Specific Potassium Binding Stabilizes pI258 Arsenate Reductase from Staphylococcus aureus*

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Arsenate reductase (ArsC) from Staphylococcus aureus plasmid pI258 catalyzes the reduction of arsenate to arsenite and plays a role in bacterial heavy metal resistance. The high resolution x-ray structure of ArsC reveals the atomic details of the K⁺ binding site situated next to the catalytic P-loop structural motif of this redox enzyme. A full thermodynamic study of the binding characteristics of a series of monovalent cations (Li⁺, Na⁺, K⁺, Rb⁺, and Cs⁺) and their influence on the thermal stability of ArsC was performed with isothermal titration calorimetry, circular dichroism spectroscopy, and differential scanning calorimetry. Potassium has the largest affinity with a $K_b$ of $3.8 \times 10^9$ M⁻¹, and the effectiveness of stabilization of ArsC by monovalent cations follows the binding affinity order: $K^+ > Rb^+ > Cs^+ > Na^+ > Li^+$. A mutagenesis study on the K⁺ binding side chains showed that Asn-13 and Asp-65 are essential for potassium binding, but the impact on the stability of ArsC was the most extreme when mutating Ser-36. Additionally, the thermal stabilization by K⁺ is significantly reduced in the case of the ArsC E21A mutant, showing the importance of a Glu-21-coordinated water molecule in its contact with K⁺. Although potassium is not essential for catalysis, in its presence the $k_{cat}/K_M$ increases with a factor of 5. Altogether, the interaction of K⁺ with specific residues in ArsC is an enthalpy-driven process that stabilizes ArsC and increases the specific activity of this redox enzyme.

It has been known for a long time that cations serve a variety of functions in proteins. They play a role in protein stabilization and/or are involved in catalytic processes of enzymes. Some of the questions to be answered in studies of ion-protein interactions are still those posed by Scatchard (1) in 1949: how many, how tightly, where, and why? Although the combination of kinetic, thermodynamic, and structural studies can provide these answers, complete studies are relatively rare, and often only partial answers are available. The analysis of metal binding sites in proteins is also becoming of considerable interest because of efforts to create novel metal binding sites, either by de novo design or by redesign of existing scaffolds (2, 3). Transition metal-containing enzymes and proteins that bind divalent Mg²⁺ and Ca²⁺ ions are well documented (4–12). Na⁺ and K⁺ mostly associate with proteins rather weakly, and as bulk electrolytes they are predominantly involved in surface charge stabilization. Sometimes, however, they are involved in protein stabilization and/or activation through binding to specific, well-defined sites (13–18). Overall, the structural chemistry and the binding of Na⁺ and K⁺ remains for the most part poorly documented, presumably because of the relative rareness of high-affinity binding sites for monovalent ions.

Here, we present an in-depth study of a well-defined K⁺ site in arsenate reductase of Staphylococcus aureus (ArsC). ArsC enzymes are part of the arsenic detoxification system of various organisms. They catalyze the reduction of arsenate to arsenite, which is then extruded from the cell. ArsC from S. aureus plasmid pI258 is representative for enzymes from the Gram-positive group of bacteria and has a tyrosine phosphatase (PTPase) I fold typical for low molecular weight (LMW) PTPases (19). From the Gram-positives, ArsC from Bacillus subtilis has also been structurally characterized (20). Both enzymes have a conserved CX³R P-loop signature motif. There is no sequence, and fold homology between ArsC from S. aureus and ArsC from Escherichia coli plasmid R773 (Gram-negative representative) and also the reaction mechanisms differ (21). Recently, another type of arsenate reductase (Acr2p) has been identified from the eukaryotic yeast Saccharomyces cerevisiae (22). Acr2p also contains a P-loop signature but is homologous to the human cell cycle control phosphatase Cdc25a (23). The reaction mechanism of pI258 ArsC had first been proposed on the basis of the structures of the reduced and oxidized forms (19) and has recently been thoroughly documented with the x-ray structures of all the key intermediates along the reaction pathway (24). We have observed a potassium ion bound to the protein in all our x-ray structures of pI258 ArsC. The fact that potassium was systematically observed in contact with the same residues in ArsC suggested specific binding. Furthermore, a definite protein stabilizing effect of K⁺ ions was observed. The coordination around the potassium ion was analyzed on the basis of high-resolution structural data (1.1 Å) for the reduced form of the protein (19). To determine the contributions of the residues involved in binding to the stabilization of the protein, a set of mutants of the ligating residues substituted by Ala was constructed. Thermal denaturation of wild type and mutants was systematically studied by circular dichroism spectrophotometry and differential scanning calorimetry (DSC). Binding of the first series of monovalent cations

