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Application of shielding boronate affinity chromatography in the study of the glycation pattern of haemoglobin

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Abstract

Human haemoglobin (Hb) may appear in a number of glycated species. The glycation pattern of Hb using shielding boronate affinity chromatography (SBAC) has been studied in the present work. SBAC is a novel separation technique, which eliminates nonspecific boronate–protein interactions by introducing a so-called shielding reagent [1,2]. Two samples from Bio-Rad (Lyphochek®)—one from normal persons' blood with relatively low HbA_{1c} level (HbL) and the other from diabetic patients' blood with an elevated HbA_{1c} level (HbH)—were used for the investigation. Glycated Hb (GHb) was separated from nonglycated Hb species using Tris as the shielding reagent. Two eluted peaks, eluted peak 1 (E1) and eluted peak 2 (E2), were obtained using a linear gradient elution with Tris. Several bands were observed on isoelectric focusing gel, which showed the same migration positions as Hb adducts, such as HbA_0, which is major Hb component containing two α chains and two β chains; HbA_{1c}, which is post-translational glycation on the N-terminus of the β chains of HbA_0; Foetal Hb (HbF), consisting of two α chains and two γ chains; and glutathione Hb (also called HbSSG), which is the result from thiol-disulphide interchain exchange during oxidation of the thiol groups of Hb. In both HbL and HbH samples, E2 exhibited slightly higher amounts of HbF than E1. Electrospray-ionisation mass spectrometry showed that: (1) HbL-E1 was glycated with single glucose on both α and β chains while no observable glycated chains were present in HbL-E2; (2) both HbH-E1 and HbH-E2 were glycated with single glucoses on both α and β chains, however, compared with HbH-E1, HbH-E2 showed a higher relative intensity of the glycated β chain and lower relative intensity of the glycated α chain; and (3) the degree of glycation increased with increasing glycation level of the sample. The amount of HbA_{1c} presented in the eluted peaks was further determined using enzymatic digestion of glycated Hb by endoproteinase Glu-C and the subsequent separation and analysis of the digested peptides by reversed-phase high-performance liquid chromatography and capillary electrophoresis. The values of HbA_{1c}/HbA_0 of the eluted peaks, i.e. HbL-E1, HbL-E2, HbH-E1 and HbH-E2, were 0.27, 0.19, 0.50 and

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0.43, respectively. In both HbL and HbH samples, E1 contained higher amounts of HbA_{1c} than E2. This study demonstrates the structural heterogeneity of GHb as well as the possibility of using SBAC to detect glycated species of Hb.

Keywords: Shielding boronate affinity chromatography; Glycation pattern; Haemoglobin

1. Introduction

The heterogeneity of human haemoglobin (Hb) is known, as different Hb components are isolated from human haemolysate. HbA_0 ($\alpha_2\beta_2$) is the major Hb component, comprising about 90% of the total Hb. Two minor Hb components differing from HbA_0 in their non-α subunits, HbA_2 ($\alpha_2\delta_2$, 2.2–2.8%) and HbF ($\alpha_2\gamma_2$, <1%), have also been identified [3,4]. The remaining approximately 7% is distributed between other minor Hb components. Some of them are recognised as post-translational nonenzymatic modification of HbA_0, while some may be storage artefacts. Various modified Hb adducts, such as glycated Hb (GHb), acetaldehyde Hb, carbamylated Hb, acetylated Hb, xenobiotic Hb adducts and glutathione Hb adducts (HbSSG) have been described [5]. The structures of some minor Hb components have been elucidated, while others are still unclear.

From a clinical point of view, GHb is the most important Hb adduct. GHb is formed in vivo by a two-step nonenzymatic attachment of glucose or other carbohydrates to amino groups of haemoglobin (Hb) [3–5]. The first step involves the formation of a reversible Schiff base linkage between the aldehyde group of glucose and the available amino groups on the Hb molecule. The labile adduct (aldimine) then undergoes Amadori rearrangement to the stable ketoamine. The amount of GHb formed depends on the average concentration of glucose in the blood and the incubation time. Therefore, the measurement of GHb represents the average concentration of glucose in the blood, and is considered a reliable indicator in long-term diabetic control [6–9].

Glycation of Hb can occur on different Hb components as well as different modification sites of these components, and takes place through the lifespan of the erythrocytes (about 120 days). In addition to the N-terminus of the β chains, ε-amino groups of lysine residues and the N-terminus of α, γ and δ chains can also be modified by carbohydrates [10]. As a consequence of glycation, GHb is formed as a collection of different glycated species. HbA_{1c}, the ketoamine adduct of glucose at one or both N-terminal valines of the β chains of HbA_0, has gained widespread attention as it is the major component of GHb and has been identified as the glycated variant of importance for the assessment of diabetes treatment [10–12].

A variety of different methods claimed to measure GHb or HbA_{1c} are currently employed in clinical laboratories. These are based on physical, chemical or immunological principles. Ion-exchange chromatography was the first method available for the analysis of HbA_{1c} and has been widely used in clinical laboratories [13–15]. However, the method is difficult to standardise, as it is very sensitive to experimental variables, such as pH and temperature. Also, it is subject to interference from aberrant Hb species including sickle cell Hb (HbS), HbF, acetylated Hb, carbamylated Hb and labile pre-HbA_{1c}, glutathione Hb (HbSSG) and other genetic variants and chemical derivatives [10,16–18]. To circumvent this, many clinical laboratories use boronate affinity chromatography (BAC), which relies on the interaction of boronate ligands, usually *m*-aminophenylboronic acid, with hydroxy groups of glycated residues of Hb irrespective of glycation site [4,11,17–20]. In principle, multiple glycated Hb variants can be separated from nonglycated substances. However, there are reports indicating that the method does not completely separate all glycated Hb from nonglycated proteins [18,21]. Some glycated Hb species may be present in the breakthrough fractions, while nonglycated Hb can be bound to the boronate column due to nonspecific interactions and be eluted together with glycated Hb species. It has been noted that boronate ligands can

interact with nonglycated proteins, such as β-lactamase [22], subtilisin BPN [23], trypsin [24], α-chymotrypsin [25], pepsin [26], β-amylase [27] and lysine [1], which impairs the separation resolution of this method.

