EFFECTS OF SALTS ON THE SUBSTRATE SPECIFICITY OF A SUBTILISIN-LIKE HALOPHILIC PROTEASE

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We present in this work an analysis of the effects of salts on the substrate specificity of the moderated halophilic protease SR5-3, a secreted extracellular subtilase from Halobacillus sp., isolated from the high-salt environment of Thai fish sauce. We used FRET peptides derived from Abz-KLRSSKQ-EDDnp, where Q-EDDnp (glutaminyl-[N-(2,4-dinitrophenyl)-ethylenediamine]) and Abz (ortho-aminobenzoic acid) are the fluorescence acceptor and donor groups, respectively. Systematic modifications were introduced in this peptide resulting in five series of peptides that were used to explore the specificities of S3–S'2 binding sites in the absence of salt, and in the presence of 3M of NaCl or 1M Na_2SO_4. We further refined the analysis of the salt effects on SR5-3 substrate specificity using a series of peptides derived from Abz-KLRSSKQ-EDDnp in which we made a scan of Phe. The modifications on the leader sequence Abz-KLRSSKQ-EDDnp were reflected on SR5 3 hydrolytic reaction in similar way either in absence or presence of salts. The subtilase character of SR5-3 was demonstrated by its broad preference for aromatic or large non-polar substrate residues at P1 and for aliphatic amino acids at P2 and P'2. The peptides Suc-AAPF-pNa and Suc-AAF-MCA that contain amino acids only in the non-prime side were poorly hydrolyzed by SR5-3 in the absence of salt; however in the presence of salts their cleavages were highly improved. In conclusion, salts did not change the SR5-3 specificity but modulates its hydrolytic activity.