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Nanostructuring Biomaterials with Specific Activities towards Digestive Enzymes for Controlled Gastrointestinal Absorption of Lipophilic Bioactive Molecules

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ABSTRACT

This review describes the development of novel lipid-based biomaterials that modulate fat digestion for the enhanced uptake of encapsulated lipophilic bioactive compounds (*e.g.* drugs and vitamins). Specific focus is directed towards analysing how key material characteristics affect the biological function of digestive lipases and manipulate lipolytic digestion. The mechanism of lipase action is a complex, interfacial process, whereby hydrolysis can be controlled by the ability for lipase to access and adsorb to the lipid-in-water interface. However, significant conjecture exists within the literature regarding parameters that influence the activities of digestive lipases. Important findings from recent investigations that strategically examined the interplay between the interfacial composition of the lipid microenvironment and lipolysis kinetics in simulated biophysical environments are presented. The correlation between lipolysis and the rate of solubilisation and absorption of lipophilic compounds in the gastrointestinal tract (GIT) is detailed. Greater insights into the mechanism of lipase action have provided a new approach for designing colloidal carriers that orally deliver poorly soluble compounds, directly impacting the pharmaceutical and food industries.

Contents

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1. Introduction

Lipases are digestive enzymes that catalyse the hydrolysis of fat (lipid, triglyceride) into free fatty acids (FFA) and monoglycerides, molecules that can be absorbed into the bloodstream [1]. Lipases are water soluble enzymes that are active at the oil — water interface. In aqueous environments their active sites are covered by an α-helical lid (or polypeptide flap) that renders the enzyme unable to bind to isolated lipid molecules. Lipase can only hydrolyse lipids efficiently if they are present in a separate phase, such as a fat (oil) droplet. Adsorption of lipase to the oil-water interface causes the lid domain to open and exposes the active site and hence initiates lipid hydrolysis [2]. Consequently, lipid digestion kinetics can be altered by intelligent design of the triglyceride-water interface. A number of techniques have been employed to control lipid digestion, including altering lipid surface area [3,4], interfacial composition [5,6] and designing carrier particles for lipase encapsulation [7,8]. Despite this, mechanistic understanding of lipase action is still lacking and there is a high level of speculation in the literature regarding the role of key parameters on catalytic activity.

Understanding and controlling lipase-mediated digestion is of great importance for the rational design of intelligent food delivery systems and lipid-based drug formulations. Obesity is a rapidly growing concern worldwide, with over 30% of the global population are classified as overweight and obese [9]. Lipid digestion is fundamental for the absorption of fat, as well as encapsulated bioactive lipophilic compounds [10]. Therefore, the regulation of lipid bioavailability is receiving increasing attention in order to successfully manage the nutritional value of our food and combat chronic diseases, such as obesity, diabetes and heart disease [11–13]. Inhibiting lipid digestion is considered an effective means to reduce appetite and promote satiety [14]. Thus, the ability to manipulate the rate and extent of lipase-mediated digestion through colloidal engineering of the lipid-water interface provides a way to regulate excess fat absorption and energy intake.

Furthermore, current estimates suggest that 40–70% of newly discovered drug entities are poorly water-soluble [15]. Lipid-based formulations have emerged as a most promising approach to improve the bioavailability of lipophilic compounds, due to their effectiveness and versatility in enhancing drug solubility [16]. As processing of lipids in the gastrointestinal tract (GIT) is initiated, digestion products are released, stimulating the secretion of bile salts from the gall bladder, which combine together to form mixed micelles and vesicles [17]. These colloidal species further solubilise lipophilic bioactive molecules and promote their absorption into the systematic bloodstream [10]. However, oral lipid-based delivery systems are estimated to account for only 2–4% of the pharmaceutical market worldwide. This is due to a number of factors, including low stability, poor *in vitro-in vivo* correlations and limited control over release of bioactive molecules. This underlines the need for more intensive and systematic investigations on the role of lipid microstructure and interfacial chemistry on lipase activity to optimise digestion and absorption of fat and encapsulated bioactive molecules. To improve the commercial success of lipid-based formulations, greater understanding of the mechanism of lipase action is required, which will in turn provide greater control over *in vivo* drug bioavailability.

2. Lipases: enzymes active at the oil-water interface

Lipases are acyl hydrolases that naturally cleave ester bonds to break down triglycerides into free fatty acids (FFA) and monoglycerides (Fig. 1) [18]. Lipases are responsible for the digestion of nonpolar dietary fats and oils into polar lipids that can be readily absorbed into the bloodstream [19–21]. Due to the difference in polarity between the enzyme and the lipid substrate, lipases are surface active proteins that catalyse reactions at the interface between the aqueous and oil phases [22]. Thus lipase-catalysed reactions can be controlled by the interfacial composition and concentration of triglyceride droplets [23].

Lipases are also of considerable interest to other biotechnology and chemical industries due to their ability to catalyse the reverse, esterification reaction, *i.e.* triglyceride synthesis from alcohols and fatty acids [25]. The equilibrium of a lipase-catalysed reaction is governed by the aqueous content of the reaction medium, as water is a product of the esterification reaction [26]. The catalysis of esterification reactions by lipolytic enzymes presents a number of advantages over conventional chemical synthesis as the high catalytic efficiency and inherent selectivity of the enzymes result in purer products [27].

The high stability and versatility of lipases have made them the most utilised enzymes in biotechnology and synthetic chemistry applications [28]. They have enormous industrial value [29] and are used in a wide range of novel applications such as the synthesis of biopolymers and biodiesel [30]. However, this review will focus on the unique characteristics and features of physiological lipases that enable the controlled and optimised digestion of triglycerides. In this section, the structural aspects of lipases and interfacial mechanism of lipid substrate-enzyme interaction will be reviewed in detail.

2.1. Lipase lid domain structure and interfacial activation

The structural characteristic of most lipases that sets them apart from other esterase enzymes, is their lid domain that shields the active site from the aqueous environment [2]. The closed-lid conformation is inactive and unable to bind to substrate molecules. The catalytic site is mostly hydrophobic and is composed of serine, histidine and aspartic acid residues [31]. Upon interaction with an interface, the lipase enzyme undergoes conformation changes in its tertiary structure, opening the lid and exposing the nonpolar catalytic residues to the aqueous environment (Fig. 2) [32]. This structural change, known as 'interfacial activation', facilitates the adsorption of substrate molecules to the active site.

As a result of this interfacial activation mechanism, lipase-catalysed reactions are controlled by the quality and quantity of substrate at the interface [33]. Without an interface, lipase is unable to adsorb to substrate molecules due to the differences in polarity. It was determined several decades ago that lipase enzymes exhibit little to no activity when substrates are in a monomeric state or solubilised in the aqueous phase [34]. Once the substrate is added to an aqueous phase at concentrations above its solubility limit, lipolytic activity increases significantly due to the formation of emulsion droplets, which introduce an interface for lipase activation [35]. Thus, the activity is independent of molar concentration, but controlled by the concentration of substrate at the interface [36]. This is in contrast to other enzymes, such as other esterases, that act on water-soluble substrates with a Michaelis-Menten type dependence on the substrate concentration [37].

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