The Water Effect on Allosteric Regulation of Hemoglobin Probed in Water/Glucose and Water/Glycine Solutions*

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We have previously proposed a role of hydration in the allosteric control of hemoglobin based on the effect of varying concentrations of polyols and polyethers on the human hemoglobin oxygen affinity and on the solution water activity (Colombo, M. F., Rau, D. C., and Parsegian, V. A. (1992) Science 256, 655–659). Here, the original analyses are extended to test the possibility of concomitant solute and water allosteric binding and by introducing the bulk dielectric constant as a variable in our experiments.

We present data which indicate that glycine and glucose influence HbA oxygen affinity to the same extent, despite the fact that glycine increases and glucose decreases the bulk dielectric constant of the solution. Furthermore, we derive an equation linking changes in oxygen affinity to changes in differential solute and water binding to test critically the possibility of neutral solute heterotropic binding. Applied to the data, these analyses support our original interpretation that neutral solutes act indirectly on the regulation of allosteric behavior of hemoglobin by varying the chemical potential of water in solution. This leads to a displacement of the equilibrium between Hb conformational states in proportion to their differential hydration.

It is well established that differential water binding occurs between distinct conformations of a protein. Hence, whether or not differential water binding contributes to the energetic of protein reactions, as some ions and metabolites do, is a key question to fully comprehend the mechanism of biological control. The role of water on allosteric regulation had been previously investigated, using Hb oxygenation as a model, by the so-called osmotic stress method (1, 2). The concept of this method is to use solutes, which are potentially excluded from the protein surface, to set the bulk water activity $a_w$ (3). Haire and Hedlund (4), using ethylene glycol, observed that at a low concentration range ethylene glycol decreases Hb oxygen affinity while at a higher concentration causes the opposite effect. They interpreted these results as reflecting preferential binding of ethylene glycol to Hb at the low solute concentration range, whereas, at high ethylene glycol concentration range, the increased $O_2$ binding affinity of Hb was attributed to an effect of water activity on the R to T allosteric equilibrium (4).

In our former studies of the effect of water activity on Hb $O_2$ binding properties, we have used chemically different solutes like sucrose, stachyose, and polyethylene glycols to distinguish solute from water binding to Hb (1). Besides finding evidences for an indirect effect of solutes through water on the deoxy- to oxy-Hb conformational equilibrium, we have also determined quantitatively this solvation effect on Hb allosteric transition in terms of the differential number of water molecules bound between the two extreme conformations of Hb. By using two different model approaches, the Wyman linkage equation or the Gibbs Duhem equation, we found that 65–72 water molecules are linked to the binding of four oxygen molecules to human Hb under the experimental conditions used. Furthermore, this hydration change was found to be in agreement with the difference between the solvent-accessible surface areas of deoxy- and oxy-Hb computed from x-ray structures by Clothiaux et al. (5, 6).

The experimental approach and analyses originally used by us to assess and quantify the role of water on the allosteric control of Hb have been tested in several other biochemical systems (7–14). A standard linear dependence of equilibrium and kinetic free energy parameters on water activity rather than solute activity has been lately reported. It has been also found that this phenomenon is independent of the nature of the solute used to set the chemical potential of water. However, it can be dependent on solute size for certain processes used to set the chemical potential of water $\mu_w$ (11–14). In parallel with our work, these solute effects have been interpreted as a solvation effect and have been used by several authors to measure the apparent number of water molecules involved in biochemical reactions.

However, the proposed role for water in allosteric control has been recently challenged with results which indicate that sucrose has no effect on the energy of trout Hb-I oxygen binding (15). In addition, it was shown that 1.45 M sucrose causes increased dissociation of human Hb-CO into dimers, as measured by flash photolysis. In contrast to our proposition, these results were interpreted by the same authors in terms of solute-specific differential binding to Hb, i.e. that sucrose acts as a weak allosteric effector or, alternatively, that the change in the oxygen affinity of Hb is due to the decreased dielectric constant of the water/sucrose solution as compared to pure water.

