creased by addition of calculated amounts of 200 g/L glucose. Serum glucose concentrations attained by this procedure were controlled by measurement with the Beckman Glucose Analyzer.

The sensitivity to glucose (calculated for the range 5–10 mmol/L) in phosphate buffer (pH 7.4) was 10.2 ± 3.3 nA·L·mmol⁻¹ (mean of slopes calculated from each experiment ± SE; Fig. 1A). The sensitivity to glucose after exposure to human serum for 15 h was 3.7 ± 1.7 nA·L·mmol⁻¹. After reexposure to buffer, the sensitivity was 8.3 ± 2.0 nA·L·mmol⁻¹. The mean sensitivities in serum were significantly lower in serum compared with buffer before and after exposure to serum (P < 0.01; one-way ANOVA). The corresponding results obtained with flow cells in which a microporous PTFE membrane was placed between the electrode surface and the YSI membrane are shown in Fig. 1B. Again, the sensitivities for glucose (calculated for 5–10 mmol/L) were similar in PBS before (1.7 ± 0.4 nA·L·mmol⁻¹) and after (1.3 ± 0.3 nA·L·mmol⁻¹) exposure to serum. However, in contrast to the experiment without the PTFE membrane, there was no decrease of sensitivity after exposure to serum (1.8 ± 0.4 nA·L·mmol⁻¹). Statistically significant differences between the sensitivities in serum and in buffer before and after serum exposure could not be detected.

Exposure of unmodified glucose electrodes to human serum for 15 h decreased the sensitivity to glucose by 55%. When electrodes were subsequently perfused for 1 h with PBS, the sensitivity was restored almost completely. The decrease of sensitivity in serum was prevented when a microporous PTFE membrane was placed between the enzyme layer and the electrode surface. The observation that a membrane that protects only the platinum anode (and not the outer membrane or the enzyme layer) can prevent the loss of sensitivity clearly speaks against the importance of protein adsorption or enzyme inactivation in the loss of sensitivity. On the other hand, these results allow one to conclude indirectly, and therefore cautiously, that processes at the electrode surface that inhibit the detection of hydrogen peroxide, and that are possibly prevented by the protecting membrane, are more important. This is in accordance with the results from other authors that show that sensitivity to glucose in blood or serum is retained best in electrodes that exhibit minimum responsiveness to interfering substances (7). From the data presented here, direct information about electrode fouling processes cannot be derived. In the beginning of the study we planned to monitor the responses of the electrodes to hydrogen peroxide before, during, and after exposure to serum. The rapid decomposition of hydrogen peroxide in serum, however, precluded this strategy. Strictly speaking, the conclusions apply only to macrosensors for glucose measurement exposed to undiluted human serum. Additional experiments are necessary for assessment of the situation with microsensors implanted into subcutaneous tissue.

References


Capillary Zone Electrophoresis for the Diagnosis of Congenital Hemoglobinopathies, Nathalie Mario,1 Bruno Baudin,1,2 Arnaud Bruneel,1 Jacques Janssens,3 and Michel Vaubourdolle1 (1 Service de Biochimie A, Hôpital Saint-Antoine, AP-HP, 184 rue du Fbg Saint-Antoine, 75571 Paris Cedex 12, France; 2 Laboratoire de Biochimie et Glycobiologie, Faculté de Pharmacie Université René Descartes-Paris V, 75006 Paris, France; and 3 Analis, B-5000, Namur, Belgium; * author for correspondence: fax 33 1 49 28 20 77, e-mail nathalie.mario@sat.ap-hop-paris.fr)

