THE ROLE OF α-GLYCEROL PHOSPHATE IN MUSCLE RELAXATION*

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The properties of the so-called Marsh-Bendall relaxing factor system of muscle have been reviewed by Needham (1960). This factor inhibits myofibrilar and actomyosin ATPase, ATP induced tension development in single muscle fibers and the syneresis of myofibrils and actomyosin. The activity is associated with the small granule fraction of muscle sedimentable between 7,000 and 35,000 x g. Parker and Gergely (1960) have shown that when relaxing factor granules are incubated with ATP for 15 to 30 minutes and the granules removed by centrifugation (5,000 x g for 2 hours) the granule free supernatant inhibits myofibrilar ATPase to about the same extent as the granule system. This finding has been confirmed by Briggs and Fuchs (1960) who showed that the granule free soluble relaxing substance (RS) will inhibit ATP induced tension development in single muscle fibers and that RS is dialyzable. The latter finding establishes the non-protein nature of relaxing substance.

Recently Marsh (1960) has reported that α-glycerol phosphate (α-GP) has relaxing factor activity in the presence of inorganic phosphate. Since glycerol kinase is present in muscle, it is of interest to know if the granule factor contains this enzyme and whether or not RS is α-GP.

Myofibrils and relaxing factor granules were prepared as described by Gergely et al. dl-α-glycerol phosphate was synthesized by the method

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of Bailly (1916). α-glycerol phosphate dehydrogenase and DPN were obtained from the California Corporation for Biochemical Research.

The data in Table I clearly show that dl- α-GP does not inhibit myofibrilar ATPase. Inorganic phosphate is without effect. Since dl- α-GP was used, it might be argued that the d-isomer is antagonistic

TABLE I

The Effect of Relaxing Factor Granules and α-Glycerol Phosphate on Myofibrilar ATPase Activity

The ATPase assay mixture contained 20 mM histidine, 50 mM KCl, 5 mM $MgCl_2$, 5 mM ATP, 2.5 mM K oxalate and α-GP, phosphate and granules as indicated in the Table. Myofibrils, 1.0 mg protein per ml; pH 7.0; final volume 2.0 ml. ATPase activity was determined by measuring the amount of inorganic phosphate liberated in 5 minutes. The reaction was stopped by addition of an equal volume of 10 per cent trichloroacetic acid. Phosphate was determined in the protein free supernatant by the method of Fisk and SubbaRow.

Additions	Relaxing Factor Activity*		
	Without Granules	150 µg Granules per ml	50 µg Granules per ml
none	-	79.4	26.5
1.0 mM α-GP	0	80.0	28.0
1.0 mM α-GP 0.5 mM P_i	0	83.5	29.5

*Relaxing factor activity is expressed as per cent inhibition of myofibrilar ATPase activity.

to the relaxing factor activity of the l-isomer or vice-versa. If one or the other of the two optical isomers of α-GP is RS and is produced by the granules, dl- α-GP therefore would be expected to inhibit the action of the granular relaxing factor. It can be seen in Table I, that this is not the case, dl- α-GP has no effect on the action of the granular factor.

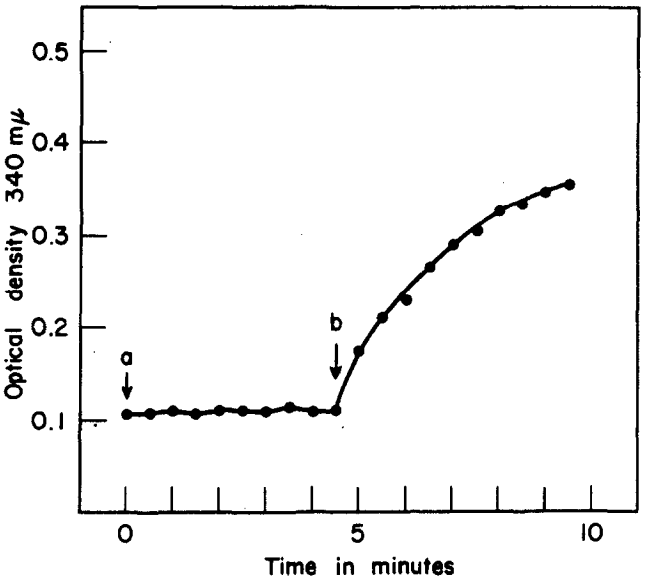

Figure 1. The glycerol kinase activity of relaxing factor granules.

The incubation mixture contained 50 mM KCl, 20 mM histidine, 2.5 mM K-oxalate, 5 mM $MgCl_2$, 1 mM glycerol, 5 mM ATP and 150 ug granule protein per ml. Final volume, 3 ml, pH 7.0. After 5 minutes incubation the pH was adjusted to 9.0 with 1 N NaOH. DPN was added to give a final concentration of 1 mM. Additions (a) 4 μg α′-glycerol phosphate dehydrogenase, (b) 3 μmoles dl-α-GP.

The possibility remains that α′-GP is an intermediate in the formation of RS. If the step involving the conversion of α′-GP to RS is rate limiting, the formation of RS will be independent of the concentration of α-GP and no potentiation of granule activity would be expected. This is the case. Neither α′-GP nor α-GP and inorganic phosphate have any potentiating effect on the relaxing factor activity of granules at a granule concentration that shows only partial inhibition of myofibrilar ATPase. However, the assumption that α′-GP is an intermediate in the formation of RS also requires that relaxing factor granules synthesize α-GP and that granules contain either glycerol or some precursor of glycerol since they produce RS in the absence of added α′-GP or glycerol. The results in Figure 1 show that granules do not synthesize α-GP from glycerol and ATP. α-glycerol phosphate was assayed spectrophotometrically as de-

scribed by Bublitz and Kennedy (1954). Granules were incubated with ATP and glycerol. After 5 minutes the pH was adjusted to 9.0 and DPN and α-glycerol phosphate dehydrogenase were added. The formation of DPNH was taken to indicate the presence of α-GP. The failure to detect DPNH formation -- as evidenced by no change in the optical density at 340 mμ -- in the absence of added α-GP indicates little or no glycerol kinase activity in the relaxing factor granules (Figure 1). The procedure is capable of detecting 0.1 umole of α-GP per ml.

The above results strongly suggest that α-GP is not identical to the soluble relaxing factor reported by Parker and Gergely since α-GP is not produced by the granular factor. In addition the results confirm the earlier finding of Bendall (1960) that α-GP has no effect on the cleavage of ATP by myofibrils.

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