GLYCATION LIGAND BINDING MOTIF IN LACTOFERRIN

Implications in Diabetic Infection

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1. SUMMARY

Lactoferrin and lysozyme are two important, naturally occurring antibacterial proteins found in saliva, nasal secretions, milk, mucus, serum and in the lysosomes of neutrophils and macrophages. Both proteins bind specifically to glucose-modified proteins bearing advanced glycation endproducts (AGEs). Exposure to AGE-modified proteins blocks the bacterial agglutination and bacterial killing activities of lactoferrin and also inhibits the bactericidal and enzymatic activity of lysozyme. Peptide mapping by AGE ligand blot revealed two AGE-binding domains in lactoferrin, and a single AGE-binding domain in lysozyme. None of these AGE-binding domains displayed any significant homology in their primary sequences; however, a common 17–18 amino acid cysteine loop motif (CX_{15-16}C) was identified among them, which we named an ABCD motif (AGE-Binding Cysteine-bounded Domain). Similar domains are also present in other antimicrobial proteins such as defesins. Hydrophilicity analysis indicated that each of these ABCD loops is markedly hydrophilic. Synthetic peptides, corresponding to these motifs in lactoferrin and lysozyme, exhibited AGE-binding activity. Since diabetes is associated with abnormally high levels of tissue and serum AGEs, the elevated AGEs may inhibit endogenous antibacterial proteins by binding to the conserved ABCD motif, thereby increasing susceptibility to bacterial infections in diabetic individuals. These results may provide a basis for the development of new approaches to prevent diabetic infections.

2. ANTIBACTERIAL ACTIVITY OF LACTOFERRIN

There is a large body of evidence supporting for an important role for lactoferrin in general defense against bacterial and viral infections. Lactoferrin together with lysozyme
are first line defense proteins in certain body compartments such as the nasal cavity and the bronchi. Plasma lactoferrin has been recognized as a marker of infection in elderly individuals. Apparently, lactoferrin has a broad spectrum of non-specific defense functions. It can kill bacteria directly or inhibit the entry of bacteria into cells. The defense functions of lactoferrin have been demonstrated on various microorganisms in vitro, including *Candida albicans*, * Legionella pneumophila*, *E. coli*, * Helicobacter pylori*, herpes simplex virus, cytomegalovirus and *Toxoplasma gondii* parasites. Lactoferrin binds directly to the bacterial surface and interacts with lipopolysaccharide. The antibacterial activity of lactoferrin is located in the N-terminal region.

3. DIABETES AND GLUCOSE-MODIFIED PROTEINS

Proteins spontaneously react with reducing sugars such as glucose in non-enzymatic reactions to form AGEs. Although the exact chemical structure of most AGEs remains unknown, they comprise a heterogeneous group of biologically reactive molecules that can be detected and quantitated by immunological methods. AGEs have been implicated in tissue remodeling, the induction of growth factors and cytokines, and atherosclerosis. Both insulin dependent diabetes mellitus and non-insulin dependent diabetes mellitus patients, as well as aging individuals show increased levels of serum AGEs, and tissue AGEs accumulate at an accelerated rate in vascular and renal tissues of diabetic patients. Because of their biochemical properties and distribution, AGEs are considered important factors in the pathogenesis of diabetic and aging complications.

4. AGE-BINDING PROTEINS

AGE-specific cell-surface binding proteins have been identified on many cell types, including phagocytic and mesenchymal cells. They were first identified on macrophages and characterized to mediate the uptake and degradation of AGEs. Interaction of AGE-modified proteins with the macrophage AGE-binding proteins not only serves to degrade AGE-proteins, but also to induce synthesis and release of cytokines and growth factors, suggesting a dual purpose: disposal of senescent AGE-modified molecules and initiation of tissue repair and protein turnover. An 80 kDa AGE-binding protein has also been isolated from pulmonary tissues while searching for AGE receptors, that was further showed to be identical to lactoferrin. It has been proposed that lactoferrin together with other AGE-binding proteins may participate in endothelial cell activation and the functional enhancement of procoagulant activity and vascular permeability. Recently, we found that lysozyme binds AGEs with a high affinity. It is interesting to us that the two AGE-binding proteins lactoferrin and lysozyme share a common biological activity—general defense.

5. COMPETITION OF AGE-BINDING BY PROTEOGLYCANS AND SUGAR CONJUGATES

Defense proteins, such as lactoferrin and lysozyme, are a group of anti-microbial proteins present at a high level in saliva, nasal secretions, mucus, serum and in the lysosomes of neutrophils and macrophages. They usually bind to and kill bacterial though