Shielding boronate affinity chromatography (SBAC), a technique that can suppress nonspecific interactions between the boronate ligand and nonglycosylated proteins, has been described in our previous work [1,2]. The technique is based on the introduction of a so-called shielding reagent, a low-molecular-mass polyhydroxyl compound that can interact with the boronate ligand, in the mobile phase during BAC. Nonspecific interactions between the boronate ligand and nonglycosylated proteins are suppressed as the shielding reagent has a stronger binding with the boronate ligand than nonglycated proteins. However, boronate–glycoprotein interactions can still take place, as the binding between the shielding reagent and the boronate ligand is weaker than that between glycoproteins and the boronate ligand. The evaluation of potential shielding reagents carried out in our previous work showed that some polyhydroxyl chemicals, such as Tris or Tris-like compounds, exhibited the highest shielding efficiency due to the formation of a tridentate complex with the boronate ligand [1,2]. Studies also showed that some shielding reagents, such as Tris, can function as competitive eluting agents when used at an increased concentration to elute the bound glycoproteins from the boronate column [28,29]. SBAC has been applied to the separation of glycoproteins from nonglycosylated proteins, as well as the separation of glycoproteins based on the degree of glycosylation [2,28,29].

The application of SBAC to study the glycation pattern of GHb has been investigated in the present work. Two samples from Bio-Rad, one from normal persons' blood with relatively low HbA_{1c} level (HbL) and the other from diabetic patients' blood with elevated HbA_{1c} level (HbH), were used. The glycated Hb species obtained were analysed using electrospray-ionisation mass spectrometry (ESI-MS) and isoelectric focusing (IEF). The amount of HbA_{1c} present in these glycated species was determined using enzymatic digestion by endoproteinase Glu-C. The resulting peptides were subsequently separated and analysed using reversed-phase high-performance liquid chromatography (RP-HPLC) and capillary electrophoresis (CE).

2. Materials and methods

2.1. Materials

Two samples, HbL and HbH (Lyphocheck®) containing 4.6 and 8.0% HbA_{1c}, respectively, were bought from Bio-Rad (Hercules, CA, USA). The value of HbA_{1c} has been calibrated by The Diabetes Control and Complications Trial (DCCT) research group. *m*-Aminophenyl boronic acid agarose (APBA agarose, product no. A-8312, 40–80 μmol APBA per ml packed gel) and sodium chloride (NaCl) were bought from Sigma (St. Louis, MO, USA). Tris-(hydroxymethyl)aminomethane (Tris) was purchased from BDH (Pool, UK). Endoproteinase Glu-C (cat. no. 1047817, EC 3.4.21.19) was a product of Roche Diagnostics (Mannheim, Germany). Potassium cyanide (KCN) was bought from Aldrich (Milwaukee, WI, USA). Merck (Darmstadt, Germany) supplied sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate monohydrate, ammonium acetate, potassium hexacyanoferrate (III) [$K_3Fe(CN)_6$] and sodium hydrogen carbonate ($NaHCO_3$). L-Glycine was obtained from Ajinomoto (Tokyo, Japan). Trifluoroacetic acid, acetonitrile (for HPLC, far UV) and methanol were bought from Acros Organics (Geel, Belgium). Acetic acid (HAc) and hydrochloric acid (HCl) were of analytical grade. All chemicals were used without further purification.

2.2. Protein assay

The concentration of Hb was analysed using the method established by The International Committee for Standardization in Haematology [30]. The principle relies on the determination of Hb as haemiglobincyanide using Drabkin's reagent (1 g $NaHCO_3$, 200 mg $K_3Fe(CN)_6$ and 50 mg KCN dissolved in distilled water to a volume of 1 l pH 8.6). A sample of Hb was diluted appropriately with Drabkin's reagent. The mixed solution was incubated

for 10 min at room temperature. Absorption was measured at 540 nm ($A_{540\ nm}$). The concentration, *C*, of Hb was calculated from the relation: *C* (mg/100 ml)=146.5 $A_{540\ nm}$.

2.3. Shielding boronate affinity chromatography of Hb

The chromatographic columns were purchased from Bio-Rad. The chromatographic system consisted of an Alitea pump C-4v (Stockholm, Sweden), a Gilson fraction collector (Middleton, WI, USA) and a Waters 484 tunable absorption detector (Milford, MA, USA). The APBA agarose, packed in a 0.7-cm diameter column to a bed height of 10 cm, was equilibrated with the loading buffer (0.1 *M* phosphate buffer+0.15 *M* NaCl+0.016 *M* Tris, pH 8.8). HbL (3.5 mg) or HbH (3.0) was dissolved in the loading buffer and applied to the column. The column was washed with the loading buffer until the absorption at 413 nm in the effluent had reached the baseline. Linear gradient elution was carried out using Tris (0–0.1 *M*) in 0.05 *M* glycine–NaOH, pH 9.0. The total elution volume was 120 ml. Finally, 0.05 *M* HAc (pH 4.5) was used to rinse the column. The flow-rate used during chromatography was 0.2 ml/min.

