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Extended Abstract
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Pharmacists 2016 : Calcitonin-like peptide from Ciona intestinalis stimulates osteoblast alkaline phosphatase activity and mineralization in MC3T3-E1 cells - Van-Tinh Nguyen - Pukyong National University

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This study examined the effects of Ciona intestinalis calcitonin-like peptide (CCLP) on osteoblast differentiation and mineralization in the culture system of MC3T3-E1 cells. The primary structures of the CCLP containing Cys-Asp-Gly-ValSer-Thr-Cys-Trp-Leu-His-Glu-Leu-Gly-Asn-Ser-Val-His-Ala-Thr-Ala-Gly-Gly-Lys-Gln-Asn-Val-Gly-Phe-Gly-Pro-NH2 was synthesized automatically using the solid phase method with fluorenylmethoxycarbonyl (Fmoc) resin. Pre-osteoblast MC3T3-E1 cells were cultured with various concentrations of CCLP (7.5, 15, and 30 μM) during the osteoblast differentiation period. To examine osteoblast differentiation, alkaline phosphatase (ALP) activity was determined by reading the absorbance at 405 nm using a spectrophotometer, and mineralization was evaluated by staining with Alizalin red S. Moreover, the expression of differentiation markers such as ALP, osteocalcin (OSC), and osteopontin (OPN) were measured using RT-PCR and Western blot analysis. The results showed that CCLP did not exhibit any cytotoxic effect on MC3T3-E1 cells even at the highest concentration (30 μM) at 2 and 5 days. CCLP also enhanced MC3T3-E1 cells proliferation, differentiation, and mineralization demonstrated by the increased expression of several osteoblast phenotype markers such as ALP, and Alizarin red S staining. In addition, the CCLP induced mitogenactivated protein kinase (MAPK) pathway in MC3T3-E1 cells. These results suggest that CCLP exerts positive effects on osteoblast differentiation and may represent a potential target for pharmaceutical development. Antacid phosphatase (ALP, ALKP, ALPase, Alk Phos) (EC 3.1.3.1), or fundamental phosphatase, is a homodimeric protein chemical of 86 kilodaltons. Every monomer contains five cysteine buildups, two zinc molecules and one magnesium iota critical to its reactant capacity, and it is ideally dynamic at basic pH environments. High mountain has the physiological job of dephosphorylating mixes. The catalyst is found over a large number of creatures, prokaryotes and eukaryotes the same, with a similar general capacity however in various auxiliary structures reasonable to nature they work in. Soluble phosphatase is found in the periplasmic space of E. coli microbes. This catalyst is heat stable and has its most extreme movement at high pH. In people, it is found in numerous structures relying upon its birthplace inside the body – it assumes a fundamental job in digestion inside the liver and improvement inside the skeleton. Because of its far reaching pervasiveness in these zones, its focus in the circulation system is utilized by diagnosticians as a biomarker in deciding judgments, for example, hepatitis or osteomalacia. The degree of antacid phosphatase in the blood is checked through the ALP test, which is frequently part of routine blood tests. The degrees of this chemical in the blood rely upon components, for example, age, sex, or blood type. Blood levels of basic phosphatase additionally increment by two to multiple times during pregnancy. This is an aftereffect of extra soluble phosphatase delivered by the placenta. Additionally, strange degrees of basic phosphatase in the blood could demonstrate issues identifying with the liver, nerve bladder or bones. Kidney tumors and diseases just as ailing health have likewise indicated anomalous degree of soluble phosphatase in blood.[8] Alkaline phosphatase levels in a phone can be estimated through a procedure called “The scoring strategy”. A blood smear is typically taken and recolored to classify every leukocyte into explicit “leukocyte soluble phosphatase files”. This marker is intended to recognize leukocytes and decide diverse catalyst action from each example’s degree of recoloring. In Gram-negative microscopic organisms, for example, Escherichia coli (E. coli), antacid phosphatase is situated in the periplasmic space, outer to the internal cell layer and inside the peptidoglycan part of the cell divider. Since the periplasmic hole is more inclined to natural variety than the internal cell, antacid phosphatase is reasonably impervious to inactivation, denaturation, or corruption. This attribute of the catalyst is extraordinary to numerous other proteins. The exact structure and capacity of the four isozymes (Int in E.coli) are exclusively equipped to gracefully a wellspring of inorganic phosphate when nature comes up short on this metabolite. The four chemicals are reliant upon the area of the tissue articulation. The four locales of tissue articulation are the Intestinal AlP, Placental ALP, Germ Cell ALP and Liver/Bone/Kidney ALP. The inorganic phosphates created by these isozymes are then bound to transporter proteins which convey the inorganic phosphates to a particular high-proclivity transport framework, known as the Pst framework, which transports phosphate over the cytoplasmic membrane. While the external layer of E. coli contains porins that are penetrable to phosphorylated exacerbates, the inward film doesn’t. At that point, an issue emerges in how to move such mixes over the internal film and into the cytosol. Without a doubt, with the solid anionic charge of phosphate bunches alongside the rest of the compound they are a lot of immiscible in the nonpolar district of the bilayer. The arrangement emerges in dividing the phosphate bunch away from the compound by means of ALP. As a result, alongside the corresponding aggravate the phosphate was bound to, this chemical yields unadulterated inorganic phosphate which can be eventually focused by the phosphate-explicit vehicle framework (Pst system) for translocation into the cytosol. As such, the fundamental motivation behind dephosphorylation by soluble phosphatase is to expand the pace of dissemination of the atoms into the cells and repress them from diffusing out. Soluble phosphatase is a zinc-containing dimeric catalyst with the MW: 86,000 Da, every subunit containing 429 amino acids with four cysteine buildups connecting the two subunits. Alkaline phosphatase contains four Zn particles and two Mg particles, with Zn involving dynamic destinations An and B, and Mg possessing site C,

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so the completely dynamic local basic phosphatase is alluded to as (ZnAZnBMgC)2 compound. The system of activity of basic phosphatase includes the geometric coordination of the substrate between the Zn particles in the dynamic destinations, while the Mg site doesn't seem, by all accounts, to be sufficiently close to straightforwardly participate in the hydrolysis instrument, be that as it may, it might add to the state of the electrostatic potential around the dynamic center.

Biography

Van-Tinh Nguyen received his PhD from the Department of Biomedical Engineering, Pukyong National University, Korea. He graduated in 2012 from the University of Chosun and his current research interests include the isolation, safety and bioavailability of bioactive materials; development of marine-integrated cells and tissue regenerative biomedical substances.

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