

Commentary: Involvement of Amino Acid Residues 423-429 of Human Protein S in Binding to C4b-Binding Protein

In this issue, Fernández and colleagues report a very interesting study on the localization of the binding site for C4b-binding protein (C4BP) in protein S. Although seventeen years have elapsed since the interaction between protein S and C4BP was first described, a number of important questions remain to be solved, including physiological ones such as why the complement regulatory protein C4BP is circulating in complex with the vitamin K-dependent anticoagulant protein S. The interaction provides a link between complement and coagulation - but why? Also biochemical aspects, such as where does C4BP bind on protein S, remain enigmatic. Different studies have suggested different regions to be involved and different groups have had difficulties in reproducing data from other groups (for references see paper by Fernández et al.). The paper of Fernández et al. provides some answers and raises some new questions. The original report by Fernández et al. used overlapping synthetic peptides to identify the region between amino acids 413 and 433 as an important binding site for C4BP. In the present study, recombinant technology is used together with synthetic peptides to further illustrate the importance of this region. In a series of elegant experiments they convincingly demonstrate the involvement of amino acid residues 423-429. It is particularly fascinating to learn that the carboxyterminal end of a peptide may be of paramount importance for its biological effect. Thus, the PSP-420 peptide ending with an amide is a much more potent inhibitor of the protein S-C4BP interaction than the same peptide ending with a carboxyl group. This illustrates the difficulties involved in using

synthetic peptides and calls for a humble and careful approach when conclusions are drawn. Indeed, it may not be valid to use peptide inhibition data to draw firm conclusions about the relative importance of different proposed binding areas in protein S. The mutagenesis approach that is described in the paper shows that certain mutations within the 423-429 region lead to a 10-fold drop in affinity of the binding of C4BP to protein S, which further illustrates the importance of this region. Based on the presented data, it stands without doubt that the 423-429 region is involved in the protein S-C4BP interaction, but at the same time it is apparent that additional sites on protein S are involved and that the interaction is complex. In this respect it is noteworthy that the phage display approach used by Linse et al. identified residues 447-460 as being involved in the binding site. In the present work, Fernández and colleagues confirm that a peptide corresponding to this region inhibits the protein S-C4BP interaction even though it is 10-fold less potent than the PSP-420-amide peptide. I hesitate to conclude that one site is more important than the other on the basis of available peptide inhibition studies. The results on record rather point to the complex nature of this high affinity interaction. Future studies combining site directed mutagenesis in the "Fernández and Linse sites" may provide additional insights into the relative importance of the different sites. However, full understanding of the interaction must await the elucidation of the three-dimensional structures of binding domains of both protein S and C4BP. Indeed, crystallization of a complex between these binding domains appears to be a worthwhile goal for future efforts.

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