In this report, we consider these two proposed hypothesis. Initially, to assess a possible role of bulk dielectric constant on

*This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Brazil). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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§Recipient of a CNPq postdoctoral fellowship.

1. In our former calculation of the number of allosteric water molecules bound to the Hb due to oxygenation, we had converted changes in osmotic pressures to changes in the chemical potential of water at the temperature of 273.2 Kelvin degrees, which resulted in 60–65 water molecules. Here, we present the corrected number using the actual temperature at which the previous measurements were carried out.
the allosteric control of Hb, we present oxygen binding experiments with human Hb in the presence of either glycerol or glucose. These solutes cause opposite changes in the bulk dielectric constant of the solution. To evaluate the hypothesis of direct allosteric solute binding, we treat our data with an extended linkage equation that accounts for the global effect of changes in the differential binding of solute and of water molecules to Hb on oxygen binding. This equation, which is developed here in detail, has recently been applied to re-analyze the effect of chloride ions in human Hb (16), where it proved to be appropriate to quantify coupled anion and water heterotropic binding to Hb. Similar equations, as the one proposed by Tanford (17) to account for hydration effects on ligand binding, have been applied to analyze the effect of water differential binding on the free energy of binding of lac-rep to DNA (18) and charged peptides to synthetic polynucleotides studied at varying salt concentrations (19).

MATERIALS AND METHODS

Experimental Procedures—Blood was collected from healthy non-smoking adults by vein puncture using heparinized syringes. All subsequent steps were performed at 4 °C. pH measurements were made at 23 °C with a Corning model 350 pH meter. Red blood cells were washed four times in 10 volumes of cold buffered (50 mM Tris-HCl, pH 7.6) 0.9% saline containing 1 mM EDTA. The packed red blood cells were lysed with 50 mM Tris-HCl buffer, pH 7.6, containing 1 mM EDTA and dialyzed twice against 4 liters of the same buffer. Cellular debris were removed by centrifugation (20,000 × g for 30 min). HbA, was purified on DEAE-Sephadex following the procedure of Williams and Tsay (20), and purity was checked by electrophoresis on cellulose acetate. The purified hemoglobin was further stripped of organic and inorganic ions by several passages through a Dowex AG 50-X8(D) column (Bio-Rad). The concentration was raised to about 8.5 mM heme using Amicon concentrators and stored in liquid nitrogen until use.

Oxygen binding experiments were performed with 60 μM (heme) hemoglobin solution in 50 mM Tris-acetate buffer, pH 7.0, at room temperature by the tonometric method (21, 22). The functional parameters P50 (O2 partial pressure at half saturation) and cooperativity (nO2) were calculated from the Hill Plot by linear regression around half saturation. P50 represents P50 obtained in the absence of neutral solutes. Hemoglobin and methemoglobin (Hb+) concentrations were estimated using the extinction coefficients of Benesch et al. (23). At the end of the experiments, Hb+ concentration was below 5%.

The solution osmolalities (Osm) of Hb samples were determined from freezing point depression measurements using a OmegA model 5002 osmometer (Precision Systems, Inc.) after O2-binding experiments. The logarithm of water activity was then obtained through the following relation:

\[ \ln a_w = \frac{\Delta}{K_w M_w} - \frac{\text{Osm}}{M_w} \]  

(Eq. 1)

where \( \Delta \) is the freezing point depression, \( K_w = 1.86 \text{ K} \cdot \text{mol}^{-1} \cdot \text{K} \) is the cryoscopic constant, and \( M_w \) is the molarity of pure water (24).

