Research Article

Immunoinformatic Analysis of Calcium-Dependent Protein Kinase 7 (CDPK7) Showed Potential Targets for *Toxoplasma gondii* Vaccine

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Received 10 April 2021; Revised 10 June 2021; Accepted 22 June 2021; Published 12 July 2021

Academic Editor: José F. Silveira

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Apicomplexan parasites, including *Toxoplasma gondii* (*T. gondii*), express different types of calcium-dependent protein kinases (CDPKs), which perform a variety of functions, including attacking and exiting the host cells. In the current bioinformatics study, we have used several web servers to predict the basic features and specifications of the CDPK7 protein. The findings showed that CDPK7 protein has 2133 amino acid residues with an average molecular weight (MW) of 219085.79 D. The aliphatic index with 68.78 and grand average of hydropathicity (GRAVY) with -0.331 score were estimated. The outcomes of current research showed that the CDPK7 protein included 502 alpha-helix, 1311 random coils, and 320 extended strands with GOR4 method. Considering the Ramachandran plot, the favored region contains more than 92% of the amino acid residues. In addition, evaluation of antigenicity and allergenicity showed that CDPK7 protein has immunogenic and nonallergenic nature. The present research provides key data for more animal-model study on the CDPK7 protein to design an efficient vaccine against toxoplasmosis in the future.

1. Introduction

*Toxoplasma gondii* is a prevalent intracellular protozoan, which can infect a broad spectrum of mammals (i.e., human) and birds [1, 2]. Oocysts are the potential infective form in the life cycle of the parasite. Feline species as the only definitive hosts can contaminate the environment by shedding unsporulated oocysts through feces [3]. *T. gondii* is transferred by water/vegetables contaminated via mature oocysts and consumption of raw or semicooked meat from infected animals, vertical transmission from infected pregnant mothers to neonates, and blood transfusion [4–7]. Approximately one-third of human society has been exposed to *T. gondii*, worldwide [5, 8, 9]. Often *T. gondii* infection among immunocompetent people is asymptomatic or demonstrates mild symptoms, whereas in immunocompromised patients, it can cause a various range of clinical symptoms [6, 9, 10]. Toxoplasmosis in immunocompromised subjects can cause repeated attacks in the brain and manifests as encephalitis [11]. Moreover, toxoplasmosis in pregnant women can cause blindness, microcephaly, and mental retardation in the infant [6, 12]. Different factors, such as host's
Figure 1: Continued.
Figure 1: NetPhos server output for CDPK7 phosphorylation sites. (a) The number of predicted sites, based on S (serine), T (threonine), and Y (tyrosine); (b) prediction diagram of CDPK7 phosphorylation sites.

Table 1: The acylation sites of CDPK7 sequence.

| ID          | Position | Peptide           | Score |
|-------------|----------|-------------------|-------|
| TGME49_228750 CDPK7 (T. gondii) | 34       | STQLSKECLKQYLKK   | 1.129 |
| TGME49_228750 CDPK7 (T. gondii) | 109      | FLIGIAYCCRTGKSD   | 1.996 |
| TGME49_228750 CDPK7 (T. gondii) | 110      | LIGIAVCCRTGKSD    | 5.494 |
| TGME49_228750 CDPK7 (T. gondii) | 187      | QNLFSPCQRTPQNG    | 0.526 |
| TGME49_228750 CDPK7 (T. gondii) | 222      | DEEDTGSCGNSNP     | 5.293 |
| TGME49_228750 CDPK7 (T. gondii) | 244      | YPEAALVCSDPFP    | 3.693 |
| TGME49_228750 CDPK7 (T. gondii) | 321      | SLDVFQGSPFP     | 0.984 |
| TGME49_228750 CDPK7 (T. gondii) | 524      | SSEASVICPQGGISP  | 2.536 |
| TGME49_228750 CDPK7 (T. gondii) | 706      | VDKIEECEFEEHGK   | 0.403 |
| TGME49_228750 CDPK7 (T. gondii) | 736      | ILSMFTECLHEEVWG   | 1.821 |
| TGME49_228750 CDPK7 (T. gondii) | 1298     | AHDPPACSGHSDP    | 5.591 |
| TGME49_228750 CDPK7 (T. gondii) | 1309     | SPRLYSCNPCPNCNL   | 1.744 |
| TGME49_228750 CDPK7 (T. gondii) | 1312     | DLSCPNCNPPLLC   | 8.015 |
| TGME49_228750 CDPK7 (T. gondii) | 1313     | LYSSCPNCNPPLLC   | 7.05  |
| TGME49_228750 CDPK7 (T. gondii) | 1319     | CCNPLCFLPFCHSY   | 2.719 |
| TGME49_228750 CDPK7 (T. gondii) | 1322     | PLLLCPCFHSRPQPL  | 2.865 |
| TGME49_228750 CDPK7 (T. gondii) | 1340     | EGRVMEGRCQGRLG   | 2.295 |
| TGME49_228750 CDPK7 (T. gondii) | 1343     | VVMCRQCGRLGSSR   | 2.929 |
| TGME49_228750 CDPK7 (T. gondii) | 1395     | DVEAGICVGGSRR    | 5.406 |
| TGME49_228750 CDPK7 (T. gondii) | 1406     | SSRVFTRCWHCGWEL  | 0.108 |
| TGME49_228750 CDPK7 (T. gondii) | 1409     | VFTURCWCGWELSK    | 1.424 |
| TGME49_228750 CDPK7 (T. gondii) | 1416     | CGWELSKCAEMLKG    | 4.272 |
| TGME49_228750 CDPK7 (T. gondii) | 1474     | GMFLECGYELLSE    | 1.626 |
| TGME49_228750 CDPK7 (T. gondii) | 1649     | TVYYLHKCIVHRD    | 1.164 |
| TGME49_228750 CDPK7 (T. gondii) | 1683     | DFGLSTLCAPNEVLH  | 1.6   |
| TGME49_228750 CDPK7 (T. gondii) | 1693     | NEVLHCPGTLAYVA    | 1.927 |
| TGME49_228750 CDPK7 (T. gondii) | 1828     | GEERTMACPVEPPTF  | 4.139 |
| TGME49_228750 CDPK7 (T. gondii) | 1829     | EERTMACPVEPPTT    | 7.362 |
| TGME49_228750 CDPK7 (T. gondii) | 2080     | PSILAPGCDSLASS    | 3.862 |
| TGME49_228750 CDPK7 (T. gondii) | 2110     | ARQDERACGTAPA EVP | 6.173 |
immune system status, genetic background, age, gender, contact with infected cats, environmental conditions, and diet and cultural habits, as well as the protozoan genotype, can affect the morbidity and mortality rate of Toxoplasma infection [13, 14].

