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Title: Extraction of hemoglobin with calixarenes and biocatalysis in organic media of the complex with pseudoactivity of peroxidase

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Abstract: The present work involves the use of p-tert-butylcalix[4,6,8]arene carboxylic acid derivatives ((t)Butyl[4,6,8]CH2COOH) for selective extraction of hemoglobin. All three calixarenes extracted hemoglobin into the organic phase, exhibiting extraction parameters higher than 0.90. Evaluation of the solvent accessible positively charged amino acid side chains of hemoglobin (PDB entry 1XZ2) revealed that there are 8 arginine, 44 lysine and 30 histidine residues on the protein surface which may be involved in the interactions with the calixarene molecules. The hemoglobin-(t)Butyl[6]CH2COOH complex had pseudoperoxidase activity which catalysed the oxidation of syringaldazine in the presence of hydrogen peroxide in organic medium containing chloroform. The effect of pH, protein and substrate concentrations on biocatalysis was investigated using the hemoglobin-(t)Butyl[6]CH2COOH complex. This complex exhibited the highest specific activity of 9.92 x 10(-2) U mg protein(-1) at an initial pH of 7.5 in organic medium. Apparent kinetic parameters (V'(max), K'(m), k'(cat) and k'(cat)/K'(m)) for the pseudoperoxidase activity were determined in organic media for different pH values from a Michaelis-Menten plot. Furthermore, the stability of the protein-calixarene complex was investigated for different initial pH values and half-life (t(1/2)) values were obtained in the range of 1.96 and 2.64 days. Hemoglobin-calixarene complex present in organic medium was recovered in fresh aqueous solutions at alkaline pH, with a recovery of pseudoperoxidase activity of over 100%. These results strongly suggest that the use of calixarene derivatives is an alternative technique for protein extraction and solubilisation in organic media for biocatalysis.

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