2.4. Enzymatic cleavage [12,31]

Approximately 1 mg of glycated haemoglobin (30 μl) was mixed with 50 μl Glu-C solution (50 μg Glu-C dissolved in 250 μl distilled water) and diluted with 50 m*M* ammonium acetate buffer, pH 4.3, to a total volume of 500 μl. The mixture was incubated at 37 °C for 18 h with gentle rolling. The digestion process was stopped by freezing the sample at −20 °C. Immediately before analysis, the frozen sample was thawed at room temperature and centrifuged at 14 000 *g* for 2 min. The sample solution can be refrozen at −20 °C and used within 2–3 months.

2.5. Analytical methods

2.5.1. Electrospray-ionisation mass spectrometry

A time-of-flight hybrid, Q-Tof, quadrupole mass spectrometer with an electrospray ion source (Micromass, Manchester, UK) was used. The sample was injected as received in a stream of 10 m*M* acetic acid onto a C_{18} column (10×1.0 mm I.D.) packed with 5 μm Vydac particles (Microchrom, Hesperia, CA, USA), at a flow-rate of 50 μl/min. The column was washed with 10 m*M* acetic acid for 5 min. The sample was eluted by injecting 50 μl acetonitrile. Data were acquired over a mass range from 150 to 2000 *m*/*z* with a 2-s integration time per spectrum. The data were analysed using MASSLYNX TRANSFORM from Micromass. The molecular mass was calculated as an average of at least four charge states.

2.5.2. Reversed phase high-performance liquid chromatography and capillary electrophoresis

A Shimadzu VP-HPLC was used for the RP-HPLC analysis. The system consisted of a double-plunger pump (LC-10ADVP), a gradient switch valve (FCA-10ALVP), a system controller (SCL-10AVP), a UV detector (SPD-10AVVP), an autoinjector (SIL-10ADVP), a degasser (GT-154), a fraction collector (FRC-10AVP) and software (Class VP 5.0) (Shimadzu, Kyoto, Japan). The sample was loaded onto a C_2/C_{18} column (μRPC HR4.6/100, Amersham Pharmacia Biotech, Uppsala, Sweden) equilibrated with buffer A (0.1% TFA in water). Gradient elution was carried out between buffer A and buffer B (0.1% TFA in acetonitrile): 0–3 min, 0% B–7% B; 3–19 min, 7%B–18.5% B; 19–19.1 min, 18.5% B–100% B; 19.1–23 min, 100% B; 23–23.1 min, 100% B–7% B; 30 min, stop run. A flow-rate of 1 ml/min was used during the entire process. The absorption was monitored at 214 nm. The fraction of interest, the first dominating peak, was collected, lyophilised and further analysed using CE. CE was performed at 20 °C on a Beckman (CA, USA) P/ACE system 5000 with SYSTEM GOLD® software (version 8.1). The sample was dissolved in 50 μl 0.01% TFA in water and injected into Beckman fused-silica capillaries (67×0.05 μm I.D.). A mobile phase of 0.1 *M* phosphate buffer, pH 2.5, was used. The absorption was monitored at 214 nm.

2.5.3. Isoelectric focusing

IEF was performed on an electrophoresis unit, LKB Multiphor 2117 (Amersham Biosciences, Uppsala, Sweden) according to Refs. [32] and [33]. An IEF gel containing a total polyacrylamide concen-

tration of 5% and ampholyte concentration of 2.4% for a pH range of 3.0–10 was prepared using PlusOne ReadyMix IEF and Pharmalyte pH 3–10 (Amersham Biosciences). Hb samples were concentrated to 3 mg/ml using Microsep centrifugal concentrators (M_r cut-off: 3000, Pall Filtration, MA, USA). The gel was pre-run at 1400 V, 50 mA, 20 W for 30 min. A 20-μl volume of the prepared samples was loaded onto the gel via application pieces (Amersham Biosciences) and the electrophoresis was run under the same conditions as for pre-run for 30 min. The sample application pieces were then removed and the electrophoresis was continued at 1400 V, 50 mA, 30 W for 30 min. After running, the gel was immediately immersed in the fixing solution (10% trichloroacetic acid–5% sulphosalicylic acid–85% distilled water) and then stained with 0.4% Coomassie brilliant blue R-250 (Merck).

3. Results and discussion

3.1. Separation of glycated Hb using SBAC

Nonspecific interactions between boronate ligands and nonglycated Hb were observed since nonglycated Hb was either entirely bound or significantly retarded in the boronate column, regardless of whether 0.02 *M* EPPS, pH 8.0, 0.1 *M* sodium phosphate, pH 8.5 or 0.25 *M* ammonium acetate + 0.05 *M* $MgCl_2$, pH 8.5 was used as loading buffer (data not shown). The addition of NaCl enhanced the interaction of nonglycated Hb with the boronate ligand.

