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Old products, new applications? Considering the multiple bioactivities of plastein in peptide-based functional food design

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For over a century, plastein has remained mostly cryptic with inconclusive proof of their formation mechanisms. Although initial interest in the nutritional application of plastein decreased by the mid-1980s, the last decade has seen a reemergence of plastein as bioactive agents. It is thought that sequence variations due to protease-induced peptide modification during plastein formation are associated with the enhancement of bioactivities such as angiotensin converting enzyme inhibition (antihypertensive), calcium-chelating (anticoagulation), antioxidant and cytoprotective activities. Moreover, the clustering of hydrophobic residues during peptide aggregation has enabled exploration of plastein as peptide debittering agents, and as a bile acid sequestrant with potential endogenous cholesterol-reducing role. This article presents plastein from different perspectives with emphasis on their chemistry, bioactivity and prospective functional food applications.

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Introduction

Proteases hydrolyze peptide bonds of proteins resulting in low-molecular-weight peptide and amino acid products. However, higher concentrations of the hydrolyzed proteins or peptides, when incubated with the appropriate protease, can result in the formation of a less soluble aggregated macromolecular structure known as 'plastein'. First discovered in 1886, the precise mechanism of plastein formation has remained unclear throughout the turn of the century. Although some proposed formation mechanisms have been recently described in the literature [1••], a number of studies are contradictory and the role of proteases in the process remains ambiguous. Conclusive evidence is absent as of yet to validate whether plastein formation is an entropy-driven and hydrophobicity-driven process, or a protease-assisted covalent modification mechanism, or both. Essentially, the plastein preparation involves three steps [1••]:

(i) Low concentration (3–5%, w/v) protein hydrolysis.
(ii) Concentrating protein hydrolysates *via* lyophilization.
(iii) Incubation of concentrated (30–50%, w/v) hydrolyzed protein with the appropriate protease.

During the second half of the last century, there was tremendous interest in the nutritional application of plastein [2–4]. However, the challenges associated with high input and low yields appear to have limited their commercial application. Rekindled research efforts toward plastein in the last decade have focused on plastein bioactivity and possible health benefits.

Highlights of plastein design and properties

Some studies have referred to the key roles of new covalent bond formation during plastein reaction, and others reported a formation model based on hydrophobic interactions. Andrews and Alichanidis found that plastein reaction is a purely aggregation mechanism [5]. In their study, no significant qualitative differences was observed in the composition of the starting material (hydrolysates) and product (plastein) using small angle neutron scattering and gel filtration. In contrast, some studies reported that transpeptidation and condensation are the major mechanisms that drive plastein formation [2–4,6]. Using synthetic dipeptides as a model system, Stevenson and colleagues demonstrated that peptide bond synthesis can occur with proteases resulting in the formation of aggregate products consisting of newly formed oligopeptides [6]. Earlier, Tanimoto *et al.* [7] identified the role of a serine residue, located in α-chymotrypsin catalytic site, in forming peptidyl-enzyme ester during plastein reaction. The peptide–enzyme intermediate is susceptible to nucleophilic attack by the free amino group of other peptides, with a histidine residue in the catalytic site of the enzyme helping in the process. In 1972, Hofsten and

Lalasidis described the reactions that occur during plastein formation as a type of rearrangement process in which the reaction equilibrium shifts based on the generation of peptides, tending toward the formation of insoluble complexes [8]. However, it can be argued that these complexes can also be generated as a result of hydrophobic interactions.

A recent study by Liu and colleagues on Alcalase-induced plastein formation from whey protein hydrolysates demonstrated that the peptide aggregation followed a two-step mechanism [9•] as follows (Figure 1):

(i) The peptide building blocks for aggregation are initially formed *via* transpeptidation, resulting in the formation of hydrophobic cores with stable conformations. This is a variable step observed to occur between 30 min [9•] and 3 hours of reaction [10];
(ii) Once formed, the building blocks then interact with one another and aggregate with time to form plastein, and no new blocks are formed at this stage.

