In conclusion, it may be pointed out that the findings in this work with regard to the effect of molecular structure on mobility in phenol (Fig. 1) are in agreement with previous observations that mobility is decreased by the replacement of a hydrogen atom by a hydroxyl group.

I wish to thank Prof. W. SHIVE, Dr. K. PFISTER, Dr. W. A. BOLHOFER and Dr. H. W. BUSTON for their gifts of amino acids used in this investigation.

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Received March 28th, 1956

Preliminary Notes

Contribution of reducing agents to electrophoretic lack of homogeneity of crystalline ribonuclease

The heterogeneous nature of crystalline ribonuclease remains a current problem, despite recent studies which have shed much light on the mechanism of action of this enzyme. Crystalline ribonuclease has been shown to be resolvable into two or more components by partition[1], as well as by ion exchange resin[2] chromatography. Although electrophoresis[3] indicated apparent homogeneity of the enzyme protein, zone electrophoresis on starch[4] showed that crystalline ribonuclease is not electrophoretically homogeneous.

The present investigation with paper electrophoresis revealed that crystalline ribonuclease heterogeneity depends on the degree of the reduction of ribonuclease. The reduction products retained ribonuclease activity against yeast ribonucleic acid, although their electrophoretic mobilities were different.

Five mg of crystalline ribonuclease (Armour), recrystallized twice by the KUNITZ[5] procedure, were incubated separately with each of the following: reduced glutathione, cysteine, histidine, and ascorbic acid, in phosphate buffer at 50° C, for 1 hour. Aliquots of 15 μl were applied for electrophoretic mobility determination. Each sample was applied to three separate strips of filter paper run in parallel.

Electrophoresis was accomplished in the LKB 3276 electrophoresis equipment, using 40 × 410 mm Schleicher and Schüll No. 2043B (120 g/m^2) filter paper, or Whatman No. 1 filter paper. Phosphate buffers of pH 5.64, 6.50, 7.00, and 7.80, ionic strengths 0.36 or 0.10, were used.

All experiments were performed at room temperature (about 18° C), with a potential of 200 volts across the filter paper, and a current of 8 mA, for 48 hours.

After electrophoretic separation, the 3 ionograms were dried. One was stained with bromphenol blue to locate the position of the different fractions of the enzyme. These enzyme fractions were cut from the remaining 2 filter strips, eluted in phosphate buffer, and the activities of the eluates determined.

Ribonuclease activity was measured by following the optical density of methylene blue (0.20 mg/ml) and ribonucleic acid (5 mg/ml) at 5500 A, as a function of time, after the addition of the different ribonuclease fractions[6].

Table I lists the distribution of enzymic activity of the ribonuclease fractions separated by paper electrophoresis. Four fractions were recovered in the aliquot of ribonuclease incubated with reduced glutathione. The mechanism of this interaction is obscure, but it results in the liberation of enzymically active protein fragments.

TABLE I

RIBONUCLEASE ACTIVITY OF THE DIFFERENT ACTIVE PROTEIN FRACTIONS SEPARATED BY PAPER ELECTROPHORESIS*

	Original per cent	*Fractions separated by electrophoresis*				*Recovery per cent*
		A	*B*	*C*	*D*	
Ribonuclease	100	89	9	—	—	98
Ribonuclease incubated with reduced glutathione**	180	120	22	15	17	96
Ribonuclease incubated with ascorbic acid**	150	130	—	—	15	96
Ribonuclease incubated with glycine**	125	110	9	—	—	95
Ribonuclease incubated with cysteine**	155	120	25	—	—	93
Ribonuclease incubated with histidine**	145	130	12	—	—	97

* The results are reported as per cent of the original ribonuclease activity, original ribonuclease activity taken as 100%.

** All reducing agents are added to a final concentration of 0.01.

The active protein fractions A, B, C, and D recovered from the ribonuclease-reduced glutathione incubation may be the same as those separated by column chromatography and described by LEDOUX[7].

The relative quantities of the active fractions A, B, C, and D, in commercial ribonuclease samples depend on the degree of ribonuclease reduction, and determine their enzymic activity. In a previous communication[8], ribonuclease (Armour) had more B fraction than other commercial ribonuclease (Worthington, Nutrition Biochemicals). Variations in nuclease activity of different *tissues* may also be due to the amounts of reduced ribonucleases present.

Electrophoretic mobilities of ribonuclease A and B (major fractions originally present in ribonuclease[8]) and of fractions A, B, C, and D obtained from the ribonuclease aliquot incubated with reduced glutathione, as a function of pH, were investigated in phosphate buffers of different ionic strengths. Isoelectric points were found to be at pH 7.8 and 7.1 for ribonuclease A and B respectively, as well as for the A and B fractions of the ribonuclease-reduced glutathione interaction. These values are identical with those reported by RAACKE AND LI[4].

The specific action of the various protein fractions of ribonuclease reduction is under study in our laboratory.

Acknowledgement. The help and interest of Dr. M. S. SASLAW, in the writing of this paper are gratefully acknowledged.

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Received March 6th, 1956