Today, treatment of toxoplasmosis with conventional drugs can just limit the proliferation of tachyzoites at the beginning of infection, while these drugs cannot eradicate cystic forms of parasites in host tissue [15, 16]. In addition, taking these medications in pregnant women can have serious side effects, such as the possibility of teratogenic effects on the fetus [17]. Hence, the discovery and design of an effective vaccine to control and prevent toxoplasmosis is very important, especially in humans and domestic animals. In this regard, various in silico-based studies suggest various antigens as suitable candidates for vaccine design [18–32]. Calcium-dependent protein kinases (CDPKs) are a class of serine/threonine kinases that express in apicomplexans, ciliates, and plants [33]. In T. gondii as a member of the apicomplexan parasites, several CDPKs have been identified involving in critical functions in the different stages of the life cycle of parasite, including gliding motility (surface translocation), entry into (invasion), and exit from (egress) of host cells [34]. The CDPK7 is a crucial enzyme for division, growth, and maintenance of structural integrity of the Toxoplasma centrosome. As a result, TgCDPK7 knockdown is suggested as an important goal in achieving the right vaccine [35].

Computer-aided evaluation of different T. gondii proteins involved in various stages of life cycle can open new doors towards recognizing potent vaccine candidates through identification of highly immunogenic, nonallergenic, and nontoxic B- and T-cell epitopes [36]. Thereby, the present in silico study was performed to evaluate the crucial biochemical features and immunogenic epitopes of the CDPK7 protein by means of different bioinformatics servers.

2. Methods

2.1. CDPK7 Sequence. For this purpose, ToxoDB online website was used to obtain the whole amino acid sequence of T. gondii CDPK7 protein.

2.2. Physicochemical Characterization. We used the Expasy ProtParam online server to predict the physicochemical parameters of CDPK7 [37].

2.3. Prediction of Posttranslational Modification (PTM) Sites. The NetPhos 3.1 online tool was applied to predict phosphorylation location, and the CSS-Palm online server was applied to predict acylation location of the CDPK7 [38, 39].

2.4. Transmembrane Domains and Subcellular Location. The transmembrane regions and subcellular localization of T. gondii CDPK7 protein were assessed utilizing the TMHMM 2.0 and PSORT II web servers, respectively [38].
2.5. Secondary and Tertiary Structures. In this study, we employed the Garnier-Osguthorpe-Robson 4 (GOR4) online tool to forecast the secondary structure of CDPK7 protein [40]. Consequently, the three-dimensional (3D) model structures was used by SWISS-MODEL [38, 41].

2.6. The 3D Modeled Structure Refinement and Validation. GalaxyRefine was selected to develop and refine the quality of the template-based protein prediction [42]. To the Ramachandran plot validated the 3D structure of the protein, the SWISS-MODEL software was applied [43]. ProSA-web was used for evaluation of the whole quality of the model [44].

2.7. Linear and Conformational B-Cell Epitopes. We used a web-based Bcepred server to predict continuous B-cell epitopes exploiting physicochemical characteristics [45]. An online server of ABCpred was applied to predict B-cell epitopes [46]. Using the immune epitope database (IEDB), hydrophobicity [47], Bepipred linear epitope prediction [48], antigenicity [49], surface accessibility [50], beta-turn [51], and flexibility [52] were predicted. Afterwards, discontinuous B-cell epitopes were appraised by ElliPro [53] from the 3D structure of protein epitopes.

