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Hemoglobin adducts as biomarkers of alcohol abuse

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Abstract

Background: Hemoglobin can form covalent adducts with small reactive molecules such as acetaldehyde, an ethanol metabolite [1,2]. These hemoglobin adducts persist throughout the erythrocyte lifespan (~120 days), making them potential long-term biomarkers of alcohol exposure. However, conventional analytical methods, including high-performance liquid chromatography-mass spectrometry and enzyme-linked immunosorbent assay, often lack sensitivity, specificity, or detailed structural information, highlighting the need for alternative detection strategies [3]. **Objective:** This study aimed to evaluate the applicability of proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, particularly the saturation transfer difference (STD)-NMR technique, for detecting and characterizing hemoglobin-acetaldehyde adducts as potential markers of ethanol consumption. **Methods:** Human hemoglobin solutions were incubated with acetaldehyde (16 µM–20 mM) at 4 °C for 30 min. Ultraviolet-visible spectroscopy was used to assess hemoglobin concentration and oxidation state. Structural analysis was performed using 1D and 2D NMR experiments (^{1}H-^{1}H TOCSY and ^{1}H-^{13}C HSQC), while STD-NMR (saturation times 0.25–5 s) evaluated ligand–protein interaction dynamics [4]. **Results:** A novel resonance at 1.47 ppm (doublet) was identified, consistent with acetaldehyde binding to hemoglobin. The STD-NMR response showed a concentration-dependent signal increase, linear between 80 µM and 6 mM ($R^2 = 0.994$), followed by a plateau at 20 mM, indicating saturation of binding sites and confirming interaction. **Conclusions:** NMR spectroscopy enables sensitive, non-destructive detection and structural characterization of hemoglobin-acetaldehyde adducts. This approach shows strong potential for developing reliable biomarkers of chronic alcohol exposure. Further validation in complex biological matrices is ongoing to assess its analytical robustness and clinical relevance.

Keywords: hemoglobin adducts; ethanol; acetaldehyde; nuclear magnetic resonance spectroscopy

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