SC 2256

The effect of sonic vibration on exchangeability and reactivity of the bound adenosine diphosphate of F-actin*

In this communication investigations are reported on the effect of sonic vibration on exchangeability and reactivity of the F-actin-bound ADP made in connection with the study of sonically activated F-actin ATPase[1]. Sonication of sample solutions was made in the same apparatus as used in the previous study.

The first experiment is concerned with the exchangeability of the bound ADP of F-actin when it is placed in the sonic field. The bound ADP of F-actin is known to be non-exchangeable with added ATP or ADP when left standing[2]. A [^{14}C]ADP-labeled F-actin was sonicated in the presence of a large concentration of ATP. It was

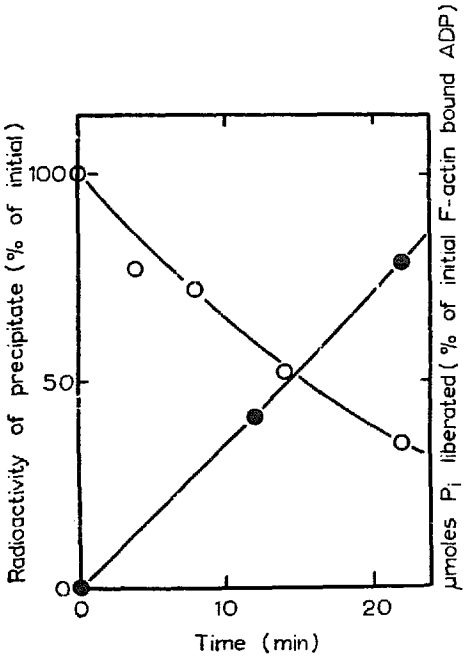

Fig. 1. The effect of sonic vibration on F-actin ATPase activity and exchangeability of the F-actin-bound ADP. According to the same method as used by MARTONOSI[3] was prepared a [^{14}C]ADP-labeled F-actin solution which contained 2.4 mg/ml (42 μM) actin, 0.15 M KCl, 2 mM $MgCl_2$ and 10 mM Tris–HCl (pH 8.1). (This sample solution was found to be contaminated by 16μM excess nucleotide.) After adding 0.05 vol. of ATP (final concentration 0.5 mM), the sample solution was sonicated at 18°. At intervals 2-ml aliquots were removed from the vibrating container and centrifuged at 100000 × g for 3 h after dilution with 10 ml solvent containing 0.1 M KCl, 2 mM $MgCl_2$ and 0.5 mM ATP, and the radioactivity of the precipitates was measured according to a conventional procedure. The activity due to the supernatant contained in the precipitate was less than 5% and the decrease in amount of the bound ADP during sonication was negligible. In parallel, ATP splitting was followed by measuring liberation of phosphate[1]. O, radioactivity of precipitate; ●, liberated P_i.

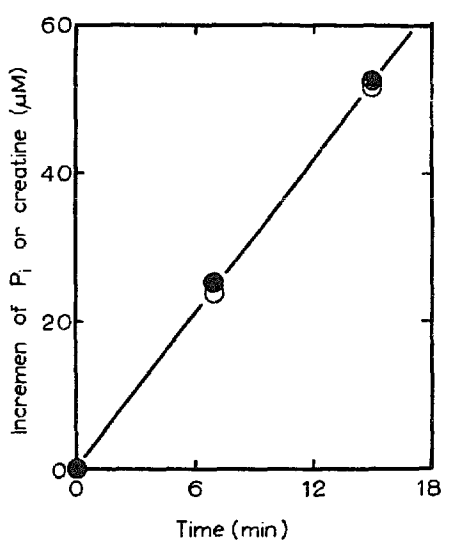

Fig. 2. Splitting of ATP and phosphocreatine induced in a mixture of F-actin and creatine kinase by sonication. In the presence of 2 mM $MgCl_2$ and 5 mM Tris–HCl (pH 8.3) a mixture of F-actin and a small amount of creatine kinase was treated with Dowex-1 for removing excess nucleotide. However, a trace of excess nucleotide remained in the solution. Sample solution contained 4.3 mg/ml (75 μM) actin, 0.33 mg/ml creatine kinase, 0.15 M KCl, 2 mM $MgCl_2$, 10 mM Tris–HCl (pH 8.3) and 32 μM excess nucleotide. After adding 0.6 mM ATP or 0.52 mM phosphocreatine, the sample solution was placed in the sonic field at 18° and liberation of P_i or creatine was followed. O, concentration of liberated P_i when ATP was added; ●, concentration of liberated creatine when phosphocreatine was added.

* A preliminary report of some of these results was presented by F. OOSAWA to the Conference on the Biochemistry of Muscle Contraction, 1962, Boston.

found that during sonication a marked decrease in amount of the [^{14}C]ADP bound to F-actin took place (Fig. 1). This decrease was due to a [^{14}C]ADP–ADP exchange, since no appreciable decrease in the total amount of the bound ADP was caused by the sonication. Therefore, Fig. 1 suggests that the bound ADP can be completely exchanged by prolonged sonication and the exchange reaction satisfies a simple first-order kinetics. Furthermore, it is remarkable that the exchange of bound ADP and the ATP splitting proceed at approximately the same rate. From these results, it can be concluded that the F-actin-bound ADP, when it is unmasked due to sonication, is replaced by added ATP followed by the ATP splitting, and that all constituent actin molecules of F-actin participate equally in the ATP-splitting reaction. It should be noted that the bound divalent metal of F-actin also becomes exchangeable under sonic vibration[4,5].

