ENZYMATIC LABELLING OF ACTIN AND TROPOMYOSIN WITH ^{14}C-LABELLED PUTRESCINE

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Transglutaminase, a Ca^{++}-activated enzyme first described by Waelsch and co-workers, catalyzes the replacement of the amide groups of protein and peptide bound glutamine residues by primary amines (1, 2). The replaced amide appears as ammononia. Folk and Cole have recently presented a detailed study of this reaction with particular reference to the specificity of transglutaminase (3).

Transglutaminase activity has been observed in various animal tissues such as liver, heart, kidney, spleen, lung and pancreas (1, 2, 4). We have confirmed the presence of this enzyme in rabbit and rat heart and further demonstrated transglutaminase activity in other muscle tissues such as the uterus and the skeletal muscle of the rat (5). Since this enzyme is present in muscle tissues the amine acceptor properties of some of the muscle proteins were studied.

In this communication we report the transglutaminase catalyzed incorporation of putrescine into actin and tropomyosin. Experiments are also presented which indicate that this enzyme can utilize these muscle proteins not only as amine acceptors but also as amine donors, thereby, connecting these two proteins together with covalent bonds.

MATERIALS AND METHODS

1, 4 - ^{14}C-putrescine was obtained from New England Nuclear

Corp. (Boston) containing 3.1μc/μ mole. This was diluted with non-radioactive putrescine to give a solution of 0.02 μc/μ mole.

Transglutaminase, a hundred fold purified preparation (35 mg/ml) from guinea pig liver was kindly supplied by Dr. J. E. Folk of NIDR, NIH, Bethesda, Maryland (6). Actin was prepared from rabbit muscle according to the prescription of Feur et al (7). Tropomyosin was prepared according to Bailey (8). Both of these protein preparations were stored in the lyophylized state. Labelling was determined by counting the radioactivity in the Packard Model 314 liquid scintillation counter.

RESULTS

The time course of the incorporation of ^{14}C-labelled putrescine into actin and tropomyosin. In the experiment with actin 28 mg lyophylized G-actin (containing 10% tropomyosin) was dissolved in 3.0 ml distilled water. To this solution the following ingredients were added: 1.0 ml 0.1 M tris-acetate buffer of pH 8.0, 1.0 ml 0.2 M reduced glutathione solution, 0.2 ml).1 M $CaCl_2$ solution, and 0.2 ml 0.1 M putrescine (0.02μc/μ mole). The pH was checked and the total volume was made into 6.0 ml. The solution was placed in a waterbath at 37° C.

The reaction started with the addition of 54μl enzyme. At various intervals 0.6 ml samples were removed to 10 ml of 5% TCA solution. Ten ml TCA solution to which 0.6 ml of reaction mizture and 6μl of enzyme were added served as the zero time control. The precipitates from the TCA solutions were collected on Millipore filters and washed twice with 10 ml protions of 5% TCA solution. The collected precipitates were subjected to counting in 10 ml of Bray's solution.

Each sample thus contained 0.024μ mole actin. A count of 600 per minute in the precipitates represented the binding of one

equivalent of putrescine.

The experiment with tropomyosin was similar except that the reaction mixture contained 27 mg tropomyosin in place of actin.

The results of these experiments are illustrated in Fig. 1. It is seen from the figure that in case of actin the incorporation

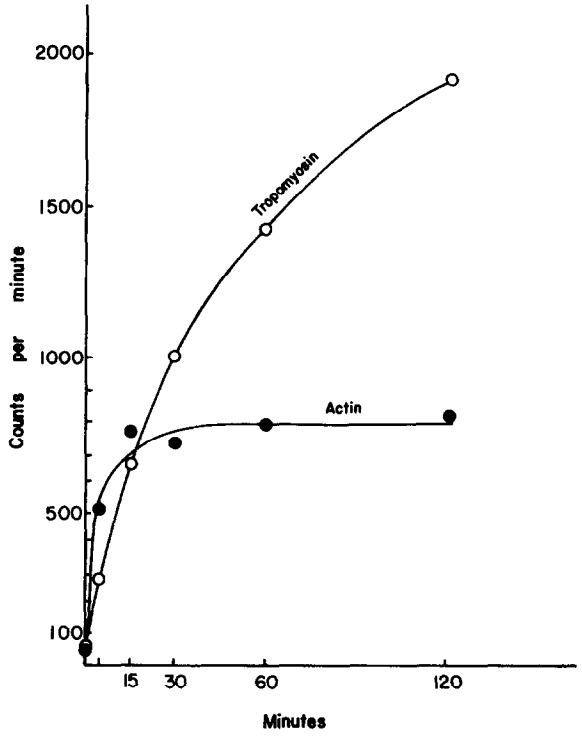

Figure 1. This figure shows the time course of the incorporation of labelled putrescine into actin and tropomyosin. On the ordinate are plotted the counts per minute per sample. 600 counts/min represent one equivalent of labelled putrescine bound to the protein. On the abscissa are plotted the times at which samples were taken out from the reaction mixture.

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of putrescine was completed in about 30 minutes whereas in case of tropomyosin the reaction was still going at 120 minutes. Actin incorporated approximately one residue of putrescine per mole, and tropomyosin more than 3 residues.

