

Ion specific effects of sodium and potassium on the catalytic activity of HIV-1 protease

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Abstract

The behavior of HIV-1 protease in aqueous NaCl and KCl solutions is investigated by kinetic measurements and molecular dynamics simulations. Experiments show cation-specific effects on enzymatic activity. The initial velocity of peptide substrate hydrolysis increases with salt concentration more dramatically in potassium than in sodium chloride solutions. Furthermore, significantly higher catalytic efficiencies ($k_{\text{cat}}/K_{\text{M}}$) are observed in the presence of K^+ compared to Na^+ at comparable salt concentrations. Molecular dynamics simulations provide insight into this ion-specific behavior. Sodium is attracted more strongly than potassium to the protein surface primarily due to stronger interactions with carboxylate side chain groups of aspartates and glutamates. These effects are of particular importance for acidic amino acid residues at or near the active site of the enzyme, including a pair of aspartates at the entrance to the reaction cavity. We infer that the presence of more Na^+ than K^+ at the active site leads to a lower increase in enzymatic activity with increasing salt concentration in the presence of Na^+ , likely due to the ability of the alkali cations at the active site to lower the efficiency of substrate binding.

Introduction

Human Immunodeficiency Virus 1 Protease (HIV-1 PR) has been the focus of intense research interest during the past two decades. Due to its indispensable role in maturation of HIV particles, it is an important target for design of anti-HIV drugs. Nine HIV-1 protease inhibitors have been approved by the FDA to date^{1,2}. However, the successful application of HIV protease inhibitors in antiviral therapy is compromised by the occurrence of resistant viral species. Therefore, continuing research of the regulation of activity and inhibition of the enzyme is still needed.

From a structural point of view, HIV-1 PR is a protein of comparatively small size and regular structure. It is an alpha/beta protein based on CATH classification³, and it is enzymatically active as a homodimer. HIV-1 PR contains all 20 naturally occurring amino acids, and the amino acid distribution is similar to that of proteins in general. There are 18 charged residues per HIV-1 PR monomer, with 10 positive (Lys and Arg) and 8 negative (Asp and Glu) amino acids. Rather unexpectedly, many of these residues are involved in salt bridges, forming a network of structurally closed contacts that are likely important for stabilization of the native structure of the protease.

Contacts at the HIV-1 PR dimer interface are maintained by non-polar as well as polar residues. The majority of proteins are usually most stable (but least soluble) near their isoelectric point(pI), at which the net charge of the protein is low.⁴⁻⁶ In contrast, HIV-1 PR is most stable (and most active) around pH 5-6, which is rather far from its pI of 8.66⁷.

The catalytic function of the protease is to hydrolyze peptide bonds in the native viral polyprotein transcripts, yielding mature viral proteins and enzymes⁸⁻¹⁰, including the protease itself. Two aspartates in the active site of the protease dimer play a central role in the reaction mechanism, which could be described as a nucleophilic attack of the water oxygen on the

amide of the peptide bond¹¹. The two aspartates are assumed to share a single proton at the pH optimal for the proteolytic activity of the enzyme.

Several groups have analyzed the dependence of the kinetics of peptide substrate hydrolysis on ionic strength/salt concentration. It has been shown that the presence of NaCl primarily affects the substrate binding (K_M value), although there is also an effect on the catalytic rate k_{cat} ¹². The effect of NaCl on K_M is attributed to “salting out” of the substrate onto the enzyme⁴. Wondrak *et al.* showed that the specific salt effects follow the Hofmeister series.¹³ In contrast to the “salting out” hypothesis, Szeltner and Polgar suggested another mechanism inspired by the observation that an increasing salt concentration indirectly increases the conformational stability of the enzyme. The higher affinity of HIV-1 PR for substrate is then the result of an entropic effect. This mechanism can be attributed to preferential hydration of the HIV-1 PR surface. Szeltner and Polgar state that “a substantial portion of the protective water shell is apparently released at high temperature”, which may also partially explain why the salt effects are less important for HIV-1 PR at an elevated temperature of 53 °C.⁷

In general, the effect of salt on enzyme kinetics can vary. On one hand, the activity of Human T-cell Leukemia Virus 1 Protease (HTLV-1 PR), an enzyme homologous to HIV-1 PR, was not affected by NaCl concentration in a range from 0 to 1.5 M.¹⁴ On the other hand, thermolysin represents the opposite extreme. This enzyme is remarkably activated in the presence of 1- 5 M of salt,¹⁵ its activity being enhanced 13-15 times in 4 M NaCl at pH 7.0 and 25 °C. Part of the salt effect can be linked to greater solubility;¹⁶ however, this cannot fully explain the dramatic increase in activity.

