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Production of mutant streptavidin protein and investigation of its effect on the performance of streptavidin

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ABSTRACT

Objective(s): Streptavidin is a versatile protein in cell science. The tetramer structure of streptavidin plays a key role in this binding, but this form interferes with some assays. If monomer streptavidin is still capable of binding to biotin, it can overcome the limitations of the streptavidin application. So, we examined the elimination of tryptophan 120 and its effect on the function of streptavidin.

Materials and Methods: Mutant streptavidin gene was synthesized in a pBSK vector. Then it was ligated to the pET32α vector. This vector is expressed in *Escherichia coli* BL21 (DE3) pLysS host. After purification and refolding of the recombinant protein, its structure was analyzed on the SDS_PAGE gel. Recombinant streptavidin binding affinity to biotin was evaluated by spectrophotometric and HABA color compound.

Results: Mutant streptavidin gene was successfully expressed in *E. coli* BL21 (DE3) pLysS host and the purified protein was observed as a single band in the 36 kDa area. The best condition for dialysis was PBS buffer+arginine. The molar ratio of biotin/protein of mutant streptavidin was not only near but also more than standard protein. Mutant streptavidin remained in the monomeric state in the presence or absence of biotin.

Conclusion: Results of this study showed that 120 tryptophan is one of the most important factors in tetramer streptavidin formation and its deletion produces the monomer form that has a high binding affinity to biotin. This mutant form of streptavidin can therefore be used in studies requiring monovalent binding as well as in studies facing limitations due to the size of streptavidin tetramer.

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Introduction

Streptavidin is produced by *Actinomyces* bacteria called *Streptomyces avidinii* and is extensively used in biotechnological studies, assays, and processes such as detection and purification due to its high biotin-binding affinity (1). Each of the subunits of homotetrameric streptavidin consists of eight anti-parallel strands and a biotin-binding site at the end of any of the subunits. This site is covered by a flexible loop (between strands 3 and 4) and acts as a lid in the binding site. Forming hydrogen bonds and the presence of several aromatic residues (creating the hydrophobic environment required for the hydrophobic ligand) make a strong binding affinity to biotin. Hence, the binding between streptavidin and biotin is the strongest bond in nature (2).

Other unique features of streptavidin include resistance against heat, denaturants (triton and SDS), high and low pH ranges, and the proteolytic activity of some enzymes (3).

Despite the mentioned desirable features of streptavidin, its tetramer form is not appropriate for some assays, such as the monovalent detection of cell surface receptors. The tetramer form causes the accumulation of ligands; on the other hand, receptor dimerization is a common mechanism in cell signaling thus wild-type streptavidin acts as a receptor ligand and finally interferes with the results of studies (4).

Also, using streptavidin tetramer as a recombinant fusion tag interrupts the structure of proteins. To overcome these restrictions, studies have developed monomeric streptavidin forms by the replacement of mutations of essential amino acids with other amino acids (5). The mutant forms of streptavidin with replacement mutations have limitations in stability, binding affinity, and *in vitro* use. It is therefore necessary to design a monomer by eliminating mutations to improve binding properties. One of the most effective factors in the high binding tendency of streptavidin to biotin is the reaction of tryptophan 120 with biotin of the adjacent subunit, which is effective in forming the tetramer form.

In this study, by removing the amino acid tryptophan 120 in the mature sequence of streptavidin, this mutation was investigated in the binding of subunits, tetramer formation, and its effect on the affinity of binding to biotin as well as the correct refolding of the protein.

According to the importance of the subject, the purpose of this study was the elimination of tryptophan 120 and examined its effect on the formation of monomer form and the biotin-binding affinity. Also, it was attempted to produce a high-affinity streptavidin monomer by dialyzing the recombinant protein in different situations.

Materials and methods

Materials and chemicals

All chemicals used in the manufacture of buffers, solutions, and other steps are provided by Merck and Roche. Protein and DNA Ladder are provided by Thermo Scientific.

Bacterial strains

Escherichia coli DH5α was used as the recombinant protein cloning host, *E. coli* BL21 (DE3) pLYs*S* was

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MDPSKDSKAQVSAAEAGITGTWYNQLGSTFIVTAGADGALTGTYE
SAVGNAESRYVLTGRYDSAPATDG
SGTALGWTVAWKNNYRNAHSATTWSGQYVGGAEARINTQWLLTS

Figure 1. Mutant streptavidin gene

employed as the recombinant protein expression host, and pET32a was applied as the recombinant expressive vector.

