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Short Communication

Capillary electrophoretic separation in both H_2O- and ^2H_2O-based electrolytes can provide more information on tryptic digests

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ABSTRACT

Capillary electrophoresis of a tryptic digest of cytochrome *c* in ^2H_2O-based buffer solutions has been shown to give complementary information to that obtained in H_2O-based electrolytes of the same acidity.

We recently [1] introduced the use of ^2H_2O-based buffer solutions in capillary electrophoresis (CE). We found that a higher resolution can be obtained if H_2O is replaced by ^2H_2O in the electrolyte solutions. The increase in resolution is thought [1] to be due to a lowering of electroosmotic flow in ^2H_2O-compared to H_2O-based buffer solutions.

Electroosmotic effects mainly occur due to negative charges arising from the ionisation of the surface silanol groups on the inside wall of the capillary [2]. Control of electroosmotic flow can have a beneficial effect on both the separation efficiency and the resolution in CE.

As the magnitude of electroosmotic flow is greatly dependent on the pH of the electrolyte in CE (electroosmosis is suppressed in acidic pH values) we have analysed the tryptic fragments of cytochrome *c* at a pH close to neutral (pH 7.81) and in an acidic medium (pH 2.95), and have carried out measurements in buffer solutions of similar acidity ($p^2H = 7.83$ and 2.95), but replacing H_2O by ^2H_2O. Results (including experimental details) are summarised in Figs. 1 and 2. CE instrumentation described in ref. 1 was used to obtain these data.

The tryptic digest maps of cytochrome *c* are very different when CE is run in ^2H_2O or H_2O solution at a a^2H or pH around 7.8. In agreement with our previous observations [1,3], migration times in ^2H_2O are longer than the corresponding values in H_2O-based buffer solutions. A striking feature of the comparison shown in Fig. 1 is the considerable improvement that occurs in ^2H_2O, especially before the negative

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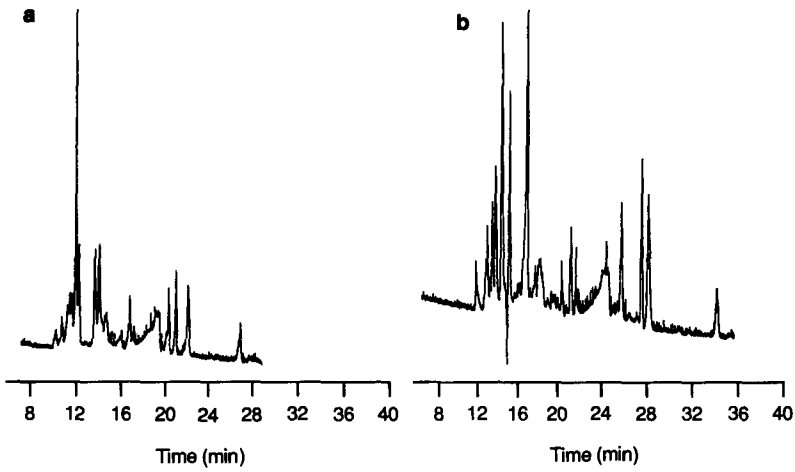

Fig. 1. CE separation of fragments from the tryptic digest of bovine cytochrome *c*. (a) pH 7.81; (b) p^{2}H 7.83. Buffer, 20 m*M* NaH_2PO_4/Na_2HPO_4 in H_2O (a) or ^2H_2O (b); capillary, 95 cm (effective length 70 cm) × 50 μm I.D., separation voltage, 20 kV; current, < 50 μA; injection voltage, 3 kV for 1 s; detection, UV at 200 nm; temperature, ambient.

peak. The latter signal is due to H_2O as samples were electrokinetically [4] withdrawn from H_2O-based buffer solutions in all experiments. The occurrence of this negative peak is convenient as it marks the point of migration of uncharged (or neutral) peptide fragments. Positively and negatively charged fragments are expected to migrate before and after this signal, respectively.

The tryptic digest maps in Fig. 2 show that differences between electropherograms in H_2O- and ^2H_2O-based buffer solutions at an acidic pH are not as significant as those observed at higher pH values. Although electroosmotic flow is largely suppressed in acidic pH values, migration times in ^2H_2O are still about 15% longer

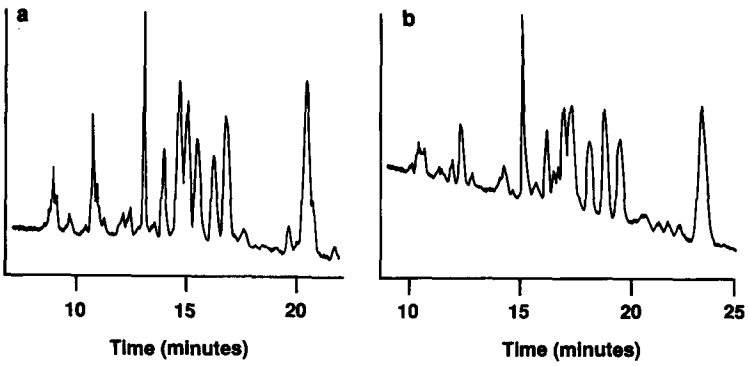

Fig. 2. CE separation of fragments from the tryptic digest of bovine cytochrome *c*. (a) pH 2.95; (b) p^{2}H = 2.95. Buffer, 20 m*M* NaH_2PO_4/HCl in H_2O (a) or ^2H_2O (b); capillary, 72 (effective length 50 cm) × 50 μm I.D.; separation voltage, 15 kV; injection voltage, 20 kV for 3 s; detection, UV at 200 nm; temperature, ambient.

than in H_2O-based electrolytes. The pattern of the digest maps in Fig. 2a and b are very similar. However, new peaks previously unresolved in the H_2O-based buffer are clearly visible in ^2H_2O. This may be largely due to the different pI values that can arise in H_2O and ^2H_2O solution [5].

In conclusion, we have again shown that CE separation of peptides in ^2H_2O solution can provide analytical information not provided by similar experiments carried out in H_2O-based electrolytes. We think that CE experiments carried out in both ^2H_2O and H_2O can give a more complete picture of the fragmentation pattern of a tryptic digest than in CE experiments in H_2O alone.

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