DENTAL MATERIALS 30 (2014) 848–860

Available online at www.sciencedirect.com

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Biodegradation of composite resin with ester linkages: Identifying human salivary enzyme activity with a potential role in the esterolytic process

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ARTICLE INFO

Article history:
Received 5 March 2014
Received in revised form
20 May 2014
Accepted 21 May 2014

Keywords:
Composite resin
Saliva
Albumin
Zinc-α2-glycoprotein
Esterase
Biodegradation
Hydrolysis
BisGMA
Methacrylic acid

ABSTRACT

Objectives. The ester linkages contained within dental resin monomers (such as Bisphenol A-glycidylmethacrylate (BisGMA) and triethylene glycol dimethacrylate (TEGDMA)) are susceptible to hydrolytic degradation by salivary esterases, however very little is known about the specific esterase activities implicated in this process. The objective of this work was to isolate and identify the dominant proteins from saliva that are associated with the esterase activities shown to be involved in the degradation of BisGMA.

Methods. Human whole saliva was collected and processed prior to separation in a HiPrep 16/60 Sephacryl S-200 HR column. The fraction with the highest esterase activity was further separated by an anion exchange column (Mono-Q (10/100G)). Isolated fractions were then separated by gel electrophoresis, and compared to a common bench marker esterase, cholesterol esterase (CE), and commercial albumin which has been reported to express esterase activity. Proteins suspected of containing esterase activity were analyzed by Mass Spectroscopy (MS). Commercially available proteins, similar to the salivary esterase proteins identified by MS, were used to replicate the enzymatic complexes and confirm their degradation activity with respect to BisGMA.

Results. MS data suggested that the enzyme fraction with the highest esterase activity was contained among a group of proteins consisting of albumin, Zn-α2-glycoprotein, α-amylase, TALDO1 protein, transferrin, lipocalin2, and prolactin-induced protein. Studies concluded that the main esterase bands on the gels in each fraction did not overlap with CE activity, and that albumin activity emerged as a lead candidate with significant esterase activity relative to BisGMA degradation, particularly when it formed a complex with Zn-α2-glycoprotein, under slightly basic conditions.

Significance. These enzyme complexes can be used as a physiologically relevant formulation to test the biostability of composite resins.

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http://dx.doi.org/10.1016/j.dental.2014.05.031
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1. Introduction

An increasing demand for esthetic restorations, continued public concern over the release of mercury from amalgam fillings, the improved mechanical properties of composite resin materials, as well as more and improved adhesive resin systems have fueled the use of composite resins in dentistry [1,2].

The combination of BisGMA and the diluent monomer TEGDMA remain two of the most common composite resin monomers still in use today due to their many advantages with respect to rapid hardening within the oral environment and ease of handling and manipulation. Both monomers contain ester linkages linking Bis-phenol-A and triethylene glycol segments to polymerizable vinyl segments. However, it is now well recognized that these ester groups are highly susceptible to hydrolysis by salivary enzymes, and can produce toxic and/or pro-biotic products such as methacrylic acid (MA), triethyleneglycol (TEG) and bishydroxypropoxyphenylpropane (BisHPPP) [3,4]. Concern over these products and their influence on the function of host cells (for example cell morbidity, cellular adhesive function and inhibition of intracellular biomolecular synthesis) and microorganisms [3–8] has been raised over the past few decades, however, there has been very little work directed towards adapting the materials toward addressing these challenges. Rather the focus of material development has remained primarily on the mechanical function of the materials [4]. One study has shown that the residue from a bis-phenol diglycidyl ether used in dental composites has resulted in allergic reactions, and much controversy has arisen around the issue of the estrogenic cell response to bisphenol A derivatives [3,9,10]. The biodegradation of composite resins has also been reported to lead to a softening of the surface layers of resins and predispose the material to mechanical wear during mastication. Once the softened surface layers are removed, newly exposed material would be prone to further degradation and this can finally lead to the failure of interfacial structures at the margins of the restoration [3,5,8,11].