Construction of gene

In the streptavidin mutated gene sequence, tryptophan translation code 120 was deleted and the BamH1 enzyme cutting site (G/GATTC) was placed at the beginning of the gene and the XhO1 cutting site (C/TCGAG) was placed at the end of the gene and codon-optimized for *E. coli* expression. The whole construct was synthesized with Biomatik Company (Cambridge, ON, Canada) and inserted into the plasmid pET32a as the expression vector. The pET32a- mutant streptavidin construct was transferred to competent bacteria *E. coli* DH5α.

Confirm recombinant plasmid

For this purpose, plasmids were purified according to the standard protocol, and digestion reaction was done to confirm recombinant plasmids with BamHI and XhoI3' (6).

Production of recombinant protein of mutant streptavidin

For expression purposes, competent *E. coli* BL21 (DE3) pLysS cells were prepared based on standard protocols and were transformed by the successfully extracted expression vector pET32a- mutant streptavidin construct. Selected *transformed* bacterial colonies grown on plates containing ampicillin and chloramphenicol were incubated with a 2 ml NB medium. Then, 300 µl of overnight culture was inoculated into 50 ml NB broth (100 µg/ml ampicillin and 34 µg/ml chloramphenicol), and incubated at 37 ˚C, 220 rpm. The cells' optical density at 600 nm was measured occasionally, and at OD_{600} nm~0.6, isopropyl thio β-D-galactosidase (IPTG) (1 mM) was added to induce protein expression. After a 4 hr incubation time, the cells were harvested by centrifugation at 5000 rpm for 20 min and the pellets were stored at -20 ˚C. SDS-polyacrylamide gel electrophoresis (SDS-PAGE12%) was applied to assay the quality of the purified recombinant protein (7).

Purification of mutant streptavidin

Denaturing conditions using 8 M urea, followed by Ni-NTA agarose resin affinity chromatography were used for purification of the recombinant proteins based on standard protocols (8). The purity of the purified protein was investigated by SDS-PAGE 12%. The quantity of protein was calculated from the following equation:

$$(1.55\times OD_{280})-(0.76\times OD_{260})=\text{amount of protein (mg/ml)}$$

Urea assay test

To ensure the absence of urea in the protein solution, a urea assay test was done with Berthelot kit (Iran) according to the kit protocol.

Refolding of the purified protein

Using urea for protein purification denatures the active folding of proteins. Accordingly, the refolding process was done by applying the prepared dialysis tubing with a 10 K molecular weight cut-off (MWCO). The dialysis process carried out in basic buffers included PBS, borate, HEPS, MOPS, and Tris-HCl at 4 ˚C for 24 hr. The additive compounds were prepared according to Table 1. Samples (1-2 ml) were loaded into the dialysis membrane followed by embedding into the chambers containing 300 ml external dialysis buffer with gentle agitation at 4 ˚C. The dialysis buffer was changed after 2 hr and dialyzed for 24 hr. Finally, dialyzed protein samples were maintained at 4 ˚C for the next stages of the project. Then, the refolded protein was analyzed by SDS-PAGE (12%) (9). The quantity of protein was calculated from the following equation:

$$(1.55\times OD_{280})-(0.76\times OD_{260})=\text{amount of protein (mg/ml)}$$

For refolding of streptavidin protein with pI=4.6, we selected buffers with a pH range of neutral to alkaline (9).

Assessing the biotin-binding affinity of the dialyzed streptavidin

To assess the effect of dialysis on the structure and performance of the produced streptavidin, streptavidin binding affinity to biotin was measured after each dialysis using the HABA dye (4'- hydroxyazobenzene-2-carboxylic acid) method, in which HABA binds to streptavidin and produces a yellow complex. In this reaction, also biotin binds to streptavidin to compete with HABA and consequently reduces the absorption of streptavidin at the wavelength of λ_{500}. The biotin-binding affinity of standard streptavidin was assessed at different concentrations. Finally, molar

Table 1. Sample dialysis conditions in different buffers in the presence of various types additives

	Dialysis buffers	Additives
1	PBS 10mM	-
2	PBS 10mM	Arginine 0.1M
3	PBS 10mM	Proline 0.1M
4	PBS 10mM	Glycine 0.1M
5	PBS 10mM	Arg-HCl 0.1 M
6	Borate 100mM	-
7	Borate 100mM	Arginine 0.1M
8	HEPES 20mM	Arginine 0.1M
9	MOPS 20mM	Arginine 0.1M
10	Tris Hcl 20mM	Arginine 0.1M
11	Tris Hcl 20mM	Arginine 0.1M+ NaCl (0.5 M), Triton X100 (0.5%), EDTA (5 mM)

ratios of biotin to the protein of the dialyzed streptavidin and standard streptavidin were compared with each other. Reduction in the absorption was calculated using the following equations (10):

$$\Delta A=(0.9 \times OD_1)+OD_3-OD_2$$

OD_1=OD reading of streptavidin+HABA mixture

OD_2=OD reading of streptavidin+HABA+biotin mixture

OD_3=Blank (OD reading of streptavidin+HABA+water mixture)

