'Fingerprinting' of 1-Dimethylaminonaphthalene-5-sulphonyl-Labelled Protein Digests

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Schmer & Kreil (1967) have reported a very sensitive method for peptide 'mapping' of proteins by reaction of hydrolysates with DNS* chloride (Gray & Hartley, 1963), followed by two-dimensional thin-layer chromatography. In applying this method to ATP-creatine phosphotransferases, myokinase and cytochrome *c*, we have used the following modifications, which improve the resolution and increase sensitivity.

(i) DNS chloride is not readily soluble in acetone at concentrations of 5 mg./ml. and above. Clear homogeneous solutions are obtained, however, by shaking DNS chloride with acetone and adding bicarbonate solution to the suspension. This order of addition is essential.

(ii) The solvent combination of Schmer & Kreil (1967) did not give adequate resolution of DNS-peptides. Of the many different solvent systems tested the best combination was: first dimension: methyl acetate-propan-2-ol-aq. NH_3 (sp.gr. 0.88) (9:7:4, by vol.) (Seiler & Wiechmann, 1964); second dimension: chloroform-95% (v/v) ethanol-acetic acid (38:4:3, by vol.) (Deyl & Rosmus, 1965).

(iii) A marked increase in sensitivity was achieved by exposing the plates to dioxan vapour before examination. Quantum yields are highest in this solvent (Chen, 1967). 'Maps' could then be obtained from 0.5 μmole of protein.

The technique finally used was as follows.

Proteins were reduced and alkylated (Crestfield, Moore & Stein, 1963) and, after dialysis against NH_3 -free water, were digested for 2 hr. with 2% (w/w) trypsin at pH 8.0 and 37° in a pH-stat. The digests were evaporated to dryness *in vacuo* and dissolved in 0.1 M- NaHCO_3 . 'Dansylation' was carried out overnight with a tenfold (w/w) excess of DNS chloride in acetone-0.1 M- NaHCO_3 (1:1, v/v). The DNS-peptides were treated with Dowex 50 (Schmer & Kreil, 1967) and dissolved in 80% (v/v) acetone. Then 0.5 μmole samples were applied to thin layers (0.25 mm.) of silica gel G and solvents were run for 10 cm. in each dimension, the plates being dried for 10 min. at 100° between runs. The chromatograms were observed under an ultraviolet lamp after exposure to dioxan vapour.

Spots could be eluted from the gel with 80% (v/v)

acetone and *N*-terminal determinations could be carried out successfully on the eluted DNS-peptides by the method of Gray (1967). The best results were obtained by applying 5–10 μmoles of 'dansylated' protein hydrolysate as a band, running with the first solvent, eluting the band and applying it to another plate for running in the second solvent. The selected band was then eluted for *N*-terminal studies. With this method it was shown that the origin spots in Fig. 1 contained five peptides. Radioautography of the peptide 'maps' could also be carried out readily to locate ^{14}C -labelled peptides; 2–5 μC of labelled peptide spot gave adequate intensity after 10 days.

Fig. 1 illustrates how the method has been used to compare ATP-creatine phosphotransferases from various sources. Characteristic differences

Fig. 1. DNS-peptide 'map' of tryptic digests of ATP-creatine phosphotransferases. Very faint spots are omitted. By-products are represented by shaded spots. Spots 20 and 35 were unique to the rabbit muscle enzyme; spots 2, 11, 15 and 16 were unique to the bovine muscle enzyme; spots 19, 31 and 40 were unique to the bovine brain enzyme; spots 9, 24, 25, 28, 37 and 38 were common to the bovine and rabbit muscle enzymes but lacking in the bovine brain enzyme.

* Abbreviation: DNS or dansyl, 1-dimethylamino-naphthalene-5-sulphonyl.

between these enzymes are apparent. Good 'maps' of myokinase and cytochrome *c* were also obtained; the latter showed better resolution of spots than those obtained with the solvent systems of Schmer & Kreil (1967). The R_F values of spots were not exactly reproducible, but the characteristic patterns and intensities of spots together with by-product markers enabled 'maps' to be compared easily. The results show that the three ATP-creatine phosphotransferases were similar to each other. The bovine brain enzyme showed less similarity to the enzymes from bovine or rabbit muscle than the latter did to each other, mainly on account of spots present in 'maps' of muscle enzymes but absent from those of the brain enzyme. This supports the conclusion reached by Thomson, Eveleigh, Laws & Miles (1968) and Eppenberger, Dawson & Kaplan (1967) that ATP-creatine phosphotransferases from the same tissue in different species are more alike than those from different tissues of the same species. It is noteworthy that bovine brain and muscle ATP-creatine phosphotransferases show greater similarity on these DNS-peptide 'maps' than on conventional paper 'maps'.

The 50-fold increase in sensitivity possible with this dansyl technique will greatly extend the usefulness of peptide 'mapping' in comparative studies of enzyme structure, particularly since *N*-terminal groups of eluted spots can readily be determined.

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