β^{+}-Selective radiodetector for capillary electrophoresis

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ABSTRACT

A compact and sensitive β^{+}-selective radiodetector for use with a high-performance capillary electrophoresis system for the analysis of compounds labelled with short-lived, positron-emitting radionuclides has been developed. The detector had a linear response in the range 0–600 kcps with a noise level of 0.2 cps and an efficiency of 85%. The detection limit was found to be in the Bequerel range. The capillary electrophoresis system was used in isoelectric focusing and capillary zone electrophoresis of ^{11}C-labelled iron-free transferrin.

INTRODUCTION

Macromolecules labelled with short-lived positron-emitting radionuclides such as ^{11}C and ^{18}F (half-lives of 20.3 and 110 min, respectively) are interesting as tracers in positron emission tomography (PET) [1] for the *in vivo* study of various physiological processes.

In the development of methods for labelling proteins with ^{11}C [2], we required a complement to analytical HPLC for analysis of labelled products. High sensitivity, short analysis times and small sample consumption are features that make capillary electrophoresis a suitable tool in the analysis of compounds labelled with short-lived radionuclides. Previously, radiodetection in capillary electrophoresis has been reported for both β- and γ-emitting radionuclides such as ^{32}P, ^{14}C and ^{99m}Tc [3–6]. In this paper, a positron radiodetector for use with a high-performance capillary electrophoresis system with simultaneous mass and radiodetection is described. The application of the electrophoresis system for the analysis of labelled proteins is illustrated by isoelectric focusing (IEF) and capillary zone electrophoresis (CZE) experiments.

MATERIALS AND METHODS

Radiodetector element

The radiodetector element was constructed

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from a small plastic scintillator (2 × 4 × 4 mm, polyvinyltoluene, Nuclear Enterprise NE102A, Nuclear Enterprise, Edinburgh, UK) mounted onto a 3/8-in. (1 in. = 2.54 cm) photomultiplier tube (Hamamatzu R 2055, Hamamatzu Photonics, Japan). A slit (2 mm long, 0.5 mm wide and 2 mm deep) was made in the plastic scintillator allowing the capillary to be placed centrally in the detector, as shown in Fig. 1a. An amplifier and low-energy discriminator, developed in-house, was used for the amplification of the photomultiplier tube signals and for minimization of noise in the measurements. An equivalent electronic system can be built from commercial NIM modules (e.g. TC 242 amplifier and TC 451 single-channel analyser, Tennelec, USA). The radiodetector was mounted adjacent to the UV photodiode detector at a distance of 24 mm in a cartridge sliding on guiding rods allowing the simultaneous removal of both detectors (Fig. 1b). The distance between the detector cartridge and the end of the capillary was 20 mm. The UV detector compartment of a Bio-Rad HPE-100 microsampler capillary electrophoresis apparatus (Bio-Rad, Richmond, CA, USA) was modified to accommodate the two-detector assembly. The incident light from the UV lamp was focused through a 0.05 × 1 mm slit facing the capillary. When operating the system, the entire capillary compartment was closed by a removable housing to prevent stray light from entering the detectors. Both detectors were interfaced to a personal computer via a Beckman AI 406 interface, adjusted to accept TTL (transistor-transistor-logic) pulses from the photomultiplier tube and the analogue signal from the UV photodiode. Data were processed using a Beckman System Gold Chromatography Software Package.