To calculate the amount of biotin (µM) per ml of the reaction mixture: C= ΔA/34

The amount of biotin (mM) per ml of the sample: B=C.10

Molar ratio of biotin to protein: B/P (P is the molar concentration of the protein sample)

Optimization of protein concentration to improve dialysis with arginine amino acid

To find an appropriate protein concentration for dialysis, the protein dialysis was performed in PBS buffer in the presence of arginine at 0.1-0.5 mg/ml concentrations of the protein for 24 and 48 hr. Then, their biotin-binding affinity was assayed.

Investigation of the structure of mutant streptavidin

The dialyzed samples were analyzed on 12% SDS-PAGE gel in the presence and absence of biotin to evaluate the structure.

To compare the structural changes between wild-type and mutant recombinant streptavidin

Samples of dialyzed streptavidin in PBS buffer containing arginine and standard streptavidin were analyzed in the presence and absence of biotin on 12% SDS_PAGE gel.

Results

Digestion of recombinant plasmid pET32a- mutant streptavidin

The results of digestion show that the ligation reaction was performed correctly and the desired fragment was removed from the pET32a plasmid. The fragment was observed in front of the 477 bp band (Figure 2).

Expression of pET32a- mutant streptavidin in E. coli BL21 (DE3) pLYsS

Protein production was analyzed with SDS-PAGE. The

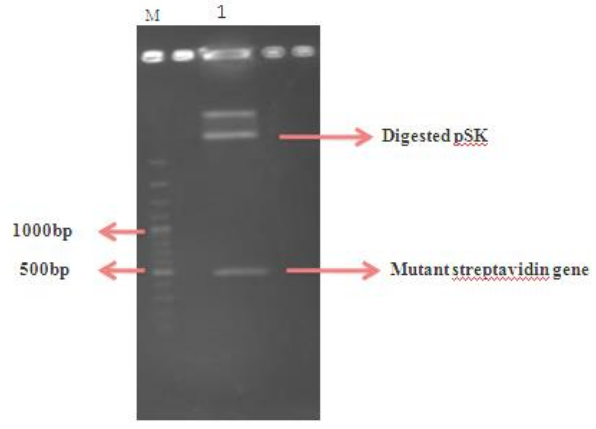

Figure 2. The results of digestion for mutant streptavidin gene
M: DNA ladder (100 bp ladder); 1: digestion reaction

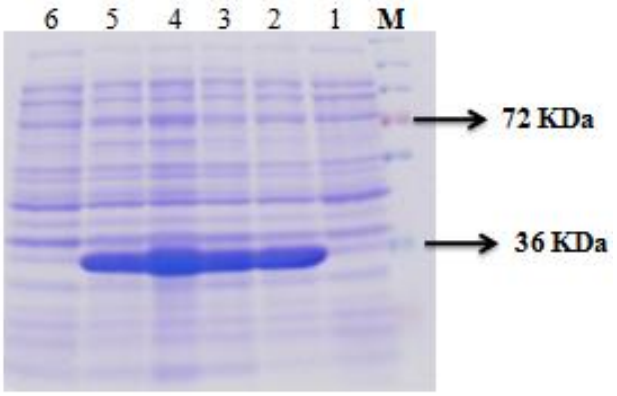

Figure 3. Expression of the recombinant streptavidin was analyzed by SDS-PAGE. M, protein marker (10 to 70 kDa protein ladder). Lane 1 and 6, the pET32a-strep before induction; Lane 2, 3, 4, and 5, the pET32a-strep after induction

produced 36 kDa protein after 2 and 4 hr of the induction are indicated with corresponding arrows in Figure 3.

Purification of pET32a- mutant streptavidin

SDS-PAGE was used to analyze the quality of the purified protein. Because of the 6Histag sequence in the amino acid end of the recombinant mutant streptavidin it was purified using the Ni-NTA kit (Figure 4).

Urea assay test

Due to the antibacterial properties of urea which can interfere with the results of *in vitro* tests, the absence of urea in the purified refolded product was confirmed by standard methods.

