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A Recombinant SurE Ortholog from *Acinetobacter baumannii*

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Abstract

Acinectobacter baumannii is a medically relevant opportunistic pathogen accounting for approximately 2 % of all healthcare associated infections in the United States. Sequence analysis of the *A. baumannii* genome revealed the presence of a 256 amino acid SurE ortholog which was expressed in *E. coli* and purified by nickel-affinity column chromatography. Polyacrylamide gel electrophoresis of recombinant SurE indicated a single Coomassie Blue staining band of approximately 26 kDa. Proteomic analysis corroborated the identity and molecular mass (27,488 Da) from the deduced sequence. Initial characterization using the artificial substrate *p*NPP indicated maximal phosphohydrolase activity at pH 5.5 in the presence of Mn^{2+} cation. Phosphate was also removed to varying degree from phosphorylated sugars, metabolites, amino acid and peptide derivatives. The SurE ortholog also exhibited 5'-nucleotidase activity removing phosphate from nucleotides AMP, CMP, IMP, UMP, dUPM, and TTP. k_{cat}/K_m values revealed UMP as the best nucleotide substrate. In summary, the SurE ortholog is a phosphatase with broad substrate specificity, the physiological significance of which remains to be established in *A. baumannii.*

Keywords: Phosphatase; SurE; 5'-nucleotidase; Acinetobacter baumannii; Characterization

Background

Acinetobacter baumannii is an opportunistic Gram-negative pathogen and member of the ESKAPE group of pathogens *(Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Enterobacter* species) [1]. The ESKAPE designation refers to an extreme drug-resistant phenotype, and ability to escape most current antimicrobial therapies. The prevalence of cases of multi-drug resistant (MDR) *A. baumannii* infection has increased worldwide and is a leading cause of increasing hospital costs and mortality [2]. This prompted the World Health Organization's assigning carbapenem-resistant *A. baumannii* the highest priority ranking for identification of new and effective drug therapies to combat it [3]. Sequence analysis of the *A. baumannii* genome revealed the presence of a 256 amino acid SurE ortholog, a large family of phosphohydrolases (nucleotidases) that dephosphorylate ribo- and deoxyribonucleoside 3' and 5'-monophosphates. Nucleotidases are wide-spread in living systems, and generally considered multifunctional, hydrolyzing phosphate esters and phosphoanhydride bonds [4-6]. We report here enzymatic characterization of an *A. baumannii* SurE ortholog, a phosphatase [7,8] with broad specificity capable of hydrolyzing phosphate from nucleotides and non-nucleotide substrates.

Methods

Cloning and Protein Expression

A gene construct from *A. baumannii* Ci79 strain encoding SurE 5'-Nucleotidase (NCBI Accession AVOD01000053.1, nt. 24056 – 24823, region encoding 256 amino acids) was synthesized with a C-terminal hexa-histidine tag, and inserted into a pET23a(+) expression vector via *NdeI/Hind*III restriction sites (Genscript, Piscataway, NY). The resulting pET23a-bearing plasmid was used to transform *Escherichia coli* Rosetta by electroporation using a BioRad (BioRad Laboratories, Hercules, CA) Gene Pulser Xcell apparatus. Transformants harboring the pET23a-construct were selected on LB agar plates containing ampicillin (100 µg/ml) and chloramphenicol (50 µg/ml). For expression of recombinant SurE protein (rSurE) an overnight culture of pET23a-transformant was used to inoculate LB broth (1 liter) while shaking at 225 RPM at 37 °C to an OD600 of 0.6. Isopropyl β-D-1-thiogalactoside (IPTG, Sigma Chemical Co., St. Louis, MO) was then added (1 mM final concentration) and incubated for an additional 4 hours after which time bacterial cells were pelleted at 4000 x g for 10 minutes at 5 °C. The supernatant was decanted, and the pellet stored at -20 °C.