The shielding concept was thus applied to BAC in order to eliminate boronate–protein interactions. The loading buffer was finally established by introducing 0.016 *M* Tris into 0.1 *M* sodium phosphate, 0.15 *M* NaCl, pH 8.8. The alkaline solution ($pH \geq 8.0$) facilitates the transition of a boronate ligand from a planar trigonal to a tetrahedral configuration, which can covalently interact with compounds containing hydroxyl groups [34,35]. The addition of a certain amount of salt (0.1–0.5 *M*) helps to suppress the nonspecific interactions. A linear gradient elution mode usually provides good results when separating heterogeneous samples, as compounds with different affinities to the ligand can be eluted under favourable conditions with continuously increasing the elution power of an eluting agent [36]. Elution was thus carried out using a linear gradient concentration of Tris from 0 to 0.1 *M* in 0.05 *M* glycine–NaOH, pH 9.0.

Under the chromatographic conditions described above, two eluted peaks, eluted peak 1 (E1) and eluted peak 2 (E2), were obtained, while a large part of loaded Hb protein was found in the breakthrough peak (Fig. 1). The analysis of these peaks was carried out using different analytical methods.

3.2. Detection of Hb species using IEF

Since charge differences resulting from post-translational glycation of β-N-terminal valine, or the variations in the primary structure of Hb e.g. HbA_0, HbA_2 and HbF, alter the isoelectric point (p*I*) of Hb molecules, the detection of different Hb components can be performed using IEF [8,32,33,37]. The typical isoelectric focusing pattern of human adult Hb can be seen in Fig. 2, lane 1. Several bands such as HbSSG, HbA_{1c}, HbA_0, HbF and sickle Hb (HbS) were detected, demonstrating the structural heterogeneity of Hb. The HbA_{1c} band was much stronger in the eluted peaks obtained from SBAC (lanes 2–4) than that in the Bio-Rad control sample (human adult Hb), indicating that HbA_{1c} was enriched during the separation process. In addition to the HbA_{1c} band, other bands corresponding to HbA_0, HbF and HbSSG were observed. These glycated Hb species can be derived from the modification by carbohydrates of ε-amino groups of lysine residues and the N-terminus of α and γ chains other than the N-terminus of β chains. Compared with E2 (lanes 3 and 5), E1 (lanes 2 and 4) contained less HbF. HbSSG adducts may be formed due to the storage of blood samples, allowing thiol disulphide interchain exchange to take place during oxidation of the thiol groups of Hb [16].

3.3. Structural analysis using MS

The transformed MS spectra of the samples investigated, HbL and HbH, are shown in Fig. 3. Both α (M_r 15 126) and β (M_r 15 867) chains were observed in HbL (HbA_{1c} 4.6% according to the manufacturer) (Fig. 2a). No detectable glycated

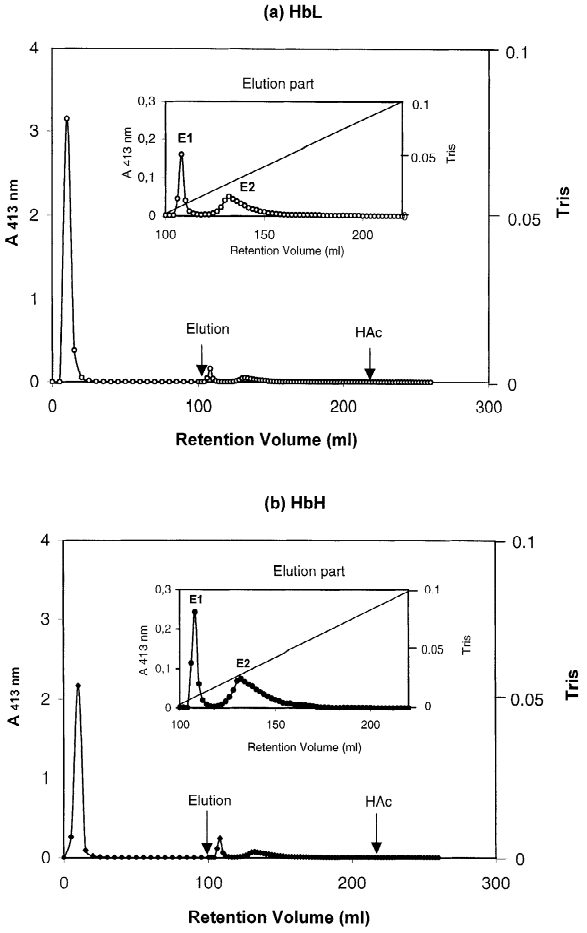

Fig. 1. Results of using shielding boronate affinity chromatography (SBAC) to separate (a) HbL and (b) HbH. Column: 10×0.7 cm I.D. Matrix: *m*-aminophenylboronic acid agarose. Buffer: loading buffer, 0.1 *M* PB+0.15 *M* NaCl+0.016 *M* Tris, pH 8.7; elution starting buffer, 0.05 *M* glycine–NaOH, pH 9.0; elution end buffer, 0.05 *M* glycine–NaOH+0.1 *M* Tris, pH 9.0. Total elution volume: 120 ml. Cleaning solution, 0.05 *M* HAc (pH 4.5). Flow-rate: 0.2 ml/min. Sample: (a) HbL, 3.5 mg; (b) HbH, 3.0 mg. Absorption was monitored at 413 nm.