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¶ The abbreviations used are: ArsC, arsenate reductase; ITC, isothermal titration calorimetry; DSC, differential scanning calorimetry; CD, circular dichroism; HEPPS, 4-(2-hydroxyethyl)-1-piperazinepropane-sulfonic acid; MOPS, 4-morpholinepropanesulfonic acid; LMW, low molecular weight.
(Li\(^+\), Na\(^+\), K\(^+\), Rb\(^+\), Cs\(^+\)) to the wild-type protein was quantitatively characterized by isothermal titration calorimetry (ITC). Additionally, we report the effect of these ions on the thermal stability of S. aureus pl258 ArsC.

**EXPERIMENTAL PROCEDURES**

**Site-directed Mutagenesis, Expression, and Purification**—pET11a arsC wild-type plasmid (25) was used as DNA template in PCR amplification with primers designed to introduce the N134A, D65A, E212A, and S36A mutations. The resulting fragments were digested with NdeI and BamHI, cloned into the pET11a vector (Stratagene, La Jolla, CA) and transformed into E. coli strain BL21(DE3) (26). The E. coli BL21(DE3) strains expressing the ArsC mutants were grown in a Luria Bertani broth pre-culture with 1% glucose and 100 μg/ml ampicillin at 37 °C. The culture was transferred to Terrific broth and was grown for 4 h at 37 °C with 0.1% glucose and 100 μg/ml ampicillin. Induction at a cell density of ~1.5 was carried out overnight with 1 mM isopropyl-β-D-thiogalactopyranoside at 30 °C. All ArsC mutants were purified as described (27). ArsC was dialyzed against a 10 mM Tris/HCl, pH 8.0, buffer solution for the CD, ITC, and DSC experiments. To prevent the oxidation of ArsC, all solutions contained 1 mM dithiothreitol.

**Kinetics**—The kinetic parameters of ArsC were determined as described (25) except for the buffer conditions, which were, respectively, 10 mM Tris/HCl, pH 8.0, 200 mM NaCl and 10 mM Tris/HCl, pH 8.0, 200 mM KCl.

**Isothermal Titration Calorimetry**—The heat effects accompanying ion binding to the reduced form of ArsC were measured at 25 °C using an Omega isothermal titration calorimeter (MicroCal). Arsenate reduction in 10 mM Tris/HCl, pH 8.0, and 1 mM dithiothreitol was titrated with a XCI solution (X = Li, Na, K, Rb, Ca) in the same buffer using a motor-driven 250-μl syringe. The salt concentrations in the syringe were 15 mM, whereas the ArsC concentrations in the titration cell were about 0.4 mg/ml. For the different salts, the protein concentration was each time 200 mM in a 10 mM Tris/HCl, pH 8.0, buffer solution. The salt concentrations in the syringe were 15 mM, whereas the ArsC concentrations in the titration cell were about 0.4 mg/ml.

