# Zinc Binds and Regulates the Activity of the Natural Anticoagulant Protein Z

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## INTRODUCTION

Calcium is an essential cofactor for most of the blood coagulation proteases. It activates clotting factors and mediates the binding of tenase complexes to the phospholipid surfaces expressed by activated platelets. Multiple studies have reported that bivalent metals such as $Zn^{2+}$, $Mg^{2+}$ , $Mn^{2+}$ can also bind to several coagulation factors and modulates their activity. Zinc, the second most abundant transition metal in the body, is thus shown to be an important regulator of fVIIa, fXII, fXI, PS, APC. Zinc deficiency is associated with impaired platelet aggregation, and cutaneous bleeding and platelet dysfunction in some cancer patients.

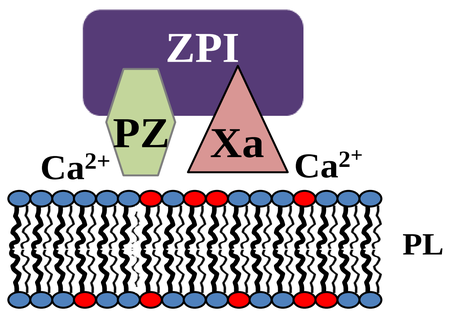


**Fig 1. Ternary complex of PZ-ZPI-fXa on lipid membrane in presence of $Ca^{2+}$**

Protein Z (PZ) is a natural anticoagulant which acts as a cofactor of protein Z dependent protease inhibitor (ZPI) in inhibiting fXa in presence of procoagulant $Ca^{2+}$ and phospholipid membrane (1). PZ, bound to membrane, positions linked ZPI in close proximity of fXa, also bound to the same membrane. Presence of $Ca^{2+}$ is required for PZ to bind to membrane and thus $Ca^{2+}$ is a crucial regulator of PZ activity. However, it is not known if other bivalent metal ions can also bind to PZ to facilitate its binding to membrane. The current study is therefore aimed to investigate the role of bivalent metal ions such as Zinc and Magnesium in regulating the activity of PZ.

## RESULTS

### Binding of Zn to PZ in absence and presence of $Ca^{2+}$

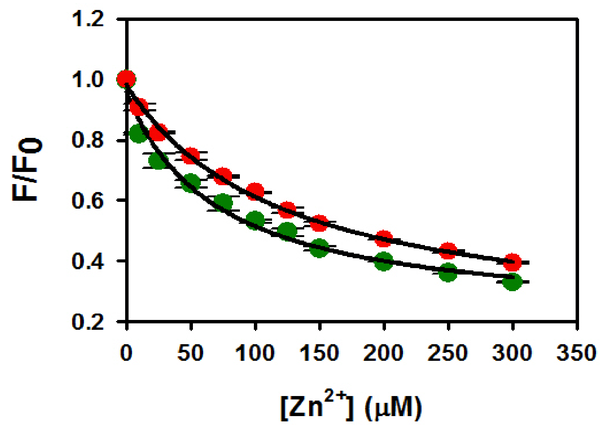


**Figure 2. Binding of $Zn^{2+}$ to PZ in absence (●) and presence ( ● ) of $Ca^{2+}$.** Binding was determined by measuring the intrinsic tryptophan fluorescence of PZ with increasing concentrations of $Zn^{2+}$. The $K_d$'s were measured to be 117±11 μM and 65 ± 13 μM in absence and presence of $Ca^{2+}$ respectively

## RESULTS

### Table 1. Soluble Phosphatidylserine (C6PS) binding to PZ in presence of different concentrations of $Zn^{2+}$

| Protein | Titrant | Metal ion | $K_d$ (μM) |
|---|---|---|---|
| PZ | C6PS | 5 mM $Ca^{2+}$ | ~ 48 |
| PZ | C6PS | 500 μM $Zn^{2+}$ | 160 ± 23 |
| PZ | C6PS | 1 mM $Zn^{2+}$ | 147 ±17 |
| PZ | C6PS | 3 mM $Zn^{2+}$ | 94 ± 12 |

