Journal of Chromatography, 166 (1978) 344–346

CHROM. 11,314

Note

Separation and detection of tetrahydropterins

BRUCE M. WATSON* and R. G. H. COTTON**

Genetics Research Unit, Royal Children's Hospital, Research Foundation, Flemington Road, Parkville, Victoria 3052 (Australia)

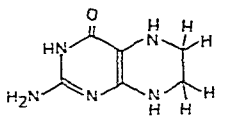
(Received July 5th, 1978)

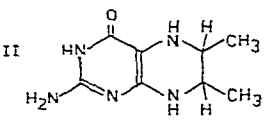
The lability of tetrahydropterins to oxidation has hindered their separation and detection. Since it is generally the reduced forms of pterins which are biologically active[1] it was considered desirable to develop a simple method of separating and detecting tetrahydropterins. Previous work in our laboratory suggested that high-voltage electrophoresis (HVE) was a good technique for accomplishing the separation[2].

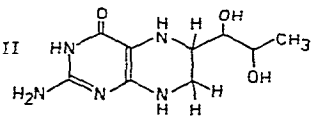
The detection method for the tetrahydropterins was based on the observation by Gunlack *et al.*[3] that 3-[4,5-dimethylthiazolyl-2-]-2,5-diphenyltetrazolium bromide (MTT) was reduced to an insoluble form by tetrahydrofolate. An earlier detection method[4] relied on a UV absorbing spot becoming fluorescent on oxidation of the tetrahydropterin.

EXPERIMENTAL

Chemicals and reagents

I 

II 

III 

Tetrahydropterin (I) was obtained from ICN Pharmaceuticals (Plainview, N.Y., U.S.A.), tetrahydro-6,7-dimethylpterin (II) was obtained from Aldrich (Milwaukee, Wisc., U.S.A.) and tetrahydrobiopterin (III) was a gift from Roche (Welwyn City, Great Britain). Solutions of tetrahydropterins were prepared immediately prior to application unless otherwise stated. MTT was obtained from Sigma (St. Louis, Mo., U.S.A.). The MTT staining reagent was prepared by dissolving 5 mg of MTT in 100 ml of 1.0 *M* sodium carbonate solution immediately before use.

* Present address: Baker Medical Research Institute, Commercial Road, Prahran, Victoria 3181. Australia.

** To whom correspondence should be addressed.

Methods

HVE was performed on Whatman 3MM paper (57 cm × 46 cm) in formate: acetate buffer pH 1.9 at 5600 V for 30 min are previously described[2]. Solutions of the tetrahydropterins were applied in 1.5-cm bands under a stream of nitrogen, in a dimly lit room, immediately prior to HVE, to prevent oxidation. Quinine sulphate (4 mg/ml in water) was applied at intervals across each paper and the migration of the tetrahydropterins was expressed relative to the migration of quinine (R_Q). After HVE the wet paper was dipped in the MTT staining reagent and hung in a fume hood. Alternatively the paper was dried in an oven at 100° for approximately 10 min and the fluorescent compounds were located by viewing the paper under ultraviolet light (365 nm).

RESULTS AND DISCUSSION

The three tetrahydropterins separated very clearly by this method which is rapid and sensitive. The purple colour locating the tetrahydropterins appeared within minutes of applying the MTT stain and was stable for several hours. The contrast between the purple spots and the white background enabled as little as 0.05 μmoles of the tetrahydropterins to be detected after preliminary HVE. Greater sensitivity could be achieved by applying the sample to a smaller area of paper.

The MTT stain confirms that the tetrahydropterins are present on the paper after HVE, as the reduction of MTT is specific for tetrahydropterins rather than their oxidation products. The migration, relative to quinine, of each of the three tetrahydropterins investigated is reported in Table I. Only one purple spot was observed for each of the tetrahydropterins.

TABLE I

RELATIVE MIGRATION OF TETRAHYDROPTERINS DURING HVE

Compound	R_Q	*Colour of fluorescent oxidation products*
Tetrahydropterin (I)	0.75	Blue
Tetrahydro-6,7-dimethylpterin (II)	0.65	Blue
Tetrahydrobiopterin (III)	0.55	Yellow

When the tetrahydropterins were dried on paper after HVE, rather than located with the MTT stain, fluorescent oxidation products were formed[4]. These were observed at the same R_Q values as the tetrahydropterins and their colours are reported in Table I. No fluorescence was observed before the paper was dried. As fluorescence was only observed at the R_Q values of the tetrahydropterins, as established using the MTT stain, it was concluded that the tetrahydropterins were not significantly oxidised during application under nitrogen in the dark and during separation by HVE.

Solutions of tetrahydropterin (I) and tetrahydrobiopterin (III) were also allowed to oxidise on the paper before HVE. After the paper was dried a range of fluorescent compounds were observed at different R_Q values indicating oxidation products present before HVE. These included pterin (R_Q 0.5), biopterin (R_Q 0.35) and 7,8-dihydroxanthopterin (R_Q 0.15).

This method should find general application in the investigation of solutions of tetrahydropterins. The MTT stain confirms the presence of tetrahydropterins, which may be identified by their R_Q values, while oxidation products of the tetrahydropterins may be distinguished by their fluorescence and different migration relative to quinine.

REFERENCES

1 H. Rembold and W. L. Gyure, *Angew. Chem., Int. Ed. Engl.*, II (1972) 1061.
2 B. M. Watson, P. Schlesinger and R. G. H. Cotton, *Clin. Chim. Acta*, 78 (1977) 417.
3 B. G. Gunlack, G. E. Neal and D. C. Williams, *Biochem. J.*, 101 (1966) 29P.
4 I. Ziegler, *Biochim. Biophys. Acta*, 78 (1963) 219.