Prediction of protein structure of novel protein (116 kDa) from human sperm membrane

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ABSTRACT

Objective: To predict the protein similarity, biological function and structure of 116 kDa protein that was isolated from human sperm membrane of acrosome by using *in silico*. **Methods:** Predictions for 116 kDa protein similarity was done through comparative analysis of its isoelectric point (pI) and molecular weight to the UniProtKB/TrEMBL protein database. The three-dimensional structure of the protein was built using SWISS-MODEL. **Results:** The 116 kDa protein with pI 4.4 was expected to have similarities with CDH23 proteins that had pI 4.38, thus they likely had similar physical and biochemical properties. The predicted protein had an extracellular N-terminal that likely acted as a receptor, and showed cell recognition characteristic, playing a role in fertilization. **Conclusions:** The 116 kDa protein isolated from acrosome membrane has similarity on its biophysical character to the CDH23.

1. Introduction

Sperm membrane has proteins that are specific and immunogenic[1]. Membrane proteins in the sperm anterior play a role in their interaction with the egg, especially with protein ZP3. Membrane proteins that are located on the equatorial head play a role in sperm–egg membrane fusion[2]. In general, a protein is located in the membrane largely as the result of biosynthesis by the spermatozoa themselves, so it is believed by some researchers that this specific protein is solely owned by a sperm[3,4]. Due to the properties of the sperm membrane protein as mentioned above, it has promising potential for immunocontraception.

The developed immunocontraception is a protein isolated from membranes of human sperm with the use of Tween-20 detergents, which is a protein with a molecular weight of 116 kDa. By using two dimensional electrophoresis to specify isoelectric point (pI) of the protein, it has been established that 116 kDa protein has a pI of 4.4[5,6]. By staining with fluorescence rhodamine and through observation using a Confocal Laser Scanning Microscope (Olympus FV-1000, Argon Laser), protein 116 kDa-seen expressed in the inner acrosomal membrane, posterior head, neck and midpiece in human sperm-most likely plays a role in fertilization.

Information on the 116 kDa protein isolated from human sperm membrane is not yet available on a database. Hence, to understand the functional biology of protein molecules 116 kDa more accurately, especially its fertilization function, it is necessary to study the three-dimensional structure of this protein. A three-dimensional structure will be able to provide an overview of proteins in the cell membrane[7,8]. A technique with a web-based program that makes a comparison with the existing protein structure may predict the structure of 116 kDa protein.

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2. Materials and methods

2.1. Protein identification

A protein on the sperm membrane with the characteristics of 116 kDa. pI 4.4 protein-kinase activity was previously identified[14]. And the protein-kinase activity was not performed. The protein was then predicted using "TagIdent tool" software[9]. Analysis was performed based on pI and molecular weight as well as the properties of non-kinase proteins in the UniProtKB/TrEMBL database.

2.2. Protein structure and function

Three-dimensional protein structures were modeled using SWISS-MODEL, the software modeling for three-dimensional structure of proteins that runs using the principle of fully automated protein structure homology-modeling server, and is accessible via the ExPASy web server[10]. The protein model was visualized using Discovery Studio Viewer software. The function of the protein is subsequently tracked through UNIPROT database and Superfamily 1.7 in the Hidden Markov Model (HMM) library and genome assignment server. HMM is a software for predicting the biological function of proteins based on the domain function of the structure[11].

2.3. Orientation of cell membrane proteins

The orientation of proteins in a cell membrane was predicted with orientations of proteins in membranes server. This software calculates the rotational and translational positions of transmembrane and peripheral proteins in membranes based on the three-dimensional structure of the protein. It can be applied to newly determined experimental or theoretical models of protein structures. Many examples of proteins that exist in the Protein Data Bank have been recalculated using this orientations of proteins in membranes software[12]. Subsequently, protein models were visualized using Discovery Studio Viewer software.

3. Results

Predictions for the 116 kDa protein sequences were performed through comparative analysis of the proteins in the UniProtKB/TrEMBL (48,744,721) database. The 116 kDa protein had pI of 4.4; the molecular weight of 116 kDa and its properties as a non-protein kinase in human sperm membrane were expected to have similarities with CDH23, which had a pI of 4.38 and a molecular weight of 116.612. pI varies for different proteins, based on the difference in the constituent amino acids, whereas each amino acid had its own characteristics. Hence, the similarity of the pI owned by 116 kDa protein with that of CDH23 protein, indicated that there may be similarities in its physical and biochemical properties. Amino acid

sequences of CDH23 were shown in Figure 1.


