Hydration and Allosteric Transitions in Hemoglobin*

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Sucrose and other neutral solutes reduce the oxygen affinity of human hemoglobin. This effect was attributed by Colombo et al. (Colombo, M. F., Rau, D. C., and Parsegian, V. A. (1992) Science 256, 655–659) to a stabilization of the deoxy-T quaternary state of hemoglobin A, via a reduction of the activity coefficient of water. This was correlated to crystallographic results which showed that a significant surface area at the $\alpha_1\beta_2$ interface, which is exposed to the solvent in the oxy-R state, is buried in the deoxy-T state.

We show that sucrose has no effect on the oxygen affinity of trout hemoglobin I, which is cooperative in oxygen binding but lacks heterotropic effects, and that in spite of the large buried surface exposed to solvent upon dissociation of human hemoglobin into $\alpha_\beta$ dimers, sucrose leads either to an increased dissociation of hemoglobin A-CO into dimers or to no effect at all (in the presence of inositol hexakisphosphate).

These results may demand a reconsideration of the hypothesis extensively discussed by Colombo et al.

Colombo et al. (1), who described the effect of different neutral solutes (sucrose, stachyose, polyethylene glycol-150, and polyethylene glycol-400) on the oxygen affinity of human hemoglobin, noticed that $P_{50}(O_2)$ increases linearly with increase in osmotic pressure, and that this effect is almost independent of the chemical structure of the solute. These authors proposed that the observation is best explained as a consequence of the reduction in the activity coefficient of water, leading to a destabilization of the high affinity quaternary state of Hb, because the transition from deoxy-Hb to liganded-Hb is associated to the exposure of an area (500–700 Å$^2$) buried in the larger $\alpha_1\beta_2$ interface of deoxy-state Hb (2, 3). This result has been presented as a first evidence for modulation of allosteric quaternary conformational transitions by water, with obvious implications for the in vivo function of allosteric enzymes, given the non-ideality of the intracellular milieu.

The hypothesis extensively discussed by Colombo et al. (1) is challenged by the experiments reported below, which deal with the effect of sucrose on the functional properties of human and trout I hemoglobins. Our data confirm that the oxygen affinity of human HbA at pH = 7.0 decreases as the sucrose concentration is increased; however, we observe that: (i) sucrose has no effect on the $O_2$ affinity of trout HbI, which displays cooperative oxygen binding but lacks heterotropic effects (4); (ii) in HbA the effect is independent of pH (6.0, 7.0, and 9.2) and inositol hexakisphosphate (IHP); and (iii) addition of 1.5 M sucrose leads to a significant increase in the tetramer-dimer dissociation constant of HbA-CO, or to no effect at all (in the presence of IHP). These results are difficult to reconcile with the hypothesis of a destabilization of the R state because of differential hydration, especially considering that upon dissociation into $\alpha_\beta$ dimers (3, 5–7) a very large intersubunit surface area becomes exposed to the solvent. Our data are better described in terms of a more classical viewpoint, i.e. that sucrose acts as a "weak" allosteric effector (somewhat like protons or organic phosphates) or, alternatively, that the salt bridge interactions are strengthened due to the decrease in the dielectric constant of the medium upon addition of sucrose, as suggested by Cordone et al. (8) to account for the effect of alcohols.

EXPERIMENTAL PROCEDURES

Human hemoglobin, prepared from freshly drawn blood was stripped from organic phosphates by a double passage through a column of mixed bed ion exchanger (Bio-Rad AG 501-X8) equilibrated with bidistilled water. Trout hemoglobin component I (trout HbI) was purified following the published procedure (see Ref. 4).

RESULTS AND DISCUSSION

As shown in Fig. 1, sucrose reduces the oxygen affinity of HbA, in agreement with Colombo et al. (1). At a concentration of 1.1 M sucrose, the decrease in affinity is of approximately 25% ($P_{50}$ is 1.35 times larger); the median Hill coefficient is not affected by sucrose. Although it was not possible to titrate the effect of the sugar, analysis of the data (according to Wyman, Ref. 9) yielded $\approx 0.5$ mol of sucrose/heme as the minimum estimate for the oxygen-linked binding sites. At pH 7.0 no competition between IHP (3 mM) and sucrose was observed; moreover, any correlation between the effect of sucrose and the overall charge of HbA was excluded by experiments at pH 9.2 and 6.0, which showed almost the same trend observed at pH 7.0 (also in Fig. 1).

The oxygen affinity of trout HbI is insensitive to known allosteric effectors, though maintaining a cooperative behavior in ligand binding (4). The three-dimensional structure of trout HbI has not been determined, but several lines of evidence indicate that in this Hb the quaternary conformational change involves a substantial reorganization of the subunit contacts, similar to that of HbA, based on: (i) comparison of the conserved residues at the $\alpha_1\beta_2$ interface in both quaternary states (10, 11), (ii) perturbation of the optical
spectrum of Tp^eq at the same interface, and (iii) spectral changes and dynamic properties of the R,-To quaternary conformational change following laser photolysis (11). As shown in Fig. 1, sucrose up to 1.5 M has no effect at all on the oxygen affinity of trout Hbf.