2.8. MHC-I and MHC-II Epitopes. To this aim, we used the IEDB website to evaluate the half-maximal inhibitory concentration (IC$_{50}$) values of peptides that bind to the main histocompatibility complex (MHC) class I and class II molecules for CDPK7 [54, 55]. All predicted epitopes were then
Figure 4: SWISS-MODEL server output. (a) Computed 3D model; (b) global quality estimate; (c) comparison with nonredundant set of PDB structures; (d) local quality estimate.
evaluated in terms of antigenicity using the VaxiJen v2.0 server.

2.9. Cytotoxic T-Lymphocyte (CTL) Epitopes. We applied CTLpred online website according to 75.8% accuracy [56]. Next, all predicted epitopes were evaluated regarding antigenicity using the VaxiJen v2.0 server.

2.10. Antigenic and Allergenic Profiles. The antigenicity of the full CDPK7 sequence was estimated by VaxiJen v2.0 [57]. The allergenic profile of CDPK7 was predicted by the AllergenFP v1.0 and AllerTOP v2.0 servers [58, 59].

3. Results

3.1. General Information of CDPK7. The amino acid structure of CDPK7 was obtained from the ToxoDB server with accession no. TGME49_228750. Based on the ProtParam database, the CDPK7 protein entails of 2133 amino acid residues with molecular weight of 219085.79 D, whereas theoretical pI was 5.79. The overall number of negatively (Asp + Glu) charged residues was 209, and positively (Arg + Lys) charged residues was 178. There are a total number of 30441 atoms. The half-life of the CDPK7 was predictable at 30 hours, >20 hours, and >10 hours for mammalian (in vitro), yeast (in vivo), and Escherichia coli (in vivo), respectively. In addition, the instability index of the CDPK7 protein presented an unstable nature with a value of 53.28. In addition, the aliphatic index was calculated 68.78, and GRAVY of the protein was estimated -0.331.

3.2. PTM Sites of CDPK7 Protein. In the present research, the results exhibited that 269 phosphorylation sites (Thr: 64, Tyr: 13, and Ser: 192) (Figures 1(a) and 1(b)) and 30 acylation
| Prediction parameter | Epitope sequence |
|----------------------|------------------|
| **Hydropilicity**    | GAGGGAGGAG; KKFSDEV; KGGSVDYEE; CRGTSKDSRM; AQAHSEGNSVGRGSHGGKKEEQNL; SPOQCTRQPONGGSSGTAGA; SPGNLDEDEDEDTGSCGSSN; SLSDTSSSNERP; EQASSESEYGRFDEESGSSYSS; DHAERSN; QGFEAQPOEP; STPTSEQGTA; SASSPAGGS; DPDAGATIGAE; RPAAGGDDGSSAPAGGAGSSEAAKAEPSKPTGTG; SQOPPRG; STQSSSTQAPGS; SGGGSREP; PSRQEGSAEV; SRAETQENETG; GEGATPGDGREASLeAGQGNG; QPSKGPTKSA; QAEKDTRKQEQAKKPN; IKEEKEENEQKDV; GSGREGGS; GKSAGSPPSSRGG; TNPAHSSPRRPRD; QATGSSGASA; ARAGSGA; GQAGPENAGA; ETSQASQHTQGSPGSPSSP; GVEPKQE; AGGAGGETQPA; ASGGP; SEGPA; SEGPA; SEGGP; SPQG; SEQATG; DPTTAGA; EAAAAAGG; GPQDGGRGSDA; SIGEEGERSGSDGDVYER; DSRAPPS; GASAGSGPA; ASPSEGASAR; ARAHHDP; SGHSPDR; AGTГANSGAGGAGSADPGSSPSLEEDVEQAE; KNGSEAA; RRGGDAPRPG; SEQVGGQ; KGETVS; ANSAKEQRE; DKGKING; TDRTPNAT; EVSSSADK; VNNGSKNID; DEVHRHSTREYGEERTA; AASSPS; DPGAPS; AARTEGDTGPVEG; DEVPESS; GGEVYSID; AGRGVED; TRQGQGQQTAG; TLQDGSEGR; AAEAGPS; SASSGTQRGTRTEEPAEPARQERAC; GSPGGS |
| **Flexibility**       | LAFSTQ; QYLKKFD; KALARSPS; QKFDFKGSS; AVCCRGTDKS; QAHHSEGNSVGRGSHGGKKEEQNL; CQCTRTQNPONGGSSGTAG; AVSSPNGNLDEDEDEDTGSCGSSN; SLSDTSSSNERP; ARLQEAEASSE; RSFDEESSGA; FDHASRNPSP; GTVSTPSQEG; PAAALSR; VSASSP; PAADGDGSS; PGAGAGG; SAAGGKAEPSKPTGTSLQQPPIR; GKEEEQNL; DDEDDEEDT; SSNERPRRPL; ARLEQEAEAS; GSDGDVYERHAG; QPSKG; EASQHQTG; PTDP; TPSG; PRAETQENEE; GDAQGREA; QAAQPSKPG; LQAEKDTRKQEQAKKPN; IKEEKEENEQKDV; GSGREGGS; GKSAGSPPSSRGG; TNPAHSSPRRPRD; QATGSSGASA; ARAGSGA; GQAGPENAGA; ETSQASQHTQGSPGSPSSP; GVEPKQE; AGGAGGETQPA; ASGGP; SEGPA; SEGPA; SEGGP; SPQG; EQGPPDGRGSGA; SVLQDGSEGRR; AEAGPS; SASSGTQRGTRTEEPAEPARQERAC; GSPGGS |
| **Accessibility**     | STQLSKECLKQYLKFSDEV; VLKKYKAL; PGIDETFLQ; GELEDAEQGN; PQAAPQS55KPTSA; LQAEKDTRKQEQAKKPN; QPSKG; EASQHQTG; PTDP; TPSG; PRAETQENEE; GDAQGREA; QAAQPSKPG; LQAEKDTRKQEQAKKPN; IKEEKEENEQKDV; GSGREGGS; GKSAGSPPSSRGG; TNPAHSSPRRPRD; QATGSSGASA; ARAGSGA; GQAGPENAGA; ETSQASQHTQGSPGSPSSP; GVEPKQE; AGGAGGETQPA; ASGGP; SEGPA; SEGPA; SEGGP; SPQG; PQVSHQAQ; VSAGSDV; AARTEGDTGPVEG; DEVPESS; GGEVYSID; AGRGVED; TRQGQGQQTAG; TLQDGSEGR; AAEAGPS; SASSGTQRGTRTEEPAEPARQERAC; GSPGGS |
| **Polarity**          | KECLKQYLKFDSSDE; VLKKYKAL; RGTKSDF; GKEEEQNL; DDEDEDEET; SNNERPRLPKYPEHP; RNPSSPR; AEKSPKGT; IQLAEKDTRKQEQAKKPN; QPSKG; EASQHQTG; PTDP; TPSG; PRAETQENEE; GDAQGREA; QAAQPSKPG; LQAEKDTRKQEQAKKPN; IKEEKEENEQKDV; GSGREGGS; GKSAGSPPSSRGG; TNPAHSSPRRPRD; QATGSSGASA; ARAGSGA; GQAGPENAGA; ETSQASQHTQGSPGSPSSP; GVEPKQE; AGGAGGETQPA; ASGGP; SEGPA; SEGPA; SEGGP; SPQG; GVEPKQE; VLYKKGKHLH; EQVGGQ; KGETVS; ANSAKEQREWVD; VTQVQAAEQ; IFRATNERE; KVIDQKINGHERELLSE; RLHNP; KELDLKTE; TLQDGSEG;

