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SHORT COMMUNICATION

Separation of the ^{131}I labelled S-sulphonated A and B insulin chains by thin-layer chromatography *[1]

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The experiments were performed on the ^{131}I labelled S-sulphochains, but it was demonstrated in the course of the work that low degrees of iodination do not interfere with the chromatographic behaviour of A and B chains. The method may therefore be used for the separation of uniodinated S-sulpho chains.

Silicagel « G » Merck and Amberlite IR-120, TLC type, were used as supporting media. Thin-layer plates were prepared in the usual way with a Chemetron apparatus and developed in closed rectangular tanks lined with a filter paper. Pure A and B S-sulphochains were prepared from radio-iodinated beef insuline, according to the method proposed by BAILEY [1].

5 mg of ^{131}I labelled insulin (250 μC/mg) were dissolved in 1 ml of a 0.03 M $Na_2S_4O_6$, 0.075 M Na_2SO_3 and 8 M urea solution; the pH was adjusted to 7.6 with acetic acid and the resulting solution was kept at $+5^{\circ}$ C for 6 hours.

Aliquots of the solution were subjected to paper electrophoresis at pH = 3.2 in an acetic acid-urea buffer (1.5 M and 8 M respectively) for 18 hours at 4 V/cm. The bands corresponding to A and B chains were cut from the paper strips, and washed with ethanol to remove urea and with ether to remove ethanol.

A and B S-sulphochains were eluted respectively with 0.02 M ammonium acetate buffer, pH = 4.1, and 0.02 M phosphate buffer, pH = 8.4 and their purity was reconfirmed by paper electrophoresis.

The pure-chain solutions were freeze-dried and stored at $+2^{\circ}$ C. For each set of experiments, solutions were prepared from the dried materials and standardized on the basis of nitrogen content. Mixtures containing different amounts of the two chains were applied 2 cm from the edge of the plates and developed with various solvents.

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The position of the spots was localized by scanning the plates with an automatic device feeding a ratemeter recorder system.

Autoradiography, using X-ray type Ferrania films, was employed in order to visualize the true shape of the chromatographic spots.

Several elution mixtures were tried. Good separations were obtained on both Silicagel « G » and Amberlite IR-120 resin plates with an elution mixture of *n*-butanol saturated with a very dilute aqueous solution of formic acid at pH = 4.6. The pH value of the aqueous phase is fairly critical (4.6 $\pm$ 0.2). As found by DU YU-CANG and co-workers [2], S-sulphonated A chain, which has four strongly acid groups but no basic amino-acids, shows a solubility minimum below pH = 3 and reaches a maximum value above pH = 4.5

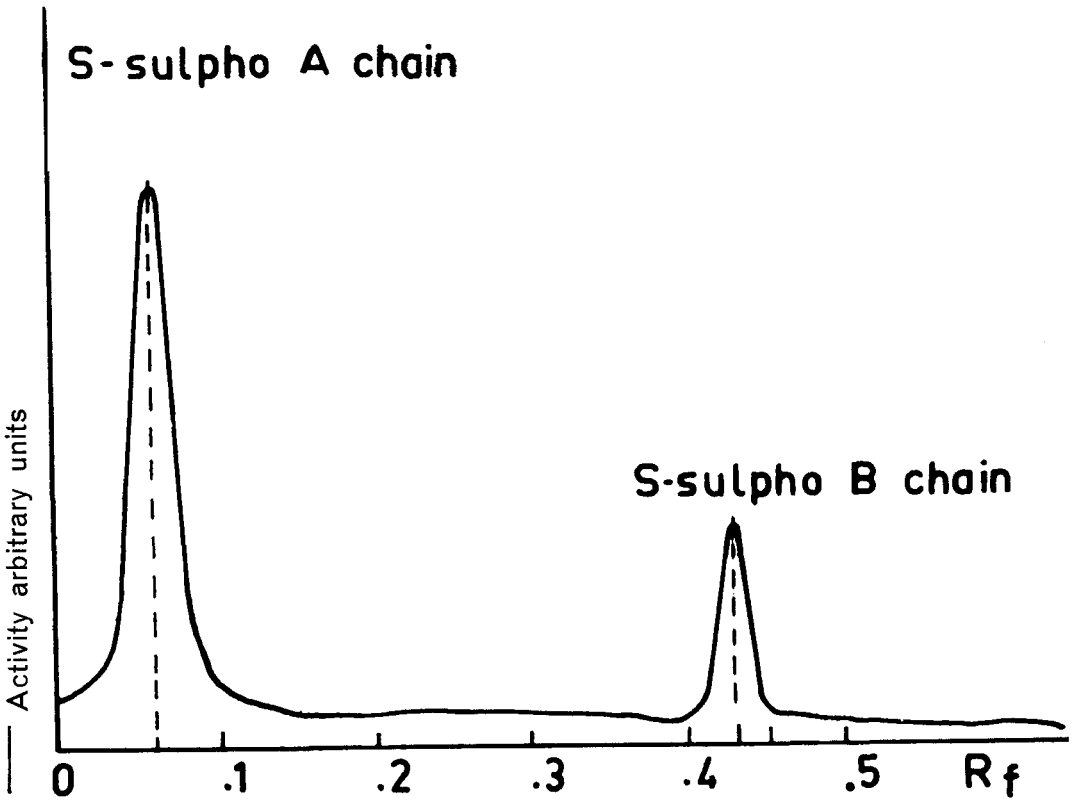
In Table I, both separation conditions and R_f values for S-sulphonated chains, insuline and iodide on Silicagel and Amberlite plates are reported.