The analysis of human hemoglobins (Hbs) is of medical importance in a number of congenital defects. The hemoglobinopathies are grouped into defective variants of Hb, such as Hb S and >600 other variants, and thalassemias characterized by abnormal expression of the genes for normal globin chains (1–3). Alkaline electrophoresis, performed historically on cellulose acetate and currently on agarose, combined with citrate agar electrophoresis at acidic pH is still widely used (4–7), but high-performance cation-exchange chromatography (HPCEC) offers superior resolution, speed, and automation (8–10). Capillary electrophoresis uses numerous separation principles and shares with HPLC the advantages of high resolution and automation, with on-line detection and direct quantification (11–15). Capillary isoelectric focusing on coated capillaries can be used to study Hb (16–21), but is slower than HPCEC (21). The first reported assays based on capillary zone electrophoresis (CZE) gave poor resolution of Hbs or were not quantitative (22–24). The aim of the present work was to evaluate a rapid CZE assay with dynamic coating of the fused-silica at alkaline pH to detect abnormal Hbs and to quantify Hbs for the diagnosis of thalassemias, and at acidic pH to confirm the identity of Hb variants.
Reagents were obtained from Analis. The alkaline kit A2 contained a hemolyzer, an initiator consisting of a polycation, and an arginine buffer (pH 8.8) containing a polyanion. The acidic kit A1 has been described elsewhere for Hb A1c measurement (25). Adult and newborn samples were collected in EDTA-containing tubes at the maternity unit of the Hôpital Saint-Antoine (AP-HP, Paris, France) and received in the laboratory for Hb analysis. Whole blood samples (20 μL) were lysed by addition of 100 μL of appropriate hemolyzing solution (Analis). Normal and abnormal controls (Lyphochek hemoglobin A2 bi-level; Bio-Rad) were stored and used according to the manufacturer’s instructions.

We used a P/ACE 5000 System with an ultraviolet/visible detector (at 415 nm) and System Gold software, Ver. 8.1, from Beckman Instruments. The Gold software quantifies the data on the basis of corrected peak areas for velocities, and values are expressed as percentages of total Hb. The separations were performed on a 25 μm (i.d.) × 24 cm (total length)/17 cm (length to detector) fused-silica capillary. The instrument was set up with the anode at the inlet end of the capillary and the cathode at the outlet. The capillary was thermostated at 26 °C. Before each electrophoresis, the capillary was first pressure-rinsed with the initiator solution (138 kPa for 3 min), and then rinsed and filled with the buffer (138 kPa for 1 min). The sample was injected for 2 s at 3.5 kPa, followed by a 15-s injection with buffer solution (3.5 kPa). The electrophoresis was performed with a constant current of 52 μA (≈16 kV) for 4 min. Between analyses, the capillary was rinsed with 0.1 mol/L NaOH and deionized water (138 kPa, 1 min each).

Quantitative data obtained for Hbs A2, F, and S on patient samples by CZE were compared with those measured by HPEC on a 100 × 5.0 mm polycatA column (Touzart and Matignon), using a method described elsewhere (21). Briefly, the separation was accomplished at a

![Diagram](A) ALKALINE

![Diagram](B) ACIDIC

| Table 1. Imprecision of Hb analysis by alkaline CZE with dynamic coated capillary. 

<table>
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<th>Low</th>
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<tr>
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* The samples used for intra- and interassay precision were different.

b Mean percentage of total Hb.

Fig. 1. Separation of Hbs A2, S, F, and A1c and A by CZE with dynamic coated-capillary (A) and superimposition of alkaline CZE profiles of Hb variants that comigrate in alkaline gel electrophoresis (B). (A, top panel), CZE at alkaline pH; (A, bottom panel), CZE at acidic pH. M, flow marker. (B, top panel), one adult with heterozygous C disease (-----) and one adult with heterozygous E disease (-----); (B, bottom panel), one adult with heterozygous S disease (-----) and one adult with heterozygous D-Punjab disease (-----).
The results of the imprecision study yielded CVs of 2.1–14% (Table 1). Measurement of Hb F was linear for values between 0.8% and 80% (not shown), as assessed with mixtures of blood. The intra- (n = 20) and interassay (n = 20 days) CVs for Hb A migration times were of 0.4% and 1.7% for alkaline gel electrophoresis, whereas it comigrated with Hb A in conventional CZE at alkaline pH and identified at acidic pH (not shown) under acidic conditions, whereas Hbs C and E were differentiated (Fig. 1B, bottom panel). However, Hb E comigrated with Hb A (not shown) under acidic conditions, whereas Hb C was separated (Fig. 1A, bottom panel). However, Hb E comigrated with Hb A (not shown) under acidic conditions, whereas Hb C was separated (not shown). Hb Hope could be detected by CZE at alkaline pH and identified at acidic pH (not shown), whereas it comigrated with Hb A in conventional alkaline gel electrophoresis.