Refolding, binding affinity, and structure of the recombinant streptavidin

In this experiment, the OD of the protein of PBS buffers containing arginine, arginine-HCL, and proline was reduced, and the highest reduction of the OD belonged to the protein of PBS buffer containing arginine. The OD reduction of the protein was negligible in PBS buffer without the amino acid and presence of glycine and its activity was lower than standard protein (Table 2; Figure 5).

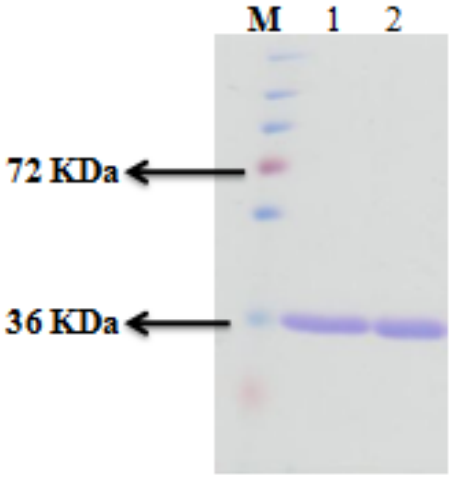

Figure 4. SDS-PAGE analysis of purification of mutant streptavidin recombinant protein
Lane 1: Protein marker; Lane 2, 3, 4, 5: fusion protein retrieved by nickel affinity chromatography

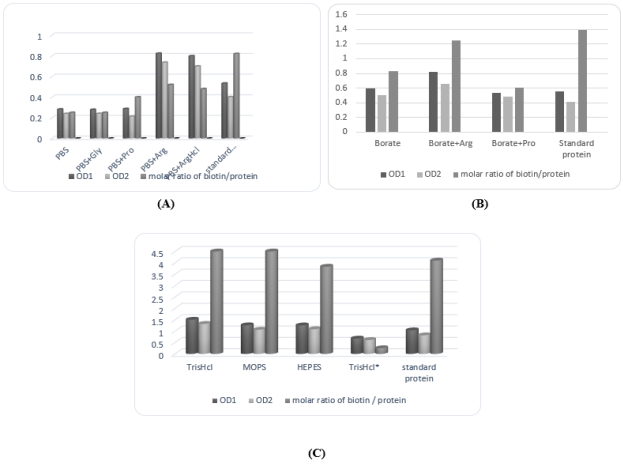

Figure 5. Comparison of OD1 and OD2 and the molar ratio of biotin/standard protein and dialysis samples in buffers. Figure A including= PBS, PBS+Glycine, PBS+Proline, PBS+Arginine -HCL+Standard protein. Figure B including=Borate, Borate+Arginine, Borate+Proline, Borate+Proline+Standard protein. Figure C including=Tris HCl+Arginine, MOPS+Arginine and HEPES+Arginine and Tris-HCl
* in presence of NaCl (0.5 M), Triton X100 (0.5%), EDTA (5 mM) and Arginine+Standard protein

The results of dialysis of the mutant streptavidin in borate buffer in the presence of arginine showed borate buffer is a good buffer for the recombinant streptavidin dialysis, as its molar ratio of biotin to protein was only slightly less than that of the standard protein. However, the borate buffer did not perform well singly. The borate buffer function without amino acid was better than the PBS buffer function without amino acid.

The dialysis of the recombinant mutant streptavidin was performed in HEPES, Tris HCl, and MOPS buffers in the presence of arginine. Tris HCl and MOPS buffers were found to be more effective and led to a higher reduction in the OD so that their molar ratio of biotin to protein was more than that of the standard protein. The HEPES buffer, however, acted like PBS. Another dialysis was performed in Tris HCl buffer in the presence of NaCl (0.5 M), Triton X100 (0.5%), EDTA (5 mM), and arginine, but no effects were observed on the refolding and function of the protein. According to these results, the best condition of dialysis was PBS buffer+arginine (Table 2; Figure 5).

Table 2. Results of reading the optical density of the dialysis samples in PBS, borate, HEPES, MOPS, and Tris HCl buffers in presence of amino acids after 24 hr

	Optical Density / Dialysis Conditions	OD1	OD2	P	Molar ratio of biotin to protein
1	PBS	0.282	0.239	0.048	0.25
2	PBS+Arg 0.1 M	0.823	0.736	0.048	0.52
3	PBS+Gly 0.1 M	0.279	0.240	0.048	0.25
4	PBS+ Pro 0.1 M	0.287	0.213	0.054	0.4
5	PBS+Arg-HCL 0.1 M	0.798	0.699	0.048	0.48
6	Standard protein	0.533	0.401	0.045	0.82
7	Borate	0.592	0.504	0.03	0.83
8	Borate+Arg 0.1 M	0.819	0.662	0.036	1.25
9	Borate+Pro 0.1 M	0.536	0.479	0.036	0.61
10	Standard protein	0.557	0.414	0.028	1.39
11	Tris Hcl+Arg	1.501	1.318	0.012	4.5
12	MOPS+Arg	1.257	1.062	0.012	4.5
13	HEPES+Arg	1.253	1.084	0.012	3.83
14	Tris-Hcl+Arg+NaCl+EDTA+triton X100	0.674	0.610	0.066	0.25
15	Standard protein	1.037	0.817	0.011	4.1

