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ON THE KINETICS OF MALEIMIDE SPIN LABELING OF HEMOGLOBIN. Janice R.Perussi, Maria H.Tinto, Valdeci Massaro, Otaciro R.Nascimento, Marcel Tabak. Inst.Fís.Quím. de São Carlos, USP, CP369, 13560, S.Carlos,SP.

Maleimide spin labeled human hemoglobin (Hb) has been used as a model system since the early days of the spin label technique. It is assumed that Hb is labelled at the two easily accessible SH-groups of the two residues cys-β93. In the present work a study was performed of the kinetics of the reaction of maleimide spin label with Hb to see if the binding to the two sites is independent and also to explore the possibility of kinetic studies by ESR. Experiments were made monitoring both the decrease of the free signal in solution as well as the increase in immobilized (bound) signal as a function of time at a constant temperature (25°C). It is observed that at low ratio of maleimide label to protein (0-1.0) the rate constant is $k=4,0x10^{-2}min^{-1}$ while at high ratios (2-4) $k=1,5x10^{-2}min^{-1}$. Measurement of the amount of label bound at the end point of reaction (24hs) allowed to obtain a Scatchard plot for the binding of maleimide. This plot permits to calculate a microscopic dissociation constant for a reaction of the Hb with maleimide=47μM, the number os sites=1,7 and the plot is indicative of independent binding. The decrease of the reaction rate with the increase in the ratio of label to protein is explained in a model assuming that the statistical factor is the limiting in this reaction.

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