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A Critical Examination of the Reaction of Pyridoxal 5-Phosphate with Human Hemoglobin Ao

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Abstract: Pyridoxylated normal adult human hemoglobin (HbAo) has been prepared using both oxygenated and deoxygenated HbAo at pH 6.8 and room temperature without the addition of Tris to produce a mixture with (**phosphorus** isotope) of 30 + or - 2 torr and a Hill coefficient of 2.3 + or - 0.1 similar to that of the isolated adult human hemoglobin from the red blood cell. **Reduction** of the pyridoxylated HbAo in the oxygen-ligated form by sodium borohydride gives unacceptable levels of methemoglobin (i.e., > 10%). Excessive foaming and methemoglobin formation can be partially avoided using deoxyHbAo. **Reduction** with sodium cyanoborohydride is much gentler and gives solutions with < 5% methemoglobin. Both **reducing** agents give products with multiple components as shown by analytical chromatography. Radioautography on the isoelectric focusing gels of HbAo treated with ¹⁴C pyridoxal 5-phosphate (PLP) shows three major bands for the cyanoborohydride-**reduced** derivatives and a much more complex mixture of labeled molecules after the sodium borohydride **reduction**. When pyridoxylated hemoglobin is prepared **reduced** without **reduction**, the preparation, after passage through a mixed-bed resin, contains 0.4 equivalents of PLP per heme, and has a P50 of 30 + or - 2 torr and an n value of 2.3 similar to the values found after **reduction**. Upon anion exchange resin chromatography, the PLP is removed, indicating that the reaction forms a reversible Schiff base. Keywords: Reprints, Pyridoxal 5-phosphate, Human hemoglobin Ao, Organic chemistry, Biochemistry, Oxygen-carrying, Erythrocytes, Conjugated proteins, Polypeptide chains, Blood chemistry. (jg) ANNOTATION: Reprint: A Critical Examination of the Reaction of Pyridoxal 5- Phosphate with Human Hemoglobin Ao.

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