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The effect of salt on the Michaelis Menten constant of the HIV-1 protease correlates with the Hofmeister series

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The effect of different types of salt on the proteolytic activity of HIV-1 protease was studied. At a similar ionic strength, the enzyme activity changed according to the salting out effect of the ions used (Hofmeister series). Kinetic studies showed that a stronger salting out effect of the ions rather than the higher ionic strength per se increased the affinity to the substrate (K_m) but in general did not alter the K_{cat} value.

HIV-1 protease; Enzyme kinetics; Salt effect; Hofmeister series

1. INTRODUCTION

The prominent involvement of virus encoded proteases (PR) in the life-cycle of retroviruses [1–3] has prompted extensive investigations of these enzymes. Due to the etiological role of human immunodeficiency virus (HIV) in the development of acquired immunodeficiency syndrome [4], studies have been focussed on the PR of this virus. Recombinant PR or chemically synthesized PR have been purified and used as sources for investigation of enzymatic properties and inhibitor studies (for review see [5–9]). High ionic strength was found to optimize the enzymatic activity by lowering the Michaelis Menten constant (K_m); the turnover number (K_{cat}) was not affected [10,11].

In this report, we describe the effect of different types of salt on enzyme kinetics.

2. MATERIALS AND METHODS

2.1. *Enzyme*

HIV-1 protease was expressed in *E. coli* as a fusion protein and purified as described elsewhere [12].

2.2. *Protease assays*

Unless noted otherwise, 10 nM of HIV-1 PR (determined by active site titration using compound 3, a potent transition state inhibitor of HIV proteases [13]) was assayed using the synthetic nonapeptide substrate, Val-Ser-Gln-Asn-Tyr-P ?-Ile-Val-Gln-NH_2, according to the procedure described earlier [14,15]. Assays were carried out in a total volume of 10 μl containing 3000 pmol of substrate, 5 μl of 2× reaction buffer (0.5 M potassium phosphate, pH 6.5, 5% (v/v) glycerol, 1 mM DTT, 1 mM EDTA and 1.5 M ammonium sulphate), and were incubated for 30 min at 37°C.

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2.3. *Kinetics*

Protease assays were carried out in buffer A (50 mM Mes, pH 6.0, 5% glycerol, 1 mM DTT and 1 mM EDTA) containing various concentrations of substrate (0.1–3 mM) and the indicated concentrations and types of salt. Lineweaver-Burk, Eadie-Hofstee and Hill analysis were used to determine K_m, V_{max} and h.

3. RESULTS AND DISCUSSION

The effect of different types of salt on the proteolytic activity of HIV-1 PR is summarized in Fig. 1. By adjusting the salt concentration to the same ionic strength in the assay (2.57 M), ammonium sulfate, ammonium acetate and sodium sulfate gave a 2.2-, 1.6- and 1.5-fold higher enzyme activity, respectively, as compared to KCl. Same ionic strength (1 M) of buffers containing NaCl, KBr or KI showed decreased enzyme activities corresponding to 80%, 20% and 5%, respectively, as compared to the same ionic strength of KCl. From this data, the salt effect on the enzyme activity with respect to the various anions and cations can be deduced as follows: $SO_4^{2-} > CH_3COO^- > Cl^- > Br^- > I^-$, and $NH_4^+ > K^+ > Na^+$. This represents the Hofmeister series of the salting out effect of ions [16]. For kinetic studies we used monovalent ions and a 50 mM Mes buffer, pH 6.0, instead of the multivalent phosphate buffer to avoid interpretation dealing with complex dissociation patterns.

Table I summarizes the effect of different types of salt on the kinetics of HIV-1 PR. In accordance with studies using NaCl [10,11] and ammonium sulfate [10], we found that the enzyme is more active at a high ionic