The second experiment is concerned with splitting of phosphocreatine when it is added to a system composed of F-actin and creatine kinase (EC 2.7.3.2), which is placed in a sonic field. Under the usual conditions, the F-actin-bound ADP is not reactive to the creatine kinase system[6]. The sonic experiment was made by using a sample solution containing a trace of excess nucleotide. (Attempts to prepare sample solution free from excess nucleotide failed.) It was found that under sonication the phosphocreatine splitting took place at the same rate as the ATP splitting when ATP, instead of phosphocreatine, was added to the system (Fig. 2). It may be concluded from this result that the unmasking of bound ADP due to the vibration is the rate-determining step in the whole reaction and takes place independently of the concentration of added ATP. The sonically activated F-actin ATPase is saturated at extremely low ATP concentrations[1].

The possibility that the unmasked ADP might be rephosphorylated before dissociating from the protein was examined. A system composed of F-actin and creatine

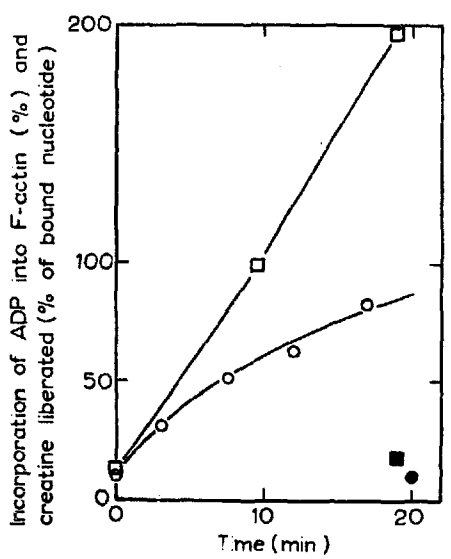

Fig. 3. Rephosphorylation of the ADP of F-actin by creatine kinase and phosphocreatine during sonication. A mixture of F-actin and creatine kinase was dialyzed against 4 mM $CaCl_2$ and 7.5 mM Tris–HCl (pH 8.2) for 4 days at 0° and then treated with Dowex-1 (1 g wet resin per 10 ml solution) at room temperature for 30 min. After adding [^{14}C]ATP and phosphocreatine, the sample solution was placed in the sonic field at 20°. Reaction mixture contained 4.4 mg/ml (78 μM) actin, 5.0 mg/ml creatine kinase, 4 mM $CaCl_2$, 7.5 mM Tris–HCl (pH 8.2), 56 μM [^{14}C]ATP (total counts/min $6.6 \cdot 10^5$) and 1 mM phosphocreatine. At intervals 3-ml aliquots were removed and free nucleotide contained in them was removed by the treatment with the resin. After removal of the resin by filtration, the radioactivity and nucleotide content of the filtrates were measured. Level of the incorporation of [^{14}C]ADP into F-actin was expressed by a ratio of the activity to the nucleotide content. Equilibrium level of the incorporation was determined by measuring the activity and nucleotide content of an aliquot which was not treated with Dowex-1. Percentage level of the incorporation was plotted. ○, during sonication; ●, untreated. In parallel, phosphocreatine splitting was followed by measuring creatine liberation. The result was expressed by 100 × (the increase in creatine concentration)/(the concentration of bound nucleotide). □, during sonication; ■, untreated.

kinase was sonicated in the presence of a very low concentration of [^{14}C]ATP and a large concentration of phosphocreatine. Incorporation of the [^{14}C]ADP into the F-actin and splitting of the phosphocreatine were followed at the same time. If the unmasked ADP were rephosphorylated before dissociating from protein, the phosphocreatine splitting should take place without accompanying the incorporation. However, this was not realized, both reactions proceeding at approximately the same rate under the vibration (Fig. 3). We must consider, then, that, in our sonic experiments, the rephosphorylation reaction takes place in the solvent, although HAYASHI AND ROSENBLUTH[7] have recently suggested that, in the case of G-ADP-actin, the ADP bound on the actin can be directly rephosphorylated by the creatine kinase system.

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The effect of sonic vibration on myosin and actomyosin adenosine triphosphate activities

In this communication investigations are reported of the effect of sonic vibration on myosin and actomyosin ATPase activities (EC 3.6.1.3) made in connection with studies of sonically activated F-actin ATPase[1–3]. Sonication of sample solutions was made in the same apparatus as used in our actin studies.

When the sonic generator was driven, the bulk temperature of solutions in the container rose about 2° higher than that of circulating water in the 1st min and then reached a constant value within an error of $\pm 0.5°$. To obtain a constant temperature during reactions under sonication, sample solutions were sonicated for 1 min in the absence of ATP and then ATP was added to the vibrating solutions. The constant bulk temperature of sample solutions was used as a measure of reaction temperature. Practically the same ATPase activity was found for myosin whether it was placed in the sonic field or not, while for F-actin ATPase an appreciable sonic activation was observed (Table I).

Under suitable conditions, actomyosin solutions undergo clearing followed by superprecipitation by the addition of ATP. The ATP splitting takes place slowly during the clearing phase and increases in speed with the superprecipitation[4–6]. If ATP is added to the same actomyosin placed in the sonic field, the ATP splitting