Preparation of Recombinant Lysate

Pellet material was resuspended in 20 mL ice-cold lysis buffer [50 mM NaH_2PO_4 buffer, pH 8 containing 300 mM NaCl, 10 mM imidazole, and cOmplete™ Protease Inhibitor Cocktail (Sigma Chemical Co.). The pellet suspension was sonicated for 10 seconds using a Misonix Ultrasonic Liquid Processor XL-200 (Misonix, Farmingdale, NY) at an output setting of 10 watts while partially immersed in an ice slurry. Sonicated lysate was centrifuged at 6000 x g for 15 minutes at 5 °C. Following decanting of supernatant, 20 mL lysis buffer containing 8 M urea was added to the pellet and sonicated a second time. The sonicate was allowed to stand in an ice slurry for one hour to extract recombinant protein from bacterial inclusion bodies. His-tagged recombinant SurE was obtained by centrifugation at 6000 x g for 15 minutes, and the resulting supernatant was subjected to affinity chromatography purification using Ni^{2+}-nitrilotriacetic acid (Ni-NTA) Agarose beads (Qiagen, Germantown, MD).

Nickel Column Purification

Ni-NTA Agarose beads (3 mL) were washed (3 times) with 2 mL cold lysis buffer and pelleted at 200 x g for 2 minutes. Recombinant protein was added to the washed beads, mixed thoroughly overnight at 5°C using an inverting rotator, transferred to a small glass column, and allowed to settle (final bed volume dimensions = 1.7 x 13 cm). The column was washed with 25 mL lysis buffer containing 8M urea followed by 20 mL cold wash buffer (50 mM NaH_2PO_4 buffer, pH 8 containing 300 mM NaCl, 40 mM imidazole, and 8 M urea). Bead-bound SurE was eluted by sequential addition of ten (1 mL) applications of cold elution buffer (50 mM NaH_2PO_4 buffer, pH 8 containing 300 mM NaCl, 250 mM imidazole, and 8 M urea). Eluates (1 mL) were collected in microcentrifuge tubes partially immersed in an ice slurry.

Removal of Urea

Recombinant SurE eluates were pooled (~ 5-7 mL), and dialyzed sequentially against 200 mL dialysis buffer (0.050 M sodium acetate buffer, pH 6.5) containing 4, 2, 1, and 0 M urea using a 500-1000 molecular weight cutoff dialysis membrane (Thermo Fisher Scientific, Waltham, MA). For each dialysis, the dialysate was allowed to equilibrate at 5°C for two hours. Dialysis against buffer containing no urea, i.e., 0 M was carried out twice after which time the contents of the dialysis bag were removed (~1 mL) to which 50 µL 10 % (v/v) glycerol was added, aliquoted (100 µL), and stored at -80 °C until used (source of purified recombinant protein used in this study).

Phosphatase Assays

All assays contained 0.16 µg affinity purified recombinant SurE protein dissolved in 0.1 mg/ml BSA and were incubated at 37 °C for 30 minutes (linear with time and protein). Apart from phosphorylated peptides (EMD Millipore Corporation, Temecula, CA) all substrates and reagents were obtained from Sigma Chemical Co.

Release of phosphate from artificial substrate paranitrophenyl phosphate (pNPP)

Generation of pNP-ol from pNPP was determined in a 300 µL reaction volume containing 2.0 mM pNPP 0.20 M MES buffer, pH 5.5, and 2.0 mM $MnCl_2$. Following incubation, reaction mixtures were immersed in an ice slurry for 5 minutes followed by addition of enzyme to the respective blanks, and heat killed at 95 °C for 5 minutes. Heat killed reactions were placed in an ice slurry for 5 minutes followed by addition of 1.7 mL 0.50 M Glycine, pH 10 buffer, and allowed to stand at room temperature for 30 minutes. Released pNP-ol was monitored at 405 nm using a Genesys 10 UV Scanning Spectrophotometer (Thermo Fisher Scientific), and quantitation achieved by extrapolation to a pNP-ol standard following subtraction of respective blank values.