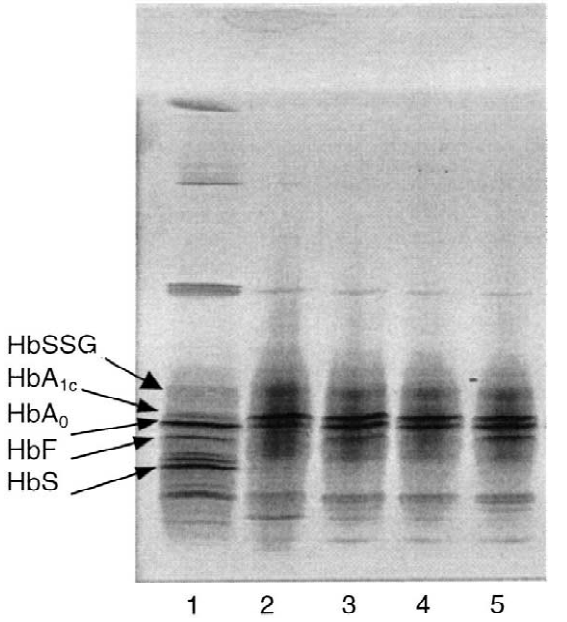

Fig. 2. Detection of Hb adducts using isoelectric focusing. An IEF gel containing a total polyacrylamide concentration of 5% and ampholyte concentration of 2.4% for a pH range of 3.0–10 was prepared using PlusOne ReadyMix IEF and Pharmalyte pH 3–10 (Amersham Pharmacia Biotech). The gel was stained with 0.4% Coomassie brilliant blue R-250. Lanes: 1=Bio-Rad control sample (human adult Hb); 2=HbL-E1; 3=HbL-E2; 4=HbH-E1; 5=HbH-E2.

chains were found, which was probably due to the low glycation level of the sample investigated. In HbH, with relatively high glycation level (HbA_{1c} 8.0% according to the manufacturer), both α (M_r 15 127) and β (M_r 15 868) chains as well as single glucose glycated α (M_r 15 289) and single glucose glycated β (M_r 16 031) chains were found (Fig. 2b). Peterson et al. have also reported similar results [38]. The glycation of both α and β chains increases with increasing value of glycated Hb obtained from clinical analysis, although not at the same rate [38].

Figs. 4 and 5 show the transformed spectra of the breakthrough peak, eluted peak 1 (E1) and eluted peak 2 (E2) arising from HbL and HbH, respectively. Neither glycated α chains nor glycated β chains were present in the breakthrough peaks of HbL or HbH (Figs. 4a and 5a), indicating that all glycated Hb species were bound to the boronate column. With regard to the eluted peak 1 (E1), both HbL-E1 and HbH-E1 contained single glucose glycated α chains (M_r 15 290 for HbL-E1 and 15 291 for HbH-E1) and glycated β chains (M_r 16 030 for HbL-E1 and 16 031 for HbH-E1), however, HbH-E1 showed a higher relative intensity of glycated β chains (Figs. 4b and 5b). In the case of eluted peak 2 (E2), no observable glycated chains were present in HbL-E2 (Fig. 4c), while HbH-E2 contained single glucose glycated α chains (M_r 15 290) as well as single

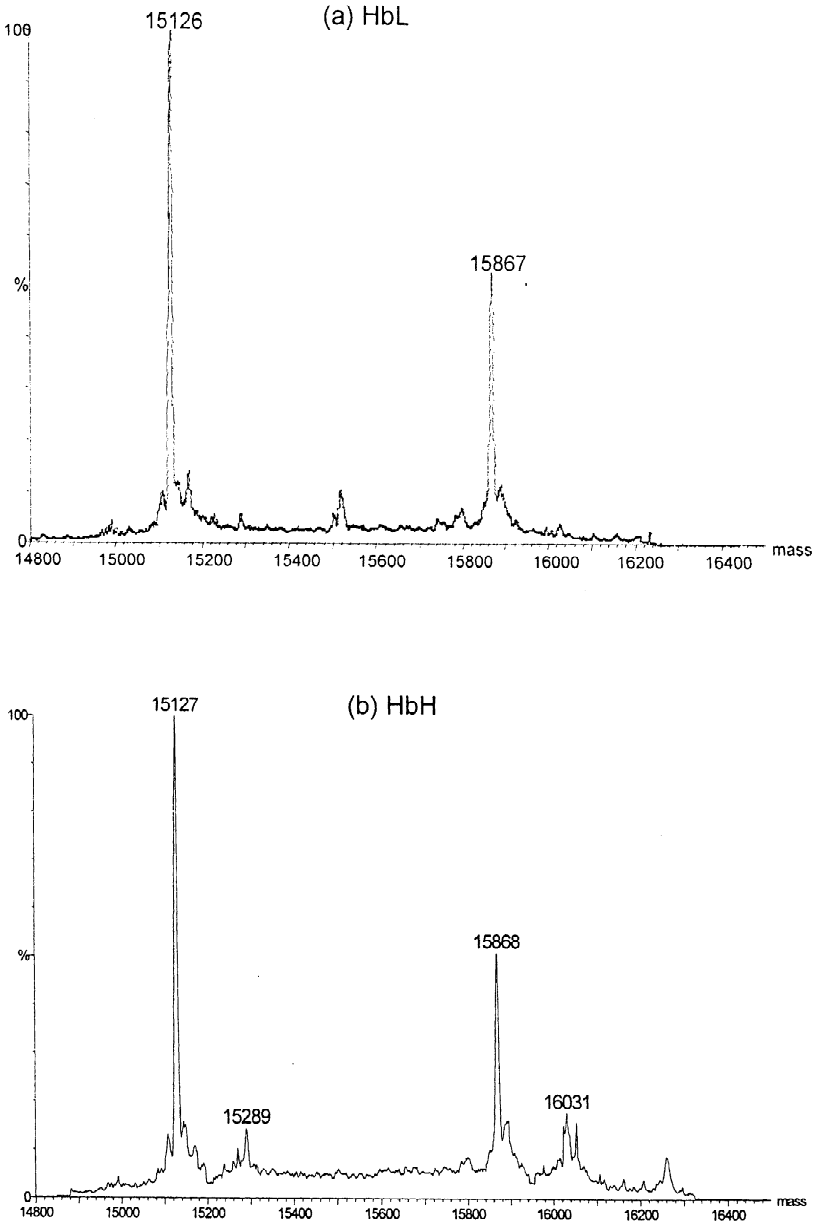

Fig. 3. Electrospray mass spectra of (a) HbL and (b) HbH.