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MGRHVATSCHVAWLLVLISGCWGQVNRLPFFTNHFFDTYLLISEDTPVGSSVTQLLAQDM
DNDPLVFGVSGEEASRFFAVEPDTGVVWLRQPLDRETKSEFTVEFSVSDHQGVITRKVNI
QVGDVNDNAPTFHNQPYSVRIPENTPVGTPIFIVNATDPDLGAGGSVLYSFQPPSQFFAI
DSARGIVTVIRELDYETTQAYQLTVNATDQDKTRPLSTLANLAIIITDVQDMDPIFINLP
YSTNIYEHSPPGTTVRIITAIDQDKGRPRGIGYTIVSGNTNSIFALDYISGVLTLNGLLD
RENPLYSHGFILTVKGTELNDDRTPSDATVTTTFNILVIDINDNAPEFNSSEYSVAITEL
AQVGFALPLFIQVVDKDENLGLNSMFEVYLVGNNSHHFIISPTSVQGKADIRIRVAIPLD
YETVDRYDFDLFANESVPDHVGYAKVKITLINENDNRPIFSQPLYNISLYENVTVGTSVL
TVLATDNDAGTFGEVSYFFSDDPDRFSLDKDTGLIMLIARLDYELIQRFTLTIIARDGGG
EETTGRVRINVLDVNDNVPTFQKDAYVGALRENEPSVTQLVRLRATDEDSPPNNQITYSI
VSASAFGSYFDISLYEGYGVISVSRPLDYEQISNGLIYLTVMAMDAGNPPLNSTVPVTIE
VFDENDNPPTFSKPAYFVSVVENIMAGATVLFLNATDLDRSREYGQESIIYSLEGSTQFR
INARSGEITTTSLLDRETKSEYILIVRAVDGGVGHNQKTGIATVNITLLDINDNHPTWKD
APYYINLVEMTPPDSDVTTVVAVDPDLGENGTLVYSIQPPNKFYSLNSTTGKIRTTHAML
DRENPDPHEAELMRKIVVSVTDCGRPPLKATSSATVFVNLLDLNDNDPTFQNLPFVAEVL
EGIPAGVSIYQVVAIDLDEGLNGLVSYRMPVGMPRM DFLINSSSGVVVTTTELDRERIAE
YQLRVVASDAGTPTKSSTSTLTIHVLDVNDETPTFFPAVYNVSVSEDVPREFRVVWLNCT
DNDVGLNAELSYFITGAAPASAHLCRPPGALPPPLPDGQPD
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Figure 1. The amino acid sequences of CDH23 (Q8N5B3_HUMAN) from UniProtKB/TrEMBL database that similar with the isolated protein from head of sperm.

The protein has been predicted involved in the biological process of spermatogenesis (superfam; superfamily 1.75, HMM Library and genome assignment server).

Three-dimensional model of the protein was built using SWISS-MODEL (Basel, Switzerland) based on amino acid sequences of CDH23 (Figure 2). The protein structure showed the transmembrane form with the extracellular part which was the N-terminal, while the part in the cytoplasm was the C-terminal. The extracellular N-terminal played a role in the receptor. Based on the biological function of CDH23, there were similarities with the function of 116 kDa protein of sperm as shown in Table 1.
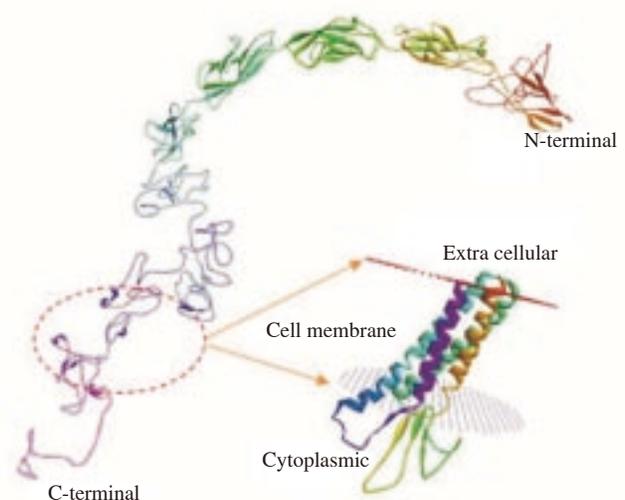

Figure 2. The 3D structure of CDH23 protein made using SWISS-MODEL.

Table 1

Biological functions of CDH23 (Q8N5B3).

Biological functions	False discovery rate (FDR)
Sexual reproduction	1.10E-07
Multicellular organismal reproductive process	8.42E-08
Biological adhesion	0
Locomotion	9.02E-10
Epithelium development	9.39E-10
Cellular component morphogenesis	1.59E-07
Cell projection organization	9.69E-07
Spermatogenesis	1.72E-07
Cell adhesion	0
Cell junction organization	2.18E-12
Establishment or maintenance of cell polarity	6.08E-08
Calcium ion binding	2.79E-12

4. Discussion

According to the UniProtKB/TrEMBL (48,744,721) database, CDH23 serves to bind with other proteins, or in cell recognition, and most likely plays a role in the fertilization process and the introduction of sperm with cells around it, either in the uterus or ovum. When compared with the 116 kDa protein, there is a similarity in the properties to the CDH23 protein. The 116 kDa protein might be expressed at the human sperm head (acrosome) and neck (midpiece). The 116 kDa protein is likely to play a role in the fertilization process. It is known that the membrane protein engages with the ZP3 protein in the anterior part during the interaction process, and that this process also likely involves outer acrosome membrane proteins, which will fuse with the membrane of the spermatozoa through exocytosis[13].

The CDH23 protein was found in cells that have mobility equal to 9.021 E-10, whereas 116 kDa protein found in human sperm has high mobility along the reproductive tract. The results of immunohistochemistry using a polyclonal antibody of 116 kDa protein showed that 116 kDa protein was not found in human cells that are immobile, such as the pancreas, kidney, spleen, prostate, heart, liver, brain and blood vessels[14]. This data suggested that the protein only expressed specifically in spermatozoa may be important for recognition or fertilization process. The CDH23 was found in the several tissues, but there has been no information concerning about the protein found in the spermatozoa. Therefore, in the study, this prediction is novel information that the 116 kDa protein from spermatozoa membrane is CDH23. Further research to elucidate the information that the 116 kDa is CDH23 is necessary to be done.

Based on the _in silico_ analysis, it is suggested that 116 kDa protein found in sperm has similar characteristics to the CDH23 protein.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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