Thermodynamic Analysis of the Linkage between Either Oxygen and Solute or Oxygen and Water Binding to Hemoglobin—The effect of a single heterotropic ligand X coupled with Hb oxygenation is typically analyzed with the Wyman linkage equation (25, 26),

\[ \frac{d \ln P_{50}}{d \ln a_w} = -\frac{\Delta n_O}{4} - \left( \frac{n_X}{n_O} \right) \frac{\Delta n_s}{4} \]  

(Eq. 2)

from the sensitivity of P50, the oxygen pressure at Hb half saturation, on \( a_w \), the activity of X. Therefore, the slope of ln P50 versus ln aw gives the difference in the number of X bound to oxy and deoxy structures of Hb, \( n_X \). This analysis is strictly correct when only the activity of a single heterotropic ligand varies, i.e. the activity of any other heterotropic ligand than X present in the solution, \( a_s \), is maintained constant. In our previous analysis of the effect of neutral solutes on Hb binding characteristics using Equation 2, we considered two extreme cases: (i) direct solute binding only or (ii) considering only the indirect effect of solute on water activity (3). We made the distinction between these two cases using different solutes and found a common dependence of ln P50 on ln aw with aw adjusted using sucrose, stachyose, or polyethylene glycol. This independence on the different chemical natures of the solutes supported the assumption of the exclusion of these solutes from protein hydration water and from any specific interaction with deoxy or oxy Hb structure.

Thermodynamic Analysis of the Linkage between Oxygen, Solute, and Water Binding to Hemoglobin—To derive a more general linkage equation to couple solute, water, and oxygen binding to hemoglobin, we recognize that the solution activities of water, \( a_w \), and of solute, \( a_s \), are not thermodynamically independent quantities; they are connected by the Gibbs-Duhem equation that, applied to a solution of m moles of solute per kg of water, is given by

\[ 55.51 \, d \ln a_w + m \, d \ln a_s = 0 \]  

(Eq. 3)

even in a presence of tens of micromolar of protein. Another Gibbs-Duhem relationship, the one including protein, can be also used to correctly describe changes in the chemical potential of a protein due to binding of solute and water (27). Solvent properties of temperature and hydrostatic pressure, the effect of \( P_O \), water, and solute binding on Hb chemical potential, \( d\mu_p \), is given by the following:

\[ -\frac{d \mu_p}{R T} = n_O \, d \ln P_O + n_s \, d \ln a_s + n_w \, d \ln a_w \]  

(Eq. 4)

where \( n_i \) accounts for the number of each of these ligands bound to Hb and \( d\mu_p \) for the change in their chemical potentials (1, 16). Combining Equations 3 and 4, we obtain the following:

\[ -\frac{d \mu_p}{R T} = [n_O] \, d \ln P_O + \left[ \left( -\frac{55.51 \, n_s}{m} + n_w \right) \right] \, d \ln a_w \]  

(Eq. 5)

enabling us to write the following Maxwell relation:

\[ \frac{\partial n_O}{\partial \ln P_O} = -\frac{55.51 \, n_s}{m} \frac{\partial n_s}{\partial \ln P_O} - n_w \frac{\partial n_w}{\partial \ln P_O} \]  

(Eq. 6)

which correlates the measured change in \( O_2 \) saturation due to changes in \( a_w \), at fixed oxygen partial pressure with changes in solute and water saturation due to changes in \( P_O \), at constant \( n_s \). If Hb oxygen affinity depends solely on either \( n_s \) or \( n_w \), it is possible to integrate this equation over the saturation isotherm to determine the differential binding of either \( n_s \) or \( n_w \).

However, to quantify the concomitant binding of solute and water, Equation 6 has to be rearranged. We start re-writing

\[ \frac{\partial n_s}{\partial \ln P_O} = -\frac{55.51 \, n_s}{m} \frac{\partial n_w}{\partial \ln P_O} - n_w \frac{\partial n_w}{\partial \ln P_O} \]  

(Eq. 7)

\[ \frac{\partial n_w}{\partial \ln P_O} = -\frac{55.51 \, n_s}{m} \frac{\partial n_s}{\partial \ln P_O} - n_w \frac{\partial n_w}{\partial \ln P_O} \]  

(Eq. 8)

