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Cloning, Expression and Characterization of the Gene Encoding the Enolase from *Fusobacterium nucleatum*[1]

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Abstract—The gene encoding enolase from *Fusobacterium nucleatum* (*Fn*ENO) was cloned and analyzed for the first time. The gene comprises of 1302 nucleotide base pairs and encodes 433 amino acids. The gene sequence alignment demonstrated the presence of several distinct insertions and deletions, compared with the human enzyme. The gene for recombinant *Fn*ENO was inserted into the pLATE 31 vector system and expressed in *E. coli* BL21(DE3) cells as a soluble protein. The protein was purified by affinity chromatography using a Ni-NTA agarose matrix and shown on SDS-PAGE to be a 46 kDa protein. The molecular weight of the octameric form of the purified recombinant protein was determined as being 375 kDa by size exclusion chromatography. Optimal enzyme activity was observed at pH 8.5 and the enzyme remained stable at a range of different temperatures from 30 to 60°C. Using 2-phosphoglyceric acid as substrate for the purified enzyme, K_M, k_{cat} and k_{cat}/K_M were determined as 0.48 mM, 20.4 $s^{-1}$and 4.22 × 10^4 $M^{-1}s^{-1}$, respectively. Potential drug binding sites of *Fn*ENO were detected using homology modeling. These data could facilitate the design of new inhibitors of *F. nucleatum* which has already been shown to be resistant to several known antibiotics.

Keywords: *Fusobacterium nucleatum*, enolase, periodontal diseases, kinetic characterization, homology modeling

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Periodontal diseases result from bacteria in dental plaque which initiate the host inflammatory response. Among the numerous bacterial species present in plaque, the Gram-negative, anaerobe *Fusobacterium nucleatum* commonly exists in the human oral microflora and is regarded as key bacteria in human periodontal disease pathogenesis [1]. *F. nucleatum* interacts with other Gram-negative and Gram-positive bacteria by coaggregation and is a bridge enabling the colonization of pathogens on the tooth surface [1, 2]. It is known that certain *F. nucleatum* strains have developed resistance to several antibiotics [3, 4]. Therefore, alternative drugs would be required for elimination of infections where *F. nucleatum* is the causative organism.

Target molecules such as proteins, enzymes, and nucleic acids exert important biological functions, and have been selected as targets for new drug discovery strategies [5]. Indeed enolase is an enzyme that catalyzes a reversible reaction in glycolysis metabolism, dehydration of 2-phospho-D-glycerate to phosphoenol pyruvate, a pathway common to both anaerobic and aerobic respiration [6]. Also, plasminogen-binding and fibronectin binding are features of enolase acting as a cell surface receptor and contributes to the penetration of pathogens in host tissues. Furthermore the enzyme can induce immunogenic response as a surface antigen [7, 8].

The cloning and analysis of the enolase from *F. nucleatum* (EC 4.2.1.11) has not previously been reported, especially in relation to its potential for drug design. In the present study, the enolase enzyme was structurally and kinetically characterized due to its biological relevance and importance in disease pathogenesis. This study provides data which can be used to underpin the design of enzyme inhibitors which have the potential for future pharmaceutical use.

MATERIALS AND METHODS

Reagents and cells. Long PCR enzyme mix was purchased from Thermo Scientific (USA) and *Taq* DNA polymerase was obtained from Thermo Fisher

[1] The article is published in the original.