Extraction of myosin.

by

F. GUBA AND F. B. STRAUB.

If rabbit's muscle is extracted with salt solution an actomyosin of varying activity is obtained. In order to prepare pure myosin it is essential to start from a possibly inactive material.

We have studied the amount and the activity of the extracted myosin under varying conditions. The mode of mincing, the pH, the salt concentration and the time of extraction were varied. Activity was determined by the method of STRAUB[1], myosin contents were calculated from the viscosity in presence of ATP.

We find that the extraction of coarsely minced rabbit's muscle for 10 minutes at 0° with 3 volumes of a phosphate-KCl mixture of pH 6,5 (which contains 0,15 *M* K-phosphate buffer of pH 6,5 and 0,3 *M* KCl) will give an actomyosin of low (3 %) activity.

1. The effect of mincing and time of extraction. In the first series the muscle tissue was minced through an ordinary meat chopper only* (= coarse mincing), in the second it was minced first through the meat chopper and then minced again through a Latapie mincer (= fine mincing). In both cases the mince was extracted at 0° with 3 volumes of a 0,6 *M* KCl solution under constant stirring for different lengths of time, as indicated in Table I.

* Diameter of holes: 2 mm

Table I.

Time of extraction minutes	Coarse mincing		Fine mincing	
	% activity	mg myosin/ml	% activity	mg myosin/ml
5	4,5	12	7	15,6
10	4,8	3,8	9	16,5
15	5,0	15,5	13	17,2
20	6,6	17,7	20	18
25	9,0	20,2	22	19
60	—	—	40	—
120	—	—	80	—

Table I. shows that there is a rise in the activity of the extracted myosin on prolonged extraction. We therefore extract the muscle tissue only for 10 minutes. Fine mincing results in somewhat more myosin being extracted, but the activity of the extracted myosin is in this case more than twice of that obtained from coarsely minced muscle. It is further seen from the table that even from the coarsely minced muscle KCl extracts a fairly active myosin. This extracting agent is not satisfactory also because the results are not strictly reproducible and show considerable variations.

2. *The effect of salt concentration.* The muscle was minced through a meat chopper and extracted for 10 minutes at 0° with 3 volumes of the KCl concentration indicated in Table II.

Table II.

Salt concentration of extracting fluid, *M*	% activity	mg myosin/ml
0,2	—	0,8
0,3	17	3,5
0,4	10	8,7
0,5	11	10
0,6	12	11,8

Table II. shows that a minimal KCl concentration of 0,4 *M* is needed to extract myosin. It is to be seen moreover that the activity of the extracted myosin has a minimum value at 0,4 *M*.

The effect of pH. The muscle tissue was extracted at 0° for 10 minutes with 3 volumes of a phosphate-KCl mixture. This contained 0,15 *M* phosphate (KH_2PO_4 and K_2HPO_4) of varying pH and 0,3 *M* KCl.

Table III.

pH	% activity	mg myosin/ml
5,9	12	4,3
6,3	7	10,5
6,5	3,5	12
7,0	4,5	10,7
7,5	5	10,3

The activity was found to be 15—20 % when the muscle tissue was extracted with WEBER's solution* under similar conditions and 1% activity if it was extracted similarly with a 0,6 *M* KCl buffered to pH 10 with borate buffer.

Phosphate ions seem to have a depressing effect on the activity of the extracted myosin as extracts made without phosphate at the same pH and ionic strength are always more active than those prepared with phosphate. That the low activity is due to the lower actin content and not to the phosphate present is shown by the fact that the myosin of the phosphate extract, if precipitated and redissolved in a phosphate free KCl solution, will show the same low activity.

4. Salt concentration of phosphate-KCl mixtures. The extraction was made at 0° for 10 minutes with 3 volumes of phosphate-KCl mixture. The latter always contained 0,15 *M* phosphate of pH 6,5 and varying concentrations of KCl.

Table IV.

M KCl	% activity	mg myosin/ml
0,1	19	2,8
0,2	3	7,1
0,3	3	11,9
0,4	4	12,8

The table shows that apart from the phosphate 0,3 *M* KCl is needed to give optimal results. In a great number of experiments identical results were obtained.

References.

1. *F. B. Straub,* These studies **2**, 3, (1942).

* This solution contains 0,6 *M* KCl, 0,01 *M* Na_2CO_3 and 0,04 *M* $NaHCO_3$.