Since \( n_w \) depends only on \( P_w \) and \( a_w \), \( n_w = n_w(P_w, a_w) \), we take its differential at constant \( n_w \), which rearranged gives

\[ \frac{\partial n_w}{\partial \ln a_w} = -\frac{55.51 \, n_s}{m} \frac{\partial n_s}{\partial \ln a_w} - n_w \frac{\partial n_w}{\partial \ln a_w} \]  

(Eq. 9)

Inserting Equations 8 and 9 into Equation 6 produces the following:

\[ \frac{\partial n_s}{\partial \ln P_O} = -\frac{55.51 \, n_s}{m} \frac{\partial n_w}{\partial \ln P_O} - n_w \frac{\partial n_w}{\partial \ln P_O} \]  

(Eq. 10)

Integrating Equation 10 from fully deoxygenated to fully oxygenated state of Hb, following Wyman and his definition of the median ligand activity, \( P_m \) (25, 26), we find the relationship we have used to analyze concomitant changes in solute and water binding upon allosteric regulation, as shown in the following example:

\[ \frac{d \ln P_{50}}{d \ln a_w} = 4 \left( 55.51 \, \Delta n_s + \Delta n_w \right) \]  

(Eq. 11)

Thus, the slope of a plot InPm (or to a good approximation InP50, the logarithm of the oxygen pressure at half saturation) versus Inaw reflects the apparent difference in the number of solute and water molecules (\( \Delta n_s \) and \( \Delta n_w \), respectively) bound to the fully oxygenated and fully deoxygenated hemoglobin molecules. These same results can be obtained from the plot of ln Pm versus ln aw as a consequence of the interdependence of dlnaw and dlnaaw expressed by Equation 3. In such a case, Equation 11 becomes the following:
RESULTS

The effect of glucose and glycine on Hb oxygen affinity in 50 mM bis-Tris/acetate, pH 7.0, expressed as \( \ln(P_{50}/P_{50}^o) \), is presented in Figs. 1–3 representing three distinct linkage plots.

First, we considered a linkage between \( O_2 \) and heterotropic water binding by plotting \( \ln(P_{50}/P_{50}^o) \) versus \( \ln(a_w) \), the logarithm of water activity varying glucose (Fig. 1A) and glycine (Fig. 1B), both in a \( Cl^- \)-free buffer solution. Measurements carried out with Hb in the presence of 0.1 M NaCl and glucose are shown in Fig. 1C. We also measured the effect of glycine with Hb in a buffer containing 0.1 M NaCl (data not shown). The observed effect on \( \ln(P_{50}/P_{50}^o) \) was in this case much smaller than that shown in Fig. 1B, measured also with glycine but in the absence of \( Cl^- \). This effect is probably caused by a decrease on the activity coefficient of ion chloride with added glycine (27).

The plots in Fig. 1, A–C, show that \( \ln(P_{50}/P_{50}^o) \) varies linearly with changes in the chemical potential of water, in agreement with our previous report on the effect of sucrose, polyethylene glycols, and stachyose on Hb \( O_2 \) affinity (1). Following the Wyman linkage equation (Equation 1), the rate of change of \( \ln(P_{50}/P_{50}^o) \) on \( \ln(a_w) \) gives the apparent number of water molecules \( (D_n w) \) involved with the allosteric transition from the fully deoxygenated to fully oxygenated state of Hb. The measured hydration change for each set of data is shown in the third column of Table I and appears to be constant within the experimental error and independent of the chemical differences between glycine and glucose.

Fig. 2 illustrates the same data shown in Fig. 1, A–C, plotted as a function of the logarithm of solution osmolality, a quantity which is directly proportional to the solute activity. In such a case, the analyses of these plots based only on the Wyman linkage equation (Equation 1) could be interpreted as reporting a weak allosteric solute binding since we assume no energetic contribution from water differential binding promoted by the indirect effect of solutes on the chemical potential of water. However, these data can be better analyzed by the extended linkage Equation 12, which avoids any assumption about binding identity (water, solute, or both). This allows us to quantify the separated contribution of solute and water binding to the regulation of \( O_2 \) binding to Hb. The continuous lines drawn in Fig. 2, A–C, represent the best fit of the data to Equation 12. The adjusted parameters \( D_n w \) and \( D_n s \), the heterotropic contribution of solute and water binding to the control of Hb oxygenation, respectively, are also shown in Table I.

