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The binding of C-reactive protein, in the presence of phosphoethanolamine, to low-density lipoproteins is due to phosphoethanolamine-generated acidic pH

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Dear Editor,

We recently reported that C-reactive protein (CRP), when present with the small-molecule compound phosphoethanolamine (PEt) (Sigma-Aldrich), acquired the capability to bind to native low-density lipoprotein (LDL), and that PEt enhanced the binding of CRP to enzymatically-modified LDL (E-LDL) [1-3]. Subsequently, we found that, in the presence of PEt, CRP bound to a variety of other proteins. We randomly selected fibronectin, IgG, leptin, FcγRIIa, activated complement component C3b, complement protein factor H, serum amyloid P component, oxidized LDL, lipoprotein (a), amyloid precursor protein α and amyloid β peptide (A β)(Sigma-Aldrich, St. Louis, MO), and determined the binding of CRP to these proteins. CRP did not bind to any of these proteins. However, in the presence of PEt, CRP bound to all of these proteins. As a representative, we present here the data on the binding of CRP to A β (Fig. 1A).

During our attempts to understand the PEt phenomenon, we got hints that the addition of PEt to 10 mmol/l Tris-HCl, 150 mmol/l NaCl, pH 7.2 (TBS) containing 0.1% gelatin, 0.02% Tween-20 and 2 mmol/l CaCl₂ (TBS-Ca), the buffer which we used in the experiments, might be lowering the pH, and, indeed, that was the case (Fig. 1B). Accordingly, we determined the binding of CRP to E-LDL as a function of pH and found that the binding of CRP to E-LDL was dramatically enhanced at acidic pH (Fig. 1C). Additional experiments confirmed that the previously reported enhancement in the binding of CRP to E-LDL in the presence of PEt was solely due to acidic pH and not due to the binding of PEt to CRP.

At acidic pH, CRP also bound to many other proteins that we examined so far, including A β (Fig. 1D). The binding of CRP to an array of unrelated proteins, at acidic pH, was puzzling. We hypothesized that CRP, at acidic pH, recognized a pattern. We noticed that the only component that was common in our experiments was the technique, the ELISA-based solid-phase ligand-binding assay, in which we immobilized the protein ligands on polystyrene microtiter wells (see legend to Fig. 1). Because the immobilization of proteins results in

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conformational alteration of proteins, in addition to deposition and aggregation of proteins on the walls of the wells, our data suggest that, at acidic pH, CRP binds to deposited, aggregated and conformationally altered proteins, irrespective of the identity of the protein. If this interpretation is correct, then the enhancement seen in the binding of CRP to E-LDL at acidic pH is likely due to the property of CRP to bind to deposited, aggregated and conformationally altered proteins. It is important to note, however, that the acidic pH is not a requirement for the binding of CRP to E-LDL and that CRP prevents formation of E-LDL-loaded macrophage foam cells at normal physiological pH [2,3]. It is also important to note that when sucrose density gradient ultracentrifugation was used to evaluate the binding of CRP to native LDL in serum, in the presence of PEt, and hence at acidic pH, CRP bound to native LDL, but not to HDL [1].

Collectively, our findings suggest that CRP has two native pentameric states: one at normal physiological pH and another at acidic, pathological pH. At acidic pH, CRP changes its conformational state to gain functions distinct from the functions of CRP at normal physiological pH. The significance of the binding of CRP, at acidic pH, to deposited, aggregated and conformationally altered proteins is currently under investigation.

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Fig. 1.

CRP is functional at acidic pH. (A) CRP binds to amyloid β protein fragment 1-38 (A β) in the presence of phosphoethanolamine (PEt). Microtiter wells were coated with 10 μ g/ml of A β in 10 mmol/l Tris-HCl, 150 mmol/l NaCl, pH 7.2 (TBS). The unreacted sites in the wells were blocked with TBS containing 0.5% gelatin. CRP, in increasing concentrations, with and without 10 mmol/l PEt, diluted in TBS containing 0.1% gelatin, 0.02% Tween-20 and 0.1 mmol/l CaCl₂ (TBS-Ca), was then added to the wells. After incubation for 2 h at 37°C, the wells were washed with TBS-Ca. Bound CRP was detected using an affinity-purified polyclonal anti-CRP antibody, as described previously [2]. (B) Addition of PEt to TBS-Ca decreases the pH of TBS-Ca. (C) Binding of CRP to E-LDL is enhanced at acidic pH. Microtiter wells were coated with 10 μ g/ml of E-LDL in TBS. After blocking, CRP (10 μ g/ml), diluted in TBS-Ca adjusted to various pH, was added to the wells. After incubation for 2 h at 37°C, bound CRP was detected as in A. (D) CRP binds to A β at acidic pH. As in C except that the microtiter wells were coated with 10 μ g/ml of A β .