The increased resolution by CZE compared with solid phase separation methods for the diagnosis of hemoglobinopathies. The imprecision for quantitation by alkaline CZE was satisfactory, although the CV reached 14% for low concentrations of Hb F. However, the precise quantification of higher concentrations of Hb F and of high Hb A2 was adapted to the diagnosis of β-thalassemias. The detection limit of Hb F by CZE at alkaline pH was <0.8%, which is the lowest point of the linearity curve, but >0.5%, which is the lowest concentration detected in adult samples. The linearity of Hb F measurement on a wide range of concentrations, in particular from low concentrations, makes the CZE assay adapted to samples from both adults and newborns. Thus, CZE is more convenient than agarose gel electrophoresis, which does not allow Hb F measurements below 10%. Quantitative values for Hb A2, Hb F, and Hb S were highly correlated with HPCEC values. The Hb A2 concentrations were substantially higher when measured by CZE, which could be related to the differences for the separation of glycated or otherwise posttranslationally modified Hb A2 forms (21, 27).

In conclusion, CZE at alkaline pH is rapid, precise, and gives high resolution, and is suitable for the screening of hemoglobinopathies, whereas CZE at acidic pH is better suited for confirmation of qualitative abnormalities. The capillary electrophoretic study of Hbs at both alkaline and acidic pH corresponds to an improvement of the historical scheme for the diagnosis of hemoglobinopathies by allow-
ing accurate quantification and complete automation. In particular, the profiles were obtained in 7 min, including rinses, which is competitive to HPCEC on dedicated analyzers. Moreover, capillary electrophoresis systems are robust and could replace HPLC in clinical chemistry or hematology laboratories.

References


The monitoring of glycemic control in diabetic subjects has been greatly improved by the development of analytical methods for the measurement of glycohemoglobin concentrations, which reflect average serum glucose concentrations 4–8 weeks preceding the analysis. In particular, because Hba1c is considered one of the most powerful markers in such evaluations (1), optimized conditions for its determination still remain one of the major goals of clinical chemistry, as recently emphasized by Miedema (2).

The development of methods without the limitations inherent in chromatographic, electrophoretic, and immunological procedures (3) is certainly of great interest. These methods may be used to measure Hba1c directly and/or to determine the extent of glycation of the “universal calibrator”. Choosing between these two different approaches is necessarily related to the feasibility (and to the price/performance ratio) of each method. In this context, Roberts et al. (4) recently showed the potentialities of electrospray ionization mass spectrometry (ESI/MS) for quantifying glycohemoglobin. ESI mass spectra from the analysis of whole blood samples were used to evaluate the percentage of glycohemoglobin directly, and comparison of the ESI data with those from established affinity chromatographic procedures showed a particularly good linear relationship. These evaluations were based on the four ionic species detected in ESI conditions after baseline subtraction, i.e., glycated and nonglycated a- and b-globins.

A different, highly specific approach for unequivocal Hba1c evaluation, which was proposed recently by Kobold et al. (5), is based on enzymatic cleavage of hemoglobin by an endoprotease. This treatment yielded the b-N-terminal peptides of Hba1c and Hba0, and their separation and quantification was performed by ESI/MS and capillary electrophoresis. This procedure may be used as a reference method for Hba1c measurement. In previous investigations (6–10), we demonstrated that matrix-assisted laser desorption/ionization (MALDI) (11) MS is a valid analytical tool for determining the extent of glycation of in vivo and in vitro glycated proteins.

The MALDI technique is based on the interaction of a laser beam with a solid state sample composed of a