Surface Plasmon Resonance Imaging Studies for Proteolytic Hydrolysis of Proteins

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Hydrolysis of various proteins was simultaneously studied using a surface plasmon resonance imaging technique. Poly-L-lysine has been found to be completely resistant to hydrolysis, while bovine serum albumin, casein, and lactate dehydrogenase are hydrolyzed at different rates under the attack of pepsin.

To study the degree of digestibility in stomach, considerable efforts were devoted to the investigation of protein hydrolysis in vitro.[1,2] As an enzyme in stomach, pepsin initiates the digestion of proteins by splitting them into smaller pieces, and it is also used in the preparation of cheese and other protein-containing foods.[3] In addition, pepsin generally digests proteins during the proteomic analysis, converting protein to peptide in order to infer the sequence of amino acid in the peptide chain.[4] It has been shown that pepsin is most efficient in cleaving bonds involving aromatic amino acids, phenylalanine, tryptophane, and tyrosine.[5] Many methods have been developed to study the proteolysis,[6,7] but it is difficult to simultaneously study hydrolytic processes of various proteins in real time under the same system. As a high throughput surface plasmon resonance (SPR) technique, SPR imaging has been known for years as a very useful means for detecting biomolecular interactions[8–10] and sensing DNA and protein.[11–15] In this study, we employed SPR imaging to study the proteolysis of proteins and in situ monitor the hydrolysis process.

Bovine serum albumin (BSA), poly-L-lysine (PL, MW, 14,000), casein, and lactate dehydrogenase (LDG) were chosen in this study. A thin gold film (47 nm) with an underlayer of chromium was first modified with a self-assembled monolayer (SAM) of a carboxylic acid-terminated alkanethiol mercaptoundecanoic acid (MUA). SAM was immersed into an aqueous solution consisting of 1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide hydrochloride and *N*-hydroxysuccinimide (NHS) to form NHS ester monolayer. The proteins at the same concentration (0.1 mg/mL) were spotted the modified gold surface, where the proteins were immobilized by reacting with NHS ester monolayers.[16–18] The rest part of surface, without the immobilization of proteins, was then blocked by ethanolamine. The prepared protein array was fixed on the bottom of prism of SPR imaging setup and then SPR images were recorded with computer in real time.[19] Obtained image of protein arrays is shown in Figure 1, where the degree of reflected intensity indicates various SPR responses to the corresponding proteins. The proteins are observed as bright spots with different reflected intensity.

Proteins can be cleaved under the attack of proteolytic protease. Thus, the reflected intensity varied with the reaction time when protein array was exposed to 2 mg/mL pepsin solution (pH 1.5) at room temperature. The intensity obviously decreased with the protein array being exposed to pepsin solution, and especially, casein and LDG decreased rapidly to 50 and 70% within 1 min, respectively. The degrees of proteolysis were investigated by successive incubation in the pepsin solution. After 15 min of incubation, with the breaking of a few peptide bonds, an irreversible unfolding occurred, which led to more exposed peptide bonds and the polypeptide chains were, therefore, extensively degraded to small peptides. The reflected intensity of BSA decreased to 50%, and casein and LDG continuously decreased; as a result, some bright protein spots became dark spots. Further increasing the hydrolysis time to 25 min, the bright spots of casein and LDG completely disappeared and replaced by dark spots as shown in Figure 2. After long scale (11 h), no changes of the reflected intensity could be monitored by SPR imaging and the spots of BSA became very dim. In comparison with the relatively rapid hydrolysis of casein and LDG, no change of the reflected intensity of poly-L-lysine was observed. No significant cleavage of covalently attached poly-L-lysine chain was actually found, strongly indicating that the poly-L-lysine could not be cleaved by the pepsin under the experimental conditions used in this study. Pepsin exhibits a rather large specificity for peptide bonds.[20] To the best of our knowledge, no hydrolysis of poly-L-lysine has previously been observed. The rate of proteolysis was different under this condition; LDG has the highest rate, while BSA has the lowest. Figure 3 shows the hydrolytic

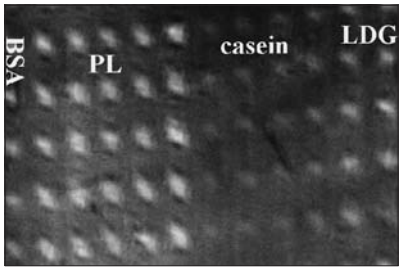

Figure 1. In situ SPR image in a phosphate buffer solution. From left to right: BSA (line 1), PL (line 2–6), casein (line 7–10), and LDG (line 11 and 12).