Dissociation of tetrameric Hb drastically increases the solvent accessible surface area, since the $\alpha_2\beta_2$, $\alpha_2\alpha_2$, and $\beta_2\beta_2$ interfaces (buried in the tetramer) are exposed to solvent in the $a^2$ dimer. Using the coordinates of oxy- and deoxy-HbA from Refs. 12-14, the buried surface area exposed upon dissociation of the R state tetramer was calculated to be 1920 Å$^2$ (3). Thus the dependence of the dissociation constant on sucrose concentration should represent a stringent test to discriminate the hypothesis of Colombo et al. (1), from other mechanisms, such as the direct binding of the sugar; in fact, the former hypothesis predicts that sucrose should hinder the dissociation.

The effect of sucrose on the tetramer-dimer dissociation equilibrium of HbA-CO has been assessed at pH 7.0. The equilibrium constant of dimeric and tetrameric HbCO was measured using the flash photolysis technique, which exploits the different reactivity toward CO of the two oligomeric states (15). Experiments carried out at different Hb concentrations (Fig. 2) show that sucrose (1.45 M) has no effect on the tetramer-dimer dissociation equilibrium of HbA-CO in the presence of 1 mM IHP; in contrast, in BisTris/acetate or in BisTris/chloride, sucrose increases the dissociation of tetrameric HbA-CO into dimers. This experiment demonstrates that the effect of sucrose on the properties of Hb cannot depend exclusively on the reduction of water activity.

In addition we notice that the total surface area exposed to solvent in the two quaternary states of HbA should be the thermodynamically significant quantity to assess if differential hydration is the correct explanation for the reduced oxygen affinity of HbA in the presence of neutral solutes. The total area of the water-exposed surface of oxy- and deoxyhemoglobin is difficult to assess, in view of the disorder of some external amino acid side chains. Nonetheless, calculation of this area, carried out by Dr. B. Vallone using the molecular graphics package "Brugel" (16), suggests that the deoxy-T state of Hb, rather than the oxy-R state, has a larger exposed surface area; if so, a decrease in the activity coefficient of water should increase rather than decrease the oxygen affinity of HbA.

In their calculation on the number of water molecules differentially bound by the two quaternary states, however, Colombo et al. (1) called attention on the accessible surface area (500-700 Å$^2$) which is buried in deoxy-Hb and becomes (as a result of ligation) exposed at the $\alpha_2\beta_2$ and the $\alpha_2\alpha_2$ interfaces (2, 3); they discussed, but eventually neglected, the exterior of the molecule and the $\beta_2\beta_2$ interface. Their estimate indicated an agreement between modeling (55-90 H$_2$O molecules) and analysis of the oxygen binding experiments (60-65 H$_2$O molecules), which, although remarkable, may be fortuitous.

Direct binding of sucrose to hemoglobin, by analogy with other allosteric effectors would account for a decrease in oxygen affinity; this hypothesis was suggested by Haire and Hedlund (17), who studied the effect of ethylene glycol, but considered improbable by Colombo et al. (1) on the basis of the better correlation between the oxygen affinity and osmotic pressure. As far as we are aware, there is no direct evidence for binding of polyols to hemoglobin; moreover, in view of the high concentrations to be employed, it is likely that the binding site(s), if any, may not be unique. Nevertheless this hypothesis is not contradicted by our experimental data, because, for example, an increased dissociation into dimers may be explained if the partially apolar $\alpha_2\beta_2$ interface exposes additional sucrose binding sites.

As a possible alternative interpretation (suggested by M. F. Perutz, Cambridge, United Kingdom), the effects of sucrose may be related to the decrease in dielectric constant of the medium upon addition of the sugar (at 1.5 M sucrose, $D = 67$ as compared to $D = 79$ for pure water at 25 °C; Ref. 18). The dependence of oxygen affinity of HbA on the dielectric constant in alcohol/water mixtures was proposed and discussed at length by Cordone et al. (8) (see also Ref. 19). The decrease in oxygen affinity of HbA (Fig. 1) may be due to a (small) stabilization of the low affinity structure, possibly via some of the more accessible salt bridges (e.g., those involving His$^{a146}$; Ref. 12); this may also account for the lack of an effect of sucrose on the oxygen affinity of trout Hbf where some of the
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canonical salt bridges stabilizing the deoxy-structure are absent (such as that involving the C-terminal His of the β chains, which in trout HbI is a Phe; see Refs. 4 and 10). Moreover, such an interpretation does not contrast with the enhanced dissociation into dimers induced by sucrose, given that the solubility of the non-polar side chains at the αβ interface would be enhanced by a decrease in the dielectric constant of the medium.

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REFERENCES