The protease-induced modification of peptides during plastein reaction has been correlated with a decrease in free amino nitrogen [11]. This is suggestive of the synthetic role of proteases under appropriate conditions. Higher temperatures during plastein reaction were observed to increase plastein formation rate, but it also resulted in decreased plastein yield [12]. The protease used in initial protein hydrolysis is recommended for use in plastein generation [12]. Using a different protease can lead to further hydrolysis, which is not considered apt for increased plastein yield. Furthermore, substrate concentration plays a major role in plastein reaction. Exponential increase in plastein yield was observed with an increase in substrate concentration [13], and lower concentrations of substrate resulted in further hydrolysis instead of aggregation [14].

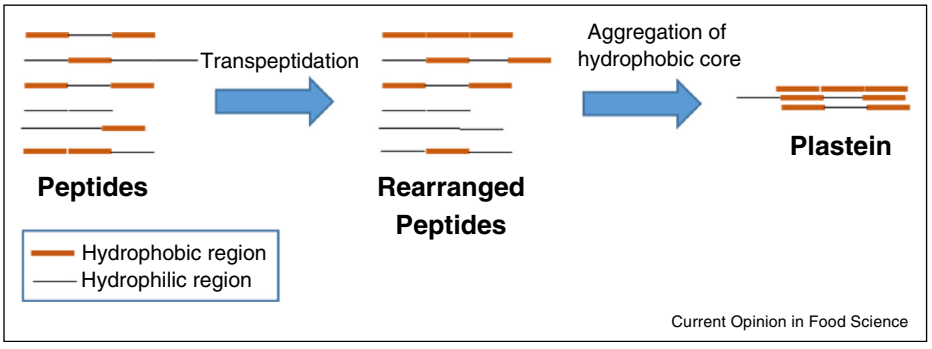
Emerging evidence of plastein bioactivity

Plastein recently reemerged in the literature with the initial report of bioactivity by Zhao and Li in 2009 [11]. Since then, plastein has been produced and assessed for a number of biological activities [15,16•,17,18,19•,20–23]. Although peptides are known to exhibit bioactivities due to their inherent amino acid sequence and composition [24], it is increasingly becoming apparent that structure modification by plastein reaction can lead to enhanced peptide bioactivity (Figure 2).

Metal chelating capacity

Metal chelating agents can serve as nutrient delivery, antioxidative, antimicrobial and anticoagulating agents, making them valuable in a wide range of applications. Recently, *in vitro* anticoagulation and anti-platelet aggregation have been reported for soy protein-derived plastein, and the aggregates were thought to act *via* calcium chelation [18]. The study found that the protease-modified soy hydrolysates had higher calcium chelating activity and inhibited calcium precipitation than the parent hydrolysate. It is worth noting that, for the antithrombotic property to be replicated *in vivo*, the plastein would need to be absorbed intact into systemic circulation. This constitutes a major constraint in translating plastein as a physiologically functional calcium chelator considering its large structure, which will impede transport across the enterocytes. Contrary to the findings, our group recently reported that plastein reaction led to a decrease in iron(II)-chelating capacity of casein hydrolysates [16•]. We thought that this could have resulted from inaccessibility of the metal-binding residues within the peptide aggregate structure for coordinate complex formation. Moreover, the amount of negatively charged amino acid (aspartate and glutamate) residues were found to decrease following plastein reaction [9•,19•]. This can partly explain the reduced capacity of the casein plastein to chelate iron(II), since the anionic amino acid residues can contribute to metal binding *via* ionic bonding. The mechanism behind the enhanced calcium-chelating capacity of casein plastein reported by Zhang and Zhao [18] remains unclear, especially considering that the aggregates were found to possess weaker surface anionic charge

Figure 1



The two-step process proposed for plastein formation involving transpeptidation and hydrophobicity-induced peptide aggregation.