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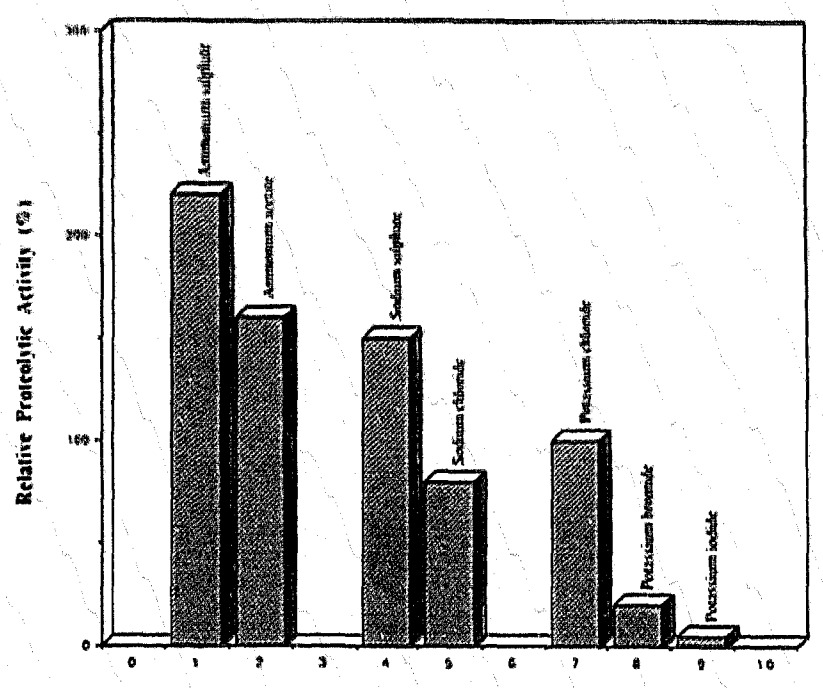

Fig. 1. Effect of different types of salt on the proteolytic activity of HIV-1 protease. The proteolytic activity is shown as a relative value (percent of the activity obtained with KCl). $(NH_4)_2SO_4$, NH_4CH_3COO, Na_2SO_4 and NaCl were assayed at a total ionic strength of 2.57 M in buffer containing 0.25 M potassium phosphate, pH 6.5, 5% glycerol, 1 mM DTT, 1 mM EDTA, and were compared with assays using potassium chloride under identical conditions having the same ionic strength. KCl, KBr and KI were assayed at an ionic strength of 1 M in a buffer A. Samples were incubated for 30 min at 37°C.

strength of KBr and KCl by lowering the K_m of the substrate, the turnover number (K_{cat}) was not affected. By increasing the ionic strength from 0.5 to 1.5 M, a 2–3-fold decrease of K_m was observed with KBr, KCl and CsCl. However, a more pronounced effect was seen at the same ionic strength (0.5 M) by comparing the K_ms of KCl, KI and KBr. With respect to KCl, the use of KI and KBr increased the K_m by a factor of 53 and 7, respectively. Both KI and KBr have a lower salting out effect following the order KCl > KBr > KI. As expected for cations, only a weak effect on the K_m was observed (KCl < CsCl < LiCl). Therefore, the salting out effect rather than the ionic strength is responsible for increasing the enzymatic activity by lowering the K_m. This suggests that the enzyme/substrate binding is mostly due to hydrophobic interactions.

Table 1

Salt effect on the kinetics of HIV-1 protease

salt	K_m [mM]		K_{cat} [s^{-1}]	
	A	B	A	B
KI	63	n.d.	12	n.d.
KBr	8.1	4.5	12	12
KCl	1.2	0.40	12	12
CsCl	1.6	0.71	5	15.5
LiCl	1.8	5.0	12	12

column A = tested at a salt concentration of 0.5 M; column B = tested at a salt concentration of 1.5 M; n.d. = not determined

In contrast to the other salts assayed, an increased LiCl concentration also increased the K_m from 1.82 to 5.0 mM. At 0.5 and 1.5 M LiCl, a Hill coefficient of 1.4 was determined. Since there was no change of the catalytic rates (K_{cat}) it is possible that LiCl affects the substrate rather than the enzyme (e.g. precipitation of substrate). With the exception of CsCl, the concentration and the type of salt used had no influence on the catalytic rate (K_{cat}) of 12 s^{-1}. At a concentration of 0.5 M, Cs^+ decreased the catalytic rate to 5 s^{-1}, and this value increased with higher CsCl concentration (1.5 M) to 15.5 s^{-1}. It is most likely that Cs^+ affects proteolysis by interaction with the enzyme in a concentration dependent manner. Changes of Cs^+ concentration may lead to conformational changes in the flap region of the HIV-1 protease thereby influencing its catalytic properties. A high flexibility of the 'flap' region of the retroviral PR has been observed [6,17], which is stabilized by hydrogen bonds [18,19]. However, a Hill coefficient of 1.5 was found at a salt concentration of 0.5 M CsCl, which may be caused by tetramer formation at the lower Cs^+ concentration.

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