Labelled materials

[11C]Carbon dioxide was prepared by the $^{14}N(p, \alpha)^{11}C$ nuclear reaction using a nitrogen gas target and the Scanditronix MC-17 cyclotron at the Uppsala University PET Centre. Sodium [11C]carbonate for detector measurements was obtained by trapping the [11C]carbon dioxide in 5 M NaOH. [18F]Fluoride$_{(aq)}$ was obtained by the $^{18}O(p,n)$ ^{18}F nuclear reaction using ^{18}O-en-
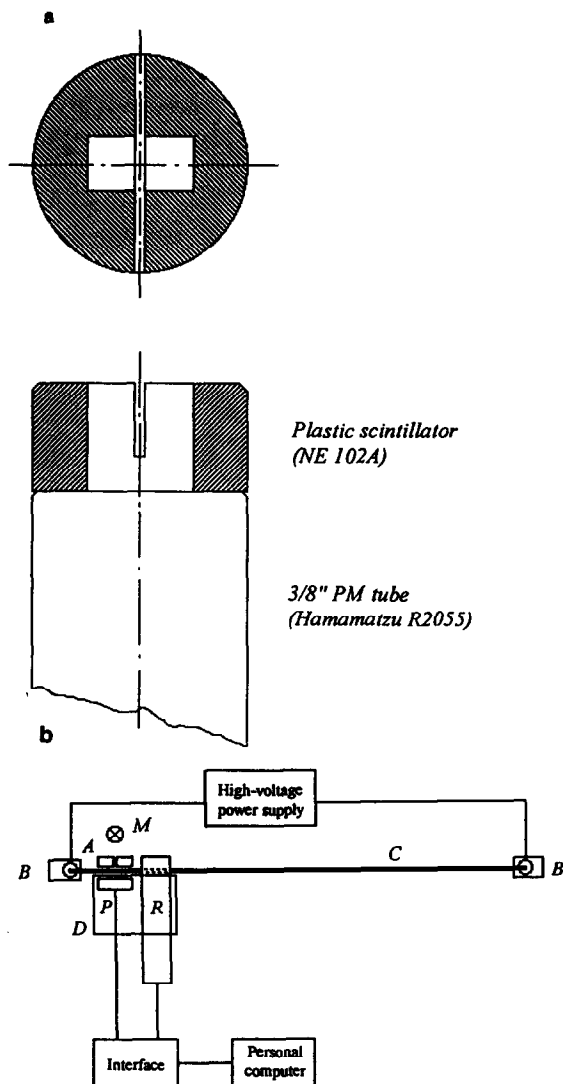

Fig. 1. (a) Scheme of the radiodetector element. The plastic scintillator (2 × 4 × 4 mm) was connected to the photomultiplier (PM) tube using optical grease. (b) Scheme of the detector assembly: B = buffer electrodes; M = UV lamp and monochromator; A = aperture (0.05 × 1 mm); P = photodiode detector; R = radiodetector; D = removable detector cartridge; C = capillary; ″ = inch.

riched water as target material. [11C]Cyanogen bromide was prepared from hydrogen [11C]cyanide according to a general procedure described elsewhere [7]. 11C-labelled iron-free transferrin was obtained by treating iron-free transferrin (Behring Werke, Marburg, Germany) with [11C]cyanogen bromide according to a procedure described in detail elsewhere [2].

[^{11}C]Cyanogen bromide was transferred in a stream of nitrogen gas to a receiving vessel containing 300 μl of 50 mM borate buffer, pH 7.5. When the radioactivity had reached a maximum, the [^{11}C]cyanogen bromide solution was added to a solution of the protein (0.1–10 mg in 100 μl of 50 mM borate buffer, pH 7.5). The reaction mixture was incubated at 40°C for 5 min, and then diluted with sterile water (2.1 ml). The crude product was desalted on a Sephadex G25 cartridge (PD-10, bed volume 9 ml, Pharmacia LKB, Stockholm, Sweden) using sterile water as the eluent.

Detector performance tests

Radiodetector linearity was tested by placing the detector with the plastic scintillator end resting on a vessel (outside the modified Bio-Rad equipment) containing 11.2 GBq (304 mCi) of sodium [^{11}C]carbonate in a light-shielded lead pot. Data collection was started and the radioactivity was left to decay overnight (16 h).