Table 3. Results of reading the optical density of the dialysis streptavidin at PBS buffer in the presence of arginine 0.1 M at different concentrations of the protein during 24 and 48 hr

	Optical density / Dialysis conditions	OD1	OD2	P	Molar ratio of biotin to protein	Time (Hr)
1	PBS+Arg 0.1 M - 0.1*	0.623	0.53	0.006	4.6	24
2	PBS+Arg 0.1 M - 0.1*	0.675	0.577	0.012	2.6	48
3	PBS+Arg 0.1 M -0.2*	1.22	1.076	0.012	3.5	24
4	PBS+Arg 0.1 M -0.2*	0.7	0.617	0.012	2.08	48
5	PBS+Arg 0.1 M - 0.3*	1.170	1.030	0.012	3.16	24
6	PBS+Arg 0.1 M - 0.3*	1.185	1.018	0.012	3.83	48
7	PBS+Arg 0.1 M - 0.4*	1.249	1.114	0.018	2.22	24
8	PBS+Arg 0.1 M - 0.4*	1.159	1.025	0.024	1.5	48
9	PBS+Arg 0.1 M - 0.5*	0.664	0.574	0.006	4.3	24
10	PBS+Arg 0.1 M - 0.5*	1.22	1.076	0.006	3.5	48

* Indicates the concentration of the protein (mg/ml)

Optimization of protein concentration to improve dialysis with arginine amino acid

All of the dialyzes showed reduced OD. At 0.4 mg/ml concentration and time (24 hr) higher molar ratio of biotin to protein compared with standard protein were observed. Also, this ratio in concentrations of 0.3 and 0.4 mg/ml during 48 hr had less difference than the standard protein. However, the most difference was observed in concentrations of 0.1, 0.2, and 0.5 mg/ml in 48 hr (Tables 3 and 4 and Figures 6 and 7).

Investigation of the structure of mutant streptavidin

The results showed that monomer structures were retained in all cases of the recombinant mutant streptavidin. (Figure 8).

To compare the structural changes between wild-type and mutant recombinant streptavidin

The mutant streptavidin maintained the monomer

Table 4. Results of reading the optical density of standard streptavidin at the 0.1-0.5 mg/ml concentrations of the protein

Optical density / Standard protein concentration	OD1	OD2	P	Molar ratio of biotin to protein
0.1 mg/ml	0.469	0.3	0.005	8.2
0.2 mg/ml	1.037	0.817	0.011	4.1
0.3 mg/ml	0.563	0.446	0.017	1.82
0.4 mg/ml	0.556	0.410	0.022	1.81
0.5 mg/ml	0.557	0.414	0.028	1.39

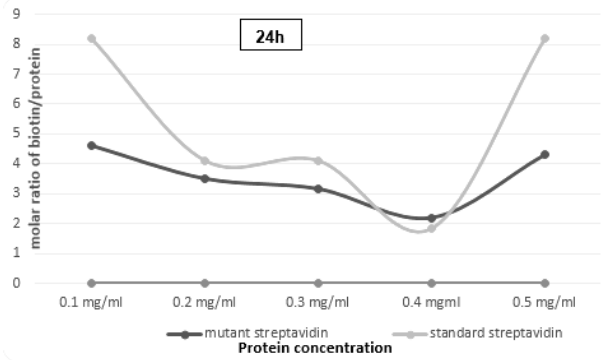

Figure 6. Comparison of the molar ratio of biotin to the protein of the dialysis streptavidin in PBS buffer, in presence of arginine and at 0.1-0.5 mg/ml concentrations of the protein during 24 hr. The molar ratio of biotin to the protein of 0.4 mg/ml concentration was more than that of standard streptavidin

state in the presence and absence of biotin. In both cases, it formed a band in the region of 36 kDa. However, standard streptavidin formed a monomeric structure in the absence of biotin and the presence of biotin, it became an aggregation of subunits and formed a band in the region of 55 kDa (Figure 9).

