Direct intracellular measurement of deoxygenated hemoglobin S solubility

Mary E. Fabry, Laurephile Desrosiers, and Sandra M. Suzuka

The solubility of deoxygenated hemoglobin S (HbS), which is the concentration of fully deoxygenated HbS in equilibrium with polymer (CSAT), is a factor that determines in vivo polymer formation. However, measurement of CSAT is usually performed under conditions that are far from physiological. In solution studies of HbS by Benesch et al., it was demonstrated that p50, the point at which hemoglobin is half-saturated with oxygen, is proportional to the amount of polymer formed and that it may be used to measure CSAT. This method has been extended to measure CSAT in intact red cells by varying extracellular osmolality, which, in turn, varies intracellular hemoglobin concentration. This method measures intracellular CSAT under physiological conditions with intact red cell contents and can be applied to human and transgenic mouse red cells. The principle is demonstrated by measuring p50 as a function of extracellular osmolality for AA, SS, and AS red cells. (Blood. 2001;98:883-884)

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Study design

Blood samples

Heparinized venous blood was obtained with informed consent from patients with SS and AS who were enrolled in Heredity Clinic of the General Clinical Research Center. Genotype was confirmed by hemoglobin electrophoresis and isoelectric focusing. None of the patients with sickle cell disease had been transfused within the prior 3 months. AA blood was drawn from hematologically normal donors after informed consent.

p50 measurements

High- and low-osmolarity buffers containing 10 mM HEPES, 5 mM KCl, 5 mM glucose and NaCl, which was added to adjust the osmolality to 160 or 450 mOsm, were prepared. The buffers had a pH of 7.4 at 37°C, and intermediate osmolalities were prepared by mixing the 2 buffers and

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measuring osmolarity with a MicroOsmette (Precision Systems, Natick, MA). Cells were washed 3 times with buffer and allowed to equilibrate for a standardized period, and aliquots were removed for measurement of p50 using a Hemoscan (Aminoco, Silver Spring, MD) on the slowest change in percentage oxygen program to allow the equilibration of polymer and solution phases. Hemoscan (Aminoco) measurements were made by plotting p50 versus extracellular osmolarity for AA, SS, and AS red cells. The CSAT for this patient, who had 9.7% HbF, was 18.2 g/dL. The patient with AS did not have the α-thalassemia trait and had an estimated CSAT of 32.2 g/dL. Under fully deoxygenated conditions, the traditional method of measuring CSAT yielded values of 15.8 g/dL for purified SS.5

### Results and discussion

The transition from polymer-free red cells to red cells with polymer occurs within the range of osmolarities tested for AS red cells (Figure 1A). Within the limits of error, a straight line with a slope of nearly 0 and a p50 of 23 mm Hg was found for AA red cells and for AS red cells at osmolarities less than 280 mOsm. This is expected because the p50s for HbA and HbS are the same in dilute Hb solutions. A straight line with a slope of 0.089 ± 0.01 mm Hg/mOsm, with r² = 0.94, was found for SS red cells. For SS cells, p50 versus osmolarity is linear to the lowest osmolarity tested; the line intersects the low-osmolarity plots for AA and AS red cells at 124 mOsm, which corresponds to an MCHC of 18.2 g/dL (Figure 1B), with a maximum value of 23 g/dL at the 95% confidence interval. The intersection of the low-osmolarity and the high-osmolarity lines for AS cells occurs at 330 mOsm; MCHC at this osmolarity is 32.2 g/dL. These values are higher than those found in solution CSAT measurements (Figure 1), which may be attributed in part to higher pH (pH 7.4 vs pH 6.8) and, in the case of SS cells, the presence of HbF in these measurements. Patient-to-patient variation is expected and observed.

These measurements yield 2 values—the osmolarity and the MCHC at which polymer formation begins. The latter is a measure of the effect of cell contents on polymer formation, whereas the former relates both whole animal physiology (plasma and renal osmolarity) and red cell physiology (factors affecting red cell density/MCHC) to polymer formation. The onset of polymer formation in AS cells occurs at an osmolarity (330 mOsm) that is higher than physiological (280-300 mOsm), but within the range found in kidney. This is consistent with the relative clinical severity of AS (AS has only a urine-concentrating defect).

Red cell heterogeneity is a potential complication for these measurements; however, because each cell acts like an independent container of well-defined solution, the effective p50 in the presence of red cell heterogeneity (MCHC or HbF) is a linear combination all cells present. This technique may be particularly suitable for measuring relative CSAT for evaluating antisickling agents with multiple effects and for comparing different strains of transgenic mice that may express complex mixtures of hemoglobins.

### References

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