Purification of Invertebrate Hemoglobins for use as an Alternative to Donated Blood

Jack A. Dienes, Devon C. Zimmerman, Jacob J. Elmer
Villanova University, 800 E Lancaster Ave, Villanova, PA 19085

Although donated blood will always be the preferred material for transfusion, its limited availability has motivated the development of hemoglobin based oxygen carriers (HBOC's) that can be used when donated blood is unavailable. For example, donated blood is often unavailable in military situations since it requires refrigeration facilities that are impractical on the battlefield. Our goal is to develop an HBOC that does not require refrigeration, thereby satisfying this vital need. In this work, we purified and characterized the hemoglobin from several different invertebrates for use as HBOC's, including earthworms (*Lumbricus terrestris* and *Eisenia fetida*), bloodworms (*Glycera dibranchiata*), and sandworms (*Nereis virens*), as well as the novel hemoglobins of ribbon leeches (*Nephelopsis obscura*) and Alabama jumper worms (*Amynthas gracilis*). Each of the hemoglobins was highly purified using a combination of metal (zinc) precipitation and tangential flow filtration. The purity and molecular weight of each hemoglobin were then analyzed with native and denaturing PAGE gels. The oxidative stability and oxygen transport characteristics of the hemoglobin samples were then compared and used to identify the best candidate for future animal studies. The hemoglobin from *L.terrestris* and *G.dibranchiata* were identified as lead candidates, since they oxidized the least during purification (5.3% for LtEc, 8.5% for GdHb). These hemoglobins also have oxygen affinities (26.50mmHg for LtEc in Hemox buffer, 22.95mmHg for GdHb in TRIS) that are highly similar to human red blood cells (28mmHg in Hemox buffer) at body temperature (37^{o}C). We are currently comparing the oxidation rates of LtEc and NoEc to see which is more stable, and we are also comparing the oxidation rate of LtEc in TRIS buffer against the oxidation rate of LtEc in Ringer's Solution. Overall, our results show that invertebrate hemoglobins may be promising HBOC's, which further motivates the investigation of even more diverse invertebrate hemoglobins in the future.