Chromatography and Mass Spectrometric Analysis of Isoforms of Recombinant Apoaequorin

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Anion-exchange (MonoQ) column chromatography of recombinant apoaequorin carried out in the presence of Ca\(^{2+}\) revealed the presence of 2 isoforms of apoaequorin, designated A and B. The N-terminal amino acid sequences of the 2 isoforms were found to be identical. On native PAGE, the 2 isoforms moved as a doublet, with B moving slightly ahead of A. When treated with 2-mercaptoethanol and then subjected to native PAGE, isoform B moved at the same rate as A, indicating that isoform A is the reduced, and isoform B the oxidized, form of apoaequorin. When isoform A was analyzed by electrospray ionization (ESI) mass spectrometry, 2 peaks were obtained, corresponding to 1 or 3 Ca\(^{2+}\) per molecule, whereas isoform B gave 3 peaks corresponding to 1, 2 or 8 Ca\(^{2+}\) per molecule. When isoform B was treated with EDTA and 2-mercaptoethanol, and then subjected to ESI mass spectrometry, Ca\(^{2+}\) was found to be absent. From these results, we conclude that both EDTA and 2-mercaptoethanol are necessary for the complete removal of Ca\(^{2+}\), as required in the regeneration of apoaequorin into aequorin.