Table 2: Epitopes predicted in CDPK7 protein by different parameters based on the Bcepred online server.
| Prediction parameter | Epitope sequence |
|----------------------|------------------|
|                      | QLSKECLKQYLK; VEVLKKVYK; FLQYFPLPGL; VCCRGTK; MYVLFQVFDL; NLFSPQCQ; LVCVSDFVPSQQYV; YEPHPLL; YSSLSDVFQCFSPFDH; PSIDSLVS; GGSPVVLPPPVD; SRPVSVLPSRQS; SVICPQGG; PPPIVPTS; VSPPPQVPPVVVR; QKDVLDVEGIV; ECLHEEVW; FQKVKHLF; GPISVPVSPSVT; QEVTVSVSVVTV; PSITLQVTTTL; IVSKELVDFIRS; PRDLYSCPNCCNPLLLCPFCHSRYPQLTLLEGRVVMECRQCGRL; ICVGGSS; VFTRCWHC; IDGVLYK; RYYVLVDNML; FLEGCYVELLSEQVG; TVSKRLLF; LEQLYQV; GKFSIVYKGIH; ILRLLNHPNV; KETLYIVMELVR; LFDLIQQ; RLPELHVNRSOLRVYHGIVHRD; FGLSTLC; EVLHQPCGTL; YNHQVDVWSIGVIMYLLLRGRL; LIVRMLQ; IDVYQSD; CCPEVPT; LRPVSOQ; YSPSSLP; SLLNILTG; SVPSYSPSY |