**Results**

**Structural Features of the Potassium Binding Site**—During the crystallization trials we had already observed a strict requirement of K\(^+\) ions in order to obtain good quality crystals of ArsC (27). Structural analyses clearly demonstrated the presence of a bound potassium cation in all the crystal structures of p1258 ArsC (19, 24). The environment around the potassium in all these structures was essentially identical, suggesting that K\(^+\) binding is specific. In Fig. 1 the potassium binding site is depicted as observed in the structure of reduced ArsC (1.1 Å resolution data) (19). The K\(^+\) ion is coordinated by seven oxygen atoms, belonging to six ligands: two well defined water molecules, Asn-13, Ser-36, Thr-63, and Asp-65. The carboxylate group of Asp-65 is coordinated in a monodentate fashion with a K\(^+\)-O distance of 2.57 (2) Å. The remaining carboxylate oxygen is included in hydrogen bond formation with one of the water molecules. Coordinated water oxygens are located at distances of 2.54 (2) Å and 2.92 (1) Å from the K\(^+\). Thr-63 is coordinated through a main chain carbonyl oxygen (K\(^+\)-O 2.79 (1) Å), whereas Ser-36 is bidentately bound through a main chain carbonyl oxygen (K\(^+\)-O 2.75 (1) Å) and through the hydroxyl oxygen at a longer distance (3.08 (1) Å). Asn-13 is coordinated through the oxygen of the side chain amide group at a distance of 2.86 (1) Å.

**Binding Characteristics of Monovalent Cations**—The binding of K\(^+\), Na\(^+\), Li\(^+\), Rb\(^+\), and Cs\(^+\) to the reduced form of ArsC was examined by isothermal titration calorimetry (Fig. 2, a and b). The titration curves were analyzed by a 1:1 binding model (Equation 1) to obtain the thermodynamic parameters of K\(^+\), Rb\(^+\), and Cs\(^+\) binding (Table I). Further, the influence of the presence of Na\(^+\) on the binding of K\(^+\) was examined. After finishing the titration with an excess of Na\(^+\), we continued with K\(^+\). This ITC experiment showed that K\(^+\) binds to the protein with the same binding characteristics as in the absence of Na\(^+\).

As such, Na\(^+\) does not seem to compete for the potassium site.

During the analysis of the ITC curves, different models were taken into consideration. With one cation binding site observed in the crystal structure of the ArsC, the first choice was the simplest model that describes binding as a 1:1 association. The fitting of this model function was very good agreement with the experimental data for all the studied cations. However, for Li\(^+\) and Na\(^+\) the global minimum of the χ² function with respect to the two adjustable parameters (ΔH\(^D\) and K) is shallow; consequently their exact physical meaning is lost. By simulating the ITC curves for different values of model parameters, we were able to estimate that the affinities of Li\(^+\) and Na\(^+\)
to ArsC are more than one order of magnitude lower than the corresponding affinities of K⁺, Rb⁺, and Cs⁺.

In contrast, model analysis of the K⁺, Rb⁺, and Cs⁺ curves strongly suggests that ΔH‡ and K determined for a 1:1 model are reliable within the errors (squared variances) presented in Table I. Nevertheless, the physical meaning of the parameters obtained with a 1:1 model was checked by fitting the models with two or more equivalent independent binding sites, but the agreement was much better for a 1:1 model. Even for Cs⁺ with the most gradual slope of the ITC curve (compared with K⁺ and Rb⁺), the fitting of the second best model (2:1) resulted in about five times higher value of the χ² function in its minimum than in the case of a 1:1 model. For K⁺ and Rb⁺ the differences between the χ² functions for 1:1 and other models were even more pronounced.

By supposing a model with two different independent binding sites, the best fitted K for binding to the second binding site was in the case of K⁺, Rb⁺, and Cs⁺ more than one order of magnitude lower than K for the first binding site. This was tested and proved by fitting experiments with different initial guesses of ΔH and K. So, if some other binding site with K within the same order of magnitude and comparable ΔH as for the first binding site would exist, significantly different shapes of the ITC curves were expected.