We have further analyzed the correlation between changes in \( O_2 \) affinity and changes in dielectric constants. In Fig. 3, we plotted the free energy change of oxygenation (expressed as \( \ln P_{50} \)) as a function of the solution bulk dielectric constant with increasing glycerol (27) and glucose (28) concentrations. As seen in Fig. 3, the Hb-\( O_2 \) affinity cannot be attributed to electrostatic differences between R and T Hb conformations, since the changes in \( \ln P_{50} \) are in opposite directions for changes in dielectric constant below and above its value in pure water. Clearly, the dielectric hypothesis can be ruled out on the basis of these experiments.

DISCUSSION

The data shown in Figs. 1–3 clearly resolve part of the controversy raised by Bellelli et al. (15), who have challenged our interpretation of the effect of neutral solutes on Hb oxy-

Tanford (17) has originally derived an equation similar to this starting from the definition of the equilibrium constants for ligand and water binding to a macromolecule.
These authors suggested that the lowering of the solvent dielectric constant produced by sucrose promotes increased stability of the deoxy T form of Hb. The decreased oxygen affinity of Hb in neutral solutes (Figs. 1 and 2) is independent of the changes in the dielectric constant of the solution produced by glycine (27) and glucose (28) as indicated in Fig. 3. In agreement with this finding, results reported by Ackermann et al. (29) have shown that the rate constants for the reaction of carbon monoxide with HbO₂ are also insensitive of changes of bulk dielectric constant produced by addition of glycine, glycerol, and sucrose. The lack of a dielectric effect on Hb binding characteristics appears to be widespread among macromolecular reactions. Other studies of the effect of cosolvents on protein-DNA (9) and drug-DNA (30) interactions have also shown no correlation between solution dielectric constant and the energy of binding.

Furthermore, the lack of sensitivity of Hb cooperative oxygen binding toward bulk dielectric constant provides extra evidence for the exclusion of polyols, polyethers, and glycine from the protein surface, in agreement with the experimentally measured enhancement of preferential hydration of proteins in cosolutions of these neutral solutes (31). From the experimental point of view, this is an important observation since the osmotic stress method, as a tool to measure the water effect on biochemical reactions, relies on our ability to separate direct from indirect effects of solutes. The more effective the solute exclusion from protein the simpler the analyses of its effect.

Hb oxygen uptake depends on solvent and solute allosteric binding. A common allosteric mechanism controlling oxygen binding to Hb by glucose and glycine is suggested by the close superposition of the experimental points shown in Fig. 1, A–C (and Fig. 2, A–C), a characteristic already observed in our previous report on the effect of sucrose, polyethylene 200 and 400, and stachyose Hb affinity (1). These plots analyzed with the traditional Wyman linkage equation (Equation 1) offer two distinct interpretations of the effect of solutes on O₂ uptake: (a) Fig. 1 indicates the role of water, linking a fixed differential change in hydration between the structures of the fully oxygenated and fully deoxygenated state of Hb with oxygen affinity; (b) Fig. 2 indicates an identical heterotropic binding of glucose and glycine, despite their different chemical structures.
We have previously indicated that the discrimination between these two hypotheses, in favor of the water effect, is possible by using different solutes to set water chemical potential. All the solutes we tested, although chemically distinct, belong to the class of cosolvents that are excluded by protein and macromolecular surfaces. This phenomenon is supported by different experimental approaches, like hydration forces (3) and density measurements (31), as well as by equilibrium dialyses of sucrose and proteins (32). However, solute exclusion does not imply the non-existence of solute specific binding. For instance, we have shown that when titrating Hb with NaCl to measure the heterotropic chloride effect on this protein, both Cl⁻–specific heterotropic binding and water differential binding are operative in the control of the energy of O₂ saturation (16). Similarly, water-cation combined effects have been considered in the interpretation of the non-linear dependence of ∆G of protein (18) and peptides (19) on salt activity. Thus, the functional profile of the linkage plots, illustrated in Figs. 1 and 2, carries considerable information about the mechanism of heterotropic action of ligand- and non-ligand-soluble species.