Specific effects of salt ions are connected with the lyotropic (Hofmeister) series, a classification of cations and anions according to their ability to precipitate proteins¹⁷. The

salting out effect is traditionally attributed to changes in the bulk water structure¹⁸. However, recent experimental and theoretical studies suggest that monovalent ions do not affect the bulk water structure beyond the first solvent shell and that the Hofmeister series should rather be rationalized in terms of ion–protein or ion-water interactions in the first hydration shell of a protein¹⁹. In this context, we have recently quantified the higher affinity of Na⁺ over K⁺ to protein surfaces by molecular dynamics (MD) simulations and conductivity measurements²⁰. We have shown that sodium binds at least twice as strongly to the protein surface than does potassium. This is primarily due to an enhanced affinity to the carboxylic groups of aspartate and glutamate with interactions with backbone amide oxygens also playing a role.

In this study we investigate the specific salt effects on HIV-1 PR by a combination of experimental and computational techniques. On the computational side, we performed MD simulations of HIV-1 PR in aqueous solutions of NaCl or KCl. The aim was to unravel specific ion affinities to individual regions of the surface of the protease and to relate them to the different influence of sodium *vs.* potassium on the PR activity. The observed specific ion affinities to different parts of the surface of the protease provide clues as to the molecular mechanism of the influence of individual salt ions on the protein. On the experimental side, we analyzed the influence of sodium and potassium ions on the activity of HIV-1 PR. We determined the initial velocities of the hydrolysis of chromogenic peptide substrates and the Michaelis-Menten constants for the enzymatic reaction at varying salt concentrations.

Materials and Methods

Molecular dynamics simulations

The database structure of HIV-1 protease (code 3HVP) was used for modeling²¹. Protonation states of individual amino acids were assigned according to pH = 6. For the pair of catalytic aspartates in the active site we considered both the deprotonated and singly protonated model according to recently published studies^{22, 23}. The resulting structure was solvated in a periodic cubic water box, employing the SPC/E water model. The system with dimensions of approximately 79 x 79 x 79 Å³ contained 12335 water molecules. The total charge of the protein (+4e) was compensated by four chloride anions.

Consequently, NaCl or KCl ions were introduced into the simulation cell to form NaCl or KCl solutions (55 ion pairs in each case, corresponding to 0.25 M solutions). Furthermore, mixed NaCl/KCl solutions were created with the concentration of each cation being 0.25 M. Two different initial ionic distributions were employed and two additional simulation cells were obtained by exchange of positions of Na⁺ and K⁺ cations in solutions. This procedure was employed to exclude potential bias introduced by the initial positions of the cations.

MD simulations consisted of minimization, heating to 300 K, 0.5 ns of equilibration, and 10 ns production runs at constant temperature and pressure for simulations of HIV-1 PR. All calculations were carried out using the AMBER8 simulation package employing the parm99 forcefield²⁴. Nonbonded interactions were cut off at 12 Å. For the substrate we also employed a shorter cut off of 7.5 Å, which yielded practically the same results. Long range electrostatic interactions were accounted for using the smooth particle mesh Ewald (PME) method²⁵. Bonds involving hydrogen atoms were constrained using the SHAKE algorithm. We used a similar simulation protocol in our previous protein studies, in which we show that it provides converged results^{20, 26}.

The resulting trajectories were analyzed by means of distribution of ions in the vicinity

of the protein or substrate surface. The fraction of the simulation when a particular cation is in contact with an amino acid residue was also monitored. A contact is defined as a situation when a cation is found within 3.5 Å of the respective residue. Frequent exchanges between bulk and residue-bound ion positions were observed, which ensures good statistics.

Enzyme activity determination

Buffers

Buffer solutions were prepared by dissolving the corresponding buffer powder in HPLC-quality water and adding different amounts of NaCl or KCl (Lachema, Brno, Czech Republic p.a. grade in the case of the acetate buffer, Sigma-Aldrich, super pure grade, for all other buffers). The appropriate hydroxide or acid was used to adjust pH.

Enzymes

The expression, refolding, and purification of HIV-1 PR were performed as described²⁷. Briefly, the pET24a plasmid vector and host strain *E. coli* BL21 (DE3) (Novagen) were used for heterologous bacterial expression. HIV-1 PR was purified from inclusion bodies by solubilization in acetic acid, followed by chromatography on Mono S-Sepharose (Amersham Pharmacia Biotech). The active-site concentration of HIV-1 PR in the final preparation was determined by titration with saquinavir,²⁸ and frozen aliquots of the enzyme were stored at -20 °C.

Kinetic measurements

All kinetic assays were performed with chromogenic peptide substrates.