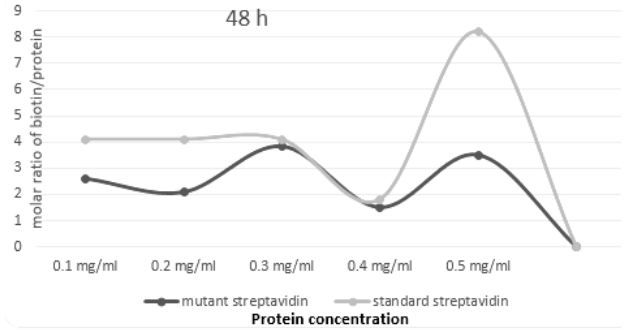

Figure 7. Comparison of the molar ratio of biotin to the protein of the dialysis streptavidin in PBS buffer, in presence of arginine and at 0.1-0.5 mg/ml concentrations of the protein during 48 hr

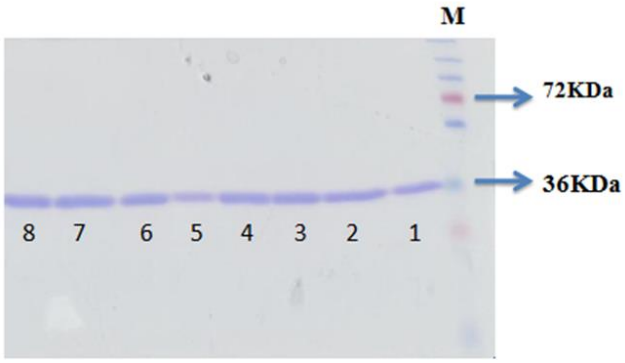

Figure 8. The monomer structure of the mutant streptavidin. M: Protein markers (10 to 70 kDa Protein Ladder), bands 1, 2, 3, and 4 of the samples analyzed in the absence of biotin, which were dialyzed in PBS, Borate, Tris HCl and MOPS buffers, respectively. Bands 5, 6, 7, and 8 of the samples were analyzed in the presence of biotin and dialyzed in PBS, Borate, Tris HCl, and MOPS buffers, respectively

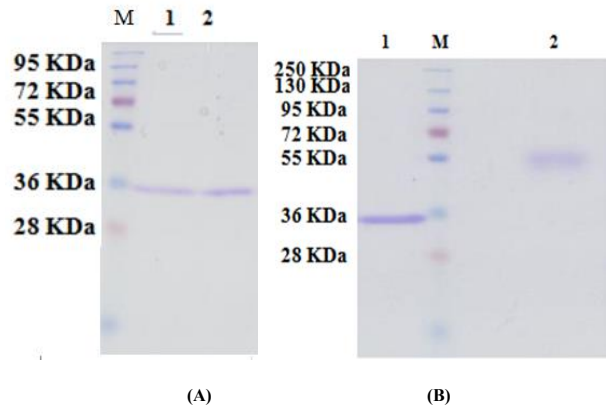

Figure 9. After dialyzing, wild-type and mutant recombinant streptavidin were analyzed on SDS-PAGE gel in the presence and absence of biotin. A: M, protein marker. Lane 1 and 2: mutant recombinant streptavidin in the presence and absence of biotin. B: M, protein marker. Lane 1: Recombinant streptavidin in absence of biotin. Lane 2: wt recombinant streptavidin in the presence of biotin

Discussion

Streptavidin has great applications in molecular sciences Because of its high biotin-binding affinity. Despite the high binding affinity of streptavidin tetramer, this form interferes with some assays. The large size of the tetramer form of streptavidin also limits its use as a sequence. As a result, the applications of this protein are limited. Efforts have therefore been made to improve the uses of streptavidin by designing monomeric variations (11). Although the studies conducted on the synthesis of the monomeric form, the produced forms through substitution mutations have shown a low binding affinity compared with the affinity observed for wide protein, and also their stability and solubility have been limited (12).

The substitution mutations used in previous studies have identified the reaction of tryptophan 120 with biotin in the adjacent subunit as one of the most effective factors contributing to the high biotin-binding affinity in streptavidin, as well as this reaction's effects on the tetramer formation (13). This study is the first effort in removing the tryptophan 120 residue from the full sequence of the streptavidin gene and examining the effect of this mutation on the formation of the tetramer, the biotin-binding affinity, and also to correct refolding of the protein.

In this report, the mutant streptavidin gene sequence was successfully expressed in *E. coli*. The recombinant protein with high purity was obtained through nickel affinity chromatography. The results of the biotin-binding affinity of the dialyzed streptavidin confirmed that removal of tryptophan 120 produced a monomeric form of streptavidin and that the molar ratio of biotin to mutant streptavidin protein was higher than standard streptavidin in some dialysis conditions.