The possible dependence of ΔH and K on concentration has been ruled out by performing ITC experiments with about two times lower and two times higher starting concentrations of ArsC in the cell. The possibility of coupling of the protonation or the deprotonation to the binding of cations to ArsC was
Potassium-induced Stability of ArsC

The Effect of Monovalent Cations on Thermal Stability and Activity—To study and quantify the stabilization by K⁺, we performed both CD melting experiments and DSC studies on oxidized and reduced wild type ArsC in buffer solutions containing either KCl or NaCl (Fig. 3). Transition temperatures and values of van’t Hoff denaturation enthalpies determined from CD data (Fig. 2) correlate well with those obtained by DSC experiments (Fig. 4, Table II). Moreover, van’t Hoff enthalpies calculated from the CD melting curves and DSC thermograms (Equations 2–4) are in good agreement with the corresponding ΔH_DSC values obtained directly from the areas under the measured DSC thermograms. This confirms that the two-state model is applicable for the description of the denaturation process of ArsC.

It should be noted that the best buffer for the purification of ArsC (Tris buffer at pH 8.0) is not ideal for this kind of experiment because it has a rather high ionization enthalpy (34). On the other hand, buffer solutions like cacodylate and phosphate characterized by low ionization enthalpies (34) cannot be used because arsenate and phosphate are stabilizing ArsC by binding in its P-loop (25), making the experiments tetrahedral oxyanion-dependent. The possible effect of the temperature dependence of the pH on the thermodynamic parameters was checked by performing DSC measurement on the wild type ArsC in the presence of potassium in the Mops buffer solution at pH 7.2. The obtained thermodynamic parameters are within the experimental errors presented in Table II.

ArsC is thermally stabilized by potassium ions (Fig. 4, Table II). The shift of the melting temperature to higher values is accompanied by an increased value of denaturation enthalpy for both the oxidized and reduced forms of the protein. The

| K⁺ | 3.8 × 10⁻³ | -11.7 | -6.8 | -4.9 |
| Rb⁺ | 3.2 × 10⁻³ | -10.8 | -6.0 | -4.8 |
| Cs⁺ | 1.0 × 10⁻³ | -7.9  | -3.8 | -4.1 |

TABLE I

Summary of the thermodynamic parameters of monovalent cation binding to ArsC at 25 °C

The relative parameter errors are: 5% for K, 4% for ΔH°, 3% for ΔG°, and 7% for ΔTΔS° values.

![CD spectra of ArsC (reduced form) in 200 mM KCl solution scanned at different temperatures. Inset shows the corresponding melting curve (θ versus T) at 220 nm.](image)

![TheEffect of Monovalent Cations on Thermal Stability and Activity — To study and quantify the stabilization by K⁺, we performed both CD melting experiments and DSC studies on oxidized and reduced wild type ArsC in buffer solutions containing either KCl or NaCl (Fig. 3). Transition temperatures and values of van’t Hoff denaturation enthalpies determined from CD data (Fig. 2) correlate well with those obtained by DSC experiments (Fig. 4, Table II). Moreover, van’t Hoff enthalpies calculated from the CD melting curves and DSC thermograms (Equations 2–4) are in good agreement with the corresponding ΔH_DSC values obtained directly from the areas under the measured DSC thermograms. This confirms that the two-state model is applicable for the description of the denaturation process of ArsC.](image)

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addition, we mutated Glu-21, which is not directly bound to K
are (together with Thr-63) in direct contact with potassium. In
ing site, single mutants were constructed in which Asn-13,
form of ArsC for the solutions containing Li
, it can be concluded that the binding of K
entalpy-driven process accompanied by negative entropy con-
values of the heat capacity change,
naK
denaturation (Fig. 4
. The complete quantitative data are listed in Table II, and the
were performed in the solutions containing NaCl w_red 47.1 46.0 73 74 72

KCl w_red 51.2 51.6 85 87 85
w_ox 51.4 50.6 75 72 73
N13A 41.5 40.9 61 62 59
S36A 41.0 39.6 63 59 60
D65A 46.0 45.3 66 61 67
E21A 46.6 45.1 74 72 73

NaCl 47.1 46.0 73 74 72
w_ox 46.1 44.8 62 63 64
N13A 41.3 40.7 60 60 56
S36A 37.9 37.5 54 55 52
D65A 46.4 45.2 65 60 66
E21A 46.1 44.2 71 67 71

a \( \Delta H_{\text{DSC}} \) is the model independent heat of denaturation obtained by integration of the corresponding DSC thermograms.