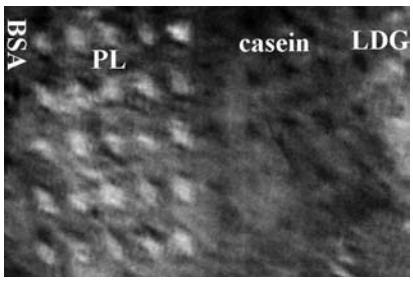

Figure 2. In situ SPR image taken from the immobilized proteins after being interacted with pepsin solution for 11 h.

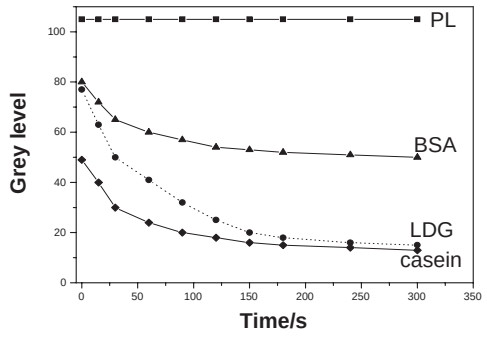

Figure 3. The changes in reflected intensity of casein, LDG, BSA, and PL during enzymatic cleavage vs. time.

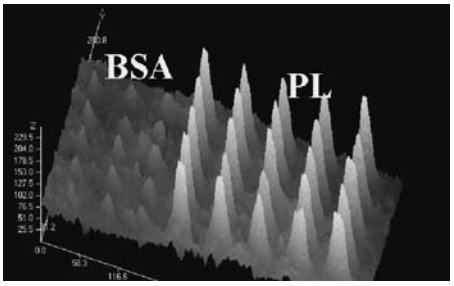

Figure 4. The 3-D SPR image taken in the phosphate buffer solution after being interacted with pepsin. The left 3 lines are BSA and the right 5 lines are poly-L-lysine. *x* and *y* represent the distance of protein spots and *z* represents the reflected intensity.

rate of these proteins, where the grey level elucidates the degree of reflected intensity. The higher grey level, the more reflected intensity. It probably suggests that more peptide bonds of LDG and casein are susceptible to hydrolysis than those in BSA. After hydrolyte being released from the proteins, some remained ones still linked on the gold surface. As we know, the SPR response is related with the amount of adsorption onto gold surface;[21] thus, reflected intensity could show the chain length of remained peptide on the gold surface on certain scale. As seen in Figure 2, the dark spots of LDG and casein show that LDG and casein were almost completely hydrolyzed, but there were still some short peptide chains linking on the gold surface.

Changing the incident angle changes SPR signal. At another incident angle which decreased 0.053° than the previous one, the spots of LDG and casein could barely be observed. However, a better contrast of BSA SPR image was obtained. Figure 4 shows a 3-D SPR image of BSA and poly-L-lysine after adjusting incident angle, it indicates that there were still long peptide chains remained on the gold surface after prolonged hydrolysis of BSA.

During the process of proteolysis, pepsin attacked the peptide bonds of proteins and some peptide bonds were broken. But not all peptide bonds of peptide linkage can be easily broken by pepsin. For instance, synthetic substrates containing Ile–Phe bond have been found to be completely resistant to cleavage by pepsin.[22] Proteins were covalently attached to gold surface by the linkage of peptide bond through the carboxyl group coupling with residue amine group of protein. However, the peptide bond on the gold surface was not broken, thus, the remained peptide still linked on the gold surface as the hydrolyte of protein. The above results have demonstrated that the approach of immobilizing protein in studying proteolysis is feasible.

In summary, a simple and convenient method has been developed to monitor the protein hydrolysis on a gold sruface in large-scale by utilizing SPR imaging. This high throughput method would provide very useful information to study the interaction among biological macromolecules.

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