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Research Article

Hofmeister effect on catalytic properties of chymotrypsin is substrate-dependent

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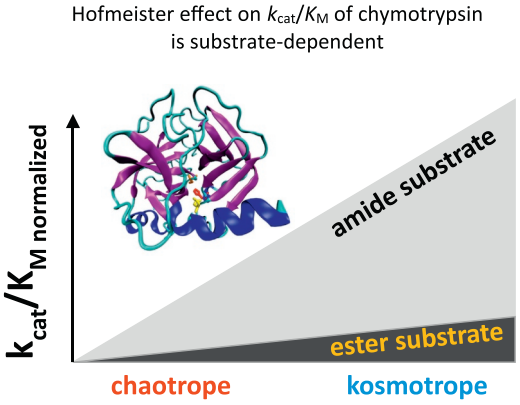
HIGHLIGHTS

- Hofmeister effect on activity and stability of chymotrypsin has been studied.
- Stability and activity of chymotrypsin increase with kosmotropicity of anions.
- Hofmeister effect on ChT catalysis is 10-fold higher for amide than for ester substrate.
- Enzyme properties are affected by direct interaction of anions with protein surface.

GRAPHICAL ABSTRACT



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ABSTRACT

Effect of Hofmeister sodium salts, sulfate, chloride, bromide and perchlorate, on catalytic properties and stability of chymotrypsin has been studied by absorbance and circular dichroism spectroscopies. To address Hofmeister effect on activity of chymotrypsin, two different substrates, N-benzoyl-L-tyrosine ethyl ester and amide N-succinyl-L-phenylalanine-p-nitroanilide, were used. Catalytic activity of chymotrypsin is dependent on salt concentration and position of anion in Hofmeister series. The enzyme activity for both substrates is only slightly affected by chaotropic anions and increases with kosmotropic nature of anions. While the trend of Hofmeister effect on chymotrypsin catalysis is similar for both substrates, the amplitude of the effect significantly differs. In the presence of 1 M sulfate, catalytic efficiency increased by ~2-fold for the ester but ~20-fold for the amide substrate. Positive correlation between stability and activity of chymotrypsin indicates the interdependence of these enzyme properties and is in agreement with recently developed macromolecular rate theory suggesting an important role of protein dynamics in enzyme catalysis. Linear dependencies of catalytic properties of chymotrypsin with partitioning of anions at bulk water/air as well as at hydrocarbon surface strongly indicate that the modulated enzyme properties are results of direct interaction of anions with protein surface.

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1. Introduction

Effect of high concentrations (> 0.3 M) of salts on numerous biophysical and biochemical processes is well-known as the Hofmeister effect [1,2]. Hofmeister effect has been particularly intensively studied in relation with colloidal systems and biomacromolecules [3–8]. In fact, the influence of high concentrations of salts on properties of biomacromolecules was originally observed in relation to protein precipitation [9,10]. This and numerous following studies led to formation of the so-called Hofmeister series of cations and anions. The typical order, the usually quoted, for the anion series is as follows [11]:

$SO_4^{2-} > OH^- > F^- > Cl^- > Br^- > NO_3^- > I^- > SCN^- > ClO_4^-$.

Anions on the left are called kosmotropes (stabilizing, salting-out anions), whereas anions on the right are chaotropes (destabilizing, salting-in anions). Chloride is usually considered the dividing line between these two types of behaviour.

It has been shown that anions, in dependence on their position in Hofmeister series, affect protein properties such as equilibrium between different conformational states [12–14], stability [15–19], aggregation/fibrilization [20–22], and protein dynamics [23–27].

Modulation of equilibrium between different conformations as well as modulation of protein dynamics has to result also in modification of functional properties of enzymes. In fact, some previous studies revealed that the effect of anions on enzyme activity usually follows Hofmeister series and the enzyme activity increases in direction from chaotropic to kosmotropic anions [19,28–35] but there are also exemptions that show nonlinear effect of anions on enzyme properties in relation to their positions in Hofmeister series [24,36].

Understanding of how Hofmeister anions modulate enzyme functions is important not only from the basic-research point of view but salt-induced activation of enzymes by more than one order in magnitude [33,37] is potentially interesting for biotechnological applications as well.

In this study, we chose α-chymotrypsin (ChT) as an example of the enzyme from the large superfamily of serine proteases [38]. Serine proteases of the chymotrypsin family are structurally very conservative since they have maintained a common fold over an evolutionary span of more than one billion years [39] with possibly similarly conservative dynamical properties of their polypeptide chain [40].

