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One-shot method for purification of multiple natural amelogenin isoforms

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ABSTRACT

Amelogenin isoforms constitute the predominant component in the enamel matrix and each amelogenin isoform executes unique role in the enamel biomineralization process. Enamel matrix derivative enriching amelogenin isoforms have also bioactive property for tissue regeneration. Despite the development of recombinant protein technology that has greatly forwarded the understanding of amelogenin properties, substantial evidences have revealed biochemical and functional difference between natural amelogenins and their recombinant form. To facilitate the study of enamel formation mechanism, more facile methodology to purify multiple natural amelogenin isoforms is pursued. Here we developed an effective one-shot method via reverse phase high-performance liquid chromatography (RP-HPLC) to purify various amelogenin isoforms from pig-derived amelogenin complex. A thorough process of chromatographic condition establishment including sample analysis on analytical scale and chromatographic condition design on preparative scale was described. Three representative amelogenin isoforms (TRAP, P148, P173) were isolated in one step and their purity was confirmed by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) and high resolution mass spectrometry.

1. Introduction

Enamel is a typical biological mineralization material with excellent physicochemical property. The development of enamel is a complicated process under the regulation of enamel matrix. Amelogenins, composing more than 90% of enamel organic matrix, play an important role in formation of enamel [1]. Amelogenins are a series of proteins derived from amelogenin gene by alternative splicing expression or proteolysis of the full-length isoform. At least 15 amelogenin isoforms have been identified [2,3]. These amelogenin isoforms differ in their supramolecular assembly property and crystal growth modulation [4–8]. The quantity and proportion of these isoforms continuously evolve with the concurrent growth of enamel crystals during the enamel formation [9], suggesting a specific role of each isoform in enamel biomineralization. In particular, full-length amelogenin is secreted by ameloblast during the early stage of mineral deposition and then is processed in a stepwise and controlled manner at its C terminus, resulting in a number of proteolytic products including the most abundant isoform lacking the hydrophilic carboxy-terminal motif (P148 or "20 kDa" in the case of porcine). Porcine amelogenins are also extracted and used for regeneration of periodontal tissue and show extensive clinical successes, but the specific bioactive amelogenin isoforms and the mechanism about how they execute their function in periodontal tissue regeneration are still obscure [10–13].

The purification of amelogenin isoforms is vital to characterize their function in enamel biomineralization and the specific activity in periodontal tissue regeneration. But the complexity of components and the close chemical and physical properties of several major fractions in amelogenins family make it difficult to obtain highly purified amelogenin isoforms from developing enamel matrix of animal sources [14,15]. Although the development of recombinant protein technology has enabled the design and purification of recombinant amelogenins that greatly forwards the understanding of their properties [16–20], substantial evidences have revealed the biochemical and functional difference between natural amelogenins and their recombinant form [21–24]. Many efforts have been made to purify natural amelogenins, but their purity is still demanding [25,26]. Mumulidu et al. separated

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P173:MPLPPHPGHPGYINFS^{P}YEVLTPLKWYQNMIRHPYTSYGYEPMGGWLHHQIIPVVSQQTPQSHALQPHHHIPMV
PAQQPGIPQQPMMPLPGQHSMTPTQHHQPNLPLPAQQPFQPQPVQPQPHQPLQPQSPMHPIQPLLPQPPLPPMFSMQS
LLPDLPLEAWPATDKTKREEVD

P148:MPLPPHPGHPGYINFS^{P}YEVLTPLKWYQNMIRHPYTSYGYEPMGGWLHHQIIPVVSQQTPQSHALQPHHHIPMV
PAQQPGIPQQPMMPLPGQHSMTPTQHHQPNLPLPAQQPFQPQPVQPQPHQPLQPQSPMHPIQPLLPQPPLPPMFS

TRAP: MPLPPHPGHPGYINFS^{P}YEVLTPLKWYQNMIRHPYTSYGYEPMGGWL

Scheme 1. Amino acid sequences of P173, P148 and TRAP.

