ELECTROPHORETIC INHOMOGENEITY OF CRYSTALLINE RIBONUCLEASE

by

I. D. RAACKE AND CHOH HAO LI

Hormone Research Laboratory, University of California, Berkeley, California (U.S.A.)

Crystalline ribonuclease has recently been shown to be chromatographically resolvable into at least two components by partition[1] and ion-exchange resin[2] chromatography. Despite the demonstration of the apparent homogeneity of the enzyme protein in experiments with *free* electrophoresis[3], the present investigation using *zone* electrophoresis on starch has been able to show that crystalline ribonuclease is not electrophoretically homogeneous.

The apparatus employed for the zone electrophoresis was a modification of the arrangement described by KUNKEL AND SLATER[4]. Reversible Ag–AgCl electrodes were used. The electrode vessels, constructed of plastic (Perspex), are composed of two compartments separated from one another by a labyrinth arrangement of partitions. The rear compartment contains a cylindrical well filled with saturated KCl solution into which the electrodes are dipped; the front compartment is in contact with the trough. Troughs of the type previously described[5], 60 cm in length, were used.

Electrophoretic experiments were carried out in a cold room at 3° for 48 to 72 hours, with a potential of about 200 volts across the trough. The starch in the trough was cut into segments of 1 cm each, extracted with 5 ml of water, and suitable aliquots were analyzed for protein concentration by the method of LOWRY *et al.*[6], and for enzymic activity by the procedure of KUNITZ[7]. Fig. 1 shows the pattern obtained when twice-crystallized ribonuclease (Armour) was submitted to electrophoresis in an acetate buffer of pH 5.2 and 0.1 ionic strength, for 72 hours at 190 volts. At least two enzymically active main components (Ribonuclease I and II) are identifiable; the other small components, which are observable in every experiment, should probably not be considered as artefacts. A sample of crystalline ribonuclease obtained from the Worthington Laboratories also gave similar patterns, except that the concentration of ribonuclease II was almost as high as that of component I. It should be mentioned that free electrophoresis of the Armour ribonuclease in a conventional Tiselius apparatus under the same conditions gave no evidence of inhomogeneity.

Ribonuclease I from the Armour preparation was isolated from segments 13–14 (Fig. 1); when it was re-run in a fresh trough under the same conditions, no evidence of contaminating components

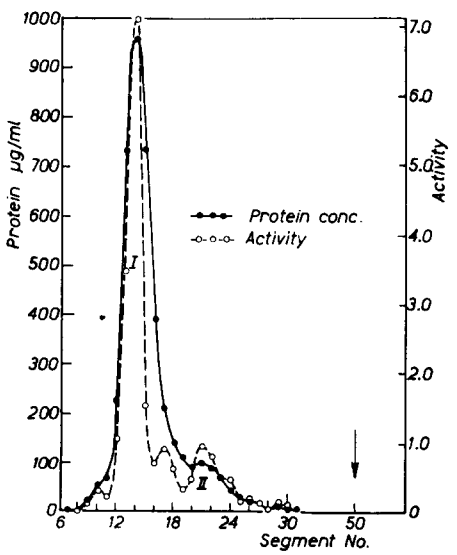

Fig. 1. Zone electrophoresis of crystalline ribonuclease (Armour) on starch in an acetate buffer of pH 5.2 and 0.1 ionic strength, for 72 hours at 190 volts. The arrow indicates the site of application of the protein solution.

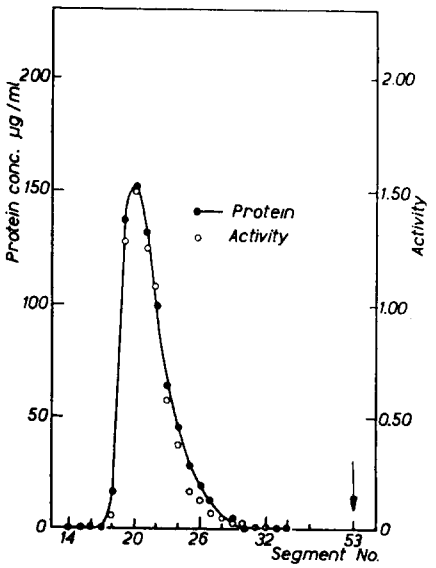

Fig. 2. Zone electrophoresis of ribonuclease I on starch in an acetate buffer of pH 5.2 and 0.1 ionic strength, for 70 hours at 195 volts. The arrow indicates the site of application of the protein solution.

was observed, as judged by the distribution of both protein concentration and specific enzymic activity (Fig. 2). The electrophoretic mobilities of ribonuclease I and II as a function of pH were investigated in borate buffers of 0.1 ionic strength, and the isoelectric points were found to be located at pH 7.8 and 7.1 respectively. The value for ribonuclease I is identical with that given by ROTHEN[8] for the crystalline ribonuclease. A detailed account of the present investigation as well as results of the application of zone electrophoresis to other biologically active proteins will be reported at a later date.

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RESTORATION OF ACETOIN AND SUCCINIC SEMIALDEHYDE FORMATION IN PIGEON MUSCLE HOMOGENATES IMPAIRED BY THIAMINE DEFICIENCY

by

C. H. MONFOORT

Laboratory for Physiological Chemistry, The University, Utrecht (Netherlands)

As reported in previous notes in this journal[1] the formation of acetoin and succinic semialdehyde (SSA) from pyruvate and α-ketoglutarate added to homogenates of various muscles of pigeons, which had been on a diet without thiamine and rich in carbohydrate[2] for 12 days, is considerably decreased as compared to the formation of these compounds in the corresponding muscle homogenates from normal pigeons. Even after 4 days of deficiency a decrease could be already observed. In view of work since then carried out in this laboratory by FRANKEN AND STAPERT[3], it now appears desirable to complete these notes by reporting the results obtained upon adding thiamine pyrophosphate (TPP) to the homogenates.

The effect of adding TPP was studied in simultaneous experiments, employing the same muscle preparations as used in the experiments which have been reported previously in another connexion[1,4].

The results concerning acetoin formation are assembled in Table I, those concerning succinic semialdehyde formation in Table II (for experimental details see MONFOORT[4]). In these tables *B* denotes breast muscle, *H* the muscle of the left ventricle of the heart, *L* a mixture of leg muscles and *n* the number of pigeons examined. The standard deviations mentioned are standard deviations of the means.

The small effect of addition of TPP on the enzymic activity of the homogenates from normal muscles may be explained either by the presence of small amounts of apo-pyruvic decarboxylase and apo-α-ketoglutaric decarboxylase in the muscles *in situ* or by the dissociation of part of the holoenzymes during the preparation of the homogenates.

The point to be stressed in particular is that the production of acetoin from added pyruvate, as well as that of SSA from added α-ketoglutarate, in the homogenates of the deficient muscles, is raised to the same level as reached upon addition of TPP to the normal homogenates. Hence the