ChT is composed of two-six stranded anti-parallel β-barrel domains, one end is included in catalysis and the other end in regulation, followed by an anti-parallel hairpin motif with small α-helix content. ChT contains catalytic triad composed of Ser195, Asp102 and His57 in the active site of enzyme. The specificity of serine proteases is usually determined at positions 189, 216 and 226, which form S1 binding pocket. The residue 189 is polar Ser in chymotrypsin and that is the reason why ChT cleaves polypeptide chains on the C-terminal side of large hydrophobic residues (Phe, Trp and Tyr). Generally accepted mechanism applies to chymotrypsin-like serine proteases, which includes process of acylation and deacylation (Scheme 1) [38,41]. Its catalytic cycle first step involves formation of a substrate-enzyme complex (ES). This is followed by the step of acylation, when the hydroxyl group of nucleophile Ser195 attacks the substrate's carboxyl group forming covalently bound tetrahedral intermediate $(ES)_{TET1}$. This intermediate is stabilized by hydrogen bond with Asp102. The final step of acylation includes the destruction of tetrahedral intermediate as a result of the release of amino group to form acyl-enzyme intermediate. The deacylation step includes the attack of hydroxyl group of water molecule on acyl-enzyme intermediate to form the second tetrahedral intermediate $(EP_2)_{TET2}$. At the final step, the destruction of tetrahedral intermediate is caused by releasing of carboxyl acid product. ChT besides playing an important role in many physiological processes has also application potential in biotechnology [42,43].

Results of previous limited studies, which addressed the effect of salts on ChT catalytic properties [28,37,44], suggested an activation effect of kosmotropic anions with significantly different extent of the activation.

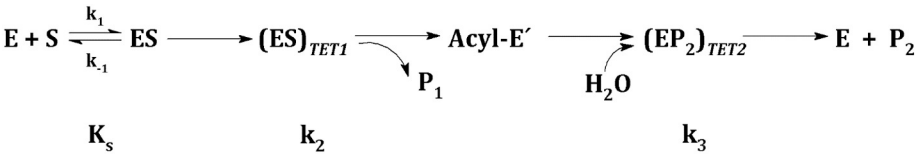
In the present work, we analyzed Hofmeister effect on ChT catalytic properties in the presence of two different substrates, BTEE and SPNA. They differ by the bond cleaved by ChT, i.e. ester bond in BTEE and amide bond in SPNA and by the rate determining steps - deacylation for BTEE and acylation for SPNA. Correlation of catalytic and stability properties of ChT with charge densities of anions suggests that hydration of anions is major determinant that modulates enzyme properties. Moreover, correlation of catalytic properties with stability of ChT indicates a role of dynamical properties of polypeptide chain in enzyme-catalyzed reactions [45,46].

2. Materials and methods

Enzyme α-chymotrypsin (ChT) (EC 3.4.21.1) from bovine pancreas, N-benzoyl-L-tyrosine ethyl ester (BTEE), N-succinyl-L-phenylalanine-p-nitroanilide (SPNA), HCl, $Na_2HPO_4.2H_2O$, $NaH_2PO_4.H_2O$, NaCl, $NaClO_4.H_2O$, NaOH, MOPS (3-(N-morpholino)propanesulfonic acid) were purchased from Sigma Aldrich. NaBr was obtained from Penta, Na_2SO_4 from Merck, and PeFabloc and protective solution PeFabloc were purchased from Roche.

2.1. Enzyme assays of activity of chymotrypsin

Kinetic measurements were performed on UV-VIS absorption spectrophotometer Specord S300 equipped by Peltier element (Analytic Jena). The concentration of ChT was determined from the absorbance at 280 nm and an extinction coefficient of 50,585 $M^{-1}\,cm^{-1}$ [37,47]. The rate of ChT-catalyzed substrate hydrolysis was measured by following the increase in absorbance at 256 nm and 410 nm for BTEE and SPNA, respectively. The temperature during all measurements was kept constant at 25 °C. Data were collected for 2 min from the start of the reaction. The initial reaction velocities were determined from the slope of the initial increase in the absorbance at the corresponding wavelength. The absorbance was converted to molar concentration using molar extinction coefficient for N-benzoyl-L-tyrosine (cleavage product of BTEE) of 964 $M^{-1}\,cm^{-1}$ at 256 nm and for p-nitroaniline (cleavage product of SPNA) of 8800 $M^{-1}\,cm^{-1}$ at 410 nm. The measurements were performed in the presence of four sodium salts at four different concentrations (0.25 M, 0.5 M, 0.75 M and 1 M). The reaction mixture contained selected concentration of a corresponding sodium salt ($NaClO_4$, NaCl, NaBr, and Na_2SO_4), and 34.5 nM ChT in 20 mM phosphate buffer, pH 7.8. Before each measurement, the buffer solution along with the enzyme was incubated for 4 min at 25 °C. The reaction was started by adding BTEE, with final concentrations in the range from 2 μM to 420 μM. In the case of SPNA hydrolysis, the reaction mixture contained 5 μM ChT in 50 mM MOPS at pH 7.5. The final concentrations of SPNA were in the range from 0.1 to 3 mM. Experimentally determined data were fitted according to Michaelis-Menten equation



Scheme 1. General scheme of serine protease catalysis.