| Rank | Sequence | Start position | Score |
|------|----------|----------------|-------|
| 1    | SSPPGTPASVVSPAAPAGAPI | 965 | 0.95 |
| 1    | EVPQAAQPSKGPTKSMALLQ | 638 | 0.95 |
| 1    | GGVSPPPPQVPPVVRASSPR | 564 | 0.95 |
| 1    | GETVSKRLLFANSAKEQREW | 1497 | 0.95 |
| 2    | DVLDVEGIVDKHEECCEFEE | 691 | 0.91 |
| 2    | EDEAQNGMNLVYPQAAQPS | 627 | 0.94 |
| 2    | GAPTVPATVPVAVISSAPP | 1922 | 0.94 |
| 3    | KNGAKLQNHGAPVATAGPP | 1839 | 0.93 |
| 4    | ALEQLYQVGEQILGHGKFSIV | 1528 | 0.92 |
| 4    | GAPSILAVGATPLAGTTPPP | 927 | 0.91 |
| 4    | FGYSASGGMVNMQHFQKVK | 821 | 0.91 |
| 4    | KDKTQRSEQKKNPSVPVQLSL | 660 | 0.91 |
| 4    | EDTQGCGSNNFPGQAQQQA | 217 | 0.91 |
| 5    | LPAAPAVASRAPAASSPSL | 1868 | 0.91 |
| 5    | DVWSIGVIMYILLLRGLPFP | 1714 | 0.91 |
| 5    | VYKGIHRATNELYAIKVIDK | 1547 | 0.91 |
| 5    | SAKEQREWVDTLRVTAKQQA | 1509 | 0.91 |
| 5    | TPYQAAPASAPGVSLSGG | 1898 | 0.89 |
| 5    | LIQQNHRLPELHVNRJSQL | 1620 | 0.89 |
| 6    | SGDARDDDVYERIAGYRICH | 1184 | 0.89 |
| 6    | VAGAPTSSAGVEKPQEVTVS | 1003 | 0.89 |
| 6    | QATGSSGAASAAAGASSVSA | 877 | 0.89 |
| 6    | RSAKLLSSRTSASSFSRSSRGM | 789 | 0.89 |
| 6    | VGSAHANAPPPSGTAPPP | 532 | 0.89 |
| 6    | LYYYRKRGDARKPRGFMLEG | 1454 | 0.89 |
| 6    | AGALAVASPVGAPLSAVGG | 916 | 0.88 |
| 6    | PAAGDGDSSAGPAGGASGE | 424 | 0.88 |
| 6    | SEAADGTVLYKKGHLLHWWQ | 1424 | 0.88 |
| 6    | KNPSVQASLKEEKEENEQ | 670 | 0.87 |
| 6    | GGDAGREASKQAFAAAGTGRG | 603 | 0.87 |
| 6    | SAGPAGGAGESAAKGAES | 433 | 0.87 |
| 7    | ASRNPSPPRVRSAOQPPTHYG | 328 | 0.87 |
| 7    | PHPLARLQEASQSESSYGR | 281 | 0.87 |
| 7    | SSNERPRRLKPYEPHILLA | 267 | 0.87 |
| 7    | TPAEVPAGSGPPGSPSIEEEV | 2112 | 0.87 |
| 7    | SLSLADGAQPAATGANTNGA | 1351 | 0.87 |
| 7    | KQEVVTVSVVTVTAGGAGS | 1016 | 0.87 |
| 7    | STVQPSRQSIATGLOGIF | 752 | 0.86 |
| 7    | LIKEEKEENEQKDVLDVEGI | 679 | 0.86 |
| 7    | LQASPHARPAAGDDGDSSAQ | 416 | 0.86 |
| 7    | AQAQGAYPEAALVCVSDFVP | 231 | 0.86 |
| 7    | GEERTMCCPEVPFTFIPKN | 1821 | 0.86 |
| 7    | RCWHCGWELSKCAEMLGN | 1405 | 0.86 |
| 7    | MSPQALDIVSKELVDFIR | 1207 | 0.86 |
| 7    | VASGSSPAAPGVTGTVTEAVA | 1044 | 0.86 |
| 7    | TQGAPGSPPVRFSGGGGS | 488 | 0.85 |
| 7    | KTAARFTSAIKRTFTSSQSS | 468 | 0.85 |
| 7    | EESSGASSYSSLSDVFQCS | 304 | 0.85 |
3.3 Transmembrane Domains and Subcellular Location. Based on the TMHMM output, no transmembrane domain was found for CDPK7 (Figures 2(a) and 2(b)). Moreover, by PSORT II, the CDPK7 subcellular site was predicted as follows: 78.3% nuclear, 8.7% cytoplasmic, 8.7% plasma membrane, and 4.3% cytoskeletal.

3.4 Secondary and Tertiary Structures. The secondary structure of CDPK7 was predicted via the GOR4 online server (Table 4). The average score of antigenicity, beta-turn, flexibility, hydrophilicity, Bepipred linear epitope prediction, and surface accessibility for the CDPK7 protein using the IEDB online server was 1.026, 1.042, 1.017, 2.396, 0.350, and 1.00, respectively (Figure 6). Five discontinuous B-cell epitopes were predicted using the ElliPro server (Table 4).

3.5 Refinement and Validation of Tertiary Structure. Protein validation by means of the SWISS-MODEL server displayed that 92.86% of residues were situated in favored regions and 1.65% in the outlier regions. According to the Ramachandran plot, there were 97.80% residues in the favored region with 0.27% residues in the outlier regions of the refined model (Figure 5).