glucose glycated β chains (M_r 16 032) (Fig. 5c). The results also showed that HbH-E1 and HbH-E2 (Fig. 5) had higher degrees of glycation than HbL-E1 and HbL-E2, respectively (Fig. 4). Although both HbH-E1 and HbH-E2 contained glycated α and β chains, compared with HbH-E2, HbH-E1 showed a higher relative intensity of glycated β chains and a lower relative intensity of glycated α chains. The MS analysis showed that both E1 and E2 contained multiple glycated adducts, which has been noted in many other reports [1,2,4–6,8–10,31]. In addition, the glycation patterns of E1 and E2 were different.

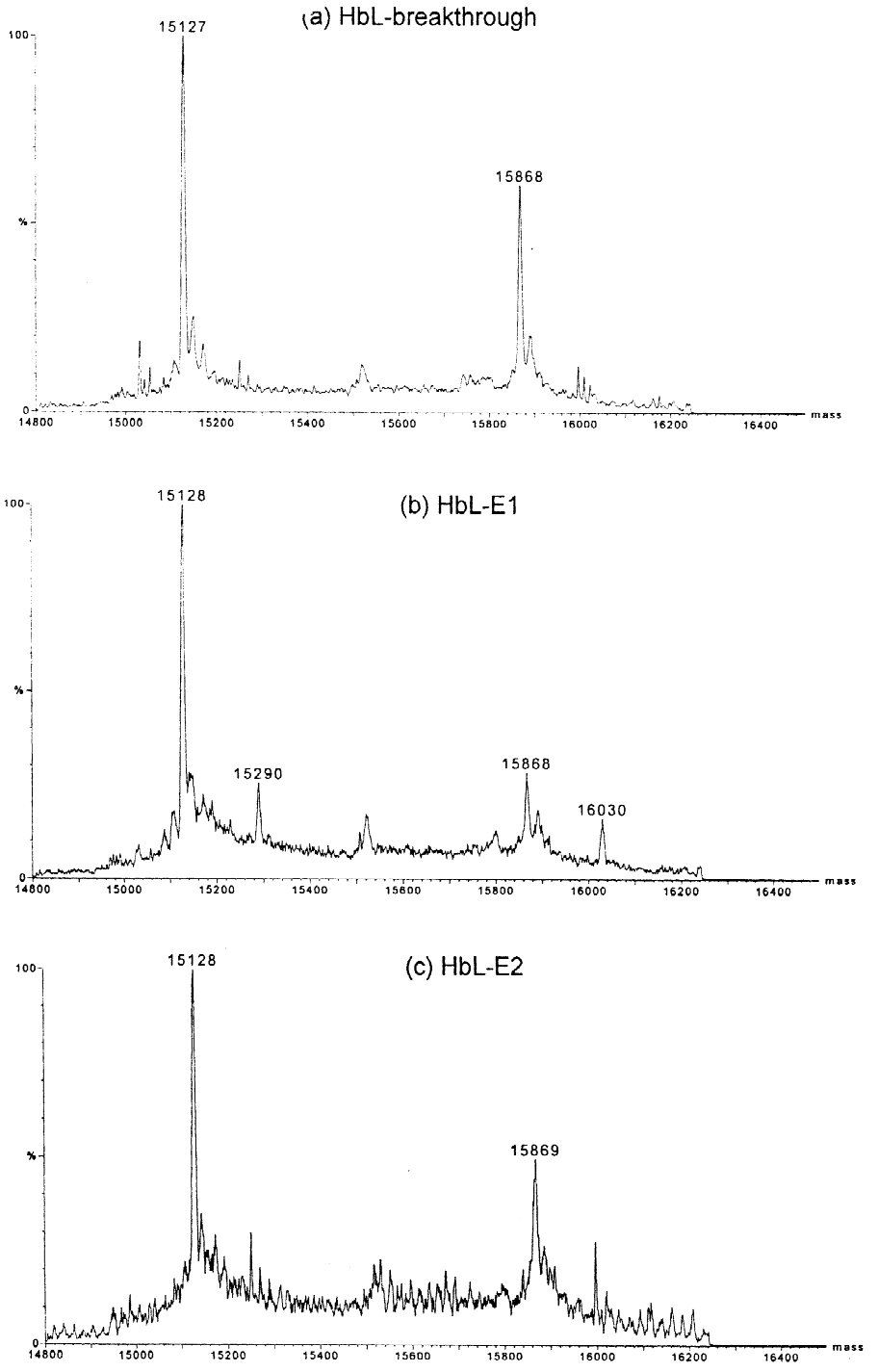

Fig. 4. Electrospray mass spectra of (a) breakthrough peak; (b) eluted peak 1 (E1) and (c) eluted peak 2 (E2) from HbL.