Human whole saliva is a complex mixture with salivary proteins and food residues. Salivary proteins have many functions such as digestive, protective, calcium and mineral homeostasis, antibacterial and antifungal function, protease inhibition, lubrication [12]. There are only a very small number of esterases reported to be found in saliva and these are typically involved in food hydrolysis and more recent years are suspected to be involved in the degradation of composite resins. Studies by the Santerre group have demonstrated that human saliva exhibits cholesterol esterase-like (CE) and pseudocholinesterase (PCE) hydrolase activities, which are able to degrade composite resin components [5,13]. However, there is still very little information published on the actual salivary enzyme proteins related to the CE-like components which are involved in the in vivo process of composite degradation.

In order to mimic salivary esterases and to apply them in assessing new products being developed, leading to enhanced function with respect to the physical stability, ultimate chemical stability of composite resins, and biological character of the degradation products from composite resin interactions with cells and bacteria, the current study has sought to isolate and analyze the predominant components in saliva and to identify the specific source of esterase activity that could be involved in catalyzing the breakdown of polymer resins. In the current study, the commercial monomer BisGMA is used as a model substrate solely for the purpose of identifying sources of esterase activity in saliva relevant to the hydrolysis of esters contained within the resin monomer components of composites. Since the susceptible linkages to chemical hydrolysis in methacrylate based polymers are equivalent to that of the monomeric form in its formulations, using the monomer in solution allows for an accelerated and easy investigation of multiple salivary enzyme species, in addition to minimizing the number of parameters that must be controlled for in the study. For example, it removes considerations of surface area, filler loading, and degree of conversion and cross-linking, all of which can affect the rate of degradation and could hinder the focus of the current study.

2. Materials and methods

2.1. *Preparation of human saliva*

Healthy human whole saliva (20 mLs) was collected in centrifuge tube from 10 donors, 2 h after breakfast and prior to lunch [14]. All human subjects provided informed consent (protocol #8469, reviewed by the human ethics committee at the University of Toronto). The saliva from each donor was thoroughly homogenized with a Polytron PT-2100 tissue homogenizer (Kinematica, Switzerland) for 1 min and centrifuged at 3700 rpm for 1.0 h at 4 °C (Allegra 6R Centrifuge, Beckman Instruments, Mississauga, ON). The supernatant was filtered through 0.8/0.2 μm syringe filters (Acrodisc, NQ105018/1, Pall Corporation, Cornwall, UK) to remove undissolved particulate. The filtrate from each donor was pooled together; quickly frozen by liquid nitrogen and then lyophilized. All dry saliva samples were stored at −78 °C until required.

2.2. *Esterase-like activity (CE) assay*

The CE-like esterase activity from the human salivary fractions was measured at 401 nm on a Beckman Coulter DU800 spectrophotometer (Beckman Coulter Inc., Fullerton, CA, USA, St. Louis, MO, USA) using 4 mM of para-nitrophenylbutyrate (p-NPB) (Sigma–Aldrich, N-9876) as the substrate [14]. One unit of esterase activity was defined as generating 1 nmol/min of p-nitrophenol from p-nitrophenylbutyrate.

2.3. *Isolation of proteins from saliva*

Saliva samples were reconstituted to a volume ratio of 10–1 with 40 mM sodium phosphate buffer, pH 7.2. Reconstituted saliva (1.5 mL) was centrifuged at 14,000 rpm for 15 min. The supernatant was injected into a High Performance Liquid Chromatograph system (Waters^{TM} 600 Controller, 996 Photodiode Array Detector) with a HiPrep 16/60 Sephacryl S-200 HR gel filtration column (Amersham Biosciences, 17-1166-01, Baie d'Urfé, PQ, Canada). Gel filtration was performed with 40 mM sodium phosphate buffer, pH 7.2, and a flow rate of

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