KARVNle*NphEANle-NH₂ (peptide 1) was assayed in 50 mM HEPES or phosphate buffer,

pH 6.0, and 2-aminobenzoyl-TINleNphQR-NH₂ (peptide 2) was assayed in 50 mM MES, pH 6.0. Kinetic assays were conducted as previously described^{12, 29} with a few modifications. Typically, 16 nmol of substrate was added to 1 ml of the corresponding buffer containing 6-7 nmol of PR and various concentrations of salt. The substrate hydrolysis was followed by the decrease in absorbance at 305 nm using a Unicam UV 500 spectrophotometer. All PR activity points were determined in duplicate. The K_M and k_{cat} values for Peptide 1 and 2 were calculated using the Michaelis-Menten equation (SigmaPlot).

Results

Computational

The ion-specific behavior of sodium and potassium was investigated by means of extensive MD simulations of HIV-1 protease in aqueous salt solutions. Both alkali cations were found to interact primarily with negatively charged side chains of Asp and Glu and, to a lesser extent, also with the backbone amide oxygens. This result, as well as the preference of these sites for sodium over potassium by about a factor of two, is consistent with our previous study of other proteins^{20, 26}. The effect is quantified in **Figure 1**, which displays the corresponding distribution functions and cumulative sums of Na⁺ and K⁺ in the vicinity of HIV-1 PR.

The results for interactions of cations with the anionic groups of Asp and Glu are further summarized in detail in **Table 1**. This table presents durations of direct contacts of Na⁺ with Asp or Glu (in percents of the total trajectory) and the ratio between these contacts for sodium *vs.* potassium. Results are presented for a mixed solution containing 0.25 M NaCl and 0.25 KCl, as well as for separate simulations of pure NaCl or KCl solutions, both at 0.25 M. We see that sodium spends about twice as much time in the vicinity of negatively charged residues than potassium. This difference is somewhat larger in the mixed solution, which indicates a certain degree of non-linear effects (competition) between the two cations. It is also worth noting that the absolute length of the contact between the carboxylate and either of the cations was found to be larger in the case of Asp compared to Glu, indicating somewhat stronger interactions with the former residue. This is partially due to the larger surface abundance of Asp *vs.* Glu pairs which can be efficiently bridged by an alkali cation.

Of particular interest in terms of specific cationic binding is the pair of Asp residues at the active site of the protease. Due to the presence of two close anionic groups the interaction with cations is particularly strong, and indeed, during most of the simulation time a cation can

be found in their vicinity. Almost exclusively, it is sodium (and not potassium) which strongly binds in the active site. This is demonstrated in Figure 2, which shows density maps of sodium and potassium at the active site averaged over the MD simulation. Both cations are present at the pair of Asp residues; however, their distributions are very different. While Na^+ is strongly localized at this site, effectively bridging the two carboxylate groups, the distribution of K^+ is much more diffuse. The localization of sodium is stronger for the deprotonated vs. singly protonated Asp pair. Nevertheless, the qualitative difference between the behavior of the two alkali cations pertains in both cases. Finally, it is worth mentioning that the preference for sodium over potassium is also revealed from test *ab initio* calculations of interaction of an alkali cation with an acetate-acetate or acetate-acetic acid pair in geometry similar to that of the active site (with water represented by a polarizable continuum model), similarly as in our previous study²⁰.

Experimental

In order to analyze the dependence of HIV-1 PR activity on individual ion concentrations, we determined the initial velocities and kinetic constants (*i.e.* K_M and k_{cat}) using a prototypic chromogenic peptide substrate derived from the processing site spanning the CA-NC cleavage site with a *para*-nitrophenyl residue introduced into the P1' position of the substrate (Peptide 1). In order to dissect the possible influence of the reaction buffer, we measured the initial velocities in phosphate and HEPES buffer, pH 6.0. The results of these experiments are depicted in Figure 3. The plots show a monotonous increase in the initial reaction velocities with increasing NaCl or KCl concentration. In both cases, the presence of KCl leads to a higher initial velocity than that observed in the presence of NaCl over the whole concentration range. Similar results were obtained in acetate buffer, pH 4.7 (data not shown).

Since peptide substrate 1 contains a Glu residue which might itself be responsible for the differences in the affinity of positively charged ions, we measured the kinetics with a second peptide substrate. Peptide 2, 2-aminobenzoyl-TINleNphQR-NH₂, is based on the p2-NC cleavage site of the HIV Gag polyprotein³⁰. The peptide lacks negatively charged residues with potential high affinity to alkaline ions and might therefore serve as an appropriate control for the possible substrate effect. The initial velocities of Peptide 2 cleavage (Figure 3C) follow a similar pattern to those calculated for Peptide 1 (Figure 3A and B); initial velocities observed in potassium-containing buffers are higher than those observed in the corresponding sodium-containing buffers.

