Solvent Perturbation and Conformational Equilibrium of Hemoglobin

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We studied the effect of organic cosolvents (monohydric alcohols and amides) on the oxygen affinity of hemoglobin (1,3). Fig. 1 shows Log P_{50} vs. the concentration of the various cosolvents. This effect can be attributed to alterations: 1) of the equilibrium between the T and R quaternary conformations; 2) of the oxygen dissociation constants (K_R and K_T); 3) of the dimer-tetramer equilibrium. The last possibility can be discarded in view of the independence of our results upon hemoglobin concentration. The oxygen dissociation constants seem not to be altered. Indeed: 1) spectroscopic data show no detectable distortions to be introduced in the proximity of the heme group in oxy hemoglobin; 2) stop-flow data show that the kinetics of replacement of O_2 by CO is not altered; 3) the dependence of Hill's constant (n_H) upon Log P_{50} follows a bell-shaped curve. Point 3 also suggests that our data can be analyzed within the frame of the Monod, Wyman and Changeux (MWC), two state model. According to MWC, $P_{50} = L^{1/4}K_R$, where $L = (T)/(R)$ in the absence of oxygen. From the above equation, and taking K_R as not affected, it follows: $4RT \ln P_{50}(c)/P_{50}(o) = RT \ln L(c)/L(o) = \Delta G(c)-\Delta G(o) = \Delta\Delta G$, where ΔG is the standard free energy difference between the R and T conformations, in the absence of oxygen. Following a phenomenological approach we separated the overall $\Delta\Delta G$ into contributions ($\Delta\Delta G_{bes}$) related to and contributions ($\Delta\Delta G_{nbes}$) not related to the variation of the solvent bulk dielectric constant. Fig. 2 shows $\Delta\Delta G_{nbes}$ as a function of the cosolvent concentration. This contribution is negative, i.e.it acts as to stabilize the R conformation and depends on the perturbants "hydrophobicity". A vant'Hoff analysis (3) showed that the $\Delta\Delta G_{nbes}$ values arise from much larger, positive and partly compensating $\Delta\Delta H_{nbes}$ and $T\Delta\Delta S_{nbes}$ values; moreover the signs of $\Delta\Delta G_{nbes}$, $\Delta\Delta H_{nbes}$ and $\Delta\Delta S_{nbes}$ indicate that these contributions reflect entropy driven processes.We attribute the $\Delta\Delta G_{nbes}$ contribution to the alteration, in the presence of cosolvents, of protein-solvent hydrophobic interactions.

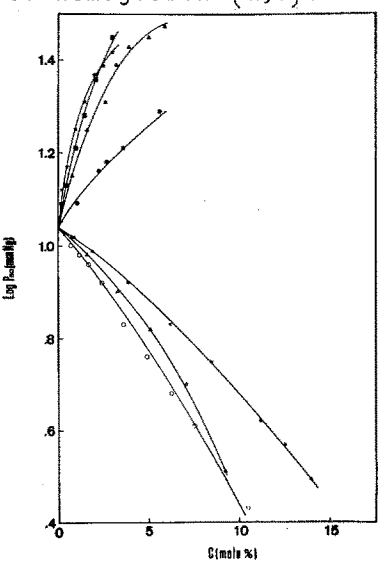

Fig. 1: Log P_{50} vs. cosolvent concentration; x Acetamide; 0 Formamide; Δ N-methylformamide; ● methanol; ▲ ethanol; ■ (2)-propanol; * (1)-propanol.

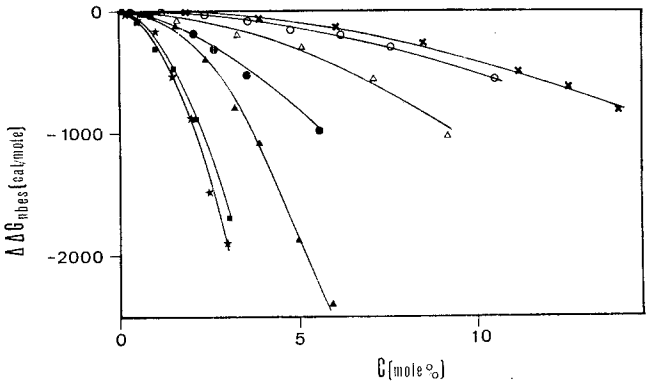

Fig. 2: Non bulk-electrostatic contribution ($\Delta\Delta G_{nbes}$) vs. co-solvent concentration; x Acetamide; 0 Formamide; Δ N-methyl-formamide; ● methanol; ▲ ethanol; ■ (2)-propanol; ⋆ (1)-propanol.

From these data we conclude that contributions coming from the interaction of the protein with the solvent medium are relevant in determining the free energy balance between the R and T quaternary conformations of hemoglobin.

1. Cordone, L., Cupane, A., San Biagio, P.L., and Vitrano, E. (1979) Biopolymers, 18, 1975 - 1988.
2. Cordone, L., Cupane, A., San Biagio, P.L., and Vitrano, E. (1980) Biopolymers (in press).
3. Cordone, L., Cupane, A., San Biagio, P.L., and Vitrano, E. (1980) Biopolymers (in press).