| \( T_{1/2} \) | \( T_{1/4} \) | \( \Delta H_{\text{DSC}} \) | \( \Delta H_{\text{CD}} \) | \( \Delta H_{\text{DSC}} \) |
|--------|--------|--------|--------|--------|
| KCl    | w_red  | 51.2   | 51.6   | 85     | 87     | 85     |
|        | w_ox   | 51.4   | 50.6   | 75     | 72     | 73     |
|        | N13A   | 41.5   | 40.9   | 61     | 62     | 59     |
|        | S36A   | 41.0   | 39.6   | 63     | 59     | 60     |
|        | D65A   | 46.0   | 45.3   | 66     | 61     | 67     |
|        | E21A   | 46.6   | 45.1   | 74     | 72     | 73     |

NaCl 47.1 46.0 73 74 72
w_ox 46.1 44.8 62 63 64
N13A 41.3 40.7 60 60 56
S36A 37.9 37.5 54 55 52
D65A 46.4 45.2 65 60 66
E21A 46.1 44.2 71 67 71

KCl 54 9 1.10^5
NaCl 35 22 2.67 10^4

Relative parameter errors are estimated to be <10%.

 overall stabilization effect is described in terms of the standard
Gibbs free energy change, \( G^0 \), corresponding to the reversible denaturation (Fig. 4c). Most stable is the reduced form of ArsC in
a K
containing solution, followed by the oxidized form in the same solution. Switching from K
to Na
ions makes both forms of ArsC substantially less stable with maintenance of the order of stability. In the oxidized ArsC form, heating of the protein up to 60 °C cannot break the disulfide bridge. This makes the probability of forming some energetically favorable contacts in its denatured state higher than in the denatured state of the reduced form. Moreover, the enthalpy-entropy compensation diagram (Fig. 4d) shows that the \( H^0 \) in the presence of K
is higher than in the presence of Na
. Together with the observation shown in Fig. 4c that \( G^0(K^+) - G^0(Na^+) \) is 1.1 kcal/mol, it can be concluded that the binding of K
is an enthalpy-driven process accompanied by negative entropy contributions. This is in full agreement with our binding studies.

We also investigated the thermal stability of the reduced form of ArsC for the solutions containing Li
, Rb
, and Cs
. From CD experiments it was not possible to obtain reliable values of the heat capacity change, \( \Delta C_p^0 \) (35). Thus, for the extrapolation of stabilities (\( \Delta G^0 \), Equation 3) to the temperatures different from \( T_{1/2} \), the average \( \Delta C_p^0 \) value obtained by DSC for the reduced form in NaCl and KCl solutions was used (1.8 kcal mol
1/(K
°C)
). The effectiveness of selected monovalent cations in stabilizing ArsC against thermal denaturation (\( \Delta G^0 \) at 25 °C) was found to follow the order: K
> (5.1 kcal/mol) > Rb
> (4.4 kcal/mol) > Cs
> (4.2 kcal/mol) > Na
> (3.6 kcal/mol) > Li
> (3.0 kcal/mol). The differences between the \( \Delta G^0 \) values are in accordance with our ITC binding experiments.

To analyze the effect of K
on the arsenate reductase activity of ArsC wild type, the \( k_{cat} \) and \( K_M \) values were determined in the presence of KCl and NaCl (Table III). A K
-stabilized enzyme resulted in a higher specificity constant as compared
as well as the highest value of \( H^0 \). However, each mutation itself introduces a perturbation to the system, and the relevant conclusions can be drawn only by comparison of the stability for the same mutant in solutions with K
and Na
. A complete loss of K
stabilization over Na
was observed for D65A and N13A. For these two mutants the values of \( T_{1/2} \) as well as the denaturation enthalpies are practically the same, regardless of whether the solution contains K
or Na
. In all cases where additional stabilization of K
with respect to Na
is observed, a positive \( \Delta H^0(K^+) - \Delta H^0(Na^+) \) difference (at given temperature) confirms that the K
binding process is enthalpically favorable.