Release of phosphate from natural phosphorylated substrates

Following incubation, all reaction mixtures were immersed in an ice slurry for 5 minutes. Enzyme was added to the respective blank reaction mixtures and heat killed. Phosphate release was monitored at 620 nm following addition of 1.0 mL BIOMOL GREEN phosphate reagent (Enzo Life Sciences, Farmingdale, NY). Respective blank values were subtracted, and released phosphate quantitated by extrapolation to a phosphate standard.

Nucleotides

Nucleotidase activity was determined using the method of Proudfoot et al. [9] as modified in this laboratory. Reactions contained 2.0 mM substrate in a final reaction volume of 200 µL containing 2.0 mM $MnCl_2$ and 0.2 M HEPES buffer adjusted to pH 7.0.

Phosphorylated sugars and metabolites:

Reactions contained 2.0 mM substrate in a final reaction volume of 300 µL containing 0.2 M MES buffer, pH 5.5, and 2.0 mM $MnCl_2$.

Phosphorylated amino acids, peptides, and protein

Reactions contained a) 2.0 mM phosphorylated amino acid, b) 0.5 mM phosphorylated peptide, or c) 0.1 mM β-Casein in a final reaction volume of 200 µL containing 0.2 M MES buffer, pH 5.5, and 2.0 mM $MnCl_2$.

Protein Determination

Protein concentration was determined using the Bradford dye-binding procedure [10] per manufacturer's recommendation (BioRad Laboratories) with BSA as protein standard.

PAGE Analysis

All electrophoresis reagents (sample buffers, running buffers, standards, precast gels, and gel stain) and equipment utilized in SDS PAGE analysis were obtained from Bio-Rad Laboratories.

Recombinant protein (~0.5-1.0 µg) and pre-stained standard ladder were added to 10 µL 2X Laemmli sample buffer under reducing conditions (2.0 % v/v β-mercaptoethanol), and denatured at 95 °C for 2 minutes. Respective samples were applied onto 4-20 % precast gradient polyacrylamide gels partitioned in 1X Tris Glycine buffer containing SDS. Electrophoresis was carried out at constant voltage (80 volts) for 90 minutes at room temperature using a Power Pac 3000 power supply, and Mini Protein II electrophoretic apparatus. Protein was stained using Coomassie Brilliant Blue R-250.

Proteomic Analysis

The predominant Coomassie Blue stained protein band (~26 kDa) resolved by electrophoresis was excised, subjected to trypsin digestion, and analyzed using a Thermo Fisher LTQ-Orbitrap Elite mass spectrometer in the Mass Spectrometry & Proteomics Core of the University of Texas at San Antonio.

Molecular Modeling

A dimer homology model of *A. baumannii* SurE was constructed using the SwissModel with *Coxiella burnetii* SurE (Protein Data Bank file 3ty2) as a template. The model had a normalized Qmean6 score of 0.72, indicating a high-quality model. Protein side chain ion pairs were identified using VMD software and Tcl measure command [11]. Distances less than 4 Å between arginine NH1 or NH2 atoms or lysine NZ atoms and aspartate OD1 or OD2 or glutamate OE1 or OE1 atoms were designated ion pairs.

Results

Expression and Purification of Recombinant SurE

His-(6x) tagged *A. baumannii* SurE was expressed in *E. coli* and purified to apparent homogeneity by nickel affinity column chromatography. The protein migrated as a species of ~26 kDa under denaturing/reducing conditions (Figure 1A). The 26 kDa Coomassie Blue stained band resolved by electrophoresis was excised and subjected to MALDI-TDF mass spectroscopic analysis. As shown in Figure 1B, five internal amino acid stretches (shown in gray) shared identical sequence homology to *E. coli* SurE protein with 29 % overall protein coverage. Assessment of size by electrophoretic migration is in close agreement with the deduced molecular mass of 27,488 Da from the protein sequence. AlphaFold 3-dimensional prediction reveals a protruding hairpin structure and C-terminal α-helix (Figure 1C).

