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SUMMARY

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Regulation of Bacterial Globin Gene Expression and Haemoglobin Production.

Haemoglobin was considered to be present only in eukaryotes, till the pragmatism was altered by WAKABAYASHI et al., in 1986, by establishing the presence of a haemoglobin-like protein in a gram-negative bacterium, *Vitreoscilla sp.* C1.

Vitreoscilla haemoglobin (VtHb) is the only well-characterized bacterial Hb but the exact role of this haemoglobin in cellular metabolism has not been conclusively proved. To understand the function of a bacterial Hb, more such proteins need to be studied. With this aim in mind, cloning of globin-like gene from related species *Vitreoscilla stercoraria* was carried out. The genomic locus responsible for the production of globin portion of *V.stercoraria* (*vstgb*) has been cloned and expressed in *E.coli*. The *vgb* gene from *Vitreoscilla* was used as a hybridization probe to screen a *V.stercoraria* genomic library constructed in cosmid pHC79. Restriction mapping and subcloning was carried out to localize the *vstgb* gene. The complete nucleotide sequence has been determined by Sanger's dideoxy method. The *V.stercoraria* Hb (VstHb) produced in *E.coli* harboring recombinant plasmid has spectral properties and subunit molecular size similar to VtHb. It also cross-reacts with the polyclonal antibodies raised against *Vitreoscilla* Hb.

Vitreoscilla sp. C1 *vgb* gene is interesting at the genetic level. It is reported to have an oxygen-responsive promoter but how oxygen level regulates the *vgb* gene is not obvious. Microaerobic mode of induction in various hosts, provided evidence for a common regulatory factor(s) involved in activation of *vgb* promoter. Primary structure analysis of *vgb* upstream regulatory region indicated the presence of a possible binding site for the transcriptional activator FNR. Further, the *E.coli* mutant lacking *fnr* gene product was not able to activate *vgb* promoter under microaerobic condition, suggesting the involvement of FNR or FNR-like proteins in modulating its activity. The possibility of a second level of control by CRP is also indicated. Oxygen responsive nature and regulatory characteristics of *vgb* promoter offers a novel system for the expression of gene in heterologous bacterial hosts in an oxygen dependent manner. *vstgb* is closely related to *vgb* but the regulation of the two genes is different. *vstgb* gene is not regulated by changes in oxygen availability. It does not have an FNR binding site but shows the presence of a consensus CRP binding site. This indicates the involvement of CAP or CRP. Repression of the transcription by addition of glucose confirms the role of CRP.

Homology of VtHb and other globins extends to helices B, C, E, F and G; helix D being absent as in α subunit of mammalian globins. Helix A is not in contact with haeme and may not be important in oxygen-binding. VtHb has conserved amino acids at important positions viz. B10, CD1 and F8. However, it has glutamine in place of histidine at E7 and leucine in place of valine at E11 position. The oxygen association and dissociation rate constants have been studied and the dissociation rate was found to be exceptionally high, resulting in a low affinity. This variation is presumably due to the presence of glutamine at E7 position. The assumption holds good in the light of the knowledge that other unicellular globins such as *E.coli* HMP, *Alcaligens* haemoprotein and Yeast haemoprotein also have glutamine in place of the usually conserved histidine. Glutamine is encoded by CAG whereas histidine by CAC/CAT. Thus, it poses the question whether glutamine in VtHb at the E7 position is just a point mutation or an evolutionarily important change. It was therefore of interest to determine the amino acid at the E7 position in the related globin, VstHb.

The analysis of the deduced amino acid sequence of *V.stercoraria* Hb shows that glutamine itself is present at the E7 position. This may lead one to believe that the amino acid substitution may be an evolutionarily important change related to the function of this protein. VstHb shows another important feature. The dissociation rate constant for oxygen for this protein is not as high as that for VtHb despite the conservation of amino acids at E7, E11, F8 and the CD1 position. Thus, one or more substitutions seen at the A12-15, C5, E3, E16, F2 and G3 position maybe responsible for the change in oxygen binding properties. The A helix of VtHb does not match that of other globins and may not play an important role in the biological function. Molecular modeling analysis indicates that the amino acid substitutions at E3 and G3 maybe responsible for the variation in dissociation rate constant and resultant affinity.

These results lead to the hypothesis that VtHb and VstHb have different functions to play in cellular metabolism. We propose that the function of *Vitreoscilla* Hb is that of an "oxygen trap" whereas that of VstHb is an "oxygen buffer".