The curved dependence of ln P₅₀ on ln (mₕ) seen in Fig. 2 is appropriately analyzed with the extended linkage (Equation 12). As described under “Materials and Methods,” this equation contains both the energetic contribution of water and solute binding to Hb oxygen affinity. The differential numbers of water (∆nₕ) and solute molecules (∆nₛ) linked with full oxygenation found by the fitting procedure are shown in the second and third columns of Table I, respectively. They show, in average, that ∆nₛ = −0.08 ± 0.06 glucose or glycine and ∆nₕ = 66.8 ± 2.8 water molecules per Hb are involved in the equilibrium between deoxy and oxy Hb. While this number of water molecules agrees with that previously reported by us (1), the number of glycine and glucose molecules coupled with the allosteric equilibrium remains near zero, independent of the chemical nature, size, and charge of these “inert” solutes. Table I also shows a slight increase in ∆nₛ measured with glucose in a Cl⁻–free medium compared to ∆nₛ measured in the presence of the anion. It is not clear, however, if this change in ∆nₛ reports some conformational change of the deoxy T structure produced by the neutralization of positive charges in the central cavity by diffusive, unbound chloride ions, as recently proposed by Perutz et al. (33). If our analysis of the data by Equation 12 does not allow a molecular interpretation of small changes in ∆nₛ it permits, however, to state that the magnitude of ∆nₛ found through this equation indicates a rather negligible specific solute binding, if it exists at all. Therefore, we found extra evidence favoring our original interpretation that the effect of the neutral solutes on Hb binding energy is indirect, i.e. through changes in the chemical potential of water and not through “weak” allosteric binding as proposed by Belliell et al. (15).

The effects of ligands acting as allosteric regulators of biochemical reactions have its importance long recognized. Without this knowledge, it is inconceivable to fully understand the mechanisms of protein regulatory interactions. While one can strip almost any ligand off macromolecules, we cannot strip solvent. Often it is difficult to maintain the activity of the solvent constant, especially when studying processes involving binding of weak ligands. The work presented here, along with others (1, 7–14, 17–19, 27), shows some strategies, by choosing appropriated inert solutes and thermodynamic models, that can be useful to reveal and quantify the role of solvent on biochemical regulation.

Acknowledgments—We are grateful to V. Adrian Parsegian and Donald C. Rau (National Institutes of Health) and to Richard Ward and Raghuvir K. Arni for critical reading of the manuscript.