The background noise level was evaluated by inserting a glass capillary (200 × 0.1 mm I.D. × 0.3 mm O.D.) filled with blank buffer (0.2 M phosphate buffer, pH 2.5) into the apparatus and then closing the detector compartment. Data were collected for 100 min using a scaler (Newport P6000, Newport Electronics, Santa Ana, CA, USA) connected to the discriminator.

The spatial resolution of the radiodetector was determined as the response to a step function. A glass capillary 200 × 0.1 mm I.D. × 0.3 mm O.D.) was partially filled with radioactivity (sodium [^{11}C]carbonate) acting as a line source. When inserting the filled part of the capillary into the detector, note was taken of the output from the detector (connected to a Newport P600 scaler). The capillary was moved 1 mm, detector output noted, etc. The capillary was taken out and the radioactivity distribution in the capillary measured by autoradiographic analysis using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA, USA).

The efficiency of the detector was tested by placing a glass capillary (200 × 0.1 mm I.D. × 0.3 mm O.D.) filled with 278 MBq ml^{-1} (7.5 mCi ml^{-1}) sodium [^{11}C]carbonate in the radiodetec-

tor and collecting data with a scaler (Newport P6000). The capillary was taken out and the total radioactivity measured in a NaI(Tl) well counter.

Capillary electrophoretic analysis of labelled proteins

The performance of the capillary electrophoresis system was assessed by experiments with ^{11}C-labelled transferrin. The labelling procedure afforded the labelled protein as a 1–2 mg ml^{-1} solution in 10 mM potassium phosphate buffer, pH 7.4. This solution was used in capillary isoelectric focusing and zone electrophoresis analysis within 5–25 min after radiolabelling.

Glass capillaries (200 × 0.1 mm I.D. × 0.3 mm O.D.) or fused-silica capillaries (200 × 0.075 mm I.D. × 0.1 mm O.D.) were internally coated with non-cross-linked polyacrylamide to reduce electroendoosmosis according to a procedure published previously [8]. Before and after runs, the capillaries were rinsed with 0.2 M phosphate buffer, pH 2.5, or 20 mM phosphoric acid and dried by aspiration. All electrophoresis experiments were performed at room temperature with a procedure analogous to previous reports [8,9].

RESULTS AND DISCUSSION

The radionuclides of interest in PET decay by emission of a high-energy positron (β^+), as illustrated for ^{11}C in eqn. 1;

$$^{11}\text{C} \rightarrow {}^{11}\text{B} + \beta^+ + \nu \tag{1}$$

where ν is a neutrino. The average energy of the positron emitted from ^{11}C is 385 keV. Thus, in principle, both the β- and γ-radiation emitted could be used for monitoring the radioactivity. In practice, β detection is preferable when considering the spatial resolution of the detector. The plastic scintillator has a high sensitivity for β detection (i.e. the full β energy of ^{11}C positrons is absorbed within 2 mm of scintillator plastic) and a low sensitivity for γ detection (hence a low background level).

Characterization of the radiodetector

The linearity of the radiodetector response was evaluated by placing the detector next to a

radioactive source which was left to decay while collecting data. The data obtained were plotted as the natural logarithm of the detector output as a function of time (Fig. 2). A regression line was fitted to the data to give a correlation coefficient (Pearson's r) of 0.9982, implying that a linear response can be expected in the interval 0–600 000 counts per second (cps).

The plastic scintillator has a very fast response to incident betas, i.e. the pulses are short (10–20 ns). The characteristics of the scintillator and the amplifier–discriminator (10–20 ns pulses, 100 ns TTL) sets an upper limit for the linearity of the order of 1 Mcps. In our application, low count rates can be expected (typically 0–10 kcps), while the limit of linearity is not considered to be a problem. Thus, a simple amplifier and a low-energy discriminator were developed, and found to give satisfactory results. If required, more sophisticated electronics can be used to increase the linear range to about 10 Mcps.

The detector background count rate was determined by collecting data from a blank sample buffer. Data were averaged over 100 min and the noise level found to be 0.2 cps. During operation, no interference from the UV lamp or the high-voltage power supply for the electrophoresis electrodes was observed.