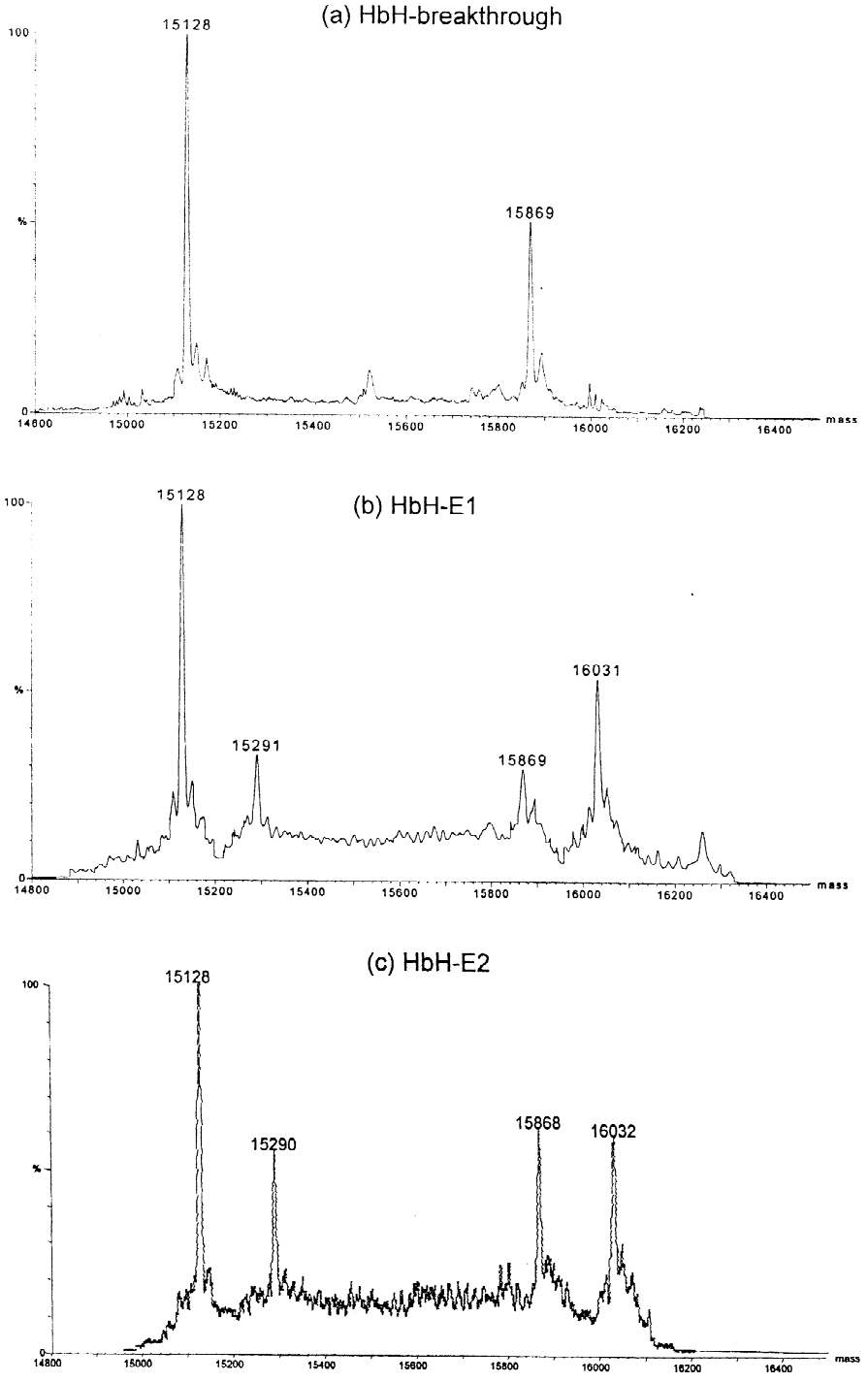

Fig. 5. Electrospray mass spectra of (a) breakthrough peak; (b) eluted peak 1 (E1) and (c) eluted peak 2 (E2) from HbH.