3.6 Predicted Linear and Discontinuous B-Cell Epitopes of the CDPK7 Protein. The predicted linear B-cell epitopes by the Bcepred are listed in Table 2. The outputs of the ABCpred server are tabulated in Table 3 (only the epitopes over scores of 0.75 are embedded in Table 3). The higher peptide score proposes the greater chance of being an epitope. The present server estimated 124 epitopes over 0.75 scores on the sequence, in which the linear epitope SSPGTPASVVSPAAGAGPI (score: 0.95) had the greatest score. Four epitopes with over 0.95 scores were as follows: “SSPGTPASVVSPAAGAGPI,” “EVPQAAQPSKGPTK-SAMLLQ,” “GGVSPPPQVVPPVGRAASPR,” and “GETVSKRLLFANSAKEQREW.” The average score of antigenicity, beta-turn, flexibility, hydrophilicity, Bepipred linear epitope prediction, and surface accessibility for the CDPK7 protein using the IEDB online server was 1.026, 1.042, 1.017, 2.396, 0.350, and 1.00, respectively (Figure 6). Five discontinuous B-cell epitopes were predicted using the ElliPro server (Table 4).

3.7 MHC-Binding Epitopes. The results are listed in Tables 5 and 6. Epitopes were assessed regarding antigenicity, and those highly antigenic epitopes were finally selected.

3.8 CTL Epitope Prediction. The high-ranked CTL epitopes predicted by the CTLpred tool for CDPK7 protein are summarized in Table 7. Epitopes were assessed regarding antigenicity, and those highly antigenic epitopes were finally selected.

3.9 Antigenic and Allergenic Profiles. The antigenic profile of CDPK7 was conducted by the VaxiJen web server with score of 0.7074 (threshold: 0.5). Based on AllergenFP and AllerTOP v2.0 analyses, the CDPK7 protein was appraised as possible nonallergen.

4. Discussion

Toxoplasmosis is a significant menace to human society as well as livestock industry [2, 8, 60]. Thus, the design and improvement of an efficient vaccine against T. gondii infection is still a great challenge for researchers against toxoplasmosis in domestic animals and humans [61]. Recently, bioinformatics tools are more focused for rational vaccine design, with some advantage, including the following: (i) time- and cost-effectiveness; (ii) accurately targeting, long-lasting immunity with favorable polarity in cellular components; and (iii) elimination of undesired responses through specific, epitope-based construct design. Nevertheless, the obtained in silico results are only theoretical data which must be confirmed using wet lab experiments inevitably [62].

It has been known that CDPK7 contributes to several functions in T. gondii such as gliding movement, host-cell invasion, and egress as well as other vital growth processes [34]. Here, we conducted a comprehensive analysis of TgCDPK7, a member of the CDPK family in T. gondii. The
Figure 6: Continued.
Figure 6: Propensity scale plots of CDPK7 protein. (a) Bepipred linear; (b) beta-turn; (c) surface accessibility; (d) flexibility; (e) antigenicity; (f) hydrophilicity. x-axis and y-axis represent position and score, respectively. The horizontal line indicates the threshold or the average score. Yellow colors (above the threshold) indicate favorable regions related to the properties of interest. Green color (below the threshold) indicates the unfavorable regions related to the properties of interest.
| Residues | Number of residues | Score | 3D structure |
|----------|--------------------|-------|--------------|
| A:V1431, A:L1432, A:Y1433, A:K1434, A:K1435, A:G1436, A:K1437, A:H1438, A:L1439, A:H1440, A:Q1441, A:W1442, A:Q1443, A:A1444, A:R1445, A:Y1446, A:Y1447, A:R1448, A:R1449, A:K1460, A:G1461, A:D1462, A:A1463, A:K1464, A:P1465, A:R1466, A:G1467, A:F1468, A:R1471, A:K1476 | 51 | 0.82 | ![3D structure](image1.png) |
| A:V1493, A:H1494, A:P1495, A:K1496, A:G1497, A:E1498, A:T1499, A:V1500, A:S1501, A:K1502, A:R1503 | 11 | 0.755 | ![3D structure](image2.png) |
| A:A1528, A:L1529, A:E1530, A:Q1531, A:L1532, A:Y1533, A:Q1534, A:V1535, A:G1536, A:E1537, A:Q1538, A:H1541, A:R1546, A:Y1548, A:K1549, A:G1550, A:L1551, A:H1552, A:R1553, A:A1554, A:T1555, A:N1556, A:E1557, A:L1558, A:V1612, A:R1613, A:G1614, A:Q1623, A:N1624, A:K1511, A:Q1513, A:R1514 | 99 | 0.677 | ![3D structure](image3.png) |
amino acid sequence of CDPK7 comprises 2133 residues with an average MW of 219085.79 D, which characterizes a suitable antigenic nature (the peptides with MW more than 10 kDa are considered as good immunogens) [63]. According to the Expasy ProtParam server, GRAVY and the aliphatic index of the CDPK7 were achieved at -0.331 and 68.78, respectively. In summary, the great value of aliphatic index means that the peptide has more stability in a broad range of various temperatures. Moreover, the low/negative value of the GRAVY factor signifies the better interaction of peptide with the molecules of water. It is efficient to identify that PTMs have a fundamental role in cell stability [64]. The acquired outcomes show that CDPK7 comprises 299 potential PTM sites (269 phosphorylation and 30 acylation positions), representing that these positions may organize protein activity.