TABLE I. Separation conditions for S-sulpho A and B chains, iodide, and insulin on Silicagel « G » and Amberlite IR-120 plates. Elution mixture : *n*-butanol saturated with an aqueous solution of formic acid having a pH value of 4.6.

	Time of run	R_f values			
		S-sulpho A chain	S-sulpho B chain	Insulin	Iodide
Silicagel « G » Merck	6 h	0.07 $\pm$ 0.02	0.44 $\pm$ 0.05	~0	0.54 $\pm$ 0.04
Amberlite IR-120 TCL type	6 h	0.16 $\pm$ 0.04	0.32 $\pm$ 0.05	0.11 $\pm$ 0.03	0.52 $\pm$ 0.01

A typical separation is shown in Fig. 1. The scan curve of the plate shows that the slight « comet » effect in autoradiography is not significant. In fact, the spots of A and B chains are developed in oversaturation conditions and less than the 3 % of the total activity of the faster component is spread along the trail. When mixtures of known amounts of ^{131}I labelled chains were deposited on the plates, the recovery, as evaluated by measuring the areas of the recorded peaks by means of a planimeter, was almost total. The successive elution of the spots from the plate proved, by paper electrophoresis, that the slower migrating component (A chain) was not contaminated by the faster (B cnain).

A sample of the reaction mixture originating from the insulin treatment with sulphite and tetrathionate according to BAILEY'S procedure, was spotted



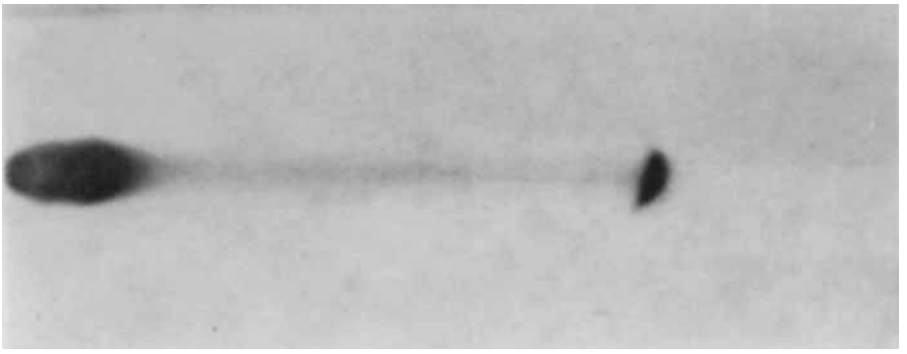


FIG. 1.
Separation of I^{131} labelled S-sulpho A and B chains, on a Silicagel G plate.

on a 20 × 20 cm Silicagel G plate together with pure S-sulpho A and B chains. Autoradiography of the plate is shown in Fig. 2.

As can be seen, the reactants used in the splitting procedure do not interfere in the separation.

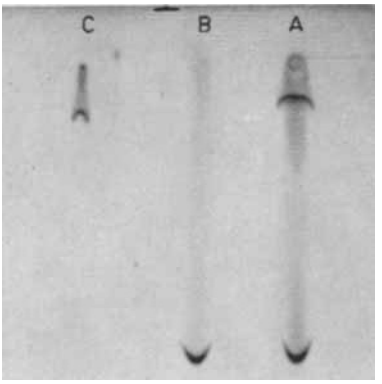


FIG. 2.

REFERENCES

1. BARLEY, J. L. — *Biochem. Journ.*, **67** : 21 (1957).
2. DU YU-CANG, ZHANG YU-SHANG, LU-ZI-XIAN, TSOU CHEN-LU. — *Scientia Sinica*, **10** : 84 (1961).