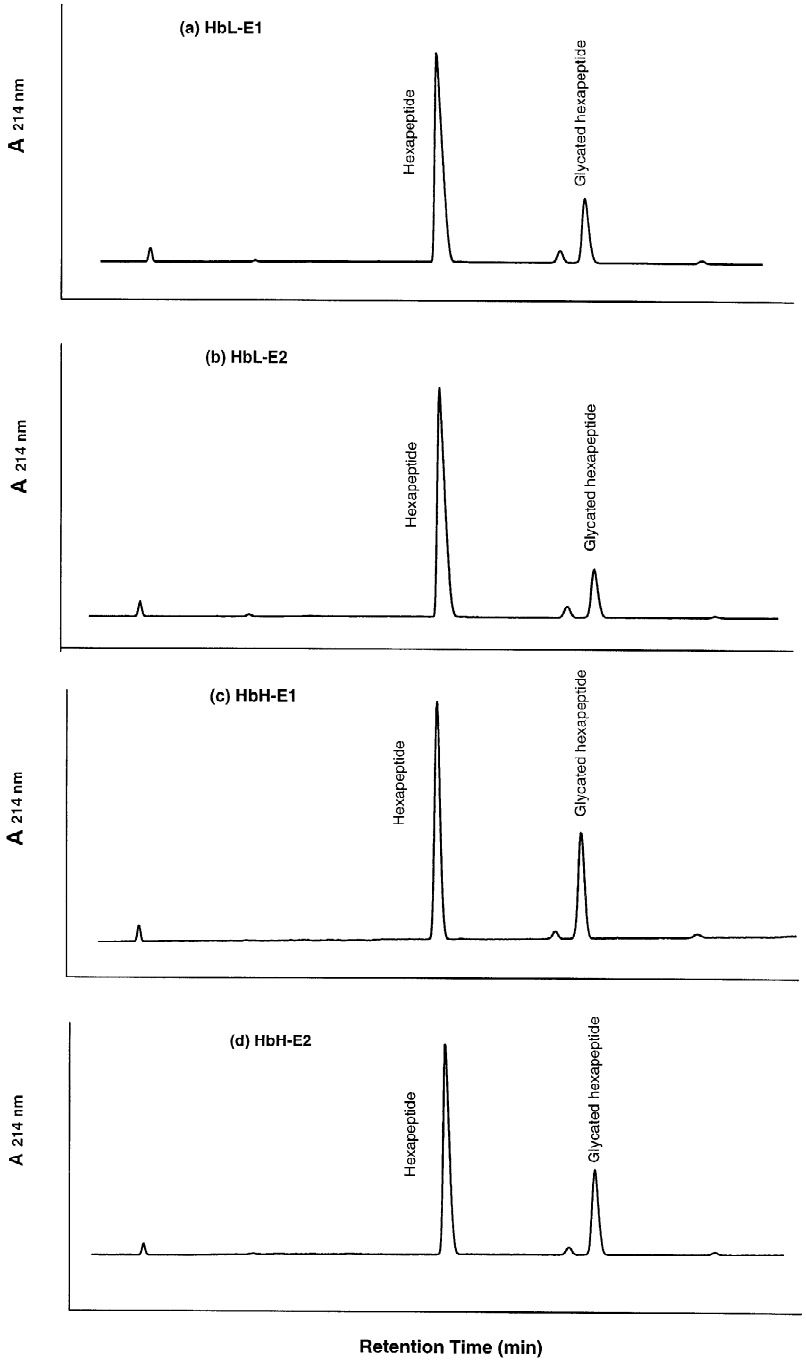

Fig. 6. Separation of glycated β-N-terminal hexapeptide from β-N-terminal hexapeptide using capillary electrophoresis. Samples detected were (a) E1 from HbL, (b) E2 from HbL, (c) E1 from HbH and (d) E2 from HbH. All samples were digested using endoproteinase Glu-C. The resulting peptides were isolated using reversed-phase HPLC on a C_{18} column.

3.4. Determination of the amount of HbA_{1c} using RP-HPLC and CE

As HbA_{1c} is an important parameter in the control of diabetes, the amount of HbA_{1c} present in the eluted peaks was further determined. A method for quantitative analysis of HbA_{1c} via β-N-terminal hexapeptides has been developed [10]. A serine protease, endoproteinase Glu-C, was used to cleave the β-N-terminal part of Hb between the two glutamic acid residues at positions 6 and 7 [10]. The cleaved hexapeptides contain only a single glycation site at N-terminal valine and can thus be used to distinguish between HbA_{1c} and HbA_0 [10]. Ammonium acetate buffer (pH 4.0) was used in the present study to limit the enzyme specificity to glutamic acid [39].

The cleaved glycated and nonglycated β-N-terminal hexapeptides were first enriched via RP-HPLC on a C_{18} column, and then separated using CE. The percentage of glycated hexapeptide (HbA_{1c}) could thus be calculated, reflecting the amount of HbA_{1c} (Fig. 6). The values of HbA_{1c}/HbA_0 were 0.27 (Fig. 6a) and 0.19 (Fig. 6b) for HbL-E1 and HbL-E2, respectively, and 0.50 (Fig. 6c) and 0.43 (Fig. 6d) for HbH.-E1 and HbH-E2, respectively. The values presented in these eluted peaks were much higher than those in nonpurified Hb sample (around 5%), confirming the IEF result that the HbA_{1c} band in these eluted peaks was much stronger than that in Bio-Rad control sample (Fig. 2). As expected, the amount of HbA_{1c} increased with increasing glycation level of the sample. However, in both HbL and HbH, E1 shows higher amounts of HbA_{1c} than E2, indicating that E1 and E2 represent different glycation patterns.

4. Conclusions

The study of the glycation pattern of Hb using SBAC combined with high-resolution analytical methods reveals multiple glycated Hb species with structural heterogeneity. SBAC improves the separation resolution by reducing nonspecific boronate–protein interactions. The study of different glycated species can be of importance in diabetes mellitus research.

5. Abbreviations

Hb	Haemoglobin
HbL	Bio-Rad sample from normal persons' blood containing 4.6% HbA_{1c}
HbH	Bio-Rad sample from diabetic patients' blood containing 8.0% HbA_{1c}
HbL-E1	Eluted peak 1 when chromatography of HbL on a shielding boronate column
HbL-E2	Eluted peak 2 when chromatography of HbL on a shielding boronate column
HbH-E1	Eluted peak 1 when chromatography of HbH on a shielding boronate column
HbH-E2	Eluted peak 2 when chromatography of HbH on a shielding boronate column
GHb	Glycated haemoglobin
HbA_0	Major Hb component containing two α chains and two β chains
HbA_2	Minor Hb component containing two α chains and two δ chains
HbF	Foetal Hb, minor Hb component containing two α chains and two γ chains
HbS	Sickle cell Hb
HbSSG	Glutathione Hb, thiol-disulphide interchain exchange during oxidation of thiol groups of Hb
HbA_{1c}	Post-translational modification of N-terminal amino group of the β chain of HbA_0 with glucose
BAC	Boronate affinity chromatography
SBAC	Shielding boronate affinity chromatography

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