The spatial resolution of the radiodetector was assessed by stepwise movement of a capillary partially filled with radioactivity through the detector (Fig. 3a). This required the detector compartment housing to be opened for each measurement. Removing the housing caused an increased background level but did not saturate the detector. Repositioning the housing quickly
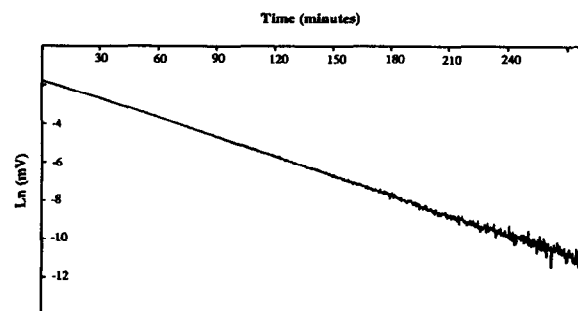

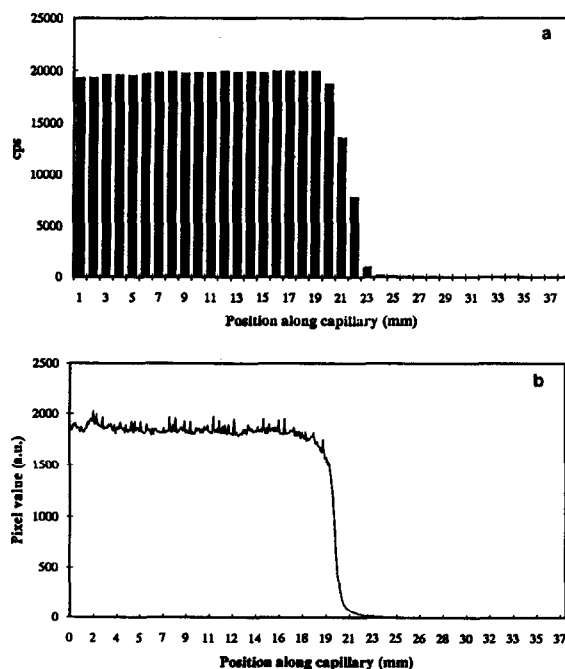Fig. 2. The time course of the decay of sodium [^{11}C]carbonate as measured by the radiodetector.

Fig. 3. (a) Plot of output from detector vs. relative position of radioactivity to detector. (b) Distribution of radioactivity in capillary obtained by autoradiography.

restored (within 5 s) the original background levels.

The detector response shown in Fig. 3a is thus a step function response (verified by autoradiography of the capillary source as shown in Fig. 3b). Assuming a Gaussian detector response to a point source, the measured data were fitted using the least-squares method. The standard deviation of the detector response function was 1.52 mm, corresponding to a full-width half-maximum value of 2.53 mm. The effective detector width using a line source was calculated to be 2.69 mm, corresponding to an effective detector cell volume of 21 nl when using a 0.1 mm I.D. capillary.

Using the value obtained above for the effective detector volume, the efficiency of the detector using a 0.1 mm I.D. capillary filled with sodium [^{11}C]carbonate 278 MBq ml^{-1} (7.5 mCi ml^{-1}) was approximately 85%. A similar value (87%) was obtained using ^{18}F$_{aq}^{-}$ (200 μCi ml^{-1}). The results are summarized in Table I.

The sensitivity of the radiodetector is best judged from the electropherograms in Figs. 4

TABLE I

DETERMINATION OF THE RADIODETECTOR EF-
FICIENCY FOR ^{11}C AND ^{18}F

After measuring the count rate using the radiodetector, the
capillary was taken out and the radioactivity measured in a
NaI(Tl) well counter.