Dialysis in PBs buffer alone had no effect on the refolding of the mutant streptavidin, but a more effective refolding was achieved in the presence of proper additives. Also, MOPS, Tris HCl, and borate buffers performed better than PBS buffer. It is noteworthy, that OD reduced more significantly in Borate buffer without amino acid compared with PBS buffer without amino acid, which may have been due to the pH of the borate buffer (pH=9). However, HEPES showed the same performance as PBS.

Arginine, arginine monohydrochloride, proline, and glycine along with NaCl (0.5 M), Triton X100 (0.5%), and EDTA (5 mM) compounds were used to facilitate refolding and reduce accumulations (14). Amino acids arginine and arginine-Hcl were effective additives for the refolding of recombinant streptavidin, but the best additive was arginine because it helped refolding at high protein concentrations. A mixture of arginine, EDTA, Triton X100, and NaCl was found to be ineffective in improving protein refolding. Arginine reacts with the side chain of most amino acids and the interaction between the arginine guanidinium group, and the tryptophan side chain is probably responsible for inhibiting protein accumulation (15). Given the isoelectric point of 4.6 for the mutant streptavidin, in this study, an alkaline pH was deemed suitable for the refolding and the gained results confirmed this deduction (16, 17).

In former studies using substitution mutations, the mutant streptavidin forms W120F (18, 19) and W120A/F (20, 21) were produced whose biotin-binding affinity was significantly reduced and no structural changes were observed. In another study, the monomeric form of

streptavidin was produced in the presence and absence of biotin by creating substitution mutations of T90A and D128A, in which T90 and D128 have a key role in the streptavidin-biotin interaction, maintaining the structure of the subunits and their interactions. So due to the lack of hydrogen bonds between biotin and T90 and D128, the biotin-binding affinity of this form was reduced by 1.3×10-8 M (22, 23). In another study, a monomeric form was produced by creating electrostatic repulsion as well as a spatial barrier that showed a reduced biotin-binding affinity (24, 25).

The obtained results showed that the deletion of tryptophan 120 prevented the formation of tetrameric form, and monomeric streptavidin was formed. According to the assay used in this study, the present findings also showed that by changing dialysis conditions, a higher biotin-binding affinity was reached compared with standard streptavidin.

In this study despite the deletion mutation, not only the biotin-binding affinity was still observed but also the recombinant mutant streptavidin maintained its monomeric form in all dialysis conditions in both the presence and absence of biotin.

It is therefore necessary to conduct further studies on this mutation. Overall, considering the mutations that cause the inactive streptavidins, the biotin-binding affinity is still observed in the macro range, which suggests very high compatibility in the binding region.

Conclusion

According to these findings, removing the tryptophan 120 alone could create the monomer form in both the presence and absence of biotin. In the dialysis conditions and based on the assessment method used in this study the binding affinity of this mutant protein was higher than that of standard streptavidin, which suggests the high compatibility of the biotin-binding region of streptavidin. As for protein refolding through dialysis the findings showed, in addition to the additives used, the type of buffer and the initial concentration of the protein affect the refolding process.

Acknowledgment

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Authors' Contributions

AS and HA designed the experiments; SSA and ED performed experiments and collected data; AS and HA discussed the results and strategy; SSA and HA approved the final version to be published.

Conflicts of Interest

Authors declare no conflicts of interest in this study.