For quantification of the dependence of Peptide 1 and 2 hydrolysis on salt concentration, we determined the kinetic constants K_M and k_{cat} at various salt concentrations at pH 6.0 (Tables 3 and 4) and pH 4.7 (data not shown). The higher activity of HIV-1 PR in the presence of K⁺ ions is documented by higher catalytic efficiencies (k_{cat}/K_M) for both salt concentrations and both peptide substrates measured at pH 6.0. Quantitatively this is more pronounced for peptide 1 (Table 3 and 4) which points to some degree of a substrate effect. Increasing salt concentration leads to a drop in the K_M value for both ions. Interestingly, the higher specific activity of HIV-1 PR in the presence of potassium and Peptide 1 at pH 6.0 could be attributed to higher turnover number (k_{cat} value). The kinetic constants describing Peptide 1 hydrolysis at pH 4.7 show a similar drop in K_M values with increasing ionic strength of the buffer (data not shown). However, the difference between sodium and potassium at this pH value is less pronounced. Kinetic analysis of Peptide 2, which does not contain any charged side chains, shows a similar pattern (Table 4); the K_M values decrease with the ionic strength and the overall catalytic efficiencies (k_{cat}/K_M) are higher in the presence of potassium [see above]. However, in this case the preference for potassium could be attributed to the

improved substrate binding, as evidenced by the lower K_M values observed in potassium-containing buffer.

Discussion

The observation that high salt concentration increases the activity of HIV-1 protease was reported as early as 1990^{12,13}. The addition of salt primarily affects the K_M value, and the increase in proteolytic activity is usually attributed to the “salting-out” of the hydrophobic substrate into the enzyme binding cleft^{4,13,31}. Furthermore, the higher ionic strength increases the strength of interaction of HIV-1 PR inhibitors with the enzyme, probably by reduction of the PR-inhibitor dissociation rate constant³¹. This is consistent with the present experimental observations (Figure 3), with the relative ordering of the cations following the Hofmeister series. Interestingly, however, the ion-dependence of HIV-1 PR activity is substrate specific - the opposite trend (*i.e.*, decreasing activity with an increase in ionic strength) was observed when a specific polyprotein substrate (immature Gag-particle) was used in the activity assay³². Simulations of various substrates aimed at rationalizing this behavior are currently being performed.

The principle aim of this study was to demonstrate and rationalize the ion-specific effect of Na^+ vs. K^+ on the enzymatic activity of HIV-1 PR. Note that the majority of previous analyses have studied the effect of different anions; in fact, most studies seem to indicate that cations have less influence on the enzyme activity than anions³³. Nevertheless, we observe a significant cation-specific influence on HIV-1 PR activity (Figure 3).

When discussing the effect of ions on enzyme activity, protein flexibility and stability are usually among factors to consider. Active site flexibility is indeed important for the

function of an enzyme. It has been shown that anions influence both the activity and flexibility of NADH oxidase (NOX), leading to a bell-shaped dependence of NOX activity on Hofmeister series anions³⁴. A similar but less pronounced effect was observed for cations³⁵. NOX (in some analogy to HIV-1 PR) can adopt both a closed and an open conformation which exist in a dynamic equilibrium. Closing of the active site plays a crucial role in the mechanism of action of the enzyme³⁵. The authors argue that both too flexible and too rigid active sites lead to NOX inactivation and that strongly hydrated cations (Li^+) stabilize the open conformation, while weakly hydrated cations (NH_4^+) favor the closed conformation. However, the present simulations do not show any sizable specific effect of salt (NaCl or KCl) on the flexibility of HIV-1 PR; similar values of root-mean-square-deviations from the initial structure were observed for both salts.

Another possible cause of ion specificity for enzymatic activity could be the difference in activity coefficients of both ions in aqueous solutions at comparable concentrations. However, in the investigated concentration range the two solutions differ in activity by <5 %, while the effect on enzymatic activity is significantly larger.

Finally, the two alkali cations differ by more than a factor of two in their affinity to the surface of HIV-1 PR, with the carboxylic side chain groups of Glu and Asp and the backbone carbonyl oxygens being responsible for this cation-specific attraction. From this point of view, HIV-1 PR behaves similarly to other proteins²⁰. The Asp pair at the active site of HIV-1 PR seems to be of particular importance in this respect. The active site Asp pair exhibits a significantly larger affinity for Na^+ over K^+ both in the deprotonated and singly protonated state. We identify this ion specificity at the active site as the most likely explanation for the lower enzymatic activity of HIV-1 PR in NaCl vs. KCl solutions of comparable concentration. This effect can be even more pronounced when we take into account the fact that the entrance of the binding site is occupied by negatively charged residues. Interactions of these negatively

charged residues with Na/K cations can modulate the electrostatic potential of the protease surface at the active site entrance and therefore influence substrate/inhibitor recognition.