**DISCUSSION**

**Specific Monovalent Cation Binding Stabilizes ArsC**—Potassium binds to the protein with a binding constant of 3.8×10^3 M
. The binding is a highly enthalpy-driven process accompanied by an unfavorable entropic contribution (Table I). Upon binding, no significant changes in secondary and tertiary structure were detected by CD. The negative entropy contribution may therefore be ascribed to lowering of the degrees of freedom because of the association reaction. An additional source of unfavorable entropy arises from the loss of conformational degrees of freedom in amino acid side chains (Asn-13 and Asp-65) during binding. The shapes of the Li
and Na
titration curves indicate that their binding affinities are much lower as compared with K
, suggesting their nonspecific binding. The ionic radii of Li
and Na
(depending on the coordination varying from 0.7 to 1.1 Å for Li
and from 1.1 to 1.3 Å for Na
) are much smaller than the K
radius (varying from 1.5 to 1.65 Å) and as such do not fit well to the geometry of the binding site. However, a significant affinity of the protein for Rb
and Cs
ions was observed (Fig. 2b). The binding characteristics of Rb
are comparable with those of K
, whereas Cs
binds to ArsC with a lower affinity and enthalpy of binding (Table I). Because the ionic radius of Rb
(1.6–1.7 Å) is close to that of potassium and the binding shows similar 1:1 characteristics, it is likely that Rb
occupies the potassium binding site. Cs
is
larger (ionic radius of 1.8–1.9 Å) and as such does not fit as well to the geometry of the binding site as K⁺ and Rb⁺ do. The stabilizing effect of the studied ions on ArsC does not follow the lyotropic series of Hofmeister (36). According to this series, Cs⁺ has greater ability to organize water molecules in a protein favorable manner. The stabilization of a protein by ions arises either by the structuring of water or by interactions with charged groups. If the degree of organization of water molecules is crucial for the cation-induced stability of ArsC, one would expect the same order as it is in the lyotropic series. This is not the case. The stabilization order follows the order of binding affinities of the studied monovalent cations (Fig. 2c), confirming that the stabilizing effect arises primarily from specific ion-protein interactions.

Enhanced Catalytic Activity of ArsC in the Presence of K⁺—A K⁺-stabilized enzyme shows a higher specificity constant ($k_{cat}/K_M$) as compared with the non-stabilized ArsC (in the presence of NaCl) (Table III). The $K_M$ is about 5 times lower as compared with the values obtained in the presence of both potassium and the stabilizing tetrahedral oxyanion sulfate (25). A low $K_M$ in the presence of potassium can only be explained because potassium binds near the catalytic P-loop, whereas sulfate has a structuring effect by binding in the P-loop. As such, the sulfate competes with the substrate arsenate. In enzymes where a tetrahedral oxyanion phosphate is binding in the active site, like fructose-1,6-biphosphatase (37) and in ribokinase (38), activation by potassium is more extreme. In these cases, potassium is directly binding in the active site to assist the reaction by facilitating nucleophilic attack or neutralizing negative charge. In p258 ArsC, the potassium binding site next to the active site P-loop has most probably a protein scaffold structuring role.

Residues for Binding Potassium—The CD and DSC melting experiments reveal that Asp-65 and Asn-13 are essential for potassium binding. By mutating those residues to Ala, we are unable to discriminate between the stability curves in the presence of NaCl or KCl (Fig. 6). Surprisingly, there is still substantial stability enhancement by K⁺ in the case of ArsC S36A mutant. Ser-36 is bidentately bound through its main chain carbonyl oxygen and side chain hydroxyl oxygen. It seems that the interaction between Ala-36 and the potassium is dominated by the main chain O-K⁺ interaction and removing the side chain oxygen has little effect on K⁺ binding. Further, from all tested mutations of residues from the potassium binding site, the S36A mutation has the biggest impact on the stability of ArsC (Fig. 6). Replacement of Glu-21 by alanine also diminished the thermal stabilization effect of K⁺. Here, the $T_{1/2}$ difference and $\Delta H^0$ difference are significantly smaller.

