Calcification at micro domains: Effect of insulin and ethanol

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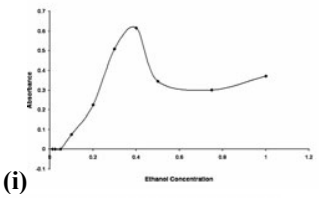
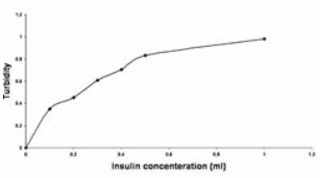
Statement of Purpose: The exploitation of the self-assembly motifs (like proteins and liposomes) for precious synthesis of nanostructures using biological molecules is a recently emerging area[1], and is known as bionanotechnology. Here we have attempted to study the effect of ethanol and insulin in crystal growth.

Methods: For mineralization experiments calcium (0.2M) in the form of calcium chloride dehydrate and phosphate (0.12M) in the form of potassium dihydrogen ortho phosphate was used[2]. The calcium to phosphate ratio was maintained as 1.6 in the solution. The aggregated particles is evaluated by checking the λ max at 350 nm using a UV Visible spectrophotometer. The quantity of crystals formed under varying conditions were evaluated by gravimetry.

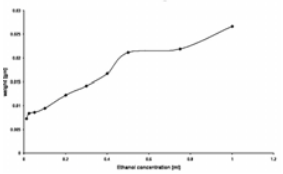
Results/Discussion: The results indicate that there is a direct relation between the ethanol concentration and calcium nucleation (Figure-1(i)) as well as insulin conformational change (Figure-1(v & vi), and eventually the calcium phosphate crystal growth(Figure-1(ii & vii) . While the insulin in its native conformation does not have any influence in crystal growth Figure-1(iv) even though it induces calcium nucleation, at higher concentrations Figure-1(iii). Here the crystals formed were not of colloidal (diameter- 100μm) range. In addition to that we have also observed that least amount of protein is getting entrapped into the crystals. This clearly indicates that the crystal is synthesized at the surface of conformationally changed protein molecules.



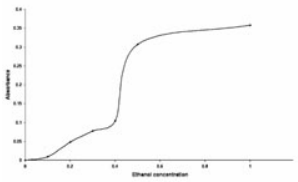
(i)



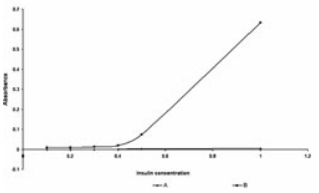
(v)



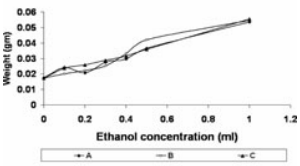
(ii)



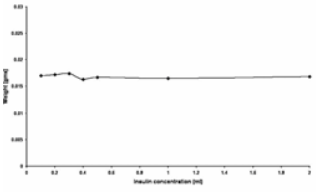
(vi)



(iii)



(vii)



(iv)

Figure-1: Effect of ethanol in inducing calcium precipitation (i), Calcium phosphate formation and crystal growth (ii). Effect of insulin in inducing precipitation (iii); calcium (A), Phosphate (B). Calcium phosphate formation and crystal growth (iv). Effect of ethanol on insulin conformational change, with respect to insulin (v), ethanol (vi) concentration. Calcium phosphate formation and crystal growth (B). Calcium phosphate crystal growth with respect to insulin and ethanol (vi), Insulin (Human Insulin 40IU/ml), three different concentrations 4IU (A), 8IU (B), 12IU (C).

Conclusions: The surface synthesis of ceramic crystals on the protein with altered conformation is enhanced by the ethanol as a co- solvent. However insulin has least effect in the crystal growth, in its native conformation. This information will be suitable for the synthesis protein-ceramic composites for various applications.

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References

(1) Mao, C. B.et. al. Science, 2004, 303, 213-217.
(2) Kaladhar. K.; Langmuir. (2004) Dec 7; 20(25): 11115-22.