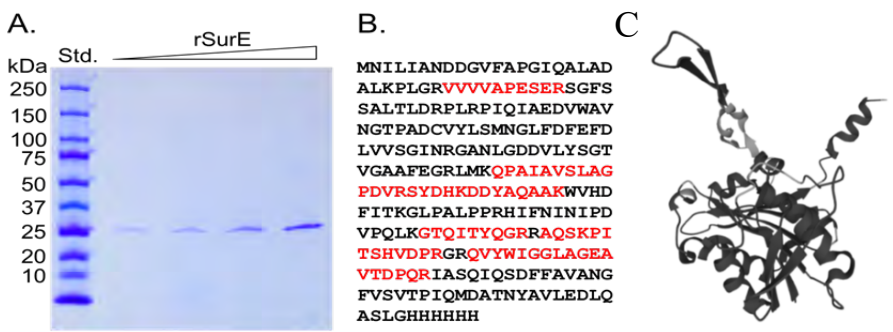

Figure 1: Recombinant SurE (rSurE). (A) SDS-PAGE analysis of increasing amounts (0.5, 1.0, 3.0, and 5.0 µg) nickel-affinity purified *Acinetobacter baumannii* SurE. (B) Proteomic sequence analysis (matching sequences are shown in gray with 29 % overall protein coverage). (C) AlphaFold predicted 3-dimensional structure. SDS-PAGE and proteomic sequence analyses were carried out as described under 'Methods'.

Dependence of hydrolysis on pH

The dependence of hydrolysis on pH was determined using the general phosphatase substrate paranitrophenyl phosphate (*p*NPP) in the presence of 2.0 mM $MnCl_2$ buffered with 0.2 M HEPES (pH 5.0-7.0) and MES (pH 6.0-7.5). As shown in Figure 2, hydrolysis of pNPP was broad but maximal at pH 5.5.

Dependence of hydrolysis on divalent cations

Shown in Table 1 is the dependence of pNPP hydrolysis on various cations at pH 5.5. Maximum hydrolysis was observed in the presence of 2.0 mM $MnCl_2$ with Co^{+2}, Mg^{+2}, and Ni^{+2} at 72, 69, and 57 percent that of Mn^{+2}, respectively.

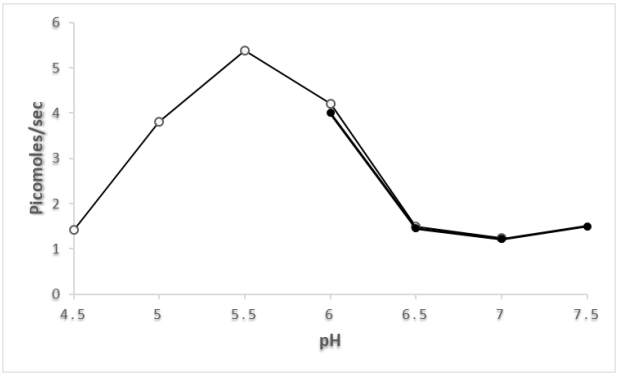

Figure 2: Determination of optimal pH for hydrolysis of pNPP. *Acinetobacter baumannii* SurE activity (pmoles pNP-ol s^{-1}) was determined as a function of pH in the presence of 0.2 M HEPES (solid circles), and 0.2 M MES (open circles) buffered reaction mixtures as previously described under 'Methods'. Each point represents the mean value of 5 replicate determinations. Error bars are not shown because replicate values varied less than 5% of respective mean values.

Table 1: Effect of divalent cations on hydrolysis of pNPP. Activity is expressed as the mean percent (5 determinations) of *Acinetobacter baumannii* SurE activity observed in the presence of $MnCl_2$ (100 %).