To predict the secondary structure of CDPK7, the GOR4 tool was recruited. The results of secondary structure of CDPK7 verified and included 502 (out of 2133) alpha-helix, 320 extended strands, and 1311 random coils. It is known that the key role of the proteins is related to their three-dimensional structure. As such, to comprehend the influences between both structures and functions, assessment of 3D structure is the key aim of expecting a protein’s nature [65].

Humoral and cellular immunity are strongly stimulated in T. gondii infection [66, 67], in such a way that the establishment of IgG antibodies avoids the protozoan from attachment to the receptors of host cell [67]. Interferon-γ (IFN-γ), CD4+, and CD8+ T cells as the main members of T cells play a dynamic role in constraining acute and chronic infection. These major cytokines prevent the reactivation of bradyzoites in the host tissue cyst [66]. Epitope prediction has critical value to evaluate the specificity of antigen. Furthermore, epitope evaluation may reveal the pathogenesis and immune process of the pathogen in design vaccine researches [65, 68]. The strength of using in silico is the detection of the component epitopes that are critical for the interaction of antibodies and antigens. Several linear B-cell epitopes were predicted by the ABCpred server, among which those epitopes above 0.9 score were of great significance to be included in multiepitope vaccine constructs. Moreover, we applied the IEDB online server to evaluate the IC50 values of peptides that link to the MHC class I/II molecules for CDPK7. According to the

| Residues | Number of residues | Score | 3D structure |
|----------|--------------------|-------|--------------|
| A:F1544, A:D1565, A:K1566, A:G1567, A:K1568, A:I1569, A:N1570, A:G1571, A:H1572, A:E1603, A:T1604, A:Y1606 | 12 | 0.645 |
| A:C1683, A:A1684, A:P1685, A:N1686, A:E1687, A:V1688, A:L1689, A:Q1691, A:P1692, A:C1693 | 10 | 0.535 |
obtained results from IEDB, the T-cell epitopes on CDPK7 have the capability to bind intensely to MHC class I and class II molecules. It is important to note that the lower IC50 values show the higher-level of affinity, which show an appropriate T-cell epitope.

Other the main stage, CTLpred is a special approach used to predict CTL epitopes, which is important in vaccine-related studies. This tool relies on elegant machine learning methods, such as ANN and SVM. We recognized the CTL epitopes using the CTLpred online database to select the top CDPK7 epitopes. The CTLpred server utilizes consensus and combined estimates, in line with these two methods [56]. Evaluation of antigenicity and allergenicity showed that CDPK7 protein has immunogenic and nonallergenic nature.

### Table 5: IC50 values for CDPK7 binding to MHC class I molecules obtained using the IEDB.

| MHC-II allele | Start-stop | Peptide sequence | Percentile rank | Antigenicity |
|---------------|------------|------------------|----------------|--------------|
| H2-Db         | 1882-1891  | SSPSSLPTPI       | 0.15           | 0.4079       |
|               | 143-152    | SNLPNLDRYM       | 0.21           | -0.4284      |
|               | 1637-1646  | SPLITTVYYL*      | 0.24           | 0.9146       |
| H2-Dd         | 647-656    | KGPNTKAMLL*      | 0.18           | 0.9201       |
|               | 1612-1621  | VRGGELFDL*       | 0.28           | 0.1770       |
|               | 1483-1492  | VGRQYGF*         | 0.64           | 0.1561       |
| H2-Kb         | 1721-1730  | IMYLIRRGL*       | 0.55           | 1.4751       |
|               | 798-807    | SSASGG*          | 1.0            | 1.3623       |
|               | 1643-1652  | VYYLHKG*         | 1.2            | 0.1373       |
| H2-Kd         | 1474-1483  | CYVELLE*         | 0.79           | 0.5079       |
|               | 1445-1454  | RYYVLVDML*       | 1.15           | 0.9079       |
|               | 822-831    | GYSASGG*         | 1.3            | 0.9579       |
| H2-Kk         | 1555-1564  | TNELYAIKV*       | 0.12           | 0.2695       |
|               | 1084-1093  | SEGPA*           | 0.75           | 0.2366       |
|               | 694-703    | DVEGIVD*         | 1.5            | 0.1511       |
| H2-Ld         | 1589-1598  | HPHYMIK*         | 3.8            | 0.2773       |
|               | 1729-1738  | RLPPP*           | 4.2            | 0.3317       |
|               | 279-288    | YEHPLILAR*       | 4.6            | 0.1027       |

*The immune epitope database (http://tools.iedb.org/mhci/). H2-Db, H2-Dd, H2-Kb, H2-Kd, H2-Kk, and H2-Ld alleles are mouse MHC class I molecules.

Ten amino acids for analysis were used each time. Low percentile rank = high level binding; high percentile rank = low level binding; IC50 values = percentile rank. * indicates potential antigenic epitopes (threshold = 0.5).

