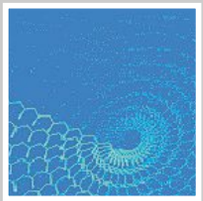
doi:10.2533/chimia.2013.685



Polymer and Colloid Highlights

Division of Polymers, Colloids and Interfaces
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Polymer–Enzyme Conjugates for Oral Drug Delivery Applications

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Keywords: Celiac disease · Mucoadhesion · Oral enzyme therapy · Polymer conjugation

Proteins, in particular enzymes, are structurally and functionally complex entities that have important pharmaceutical applications due to their specificity and high activity.[1] However, when administered orally, they can be rapidly inactivated by the denaturing conditions encountered in the gastrointestinal (GI) tract. While the stabilization of systemically administered proteins has been widely investigated, comparable efforts for proteins designed to be active in the GI tract are lacking. This is in part due to the difficulty of stabilizing a protein in an environment designed to promote its biodegradation, and, until recently, to the absence of convenient tools for monitoring enzyme activity in real time in the GI tract.

Our laboratory has recently developed a non-invasive method for monitoring the activity of proline-specific endopeptidases (PEPs) in real-time in the GI tract.[2] These enzymes are of interest as a potential adjuvant therapy for celiac disease, a severe autoimmune-based illness of the small intestine for which no pharmacological treatment is currently available.[3] For their successful use, PEPs should degrade immunogenic gluten peptides in the upper part of the GI tract, before the latter can reach the sensitive areas of the intestine. Unfortunately, orally administered PEPs are rapidly denatured in the stomach with concomitant loss of activity.[2]

To address this challenge, we have investigated the protection and retention of PEPs in the GI tract using functional polymers.[4] The conjugation of polymers to orally administered proteins has thus far only received minimal attention. Architecturally and functionally diverse polymers were evaluated to sterically protect enzymes from inactivation and to promote interactions with stomach mucin. A first generation dendronized polycation (PG1), methoxy poly(ethylene glycol) (mPEG), and poly(acrylic acid) (PAA) were conjugated to PEPs and their activity and transit evaluated *in vivo* (Fig. 1).

An exceptional enhancement of the *in vivo* performance of PEPs was achievable, both in the stomach and/or in the small intestine. PG1 strongly stabilised PEPs in the stomach, and preserved their activity at this location, due to mucoadhesion, for up to 3 h following administration. In contrast, mPEG and PAA did not promote mucoadhesion and their corresponding conjugates rapidly transited to the small intestine without displaying activity in the stomach. Nevertheless, the activity of mPEG-modified PEPs was partly regained in the small intestine.

These findings provide new insights for the development of novel therapeutic strategies based on orally administered proteins using a simple and accessible technology. Polymer functionality was demonstrated to be a key factor influencing the properties of protein–polymer conjugates, which permitted their stabilization and/or retention at different locations in the GI tract.

Received: July 4, 2013

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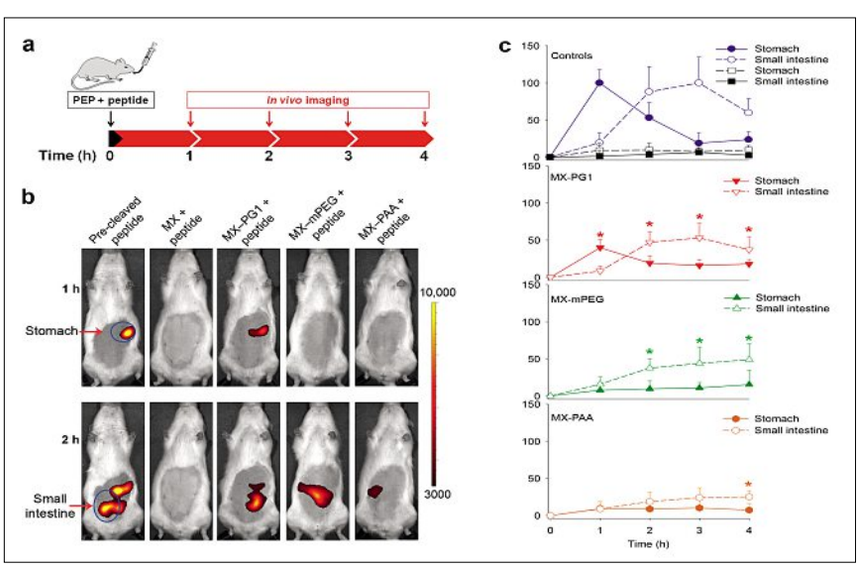

Fig. 1. *In vivo* activity of a PEP from *Myxococcus xanthus* (MX) and MX–polymer conjugates. Activity was assessed by administering a non-fluorescent peptide that becomes fluorescent upon hydrolysis by PEPs. (a) Experimental timeline for assessing *in vivo* activity after oral administration of MX or MX–polymer conjugates.[2] (b) Images show the evolution of the fluorescent signal (related to PEP activity) throughout the GI tract. (c) Relative fluorescence signal in the region-of-interest of the stomach and the small intestine with time determined from images like those in (b) (n = 6–9). Control samples were a pre-hydrolyzed peptide (blue circles) and native MX (black circles). MX–PG1 was the only conjugate to be active in the stomach. MX–mPEG was only active in the small intestine. The signal produced by MX–PAA was weak, and only different from native MX at 4 h. Figure reproduced with permission from *Nat. Chem.* **2013**, *5*, 582.