Cation	Percent $MnCl_2$ *p*NPP
None	< 5
Ba^{+2}	< 5
Ca^{+2}	19
Co^{+2}	72
Cu^{+2}	11
Hg^{+2}	< 5
Li^{+2}	< 5
Mg^{+2}	69
Ni^{+2}	57
Zn^{+2}	< 5

Comparison of kinetic constants for hydrolysis of nucleoside 5'-phosphoesters

Shown in Table 2 is a comparison of kinetic constants for hydrolysis of various nucleoside 5'- phosphoester substrates. Except for TTP, the *A. baumannii* SurE ortholog hydrolyzed 5'-monophosphate esters of AMP, UMP, 2-deoxy UMP, CMP, and IMP. No hydrolysis was observed for GMP, TMP, ADP, CDP, IDP, TDP, ATP, CTP, GTP, ITP, and UTP. Comparable micromolar K_m values, i.e., 284 +/- 83, 343 +/-38, and 476 +/- 38 for UMP, AMP, and dUMP, respectively were observed. Highest catalytic efficiency (2.88×10^3 $M^{-1}s^{-1}$) was achieved with UMP as substrate.

Table 2: Summary of kinetic constants for hydrolysis of pNPP and nucleotide substrates. K_m and V_{max} values (+/- 1 SD) were determined by Lineweaver-Burk plot analysis (five determinations) over a range of substrate concentration (0.25-10.0 mM). Mean values (V_{max} and K_m) and molecular mass (27,488 Da) were used in calculation of k_{cat} and k_{cat}/K_m.

Substrate	V_{max} (pmoles s^{-1})	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($M^{-1}s^{-1}$)
PNPP	5.38 ± 0.23	85.4 ± 17	1.85	2.19×10^3
UMP	4.81 ± 0.58	284 ± 83	0.82	2.88×10^3
AMP	4.22 ± 0.32	343 ± 38	0.73	2.13×10^3
dUMP[a]	3.90 ± 0.21	476 ± 38	0.66	1.41×10^3
CMP	6.26 ± 0.53	830 ± 156	1.06	1.29×10^3
IMP	2.82 ± 0.52	989 ± 210	0.48	0.49×10^3
TTP	2.85 ± 0.78	1282 ± 158	0.41	0.38×10^3

[a]2-deoxy. No activity was detected for GMP, TMP, ADP, CDP, IDP, TDP, ATP, CTP, GTP, and UTP nucleoside phosphates.

Hydrolysis of non-nucleotide phosphoesters

Several non-nucleoside phosphate esters were hydrolyzed, and their activity relative to that of pNPP is shown in Table 3. Although comparatively low hydrolysis (< 20 %) was observed for some substrates, moderate (~ 30 %) to very high (> 90 %) hydrolysis was observed for others. No phosphate was released from the phosphorylated amino acid phospho-L-serine or phosphoserine bearing peptide, and β-Casein (a protein containing 5 phosphorylated serine residues) substrates. Similarly, no hydrolysis of the phosphorylated amino acid phospho-L-threonine or peptide containing phosphorylated threonine was observed. In contrast, the SurE ortholog released phosphate from the amino acid phospho-L-tyrosine, and peptide containing phosphorylated tyrosine.

Table 3: Release of phosphate from non-nucleotide substrates by *Acinetobacter baumannii* SurE. Relative Activity (mean value for 5 determinations) represents the ratio of hydrolysis of phosphorylated compound to that observed for pNPP x 100.