### Table 6: IC50 values for CDPK7 binding to MHC class II molecules obtained using the IEDB.

| MHC-II allele | Start-stop | Peptide sequence | Percentile rank | Antigenicity |
|---------------|------------|------------------|----------------|--------------|
| H2-IAb        | 1109-1123  | AAGAAAAAATAAAAA* | 0.07           | 0.8045       |
|               | 1108-1122  | AAGAAAAAATAAAAA* | 0.08           | 0.8354       |
|               | 1110-1124  | AGAAAAAATTAAAAB* | 0.08           | 0.7176       |
| H2-IAd        | 1035-1049  | SETQPAMASVAGSS*  | 0.13           | 0.6766       |
|               | 1034-1048  | GSETQPAMASVAGS*  | 0.15           | 0.7059       |
|               | 1036-1050  | ETQPAMASVAGSSP*  | 0.25           | 0.6536       |
| H2-IEd        | 1451-1465  | DNMLOYYRKKGDAKP* | 0.14           | 0.6972       |
|               | 1452-1466  | NMLYYRKKGDAKPR*  | 0.14           | 0.8298       |
|               | 1450-1464  | VDNMLYYRKKGDAK*  | 0.19           | 0.6159       |

*The immune epitope database (http://tools.immuneepitope.org/mhcii). H2-IAb, H2-IAd, and H2-IEd alleles are mouse MHC class II molecules. Fifteen amino acids for analysis were used each time. Low percentile rank = high level binding; high percentile rank = low level binding; IC50 values = percentile rank. * indicates potential antigenic epitopes (threshold = 0.5).
5. Conclusion

Well antigenicity, hydrophilicity, surface accessibility, and flexibility indexes were detected for CDPK7. Hence, we recommend that a suitable vaccine should be designed and verified both in silico and in vivo by the potential B- and T-cell epitopes predicted in this study.

Abbreviations

3D: Three-dimensional
ACC: Auto cross covariance
ANN: Artificial neural network
CD: Cluster of differentiation
CDPK: Calcium-dependent protein kinase
CTL: Cytotoxic T-lymphocyte
GOR: Garnier-Osguthorpe-Robson
GRAVY: Grand average of hydropathicity
IC50: Half-maximal inhibitory concentration
IEDB: Immune epitope database
IFN-γ: Interferon-γ
MHC: Major histocompatibility complex
MW: Molecular weight
PDB: Protein data bank
pl: Isoelectric point
PTM: Post-translational modification
SVM: Support vector machine
T. gondii: Toxoplasma gondii.

Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Table 7: Predicted CDPK7 epitopes by CTLpreda.

| Peptide rank | Start positionb | Sequence     | Score (ANN/SVM)c | Antigenicity |
|--------------|-----------------|--------------|------------------|--------------|
| 1            | 280             | EPHPLLARL    | 0.83/1.3591088   | 0.0131       |
| 2            | 1716            | WSIGVIMYL    | 0.96/1.1120848   | 0.1711       |
| 3            | 1398            | GSSRVTFRC    | 0.94/1.0685326   | -0.7197      |
| 4            | 1187            | ARDDDVYER    | 0.65/1.3441588   | 0.3493       |
| 5            | 715             | SFPEFKTWL*   | 0.98/0.95345497  | 1.0485       |
| 6            | 1763            | AKDLIVRML*   | 0.98/0.89030833  | 0.8096       |
| 7            | 724             | ERNEGILSM*   | 0.65/1.0757075   | 0.5393       |
| 8            | 470             | ASRFTSAIK*   | 0.80/0.85963689  | 1.0303       |
| 9            | 1573            | ERELLRSEM*   | 0.51/1.0720792   | 0.9337       |
| 10           | 1188            | RDDDVYERI    | 0.85/0.73017891  | 0.0942       |
| 11           | 1666            | RTPNATIKL    | 0.99/0.58481613  | 0.2323       |
| 12           | 32              | KECLKQYLK*   | 0.99/0.58376856  | 1.2628       |
| 13           | 1411            | WELSKCAEM    | 0.19/1.3750392   | 0.3168       |
| 14           | 1749            | VSFDGAVWR*   | 0.96/0.59370426  | 1.2284       |
| 15           | 743             | GLQGNALYR*   | 0.99/0.54848483  | 1.4369       |

aCTLpred, available online at http://www.imtech.res.in/raghava/ctlpred/index.html. bNine amino acids for analysis were used. cThe default artificial neural network (ANN) and support vector machine (SVM) cut-off scores were set 0.51 and 0.36, respectively. * indicates potential antigenic epitopes (threshold = 0.5).

Ethical Approval

This study received the approval from the Behbahan Faculty of Medical Sciences Ethical Committee (IR.BHN.REC.1399.034).

Disclosure

The funders of this study had no role in the study design, analysis and interpretation of data, writing of the final paper, and the decision to submit the manuscript for publication. The corresponding author had access to the data in the study and had final responsibility for the decision to submit for publication.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Acknowledgments

This study was financially supported by the Behbahan Faculty of Medical Sciences, Behbahan, Iran (Grant No. 99013).

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