The effect of putrescine incorporation into actin. A reaction mixture composed similar to the above, except that the ingredients

were doubled was incubated for 2 hours at 37° C. The actin that polymerized under these conditions was centrifuged down in the preparative ultracentrifuge. The pellet was taken up in 20 ml CO_2-free distilled water which contained 3 equivalents of ATP. This solution was then dialysed for 24 hours with 3 changes of the outside solution which contained the same concentration of ATP as the solution inside the dialyzing bag. The F-actin was thus reconverted to G-actin. To test the polymerizing of this preparation KCl solution was added to an aliquot (final KCl concentration 0.1 M). Within a few minutes the solution became very viscous. Another aliquot was treated with 5% TCA and prepared as above for counting of the radioactivity. From the count it was again concluded that actin combined with one mole of putrescine per 60,000 g of actin. These experiments show that the incorporation of putrescine into a glutamine residue had no drastic effect on the ability of actin to polymerize. The effect of putrescine incorporation on the helical content of tropomyosin is under investigation.

The effect of transglutaminase on the actin-tropomyosin mixture

The experiment described below indicates that this enzyme can use these proteins not only separately as substrates to incorporate amines but also together to couple one to the other.

The composition of the actin-tropomyosin mixture was prepared as follows:

34 mg lyophylized actin (containing 3.4 mg tropomyosin) was dissolved in 5.5 ml distilled water. In a separate operation 20 mg lyophylized tropomyosin was dissolved in 3.0 ml tris-acetate buffer pH 8. To this solution were added: 0.3 ml of 0.2 M reduced glutathione, 1.0 ml of 3 M KCl solution and 0.2 ml of 0.1 M $CaCl_2$ solution.

The two solutions were mixed and left at room temperature for 1 hour. The viscosity of the solution indicated that actin became

converted to F-actin in this medium. Then, to an aliquot of 3.5 ml of this solution 0.1 ml transglutaminase solution (containing 0.1 mg protein) was added. Upon the addition of the enzyme a slow drop in viscosity was observed. In about 30 minutes the viscosity returned almost to the original value. After allowing this solution to stand for about an hour at room temperature it was left overnight in a refrigerator.

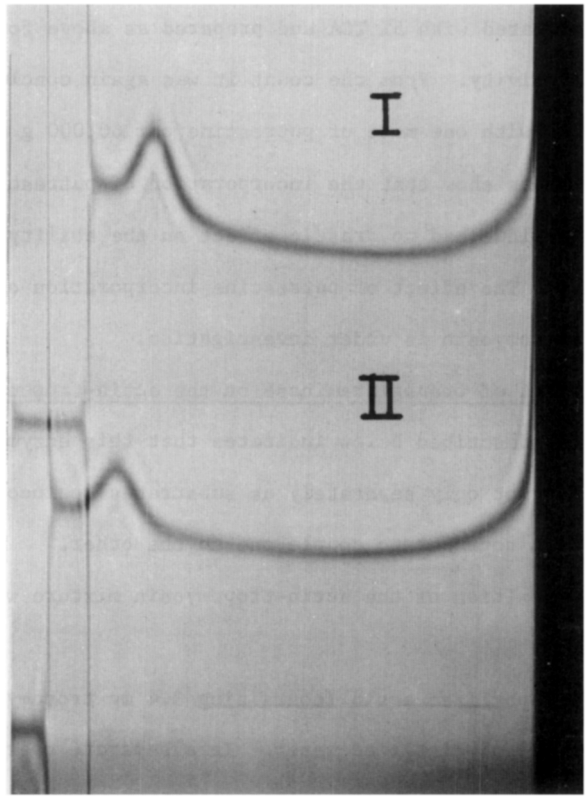

Figure 2. This figure shows the ultracentrifuge patterns of the actin tropomyosin mixtures - 39 minutes after reaching speed. Peak I represents 2.0 mg protein, peak II 1.0 mg protein. The sedimentation constant are: 3.28 S (peak I) and 2.98 S (peak II).

Before placing the solution into the analytical ultracentrifuge it was allowed to warm to room temperature. A second aliquot of the original mixture but without the addition of enzyme was treated similarly and served as a control. The two solutions were centrifuged simultaneously in two cells one of which had a wedge window. As could be expected the polymerized actin quickly sedimented to the bottom of the cells before the centrifuge reached its final speed. What remained in the supernatant sedimented with the speed of tropomyosin. Figure 2 shows the ultracentrifuge pattern of the two solutions. It is quite evident from the pattern that the sample incubated with the transglutaminase contains less protein. Planimetric measurements made on enlarged pictures of the sedimentation pattern showed that the sample incubated with the enzyme contained in the supernatant only 1, instead of 2 mg protein per ml. Thus, it can be concluded that as a result of the incubation with transglutaminase F-actin combined with more tropomyosin then in the control experiment. (9, 10).

DISCUSSION

In this paper we have demonstrated that transglutaminase can utilize actin and tropomyosin as substrates to couple, the amide, putrescine to them.

In the experiments in which actin and tropomyosin were incubated in the presence of the transglutaminase the indications were that the enzyme connected the two proteins together by utilizing the glutamin residue of one protein to act as acceptor and the amino-group of lysine of the other as the amine donor (neither of these proteins has readily available N-terminal α-amino groups).

The time course of the incorporation of putrescine into actin and tropomyosin suggests a tentative identification of the protein which in this reaction serves as the acceptor. As can be seen from Figure 1 the incorporation of the labelled amine leveled off in the

experiment in which actin was used but was still continuing in the case of pure tropomyosin at the termination of the experiment. The actin preparation used in the experiments contained 10 percent tropomyosin which could be expected to behave as tropomyosin. The fact, that in spite of tropomyosin being present the incorporation of putrescine in the actin-labelling experiment stopped, suggests that the acceptor sites of the contaminating tropomyosin were satisfied by the amino groups of actin. From these experiments we may conclude that in the combination of these two proteins, actin acted as the amine donor and the glutamine residues of tropomyosin as the acceptors.

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