Dioxygen-binding of Intact Red Blood Cells measured with the Continuous Gas Injection Apparatus

H. Sick, K. Gersonde

Abteilung Physiologische Chemie
Rheinisch-Westfälische Technische Hochschule Aachen
D-5100 Aachen, Germany (F.R.)

The dioxygen-binding parameters of red blood cells (RBC) are of particular diagnostic interest. Metabolic diseases, hormonal dysregulations, haemoglobin mutations, storage of blood and incorporation of effectors and drugs into RBC's influence the physiologic O_2 release capacity of blood.

For a rapid routine measurement of O_2 dissociation curves of RBC with O_2 half-saturation pressures in the range of 1 - 100 mmHg the continuous gas injection apparatus (Erythrox, Medizinische Biophysik e.V.,Aachen) has been developed. This method needs not more then 10 µl blood. The RBC's (hematocrit:40 %) are spread out to prepare a thin layer with not more than 25 µm thickness. In this thin layer a change in O_2 saturation of RBC is detected by photometric methods. The measuring procedure and the analysis of data are computer-controlled. One experiment inclusive evaluation of data is performed within 20 minutes.

The basic principle of this method is the continuous photometric registration of the O_2 equilibrium during a controlled exponential decay of the O_2 concentration. The decay is produced inside a gas reaction cell with a N_2 injection system. Thus the gas reaction cell is perfused by nitrogen gas and acts as a gradient mixer with a constant volume V_0. The actual O_2 partial pressure p in the gas reaction cell is described by the expression, $-dp/p = dV/V_0$ which is transformed by integration to $\ln p = \ln p_0 - V/V_0$. p_0 is the O_2 partial pressure in the gas reaction cell at the starting point of the experiment (with V = 0), V is the measured volume of injected N_2 gas.

Base-line shifts generally connected with the thin-layer technique are automatically corrected by software.

A major physical problem of the photometric determination of the O_2 saturation of RBC's is based on the fact, that a thin layer of RBC's leads to incomplete covering and therefore to an optically inhomogeneous area. The effective area of the homogeneously packed RBC's is calculated by a polynomial with Lambert-Beer terms and is used for corrections of the photometric analysis.

The calculated true O_2-binding curve of RBC exhibits properties of multiple-linked equilibria between intracellular haemoglobins, dioxygen and 2,3-bisphosphoglycerate (DPG). The analysis of the O_2-binding curve on the basis of these linked functions allows the calculation of the intracellular content of DPG bound to haemoglobin. Furthermore, the fractions of haemoglobin components with different O_2-binding properties can be estimated from the saturation dependence of the Hill parameter n (n-versus-S plot). This analysis is demonstrated for fetal and adult human RBC and for porcine RBC with highest DPG levels. In all these cases the n-versus-S plot exhibits no symmetry and no uniform change of n with saturation indicating thermodynamically different haemoglobin components in the RBC's.