REFERENCES

1. Colombo, M. F., Rau, D. C., and Parsegian, V. A. (1992) Science 256, 655–659
2. Colombo, M. F., Rau, D. C., and Parsegian, V. A. (1993) Science 259, 1336
3. Rau, D. C., Rau, D. C., and Parsegian, V. A. (1986) Methods Enzymol. 127, 400–416
4. Haire, R. N., and Hedlund, B. E. (1983) Biochemistry 22, 327–334
5. Ciofthia, C., Wodak, S., and Janin, J. (1996) Proc. Natl. Acad. Sci. U.S.A. 73, 3793–3797
6. Janin, J., Wodak, S., and Clothia, C. (1985) J. Mol. Biol. 183, 267–270
7. Kornblatt, J. A., and Hui Bon Hao, G. (1990) Biochemistry 29, 9370–9376
8. Rand, R. P., Fuller, N. L., Butko, P., Francis, G., and Nicholls, P. (1993) Biochemistry 32, 5925–5929
9. Robinson, C. R., and Sligar, S. G. (1994) Biochemistry 33, 3787–3793
10. Parsegian, V. A., Rand, R. P., Colombo, M. F., and Rau, D. C. (1994) Adv. Chem. Ser. 235, 177–196
11. Colombo, M. F., and Rau, D. C. (1992) Biochem. J. 281, 541 (abstr.)
12. Giraudier, R. S., Chen, H. H., Colombo, M. F., Choe, Y., Short, B. J., and Rau, D. C. (1995) Biochemistry 34, 14400–14407
13. Sidorova, N. Y., and Rau, D. C. (1995) Biopolymers 35, 377–384
14. Gardner, W. M., and Rau, D. C. (1995) EMBO J. 14, 1257–1263
15. Belliell, A., Brancocci, A., and Brunori, M. (1993) J. Biol. Chem. 268, 4742–4744
16. Colombo, M. F., Rau, D. C., and Parsegian, V. A. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 10517–10520
17. Tanford, C. (1969) J. Mol. Biol. 39, 539–544
18. Ha, J. H., Capp, M. V., Hohenwart, M. D., Pekerlake, M. P., and Record, M. T. Jr. (1990) J. Mol. Biol. 228, 252–264
19. Masotti, D. P., and Lohman, T. M. (1993) Biochemistry 32, 10568–10579
20. Williams, R. C., and Tsay, K. Y. (1973) Anal. Biochem. 54, 137–145
21. Rigg, A., and Woldbach, R. A. (1965) J. Gen. Physiol. 39, 585–605
22. Rosell-Faneli, A., and Antonini, E. (1958) Arch. Biochem. Biophys. 77, 487–492
23. Banesch, R. E., Benesch, R., and Yung, S. (1973) Anal. Biochem. 55, 245–248
24. Glashanite, S. (1977) ‘Termodinamica para Quimicos’, 5th Ed., pp. 460–470, Aguilar, Madrid
25. Wyman, J. (1964) Adv. Protein Chem. 19, 223–286
26. Wyman, J., and Gill, S. J. (1990) Binding and Linkage Functional Chemistry of Biological Macromolecules, University Science Books, Mill Valley, CA
27. Eddal, J. T., and Wyman, J. (1958) Biophysical Chemistry, Vol. 1, p. 368, Academic Press, Inc., New York
28. Malmberg, C. G., and Maryott, A. A. (1950) J. Res. Natl. Bureau Standards 45, 299–303
29. Ackerman, E., Berger, R. L., Blair, W. L., and Strother, G. K. (1963) Biophys. J. 3, 469–478
30. Varani, G., Della Torre, I., and Baldini, G. (1987) Biophys. Chem. 28, 175–181
31. Timashoff, S. (1993) Annu. Rev. Biophys. Biomol. Struct. 22, 67–97
32. Boll, H. B., and Breese, K. (1978) Biopolymers 17, 2121–2131
33. Perutz, M. F., Shih, D. T., and Williamson, D. (1994) J. Mol. Biol. 239, 555–560

TABLE I

| Experimental condition | Fitting to extended linkage (Equation 12) | Fitting to Wyman linkage (Equation 1) |
|------------------------|------------------------------------------|---------------------------------------|
|                        | ∆nₛ ± S.D.                               | ∆nₛ ± S.D.                            | ∆nₛ ± S.D. | r     |
| Glucose, no chloride   | 75.2 ± 1.6                               | −0.10 ± 0.02                           | 83.2 ± 2.8 | 0.99  |
| Glucose in 0.1 mM chloride | 62.4 ± 2.8                           | −0.12 ± 0.04                           | 79.2 ± 5.6 | 0.97  |
| Glycine, no chloride   | 63.2 ± 3.6                               | −0.024 ± 0.12                          | 72.6 ± 7.6 | 0.96  |

Change in the number of water (∆nₕ) and solute molecules (∆nₛ) ± S.D. in the transition of fully deoxy to fully oxy forms measured per tetramer in different solvents as estimated from Equations 1 and 12. "r" represents the linear regression coefficient.
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J. Biol. Chem. 1996, 271:4895-4899.
doi: 10.1074/jbc.271.9.4895

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