unclamping, the plate was sprayed with solution B, followed by an exceedingly light spraying (*ca.* 1 ml/300 cm^2) of 10% sodium carbonate solution. Glycoside areas appeared immediately almost at their maximum intensity. Spots appearing by this altered procedure were more compact in size but a high background colouration which soon developed masked areas of low concentration.

Both procedures have been used to detect cyanoglycosides separated on paper chromatograms and cellulose thin layers where as little as 0.005 μmoles was detected. The cyanoglycosides could not be detected on silica gel thin layers and only weakly on plates prepared from a mixture of silica gel and cellulose. No interfering substances were observed in several alcoholic extracts of plant tissue containing cyanoglycosides.

The glycosides dhurrin and taxiphyllin gave purple spots which on standing developed brown centres. This is attributed to a separate reaction of *p*-hydroxybenzaldehyde or its derivatives which are produced in the enzymic hydrolysis and may be useful in giving a partial identification of unknown glycosides.

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A method for the differentiation of histone fractions F2(a)1 and F2(a)2 by starch gel electrophoresis

The histones of calf thymus have been separated into four main fractions designated F1, F2(a), F2(b), and F3.[1] These fractions have all been characterized by total and N-terminal amino acid analyses and by starch gel electrophoresis at pH 2.3[2], which effectively separates the four groups. More recently fraction F2(a), the largest of the four groups has been subdivided into F2(a)1 and F2(a)2 by a variety of methods[3–5]. Starch gel electrophoresis at pH 2.3 does not however differentiate between these subfractions, and although it may be possible to separate them at higher pH values this is not desirable since it has been shown that histones, particularly F3, aggregate with increase in pH[6–8]. Also the proteolytic activity associated with the histones rapidly increases as the pH is raised[9].

A simple modification has therefore been made to the method described previously[2], which enables F2(a)1 and F2(a)2 to be separated in starch gel at about pH 2 and which also enables F2(a)1 to be specifically detected in a mixture of histones.

Experimental and results

Preparation of histone fractions

Histone fractions F1, F2(b) and F3 were prepared as described by JOHNS[1] using method 2. Histone fractions F2(a), F2(a)1 and F2(a)2 were prepared as described by JOHNS[5] using methods 1 and 2.

Starch gel electrophoresis

Electrophoresis in starch gel was carried out essentially as described previously[2] except for the following modification. The solution used for making the gel, for sample application and for the electrode vessels was 0.01 *N* HCl, made 0.01 *N* with respect to trichloroacetic acid, the final pH being about 1.8. This addition of trichloroacetic acid to the 0.01 *N* HCl used previously[2], appears to aggregate the histone fraction F2(a)1 sufficiently to prevent it entering the gel, but not to precipitate it since it is still readily soluble in the sample solvent.

The results obtained using fractions F2(a)1, F2(a)2 and F2(a) (*i.e.* F2(a)1 + F2(a)2) are shown in Fig. 1, together with the results using the unmodified system (0.01 *N* HCl only) for comparison. It can be seen that without using the trichloroacetic acid it is impossible to distinguish between F2(a)1 and F2(a)2 and that there

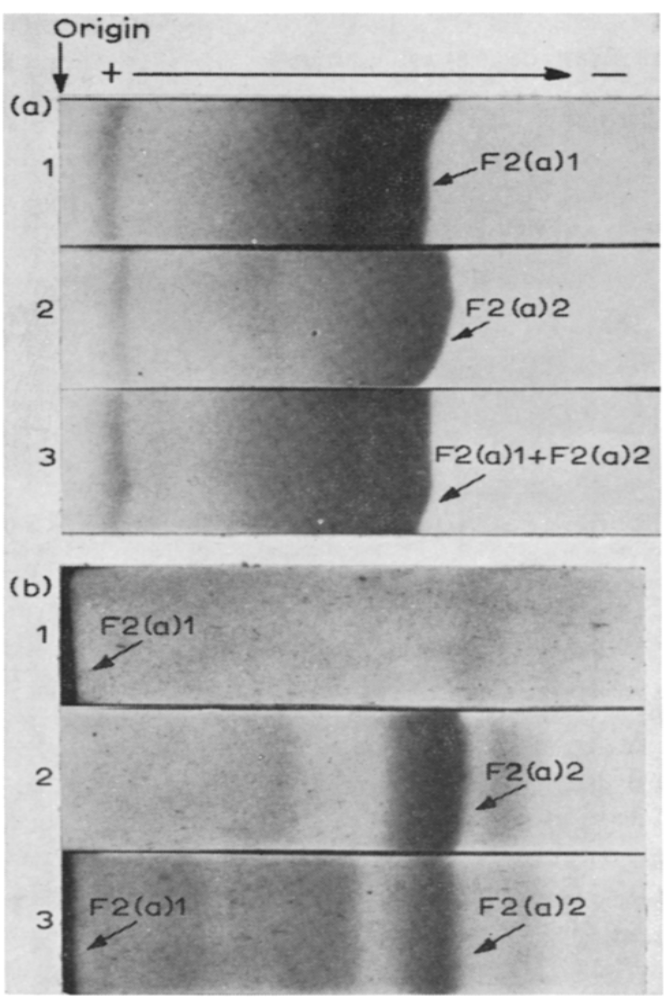

Fig. 1. (a) The electrophoresis of histone fractions in starch gel at pH 2.3 as described by JOHNS, PHILLIPS, SIMSON AND BUTLER[2]. (b) Modified method as described in the text. 1 = F2(a)1; 2 = F2(a)2; 3 = F2(a) (*i.e.* F2(a)1 + F2(a)2).

is no protein remaining at the origin. However with the modified system F2(a)1 aggregates, cannot enter the gel, and therefore remains at the origin where it stains in the normal manner. F2(a)2 migrates easily into the gel and appears to be unaffected by the trichloroacetic acid.

The other histone fractions F1, F2(b) and F3 were also tested under similar conditions and all migrated freely into the gel. Whole unfractionated histone however gave a band at the origin presumably due to its content of F2(a)1.

Discussion

The modification described above now enables the histone fractions F2(a)1 and F2(a)2 to be separated on starch gel at a low pH, and F2(a)1 to be specifically detected in whole histone or in a mixture of histones. The mechanism of the aggregation is not known, but since the other histone fractions do not aggregate under similar conditions it may be connected with the outstanding characteristics of F2(a)1 when compared with the other histone fractions. These are, a very high glycine content (approximately 15 %) and a very low proline content (1.5 %)[10]. The aggregation is unlikely to be due to its high content of arginine (13 %) since the other arginine-rich histone F3 does not aggregate under similar conditions.

It is also of interest to note that in the polyacrylamide gel electrophoresis of histone fractions described by GURLEY AND SHEPHERD[11] F2(a)1 is obviously aggregating and showing much material unable to enter the gel.

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