

Abstract

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Method for lipoprotein(a) density profiling by BiEDTA differential density lipoprotein ultracentrifugation.

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BACKGROUND: In this article, we demonstrate the analytical power of linking density gradient ultracentrifugation with affinity separations. Here we address some of the analytical challenges in the study of lipoprotein(a), (Lp(a)).

METHODS: The mean density distribution of Lp(a) was determined by a differential density lipoprotein profile (DDLp). For DDLp, the lipoprotein density distribution of a serum sample with elevated Lp(a) levels was determined by ultracentrifugation using a BiEDTA complex as a density gradient. Lp(a) was removed from a second aliquot of the same serum sample by carbohydrate affinity using wheat germ agglutinin (WGA). WGA was demonstrated to have high specificity for Lp(a) in a serum sample. This sample was ultracentrifuged to obtain a lipoprotein density distribution in the absence of Lp(a). A DDLp was obtained after subtracting the Lp(a)-depleted lipoprotein density profile from the untreated lipoprotein density profile. The DDLp methodology reported herein gives relevant information of the lipoproteins in serum such as density, isoform, and subclass characteristics.

RESULTS: Lp(a) was quantitatively isolated from serum with a recovery efficiency of 82%. Lp(a) was purified by ultracentrifugation. Lp(a) retained its inherent density (1.086 g/mL) and immunoreactivity.

CONCLUSION: The major outcome of this research was the effectiveness of using affinity separations coupled with density ultracentrifugation for the isolation of pure Lp(a) from serum and its isoform characterization based on density by DDLp.

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