Measurement number	^{11}C		^{18}F	
	cps[a]	dps[b]	cps[a]	dps[b]
1	4380	5378	162	186
2	4314	4967	162	192
3	4348	5002	177	201
4	4359	5043	149	169
Mean	4351	5098	163	187
Efficiency (%)	85.3		87.2	

[a] Radiodetector output, counts per second, decay corrected.
[b] Total radioactivity, disintegrations per second. Values were
corrected for dead-time and decay.

and 5. In the CZE experiments, a signal-to-
noise-ratio of >100 was obtained using a total
starting radioactivity of 5.05 kBq. The high
sensitivity results from the low noise level,
which, in turn, is attributable to the small size of
the scintillator. Assuming the least-detectable
signal to be twice the background noise count-
rate, the detection limit was 0.4 cps.

Least-detectable radioactivity =

$$e^{(\ln 2 + \text{ordinate value of intercept})} \qquad (2)$$

This was verified by extrapolating the linear
portion of the logarithm of a decay curve (^{11}C)
to the intercept with the background noise level.
The least-detectable amount of radioactivity was
then obtained by eqn. 2. This analysis gave as a
result a value of 0.5 cps, corresponding well to
the value above.

In a capillary of the size used in this work, the
self-absorption of the β-particles emitted from
^{11}C and ^{18}F is small (<10%). The plastic scin-
tillator volume is small (16 mm^3) and it has a low
sensitivity for absorption of high-energy photons.
No bulky lead shield is thus needed to keep the
background at very low levels. The resolution of
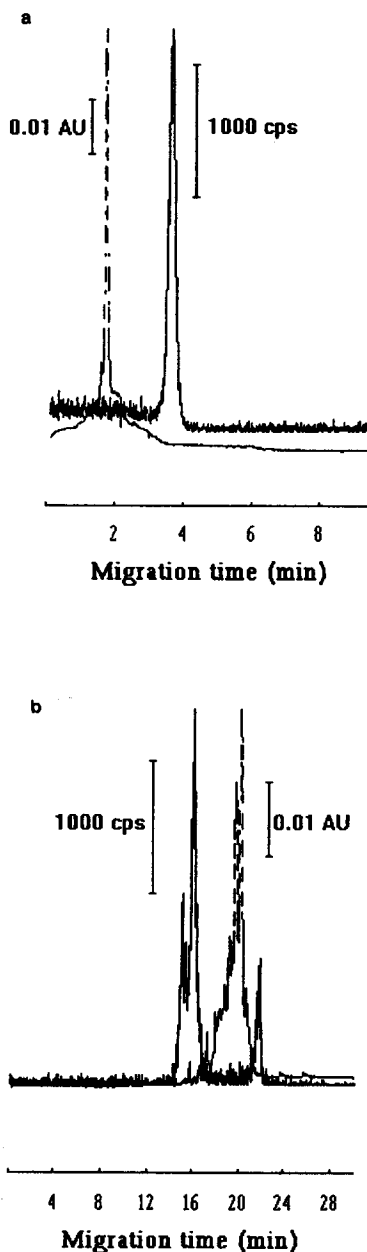the radiodetector can fairly easily be improved

Fig. 4. isoelectric focusing of [^{11}C]transferrin. Radiochannel,
solid line; UV channel, dashed line. (a) Focusing step.
Applied voltage 5 kV, UV detection at 280 nm. (b) Mobiliza-
tion step; applied voltage 7 kV. The radiochannel electro-
pherograms are decay corrected. The amount of radioactivity
in the capillary was 1.01 MBq (27.2 μCi) at the start of the
experiment, specific radioactivity 45 GBq μmol^{-1} (1.2 Ci
μmol^{-1}), direction of migration $- \rightarrow +$. Experimental condi-
tions: 200-mm glass capillary, 0.1 mm I.D.; anolyte: (a) 20
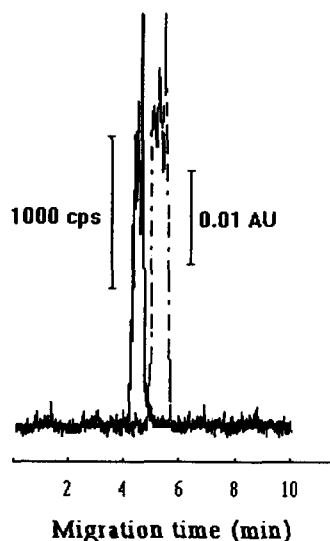mM phosphoric acid, (b) 20 mM NaOH; catholyte 20 mM
NaOH.