Conclusions

We have investigated the cation-dependence of the enzymatic activity of HIV-1 protease in aqueous solutions containing NaCl and KCl. We present kinetic measurements and a molecular dynamics analysis of the affinity of alkali cations to the protein surface. The following conclusions can be drawn from this combined experimental and computational study:

- i) The enzymatic activity of the protease increases with increasing salt concentration, and this effect is stronger in KCl-containing buffers than in buffers containing NaCl. The experimentally determined catalytic efficiency (k_{cat}/K_M) of HIV-1 PR is significantly larger in potassium vs. sodium chloride solutions at comparable salt concentrations
- ii) Molecular dynamics simulations show that Na^+ is more strongly attracted to the protein surface than K^+ . This is primarily due to interactions with COO^- side chain groups of Asp and Glu. Among those, the interaction with the Asp pair at the active site could be of particular relevance for ion-specific effects on enzymatic activity. Namely, the increased presence of sodium over potassium at the active site can lead to a decreased efficiency of substrate binding in aqueous NaCl compared to KCl. Interaction of the experimentally investigated substrates with the active site in the presence of different salt will be subject of future studies.

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Supporting Information Available: Complete Ref. 27. This material is available free of charge via the Internet at <http://pubs.acs.org/>.

References

1. Wlodawer, A.; Erickson, J. W., *Annual Review of Biochemistry* **1993**, *62*, 543.
2. Wlodawer, A.; Vondrasek, J., *Annual Review of Biophysics and Biomolecular Structure* **1998**, *27*, 249.
3. Orengo, C. A.; Flores, T. P.; Taylor, W. R.; Thornton, J. M., *Protein Engineering* **1993**, *6*, (5), 485.
4. Hyland, L. J.; Tomaszek, T. A.; Meek, T. D., *Biochemistry* **1991**, *30*, (34), 8454.
5. Hyland, L. J.; Meek, T. D., *Analytical Biochemistry* **1991**, *197*, (1), 225.
6. Miller, M.; Schneider, J.; Sathyanarayana, B. K.; Toth, M. V.; Marshall, G. R.; Clawson, L.; Selk, L.; Kent, S. B. H.; Wlodawer, A., *Science* **1989**, *246*, (4934), 1149.
7. Szeltner, Z.; Polgar, L., *Journal of Biological Chemistry* **1996**, *271*, (10), 5458.
8. Gustchina, A.; Weber, I. T., *Proteins-Structure Function and Genetics* **1991**, *10*, (4), 325.
9. Pechik, I. V.; Gustchina, A. E.; Andreeva, N. S.; Fedorov, A. A., *Febs Letters* **1989**, *247*, (1), 118.
10. Gulnik, S.; Erickson, J. W.; Xie, D., *Vitamins and Hormones - Advances in Research and Applications, Vol 58* **2000**, *58*, 213.
11. Venturini, A.; Lopez-Ortiz, F.; Alvarez, J. M.; Gonzalez, J., *Journal of the American Chemical Society* **1998**, *120*, (5), 1110.
12. Richards, A. D.; Phylip, L. H.; Farmerie, W. G.; Scarborough, P. E.; Alvarez, A.; Dunn, B. M.; Hirel, P. H.; Konvalinka, J.; Strop, P.; Pavlickova, L.; Kostka, V.; Kay, J., *Journal of Biological Chemistry* **1990**, *265*, (14), 7733.
13. Wondrak, E. M.; Louis, J. M.; Oroszlan, S., *Febs Letters* **1991**, *280*, (2), 344.
14. Ha, J. J.; Gaul, D. A.; Mariani, V. L.; Ding, Y. S.; Ikeda, R. A.; Shuker, S. B., *Bioorganic Chemistry* **2002**, *30*, (2), 138.
15. Inouye, K., *Journal of Biochemistry* **1992**, *112*, (3), 335.
16. Inouye, K.; Kuzuya, K.; Tonomura, B., *Journal of Biochemistry* **1998**, *123*, (5), 847.
17. Kunz, W.; Lo Nostro, P.; Ninham, B. W., *Current Opinion in Colloid & Interface Science* **2004**, *9*, (1-2), 1.
18. Cacace, M. G.; Landau, E. M.; Ramsden, J. J., *Quarterly Reviews of Biophysics* **1997**, *30*, (3), 241.
19. Zhang, Y. J.; Cremer, P. S., *Current Opinion in Chemical Biology* **2006**, *10*, (6), 658.
20. Vrbka, L.; Vondrasek, J.; Jagoda-Cwiklik, B.; Vacha, R.; Jungwirth, P., *Proceedings of the National Academy of Sciences of the United States of America* **2006**, *103*, (42), 15440.
21. Wlodawer, A.; Miller, M.; Jaskolski, M.; Sathyanarayana, B. K.; Baldwin, E.; Weber, I. T.; Selk, L. M.; Clawson, L.; Schneider, J.; Kent, S. B. H., *Science* **1989**, *245*, (4918), 616.
22. Nam, K. Y.; Chang, B. H.; Han, C. K.; Ahn, S. G.; No, K. T., *Bulletin of the Korean Chemical Society* **2003**, *24*, (6), 817.
23. Wittayanarakul, K.; Hannongbua, S.; Feig, M., *Journal of Computational Chemistry* **2008**, *29*, (5), 673.
24. Case, D. A.; Cheatham, T. E.; Darden, T.; Gohlke, H.; Luo, R.; Merz, K. M.; Onufriev, A.; Simmerling, C.; Wang, B.; Woods, R. J., *Journal of Computational Chemistry* **2005**, *26*, (16), 1668.
25. Essmann, U.; Perera, L.; Berkowitz, M. L.; Darden, T.; Lee, H.; Pedersen, L. G., *Journal of Chemical Physics* **1995**, *103*, (19), 8577.
26. Vrbka, L.; Jungwirth, P., *Journal of Physical Chemistry B* **2006**, *110*, (37), 18126.
27. Weber, J.et.al., *Journal of Molecular Biology* **2002**, *324*, (4), 739.

