Computational analysis of naturally occurring protein C mutants: electrostatic properties implications.

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Introduction

Activated Protein C (APC) is a vitamin K-dependent anticoagulant plasma serin protease that exerts its action through the inactivation of factors Va and VIIIa in presence of Ca++ and phospholipids. Deficiency of protein C is associated with the risk of developing venous thrombosis. APC shares homologies with other vitamin K-dependent coagulation proteins as a results of a common evolutionary pathway. The chymotrypsin-like serine proteases maintain a strictly conserved active site geometry among their catalytic Ser, His and Asp residues. The fact that this core is highly conserved both in sequence and structure among members of the serine protease family suggests that its shape has been finely tuned during evolution.
33 mutations (18 novel) in the promoter and coding regions of the PC gene were identified by PCR and sequencing in 46 patients reporting venous thromboembolic events. Here we present a computational analysis of three selected mutants (G43E, D194N, G216D) that are localized in the catalytic domain and determine a charge modification in the vicinity of the catalytic triad (fig.1).

Materials and Methods

Molecular models of aminoacidic variants were constructed starting from the X-ray structure (1) obtained from the Protein Data Bank (1aut) by residue substitution using InsightII (Accelrys Inc.) running on a SGI-O2 workstation. The lowest energy rotamer was choosen as starting side chain position, followed by energy minimization calculations consisting of 500 steps of steepest descent keeping the backbone fixed followed by 500 steps of conjugate gradient on the whole structure. Electrostatic potential calculations and histidine 57 pKa shift prediction were performed with the DELPHI software package (2). The calculation included the following parameters: ionic strenght 0.1 M, protein and solvent dielectric constant of 2 and 80 respectively and grid size 60x60x60. Electrostatic maps representation were obtained with InsightII. One half of electron charge was assigned to each ND1 and ND2 positions of histidine 57 for pKa shift calculation. The crystallographic water and ligand were excluded. The ΔpKa value was calculated from the following formula: ΔpKa= phi/2.301 where phi is the average of potentials at the two carboxyl oxygens obtained by the Poisson-Boltzman equation solution in DELPHI (3).

Results and Discussion

The three aminoacidic variants (G43E, D194N, G216D) discussed here involve residues that are highly conserved in serine protease family and are in close proximity to active site histidine 57 with a distance of about 9-10 Å for G43 and G216 and 11Å for D194. As the substitutions determine a charge modification the models were subjected to a DELPHI analysis to evaluate the change of the surface electrostatic potential. Fig.2 represents electrostatic potential map displayed on the molecular surface of mutants (right column) compared to the native structure (left column). The dashed and filled arrows indicate the position of active site histidine 57 and of mutated residue, respectively. The potential scale ranges from -5 kT/e to 5kT/e from white to black.
Mutants G43E and G216D cause a partial neutralization of histidine positive charge due to the introduction of negative residues. A more dramatic change can be observed when the negative charge of aspartate 194 is substituted by the polar group of asparagine. D194 is part of the trypsin family serine protease pattern. It is adjacent to the serine 195 of the catalytic triad, and its side chain, pointing in opposite direction respect to serine, is buried in the protein interior and forms hydrogen bonds with cysteine 191 and glycine 142. The loss of the carboxylic group results in a generalized neutralization effect at the active site level and an increased positivity of histidine 57 (dashed arrows in fig 2).
The charge modification introduced by mutations is likely to interfere with the protonation state of titrable group of histidine 57 which requires to be positively charged for the enzymatic activity. To evaluate this effect, we have calculated the pKa change of histidine 57 in the three mutants using the DELPHI potential values. Results are reported in Table 1. In the case of mutant D194N we observe a decrease in the pKa by effect of negative charge loss that is likely to destabilize the positively charged protonated histidine and decrease its pKa value.

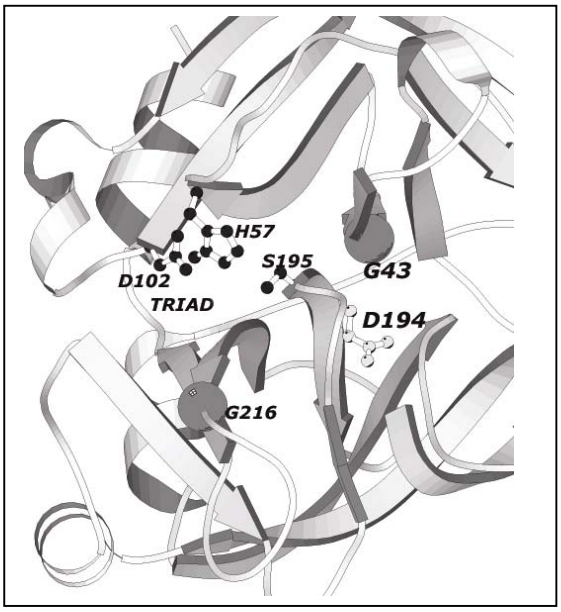

Figure 1- Protein C active site

Table 1 – Calculated Δpka at 0.1 M ionic strenght

Mutant	Residue	Atom	potential (KT/e)	ΔpKa
D194N	ASP	$O\delta^1$	1.6184	-0.695
D194N	ASP	$O\delta^2$	1.5863	
G43E	GLU	$O\varepsilon^1$	2.3166	0.91
G43E	GLU	$O\varepsilon^2$	1.8911	
G216D	ASP	$O\delta^1$	3.1057	1.066
G216D	ASP	$O\delta^2$	1.8051	

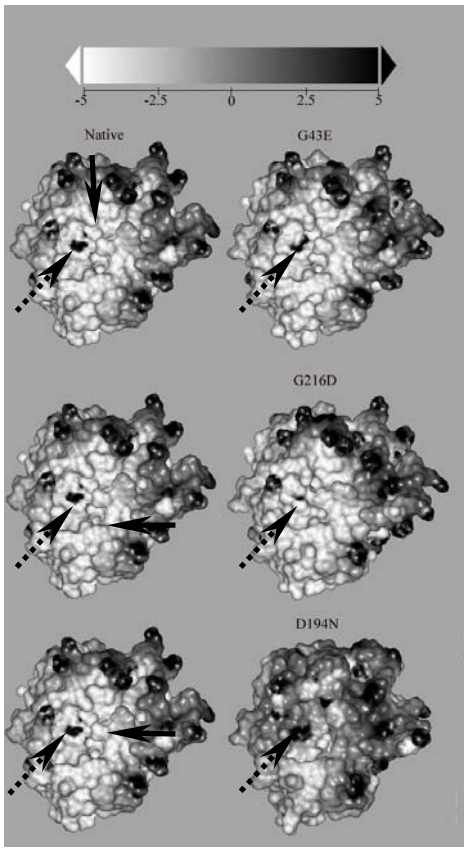

Figure 2 – Electrostatic potential map

This effect is also sharply dependent on the distance between the residues involved. In our case, distances fall within a range at which the potential effects are detectable, in agreement with literature data (3,4). For the other two mutants, where the charge is gained, we observed an increase of pKa. Our observations indicate that the all the mutants studied affect the protein C enzymatic activity possibly at a different extent. Laboratory tests in plasma of patients detected significantly reduced values of anticoagulant and amydolitic activities which are related to a reduced function. More accurate biochemical studies, using recombinant mutants, should be performed to test this hypothesis.

Acknowledgments

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