Compound	Relative Activity
Pyridoxal Phosphate	29.8
Thiamine Pyrophosphate	0.5
Thiamine Monophosphate	1.8
Phospho-L-Threonine	ND
Threonine Phosphopeptide[1]‡	ND
Phospho-L-Tyrosine	27.5
Tyrosine Phosphopeptide[2]‡	21.0
Phospho-L-Serine	ND
Serine Phosphopeptide[3]‡	ND
Phosphoethanolamine	4.1
Phosphocholine	6.5
β-Glycerolphosphate	96.7
Ribose-5-Phosphate	32.9
Galactose-1-Phosphate	6.8
Fructose-1, 6-Bisphosphate	31.0
Glucose-6-Phosphate	4.3
Mannose-6-Phosphate	16.2
Trehalose-6-Phosphate	12.8
Phosphoenolpyruvic Acid	4.1
Phosphocreatinine	ND
Phytic Acid	7.3
β-Casein	ND
NADPH	3.9

[1]KR**pT**IRR, [2]TSTEPQ**pY**QPGENL, [3]RRA**pS**VA, ‡Phosphopeptide reaction concentration = 0.50 mM. ND, no activity detected.

Discussion

The surE gene was first discovered in *E. coli* and is well-conserved among both the eubacteria and the archaea [12]. Consistent with SurE family member proteins, the characteristic N-terminal aspartate-aspartate (DD) motif (residues D8 and D9) is present in the *Acinectobacter* ortholog described here. In addition to residues D8 and D9, a total of 15 strictly conserved residues (N7, G10, P34, S39, G69, D88, S92, G93, N95, G97, N99, S107, T109, S127, and N164) have been observed among family members [13]. Sequence analysis of the *Acinetobacter* ortholog indicates 7 of the 15 to be present: N7, G10, P34, S39, G93, N95, and G97. Furthermore, the sequence motif X_3SGXNXGXN-$X_{7\text{-}8}$-S(G/A)T found in *Thermotoga maritima* and proposed to be characteristic of SurE family members is also present in the *Acinetobacter* SurE ortholog (residues 88-105) [13]. Examination of the ortholog's structure using AlphaFold reveals a protruding β-hairpin structure and C terminal α-helix. Crystal structure studies of some SurE proteins suggest that the protruding β-hairpin structure mediates formation of tetramers by assembly of two dimers [13,14].

Like the SurE proteins of *T. maritima* and *Thermus thermophilus, A. baumannii* SurE is a metal ion dependent phosphatase (pH optimum = 5.5 for hydrolysis of pNPP) in the presence of Mn^{+2} rather than Mg^{+2} [14, 15]. The metal ion dependence of the *Acinetobacter* SurE is similar to that of *E. coli*, i.e., $Mn^{+2} > Co^{+2} > Ni^{+2} > Mg^{+2}$ [9]. In contrast to the SurE proteins of *T. maritinia* and *T. thermopilus* which are activated at elevated temperatures, the *A. baumannii* SurE is inactivated at 65°C (data not shown). The lack of structural stability at higher temperatures could be attributed to the absence of multiple ion pairs which are not conserved or are only partly conserved across SurE orthologs [14]. A dimer homology model of *A. baumannii* SurE based on a *Coxiella burnetii* SurE template had 4 inter- and 9 intra-subunit ion pairs. By comparison, *C. burnetii* SurE (Protein Data Bank file 3ty2) had 6 inter- and 17 intra-subunit ion pairs, and thermally stable

T. thermophilus (Protein Data Bank file 2e6h) had 5 inter- and 16 intra-subunit ion pairs per dimer. The lack of *A. baumannii* SurE thermal stability could be attributed to the lower number of intra-subunit ion pairs which may be necessary for stabilizing and preventing unfolding of catalytically active enzyme at higher temperatures. Although, vanadate inhibits *A. baumannii* SurE hydrolysis of *p*NPP (IC_{50} = 0.8 mM, data not shown), invariant residues N99, S107, and T109 required for vanadate binding and inhibition of 1-napthyl phosphate hydrolysis (IC50 = 1.0 mM) are missing [13].