28. Konvalinka, J.; Litera, J.; Weber, J.; Vondrasek, J.; Hradilek, M.; Soucek, M.; Pichova, I.; Majer, P.; Strop, P.; Sedlacek, J.; Heuser, A. M.; Kottler, H.; Krausslich, H. G., *Eur J Biochem* **1997**, *250*, (2), 559.
29. Uhlikova, T.; Konvalinka, J.; Pichova, I.; Soucek, M.; Krausslich, H. G.; Vondrasek, J., *Biochemical and Biophysical Research Communications* **1996**, *222*, (1), 38.
30. Toth, M. V.; Marshall, G. R., *International Journal of Peptide and Protein Research* **1990**, *36*, (6), 544.
31. Porter, D. J. T.; Hanlon, M. H.; Carter, L. H.; Danger, D. P.; Furfine, E. S., *Biochemistry* **2001**, *40*, (37), 11131.
32. Konvalinka, J.; Heuser, A. M.; Hruskovaheidingsfeldova, O.; Vogt, V. M.; Sedlacek, J.; Strop, P.; Krausslich, H. G., *Eur J Biochem* **1995**, *228*, (1), 191.
33. Zhao, H., *Journal of Molecular Catalysis B-Enzymatic* **2005**, *37*, (1-6), 16.
34. Zoldak, G.; Sprinzl, M.; Sedlak, E., *Eur J Biochem* **2004**, *271*, (1), 48.
35. Toth, K.; Sedlak, E.; Sprinzl, M.; Zoldak, G., *Biochimica Et Biophysica Acta-Proteins and Proteomics* **2008**, *1784*, (5), 789.

Tables:**Table 1.** Sodium contact times and Na⁺/K⁺ contact time ratio with Asp and Glu amino acid sidechains of HIV-1 protease.

	Mixed NaCl and KCl solution		Pure NaCl or KCl solutions	
	Na/K ratio	Na contacts (%)	Na/K ratio	Na contacts (%)
Asp	2.7	53	1.7	55
Glu	2.1	12	1.6	29

Table 2. Sodium contact times and Na⁺/K⁺ contact time ratio with the Glu amino acid sidechain of the KARVL substrate.

	Mixed NaCl and KCl solution	
	Na/K ratio	Na contacts (%)
Glu	2.9	13

Table 3.

Kinetic analysis of HIV-1 PR activity dependence on salt concentration

(hydrolysis of Peptide 1 in phosphate buffer, pH 6.0). For experimental details, see Materials and Methods.

ion	K_M (μM)	k_{cat} (s^{-1})	k_{cat}/K_M ($\text{s}^{-1} \text{mM}^{-1}$)
NaCl 50 mM	120 ± 20	10 ± 1	89 ± 14
NaCl 300 mM	86 ± 10	11 ± 1	130 ± 20
KCl 50 mM	110 ± 20	16 ± 1	150 ± 30
KCl 300 mM	41 ± 2	17 ± 1	400 ± 30

Table 4.

Kinetic analysis of HIV PR activity dependence on salt concentration

(hydrolysis of Peptide 2 in MES buffer, pH 6.0). For experimental details, see Materials and Methods.

ion	K_M (μM)	k_{cat} (s^{-1})	k_{cat}/K_M ($\text{s}^{-1} \text{mM}^{-1}$)
NaCl 50 mM	34 ± 2	17 ± 2	500 ± 70
NaCl 300 mM	23 ± 1	23 ± 2	1000 ± 100
KCl 50 mM	18 ± 1	12 ± 1	690 ± 80
KCl 300 mM	17 ± 2	18 ± 2	1100 ± 160

Figures:

Figure 1: Cumulative sums (full lines) and distribution functions (dotted lines) of sodium and potassium ion distribution around HIV-1 PR.