### Model of Zn binding sites in Human PZ

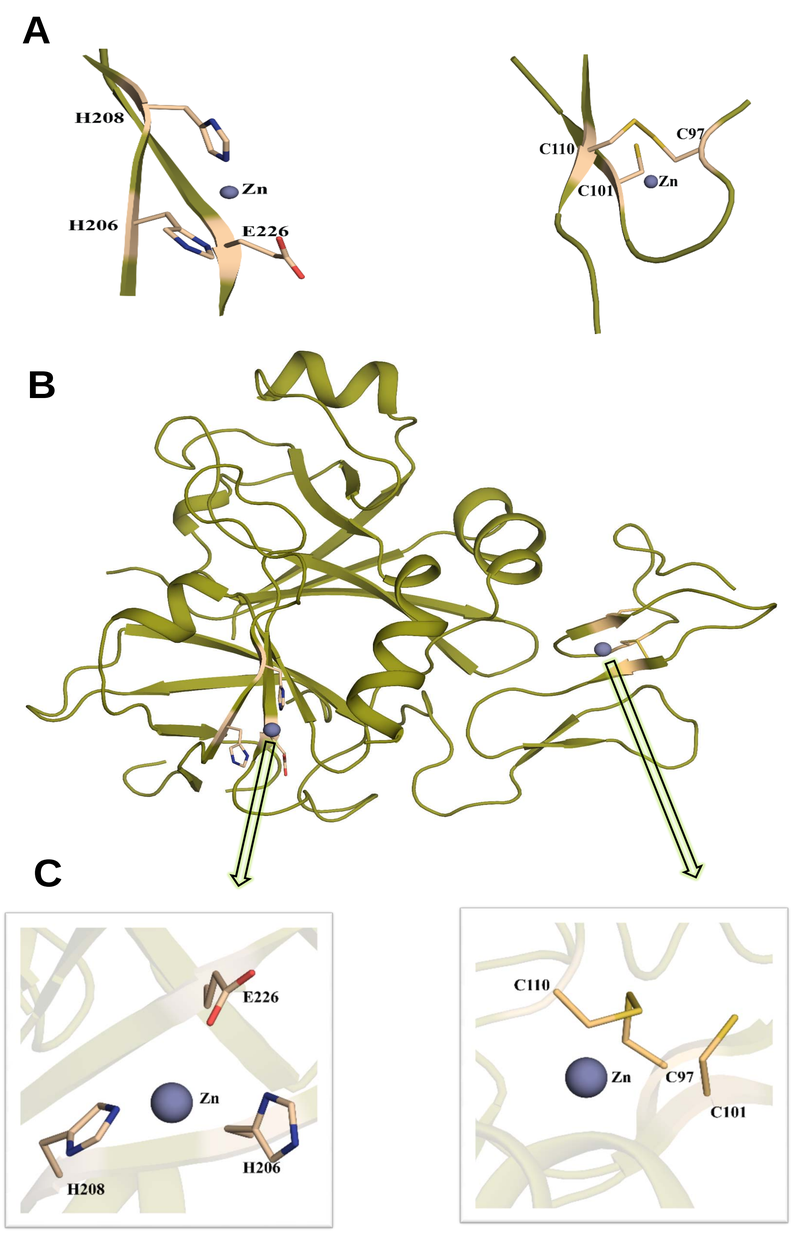


**Figure 3. Prediction of putative $Zn^{2+}$ binding sites in PZ. A.** Two $Zn^{2+}$ (blue sphere) binding sites are shown using the PDB structure 3F1S (2). **B.** Position of the $Zn^{2+}$ binding sites in the full length (Gla deleted) PZ. **C.** Cartoon representation of the two binding sites.

## RESULTS

### Effect of PZ on the inhibition of fXa activity by Antithrombin (AT) in presence of $Zn^{2+}$

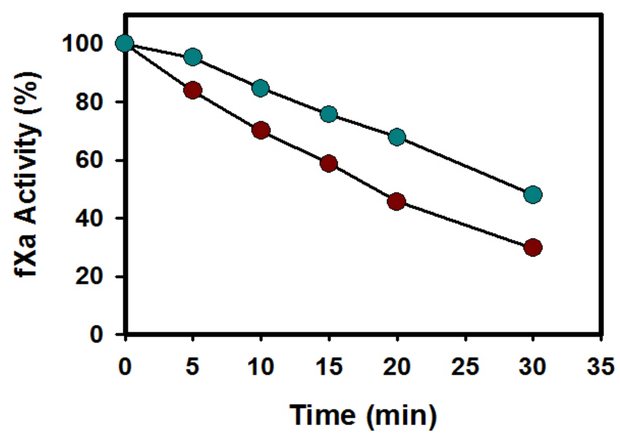


**Figure 4. Effect of PZ on the inhibition of fXa by antithrombin in presence of $Zn^{2+}$.** Reaction mixtures containing factor Xa (5 nM), 50 μM PS:PC membrane, $ZnCl_2$ (500 μM), with ( ● ) or without PZ ( ● ) (40 nM), were incubated for 5 min at before the addition of AT (3.4 mM). At the indicated times thereafter, samples were removed, and assayed for factor Xa activity.

## DISCUSSION & CONCLUSION

**We have shown for the first time that**

- PZ, an anticoagulant, can bind to both $Zn^{2+}$ and $Mg^{2+}$. Measurement of intrinsic tryptophan fluorescence spectra of PZ in presence of increasing concentrations of $Zn^{2+}$ and $Mg^{2+}$ revealed the apparent $K_d$ 's of 117±11 μM and 202±14 μM respectively (Fig 2). Addition of EDTA restored the decrease in emission maxima indicating reversible binding of the metal ions.
- Presence of $Ca^{2+}$ promoted $Zn^{2+}$ binding of PZ (Fig 2) whereas $Mg^{2+}$ binding being unaltered. $Zn^{2+}$ was also found to increase the affinity of PZ towards phospholipid in the absence of $Ca^{2+}$ (Table 1).
- Molecular modeling studies using the two crystal structures available for PZ suggested two putative Zn binding sites, His 206-His208-Glu226 and Cys 97-Cys 101-Cys 110 on PZ (3).
- It has previously been reported that, PZ protects fXa from AT-mediated inhibition by forming a stable fXa-PZ complex in presence of $Ca^{2+}$ and lipid membrane (1). Similar effects were also observed in presence of $Zn^{2+}$ (Fig 4).

## References

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