predicted to interact with R-transferase.

No coincidence of the predicted sites for heme binding and regions for protein partner interaction was found in both R-transferase and protein LIAT1. This fact may be linked with heme binding in cavities and not at the surface. Therefore, if heme doesn't cause significant conformation changes it seems unlikely that heme binding to either R-transferase or LIAT1 protein impedes their complex formation.

To check more preferable binding sites for heme in the complex of R-transferase and protein LIAT1 we performed molecular docking (by PatchDock) of heme to the complex with different combinations of protein models. Heme docking to the complex of two proteins showed that in more than 80% of results heme was bound to the protein LIAT1 and not to R-transferase. All potential heme-binding sites of LIAT1 protein were not involved into proteins interaction and so could be available for heme.

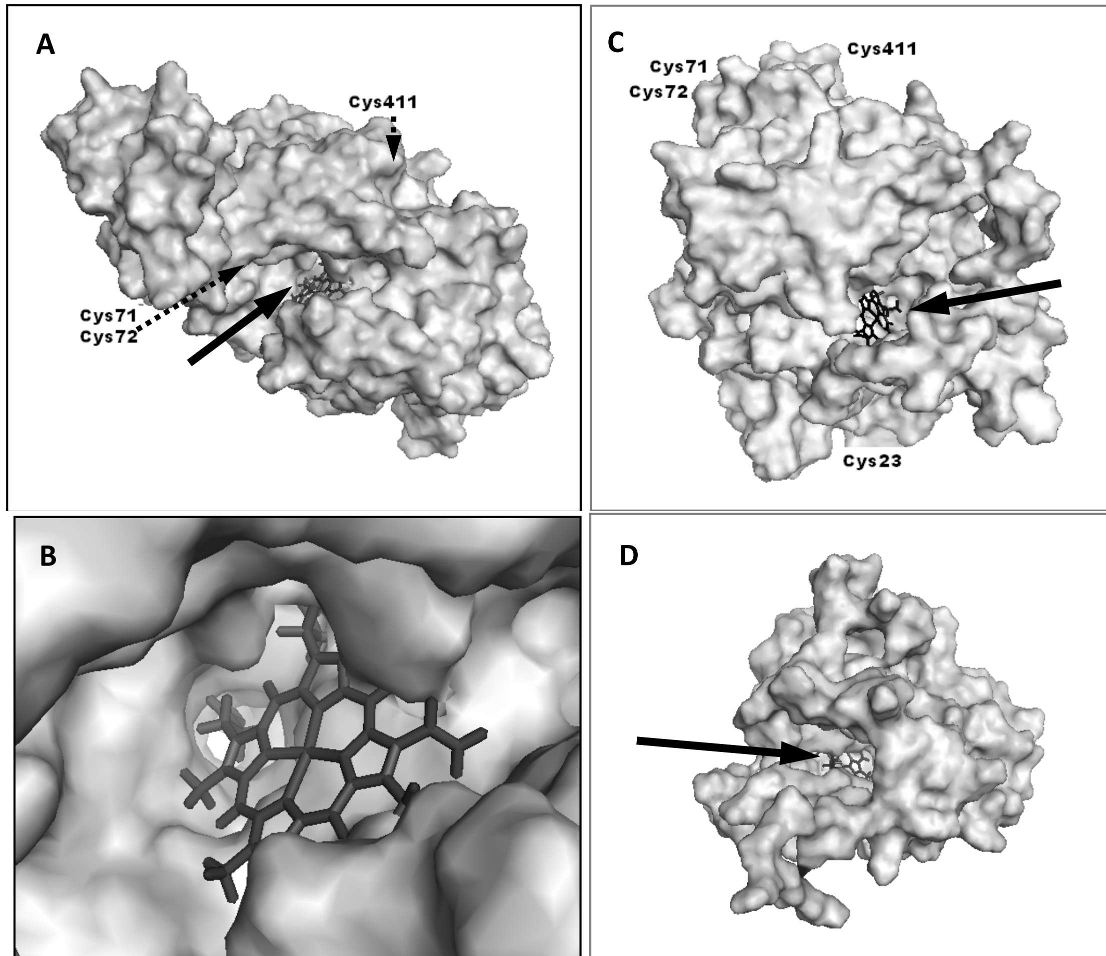


**Fig. 1. Visualization of the PatchDock predictions in PyMOL:** A – heme docking to model 5 of R-transferase – general view; B – heme placement in the cavity of R-transferase model 5; C – heme docking to model 4 of R-transferase; D – heme docking to model 2 of LIAT1 protein. Solid arrows show heme in the cavities; dotted arrows show location of cysteines in functional motifs

**Table 2.**
**The results of R-transferase and LIAT1 protein model structures docking by ZDOCK: Z-scores and amino acid regions of the contact area (within 6 Å to other chain, analyzed by SwissPDB Viewer)**

| Model | LIAT1 model 1 | LIAT1 model 2 | LIAT1 model 5 |
|---|---|---|---|
| R-transferase model 4 | Z-score=1828.5<br>R-transferase: 20AA<br>(1–12; 35–38; 258–262)<br>LIAT1: 32 AA<br>(30–40; 113–122; 198–208) | Z-score=1634.5<br>R-transferase: 36 AA<br>(1–9; 311–323; 333–346)<br>LIAT1: 62 AA<br>(28–40; 87–109; 150–171; 189; 214–228) | Z-score=1889.0<br>R-transferase: 39 AA<br>(312-350)<br>LIAT1: 49 AA<br>(37–53; 113–130) |
| R-transferase model 5 | Z-score=1103.1<br>R-transferase: 17 AA<br>(1–12; 326–330)<br>LIAT1: 14 AA<br>(113–124; 206; 210) | Z-score=1325.5<br>R-transferase: 24 AA<br>(1–14; 59–63; 109–115)<br>LIAT1: 18 AA<br>(56–60; 113–123; 177–178) | Z-score=1267.4<br>R-transferase: 21 AA<br>(1–10; 24–34)<br>LIAT1: 24 AA<br>(38–42; 113–123; 220–228) |

The active site of R-transferase has not been established yet so we can assume that high concentrations of heme could inhibit protein arginylation by three mechanisms. Firstly, heme oxidative action on R-transferase cysteines with further disulfide bond formation between Cys71 and Cys72 (Hu et al., 2008) and change of R-transferase conformation. By the other hand, heme could occupy several cavities of the R-transferase core necessary for catalysis. And additionally, higher affinity of LIAT1 protein to heme comparing to R-transferase could result in the indirect inhibition of the arginyltransferase reaction through the alterations of LIAT1 protein conformation and its consequent dissociation from R-transferase.

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