References

1. Laitinen OH, Hytönen VP, Nordlund HR, Kulomaa MS. Genetically engineered avidins and streptavidins. Cell Mol Life Sci 2006 ;63:2992-3017.
2. Chivers CE, Koner AL, Lowe ED, Howarth M. How the biotin-streptavidin interaction was made even stronger: Investigation via crystallogra-phy and a chimaeric tetramer. Biochem J 2011; 435: 55-63.
3. Dundas CM, Demonte D, Park S. Streptavidin–biotin technology: Improvements and innovations in chemical and biological applications. Appl Microbiol Biotechnol 2013;97:9343-9353.
4. Yanai I, Yu Y, Zhu X, Cantor CR, Weng Z. An avidin-like domain that does not bind biotin is adopted for oligomerization by the extracellular mosaic protein fibropellin. Protein Sci 2005;14:417-423.
5. Futch L, Pham T, Adamson B, Reilly B, Kronenfeld J, Patterson K, *et al*. It's Strong, It's Stable, It's Streptavidin. FASEB J 2015;29:LB57.
6. Molaee N, Abtahi H, Mosayebi G. Expression of recombinant streptokinase from *Streptococcus pyogenes* and its reaction with infected human and murine sera. Iran J Basic Med Sci 2013;16, 985-989.
7. Hasanzadeh L, Ghaznavi-Rad E, Soufian S, Farjadi V, Abtahi H. Expression and antigenic evaluation of VacA antigenic fragment of *Helicobacter pylori*. Iran J Basic Med Sci 2013; 16: 835–840.
8. Abtahi H, Salmanian A.H, Rafati S, Nejad G.B, Hassan Z.M. High level expression of recombinant ribosomal protein (L7/L12) from *Brucella abortus* and its reaction with infected human sera. Iran Biomed J 2004; 8:13–18.
9. Mirjamali N, Soufian, S, Molaee N, Sadoogh Abbasian S, Abtahi H. Cloning and expression of the enzymatic region of Streptococcal hyaluronidase. Iran J Basic Med Sci 2014; 17:667–672.
10. Farjadi V, Abtahi H, Zolfaghari M.R, Soufian S, Hasanzadeh L. Expression, purification and evaluation of antigenicity of CagA antigenic fragment of *Helicobacter pylori*. Jundishapur J Microbiol 2013; 6:e7367.
11. Silva FSR, Santos SPO, Meyer R, Silva ES, Pinheiro CS, Alcantara-Neves NM, Pacheco LGC. *In vivo* cleavage of solubility tags as a tool to enhance the levels of soluble recombinant proteins in *Escherichia coli*. Biotechnol Bioeng 2021;118:4159-4167.
12. Han SH, Kim HM, Lim MW, Kim J-K. Functional Expression of Soluble Streptavidin in *Escherichia coli*. J Life Sci 2015; 25:631-637.
13. Laitinen O, Hytönen V, Nordlund H, Kulomaa M. Genetically engineered avidins and streptavidins. Cell Mol Life Sci 2006;63:2992-3017.
14. Qureshi MH, Yeung JC, Wu SC, Wong SL. Development and characterization of a series of soluble tetrameric and monomeric streptavidin muteins with differential biotin binding affinities. J Biol Chem 2001;7;276:46422-46428.
15. Arakawa T, Ejima D, Tsumoto K, Obeyama N, Tanaka Y, Kita Y, *et al*. Suppression of protein interactions by arginine: A proposed mechanism of the arginine effects. Biophys Chem 2007;127:1-8.
16. Howarth M, Chinnapen DJ, Gerrow K, Dorrestein PC, Grandy MR, Kelleher NL, *et al*. A monovalent streptavidin with a single femtomolar biotin binding site. Nat Methods 2006;3:267-273.
17. Baugh L, Le Trong I, Cerutti DS, Gülich S, Stayton PS, Stenkamp RE, Lybrand TP. A distal point mutation in the streptavidin-biotin complex preserves structure but diminishes binding affinity: Experimental evidence of electronic polarization effects? Biochemistry 2010; 8;49:4568-4570.
18. Demonte D, Dundas CM, Park S. Expression and purification of soluble monomeric streptavidin in *Escherichia coli*. Appl Microbiol Biotechnol 2014;98:6285-6295.
19. Kroetsch A, Chin B, Nguyen V, Gao J, Park S. Functional expression of monomeric streptavidin and fusion proteins in *Escherichia coli*: Applications in flow cytometry and ELISA. Appl Microbiol Biotechnol 2018;102:10079-10089.
20. Chamma I, Rossier O, Lim KH, Gauthereau I, Giannone G, Park S, *et al*. Super resolution mapping of adhesion molecules in confined cellular environments using monomeric streptavidin ligands. Biophys J 2014;106:202a.
21. Jurchenko C, Chang Y, Narui Y, Zhang Y, Salaita KS. Integrin-generated forces lead to streptavidin-biotin unbinding in cellular adhesions. Biophys J 2014;1;106:1436-1446.
22. Lim KH, Huang H, Pralle A, Park S. Stable, high-affinity streptavidin monomer for protein labeling and monovalent biotin detection. Biotechnol Bioeng 2013;110:57-67.
23. Lim KH, Huang H, Pralle A, Park S. Engineered streptavidin monomer and dimer with improved stability and function.

Biochemistry 2011;11;50:8682-8691.
24. Wu SC, Wong SL. Engineering soluble monomeric streptavidin with reversible biotin binding capability. J Biol Chem 2005;280:23225-23231.
25. O'Sullivan VJ, Barrette-Ng I, Hommema E, Hermanson GT, Schofield M, Wu SC, *et al*. Development of a tetrameric streptavidin mutein with reversible biotin binding capability: Engineering a mobile loop as an exit door for biotin. PLoS One 2012;7:e35203.