5 kDa component of enamel matrix derivative [27]. Yamakoshi et al. systematically investigated and separated a number of components from porcine amelogenin complex [28,29]. Both of the studies used a combination of technologies and multiple steps. To reduce the laborious and complicate procedures, a facile method to reproducibly isolate different amelogenin isoforms with high purity is still necessary. In the case of developing porcine enamel, three amelogenin isoforms named as P173, P148 and TRAP are representative for not only their high abundance in different stages of developing enamel matrix but also characteristics of their amino acid sequences [30]. P173 is the full-length amelogenin, which consists of three domains, a hydrophilic C-terminal domain of 25 amino acids, a large hydrophobic central region comprised primarily X-Y-Pro repeat motifs (X and Y are mostly glutamine), and a tyrosine-rich N-terminal domain of 45 amino acids (referred to as tyrosine-rich amelogenin protein, TRAP) [31]. P148 differs from P173 by lacking the C-terminal domain of 25 amino acids. The detailed amino acids sequences of the three isoforms are illustrated in Scheme 1. Unlike their recombinant forms, P173, P148 and TRAP all have the first methionine and a single phosphorylated residue: serine-16 [32].

Here we report a facile method via reverse phase high-performance liquid chromatography (RP-HPLC) to purify amelogenin isoforms from natural pig-derived amelogenin complex in one step. We present the chromatographic condition design and obtained ideal separation of target amelogenin isoforms. Three amelogenin isoforms, P173, P148 and TRAP in high purity were acquired. The methodology in the process of chromatographic condition establishment may also be applicable to other mixture system.

2. Material and methods

2.1. Chemicals and reagents

Guanidine hydrochloride, ammonium sulfate and HPLC-grade trifluoroacetic acid (TFA) were purchased from Aladin Ltd, China. HPLC-grade acetonitrile was purchased from Fisher Scientific, UK. HPLC-grade water was produced by Elix 10 Ultrapure Water System from Millipore, UK.

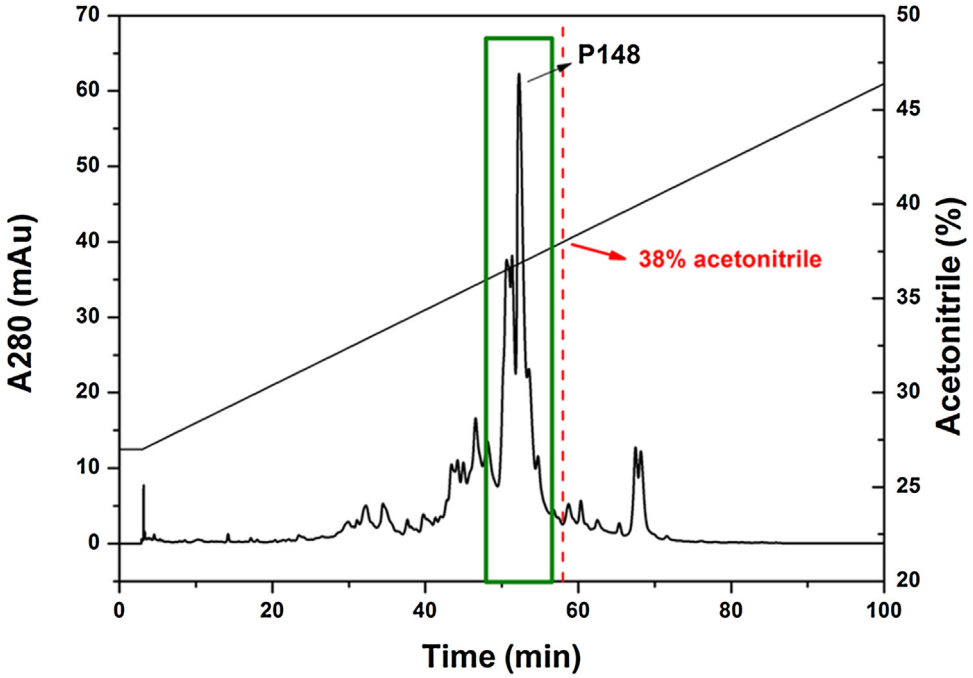

Fig. 1. Chromatogram of the linear gradient elution using the short narrow bore column (27%–47% acetonitrile in 100 min). The amelogenin isoforms mixture including P148 is marked by the green box. This mixture eluted before 38% acetonitrile.

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