The [(bathophenanthroline)$_3$:Fe^{2+}] complex as an aromatic non-polymeric medium for purification of human lactoferrin

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Abstract

We describe a non-chromatographic, ligand-free platform for the efficient purification of recombinant human lactoferrin (LF). The platform consists of a [metal:chelator] complex precipitate in the presence of osmotically active polyethylene glycol 6000 (PEG-6000). Purification is achieved in three stages. Following formation of the complex, LF is captured under neutral conditions by the aggregated complexes (Step I) a washing step follows (Step II) and then, (Step III) LF is extracted in pure form with 100 mM tribasic Na citrate buffer (pH 7). Of the four complexes investigated, [bathophenanthroline (batho)$_3$:Fe^{2+}] was determined to be the most efficient. LF is recovered with high yield (~90%, by densitometry) and purity (≥97%, by SDS polyacrylamide gel electrophoresis (SDS-PAGE)) from an artificial contamination background comprising *E. coli* lysate proteins. Purified LF is demonstrated to be monomeric by dynamic light scattering (DLS); to preserve its native secondary structure by circular dichroism (CD) spectroscopy; and, as apo-LF, to efficiently inhibit bacterial growth. Process yield is not affected by a 45-fold increase in LF concentration from 0.2 to 9 mg/mL. We provide evidence that protein capture relies on [cation:π] interactions between the lysine and arginine residues of LF with the fully aromatic [(batho)$_3$:Fe^{2+}] complexes. The use of [metal:chelator] complex aggregates is demonstrated to provide an economical and efficient avenue for LF purification.