Apart from TTP, *A. baumannii* SurE exhibited significant 5'-nucleotidase activity hydrolyzing the 5'-nucleoside monophosphate esters AMP, UMP, 2-deoxy UMP, CMP, and IMP with highest catalytic efficiency for UMP ($k_{cat}/K_m = 2.88 \times 10^3\ M^{-1}s^{-1}$). In contrast to several other characterized SurE proteins indicating 5'-GMP the preferred substrate [9,13-15], no hydrolysis of 5'-GMP as well as TMP, ADP, CDP, IDP, TDP, ATP, CTP, GTP, and UTP was observed. The comparatively lower catalytic efficiency, i.e., $10^3 M^{-1}s^{-1}$ may be advantageous in preventing intracellular nucleotide pool depletion during the stress response [16].

In addition to 5'-nucleotides, the *Acinetobacter* SurE ortholog removed to varying extent phosphate from an array of phosphorylated compounds, e.g., vitamin B_6, sugars, metabolites, and the amino acid tyrosine, the physiological relevance of which remains to be established in *A. baumannii*. The inability to remove phosphate from β-Casein could be attributed to the low substrate concentration used due to low solubility of this phosphorylated protein. Additionally, the relative rate of peptide phosphorylated tyrosine hydrolysis may be an underestimate due to the final concentration of peptide used (0.5 mM) in comparison to 2.0 mM pNPP. Phosphorylation of bacterial protein tyrosine has been shown to direct many essential cellular processes, e.g., capsule production, growth, proliferation, migration, flagellin export, adaptation to stress and production secondary metabolites [17]. Polyadenylation is a major post-transcriptional RNA modification event in bacteria marking RNAs for degradation and regulation of RNA [17]. Poly(A) polymerase I (PAP1) is the primary enzyme that polyadenylates transcripts, and Francis and coworkers have shown PAP1 tyrosine phosphorylation inhibits PAP1 polyadenylation activity under stress [18]. Thus, removal of phosphate may play a role in regulation of transcription in response to stress condition changes by *A. baumannii.*

Examination of the SurE ortholog's N-terminus reveals the absence of tandem positively charged arginine in the first 5 residues characteristic of signal peptides required for secretion. Although, a stretch of hydrophobic residues is observed followed by 2 small aliphatic residues (A18 and A20, characteristic of the signal peptide cleavage site recognized by the type 1 signal peptidase), absence of an N-terminus arginine tandem indicates that the SurE ortholog is likely located intracellularly. SignalP software (https://services.healthtech.dtu.dk) also predicts that *A. baumannii* SurE lacks a signal peptide. Like UmpH, preference for 5'-UMP supports involvement of *A. baumannii* SurE in regulation of intracellular nucleotide pools for the maintenance of DNA and RNA synthesis [19]. Considering the inability to remove phosphate from peptides containing phosphorylated L-threonine and serine, the removal of phosphate from a peptide containing phosphorylated L-tyrosine warrants further investigation into *A. baumannii* tyrosine kinase dependent processes.

Conclusion

The SurE homolog from *A. baumannii* is a phosphohydrolase with broad substrate specificity. Although similar in some respects to the nonspecific acid phosphatase [20], the SurE homolog like other SurE proteins is a nucleotidase. The physiological role of the SurE homolog and relevance to stress response, virulence, and pathogenesis of this emergent pathogen remains to be established.

Data Availability

The graphs, figures, and data presented in tables used to support the findings of this study are all included within the article and also uploaded in the figure files.

Acknowledgements

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contribution

JPC, JJY and BPA conceptualized the study. JPC, PC, LTD, and JJY carried out the laboratory investigations/determinations. RR modeled and interpreted the AlphaFold 3-dimensional depiction of the SurE homolog. JPC wrote the original draft. JPC, JJY, RR, and LTD edited the draft. All authors read and approved the final manuscript.

References

1) Rice LB. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. J Infect Dis. 2008, 197: 1079-1081.

2) Founou RC, Founou LL, Essack SY. Clinical and economic impact of antibiotic resistance in developing countries: a systematic review and meta-analysis. PLoS ONE. 2017, 12: e0189621.

3) Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibioltic-resistant bacteria and tuberculosis. Lancet Infect Dis. 2018, 18: 318-327.

4) Zimmerman MD, Proudfoot M, Yakunin A, Minor W. Structural insight into the mechanism of substrate specificity and catalytic activity of an HD-domain phosphohydrolase: the 5'-deoxyribonucleotidase YfbR from *Escherichia coli*. J Mol Biol. 2008, 378: 215-226.

5) Kohn J, Reis JL. Bacterial nucleotidases. J Bacteriol. 1963, 86: 713-716.

6) Terakawa A, Natsume A, Okada A, Nishihata S, Kuse J, Tanaka K, et al. *Bacillus subtilis* 5'-nucleotidases with various functions and substrate specificities. BMC Microbiol. 2016, 16: 249.

7) Mathiharan YK, Murthy MRN. Molecular dynamics studies on the domain swapped *Salmonella typhimurium* survival protein SurE: insights on the possible reasons for catalytic cooperativity. J Bimolecular Structure and Dynamics. 2018, 36: 2203-2311.

8) Mura C, Katz JE, Clarke SG, Eisenberg D. Structure and function of an archaeal homolog of survival protein E (SurEalpha): an acid phosphatase with purine nucleotide specificity. J Mol Biol. 2003, 326: 1559-1575.

9) Proudfoot M, Kuznetsova E, Brown G, Rao NN, Kitagawa M, Mori H, et al. General enzymatic screens identify three new nucleotidases in *Escherichia coli.* Biochemical characterization of SurE, YfbR, and YjjG. J Biol Chem. 2004, 279: 54687-54694.

10) Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 1976, 72: 248-254.

11) Humphrey W, Dalke A, Schulten K. VMD-Visual Molecular Dynamics. J. Molec Graphics. 1996, 14: 33-38.

12) Visick JE, Ichikawa JK, Clarke S. Mutations in the *Escherichia coli* surE gene increase isospartyl accumulation in a strain lacking the pcm repair methyltransferase but suppress stress-survival phenotypes. FEMS Microbiol Lett. 1998, 167: 19-25.

13) Lee JY, Kwak JE, Moon, J, Eom, SH, Liong, EC, Pedelacq, J-D, et al. Crystal structure and functional analysis of the SurE protein identify a novel phosphatase family. Nature Struc Bio. 2001, 8: 789-794.

14) Zhang RG, Skarina T, Katz JE, Beasley S, Khachatryan A, Vyas S, et al. Structure of *Thermotoga maritima* stationary phase survival protein SurE: a novel acid phosphatase. Structure. 2001, 9: 1095-1106.

15) Iwasaki W, Miki K. Crystal structure of the stationary phase survival protein SurE with metal ion and AMP. J Mol Biol. 2007, 371: 123-136.

16) Li C, Ichikawa JK, Ravetto JJ, Kuo HC, Fu JC, Clarke S. A new gene involved in stationary-phase survival located at 59 minutes on the *Escherichia coli* chromosome. J Bacteriol. 1994, 176: 6015-6022.

17) Zhang ZY. Functional studies of protein tyrosine phosphatase with chemical approaches. Biochim Biophys Acta. 2005, 1754: 100-107.

18) Francis N, Behera MR, Natarajan K, Laishram RS. Tyrosine phosphorylation controlled poly(A) polymerase 1 activity regulates general stress response in bacteria. Life Sci Alliance. 2022, 6: e202101148.

19) Reaves ML, Young BD, Hosios AM, Xu YF, Rabinowitz JD. Pyrimidine homeostasis is accomplished by directed overflow metabolism. Nature. 2013, 500: 237-241.

20) Moreno-Smiley EF, Smith D, Yu J-J, Cao P, Arulanandam BA, Chambers JP. Biochemical characterization of a recombinant acid phosphatase from *Acinetobacter baumannii*. PLoS ONE. 2021, 16: e02523772021.