Potassium Binding Sites in Proteins—Recently, the metal-ligand geometry in proteins was examined for Na⁺ and K⁺ ions and compared with observations made in the crystal structures of small molecules (39). For K⁺ in small molecules, eight was observed as the most common coordination number with seven nearly as favorable, whereas in proteins the coordination numbers tend to be somewhat lower. X-ray structures of enzymes in the Protein Data Bank (40) with a potassium ion binding site include: cytochrome c peroxidase (41), pyruvate kinase (42), ribokinase (38), fructose-1,6-biphosphatase (37). The dissociation constant of potassium in those enzymes is in the mM range. p258 ArsC has a somewhat higher affinity for potassium, giving a $K_p$ of 0.26 mM. Although in most enzymes, backbone carbonyl oxygen atoms and one or more aspartic acid residues are involved in potassium binding, no general structural rules for potassium binding sites in proteins can be deduced from this limited number of structures.

The monovalent cation potassium stabilizes arsenate reductase by binding near, but not in, the active site (P-loop). Comparison of all solved ArsC structures (19, 24) provides strong evidence that the potassium site is a specific feature of arsenate reductase from S. aureus. The most likely occupant of this site in vivo is potassium, because cells maintain an intracellular potassium concentration greater than 100 mM, a concentration range more than sufficient to saturate a site with a dissociation constant of 0.26 mM. Both the lower sodium concentration inside the cell (5–15 mM) and the fact that Na⁺ does not occupy this particular binding site show that this cation is of minor biological importance in the case of p258 arsenate reductase.

ArsC has a PTPase I fold. Although low molecular weight tyrosine phosphatase (1PNT) superimposes with a root square mean deviation of 1.4 Å for 129 pairs of Ca atoms (19), they do not possess a specific K⁺ binding site. In bovine and human LMW PTPases (43, 44), the imidazole side chain of His-72 occupies the space of the potassium binding site (Fig. 1c). More
specifically, the potassium coincides precisely with the (protonated) Ne2 of His-72, making contacts within hydrogen bonding distance with the structurally and sequentially conserved (19) Asn and Ser (Fig. 1c) and with the carbonyl oxygen of Lys-73 or Val-73 (Thr-63 in ArsC) in bovine and human LMW PTPases, respectively. The important potassium coordinating side chains of residues Thr-63 and Asp-65 are not present in LMW PTPases (Fig. 1b). In LMW PTPases, an Arg is found at the position of Asp-65, which is flipped away from this site as a had hydrogen bond acceptor for the protonated Ne2 of His-72. Potassium ions are particularly suited for replacing protonated histidine residues while maintaining similar interactions with the environment; both have a single positive charge, and the ionic radius of potassium allows it to coordinate to oxygens with bond distances similar to those of hydrogen bonds. Thus the His-K+ substitution can be very conservative structurally. In sequence alignments the His-72 of LMW PTPases is conserved and can be aligned with His-62 from ArsC. However, the different local structure (Fig. 1c) makes it possible for potassium to occupy this site and to stabilize ArsC. Thus it seems that ArsC is particularly well adapted to the cytoplasmic conditions and evolved to be most stable in the presence of relatively high potassium concentrations. This also explains the need for potassium in protocols for the purification and crystallization of ArsC (27). It might be a more general feature of cytoplasmic proteins to be more stable in potassium than in sodium salts, but this should be verified with extensive experiments with a large number of proteins.

To the best of our knowledge, this report describes for the first time a complete thermodynamic study of the potassium binding site in an enzyme in combination with structural analysis, steady-state kinetic data, and site-directed mutagenesis. We demonstrated that the stabilization of ArsC due to potassium binding is a highly enthalpy-driven process that significantly affects the stability and catalytic activity of this redox enzyme.

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