Potentiometric Titration of monomeric Hemoglobins (Chironomus th.th.) by a highly resolving Differential Titrator

N. Busch, P. Freyer, G. Steffens and H.D. Ohlenbusch
Abteilung Physiologische Chemie, Rheinisch-Westfälische Technische Hochschule Aachen, D-5100 Aachen, West Germany

The direct registration of differential titration curves using two glass electrodes is one of the most accurate methods for potentiometric titration of acids (or bases) (1,2). The essential advantage of this measuring technique is that errors in measurement caused by zero-point-drift of the glass electrodes can be completely compensated for and that errors due to drift of the slope of the glass electrodes can be adjusted. We therefore developed an apparatus for automatic registration of differential titration curves, the entire control and data registration being done by a modified microcomputer (Intel SDK-85). This technique guarantees an adjustment of measurement conditions to the measurement procedure and a good reproducibility of measurements. The relative error of the measured values $\Delta pH/\Delta q$ and q (q = titrant equivalent) amounts to 0,7 % and to 0,04 % respectively.
The differential titration curves of the monomeric components of the larval hemoglobin of Chironomus thummi thummi are measured in dependency of salt-concentration (NaCl, $NaClO_4$) as well as of "Ligands" (H_2O-Hb(III), CO-Hb(II). The titration curves in accordance to salt-concentration vary much more than it should be expected due to variation of the inter-ionic interaction alone (Linderstrøm-Lang). Moreover, with increasing salt-concentration (0,01→1 M/l) the iso-ionic pH-values are significantly shifting to alkaline values ($\Delta pH = 0{,}2 - 0{,}3$). The titration curves can be explained if one presumes a drift of proton -binding-behaviour (pK-shift) by binding anions to at least two groups for H_2O-Hb(III) and at least one group for CO-Hb(II). If one compares the titration curves of the different ligated hemoglobins under this aspect one can identify the prot olysis-group as one of the anion-binding groups for H_2O-Hb (III). The results are being discussed under the aspect of heterotropic allosteric effects particularly since a heterotropic allosteric Bohr effect for the components III and IV is known.

1. Luzzana, M., Perella, M. and Rossi-Bernardi, L. (1971) Anal. Biochem., 43, 556 - 563.
2. Busch, N. and Freyer, P. (1977) Anal. Biochem., 79, 212-216.