Figure 2: Distribution of sodium (green) and potassium (blue) ions at the surface of HIV-1 PR averaged over a simulation in the mixed NaCl/KCl solution (0.25M each salt). Insets show detailed distributions of Na⁺ and K⁺ in the vicinity of the deprotonated (left) and singly protonated (right) Asp pair at the active site.

Figure 3: Dependence of the initial velocities of the hydrolysis of Peptide 1 (panel A,B) and Peptide 2 (Panel C) by HIV-1 PR on increasing concentrations of NaCl or KCl. The kinetic measurements were performed in 50 mM HEPES (4A), phosphate (4B), or MES (4C), pH 6.0. For details, see Materials and Methods.

Figure 1

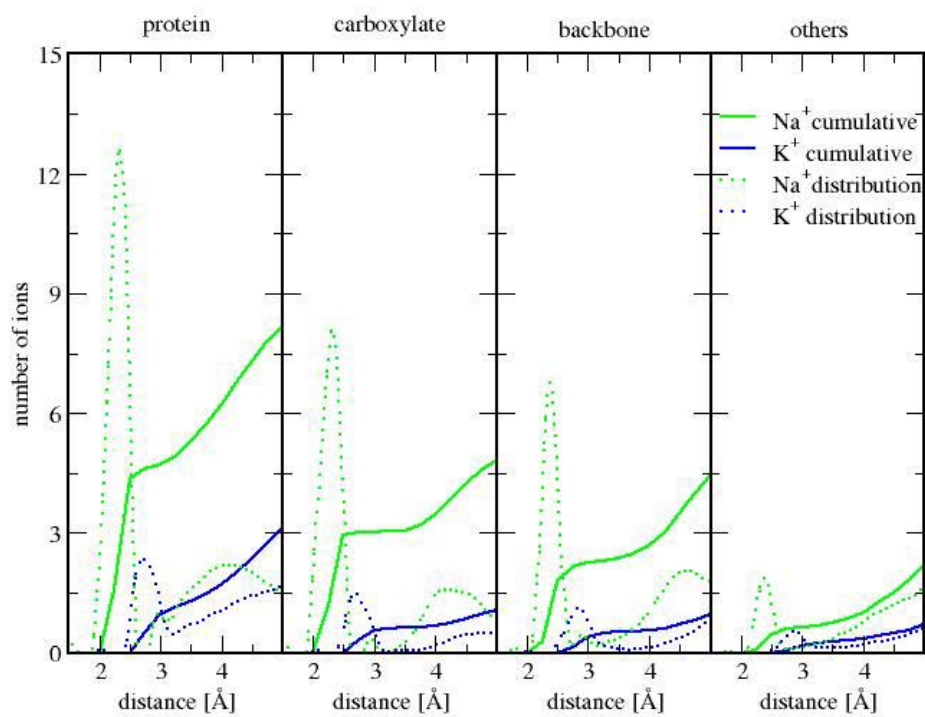


Figure 2

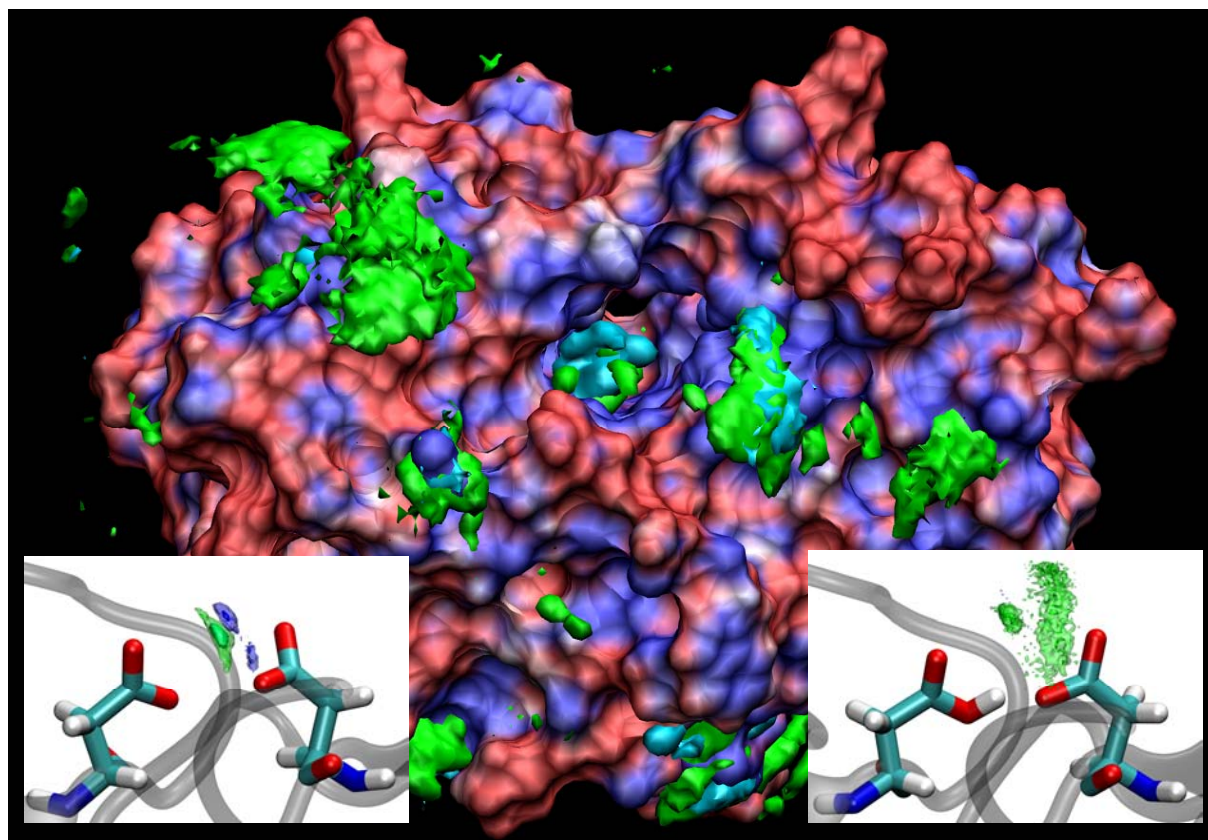
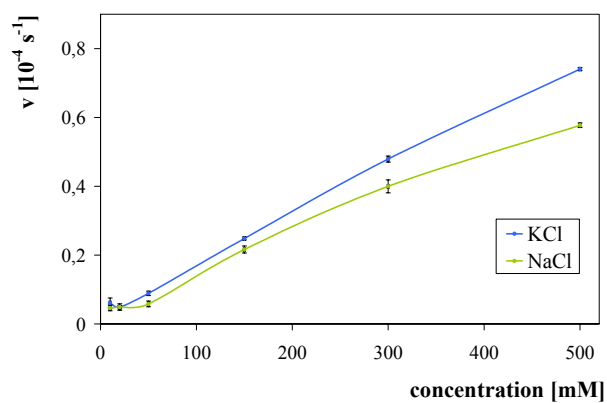
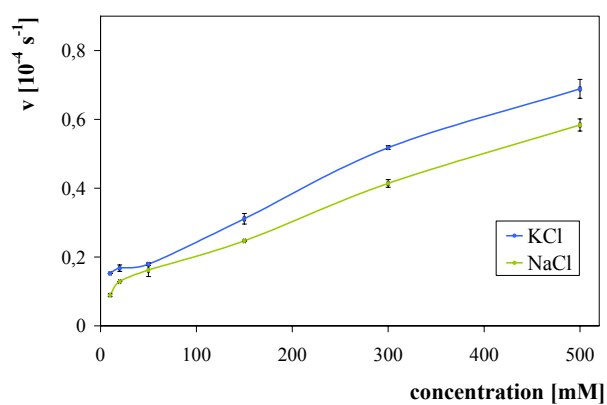


Figure 3

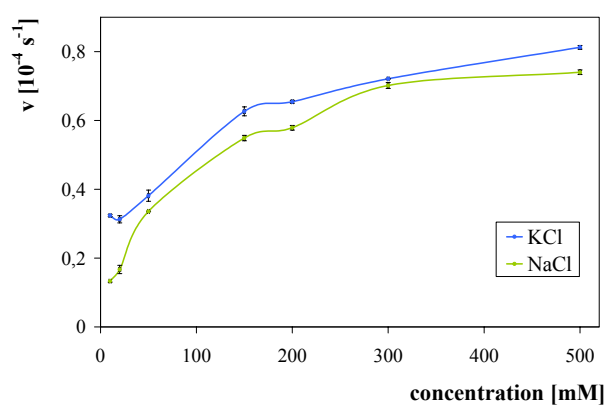
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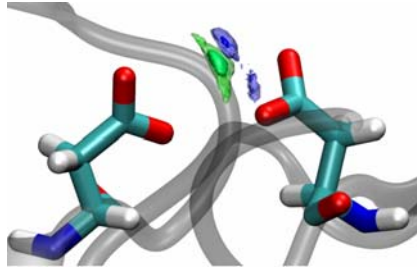
B



C



TOC Graphics



The HIV Protease active site shows dissimilar preference for Na⁺ and K⁺ binding giving an explanation of their different influence on the protease activity.