Fig. 5. Capillary zone electrophoresis of [^{11}C]transferrin. Radiochannel, solid line; UV channel, dashed line. Applied voltage 7 kV, UV detection at 280 nm. The sample was applied as 1-mm-long starting zone, with a total activity of 5.05 kBq (136 nCi), specific radioactivity 21 GBq μmol^{-1} (0.57 Ci μmol^{-1}). Experimental conditions: 200-mm glass capillary, 0.1 mm I.D.; electrophoresis buffer 18 mM Tris, 18 mM boric acid, 0.3 mM EDTA, pH 8.4; direction of migration $-\rightarrow+$.

by replacing the 2-mm-wide scintillator by a thinner one.

Electrophoresis experiments

Transferrin is a serum glycoprotein of molecular mass of 80 000 with the property of reversibly binding ferric ions, thus acting as a reservoir and transport protein for physiological iron. Human transferrin has an isoelectric point in the range 5.3–6.1, the variation in pI presumably caused by the varying carbohydrate content. Diferric transferrin has a higher pI than the apo-form [10]. Labelled transferrin was obtained by treating the protein at physiological pH with [^{11}C]cyanogen bromide according to a procedure under development in our laboratory [2]. Cyanation of protein SH groups using cyanogen bromide has been demonstrated previously [11–14]. Carbamylation of lysine residues in proteins using cyanate ion is a well-known reaction [15]. Whether the reaction of [^{11}C]cyanogen bromide with lysine residues proceeds directly to form cyanamides, or involves hydrolysis to

[^{11}C]cyanate ion with subsequent carbamylation, has not been evaluated. The ^{11}C-transferrin was obtained in 50–65% decay-corrected radiochemical yield in 5 min reaction time. The specific radioactivity of the ^{11}C-labelled transferrin used in this study was in the range 11–74 GBq μmol^{-1} (0.3–2 Ci μmol^{-1}).

An IEF experiment of a ^{11}C-labelled iron-free transferrin sample is shown in Fig. 4. The amount of radioactivity in the capillary at the start of the experiment was 1.01 MBq. In Fig. 4a, the focusing step, it can be seen that the amplitude of the noise and the baseline level of the radiochannel is higher before the peak than after. During the focusing step, the radioactivity, which was originally uniformly distributed along the capillary, is migrating towards the isoelectric point of the protein, thus giving rise to peak(s) in the radio- (and UV) detector. As the migrating components of the sample approach the isoelectric point, their migration speed decreases. Since the radiodetector is located closer to the isoelectric point, broader peak(s) results, as compared with the UV detector.

In the mobilization step (Fig. 4b), the labelled components enter the radiodetector before the UV detector, thus reversing the order of the radio and UV peaks. In Fig. 4b, the UV trace shows at least 5–7 peaks, possibly arising from the different transferrin isoforms. The radiotrace only shows two clearly resolved peaks. Whether this pattern reflects a lack of resolution in the radiodetector or a differential labelling of isoforms is not yet clear. An impurity can be seen at a migration time of 21 min. CZE of the same sample is illustrated in Fig. 5.

In conclusion, a capillary electrophoresis system with on-tube radiodetection of positron emitters has been developed. The high sensitivity and efficiency of the radiodetector allows the characterization of macromolecules labelled with short-lived, positron-emitting radionuclides. The resolving power of the instrument was illustrated by the partial resolution of transferrin isoforms.

ACKNOWLEDGEMENTS

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