Studies on the Sarcoplasmic Reticulum of Normal and Dystrophic Animals* (34116)

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The presence of increased amounts of free lysosomal hydrolytic enzymes and decreased protein concentration within the muscle cell of dystrophic animals is well established (1–4). The role of these enzymes in the mechanism of the disease is not clear (5, 6). Barany *et al.* found that among the cellular proteins proportionally the sarcoplasmic proteins were decreased most in the dystrophic muscle cell (7).

Sreter *et al.* discovered that the Ca^{2+} transport function of the sarcoplasmic reticulum was impaired in dystrophic animals (8–10). These observations led us to investigate some characteristics of the sarcoplasmic proteins and lipids and to study the effects of protease and phospholipase C on fragmented sarcoplasmic reticulum using both normal and dystrophic animals.

Materials and Methods. Dystrophic chickens were generously given by Dr. D. W. Peterson, Department of Poultry Husbandry, University of California, Davies, California. Dystrophic mice (129 B6F1-dy) were purchased from The Jackson Laboratory, Bar Harbor, Maine.

Fragmented sarcoplasmic reticulum of various sources was prepared according to the method of Martonosi and Feretos (11). Great care was taken to remove the contaminant proteins by repeated washings with 0.6 M KCl. For the determination of the amino acid composition of sarcoplasmic protein about 30–60 mg (wet weight) of sarcoplasmic reticulum was dissolved in 1 ml 90% phenol solution and the sarcoplasmic protein was precipitated by the addition of 6 ml ice-cold acetone. The dried and well washed protein was hydrolyzed by 6 *M* HCl at 110° for 2 days and the amino acid composition determined with a Phoenix Model M-7800 automatic amino acid analyzer using an automated version of the Moore and Stein technique (12). The yield of the phenol-acetone extraction procedure was 0.3 mg protein per 1.0 mg (wet weight) sarcoplasmic reticulum.

For enzymic and ^{45}Ca uptake studies the protein concentration of the sarcoplasmic reticulum was determined by the Lowry procedure (13) after the addition of 0.16% deoxycholate and heating on a steam bath (14). Aliquots containing 4 mg protein were used for the enzymic experiments and samples containing 0.2 mg protein were employed for the ^{45}Ca uptake measurements (11).

The effects of protease (from streptomyces type VI) and phospholipase C (Sigma Biochemicals) were studied at 26° in a Radiometer TTT1 + SBV + SBR 2 combination which was used as a pH stat. One milligram phospholipase C was added to an aliquot of sarcoplasmic reticulum containing 4 mg protein in 0.1 *M* KCl at pH 7.5 in 4.0 ml total volume. The digestion was allowed to proceed for 40 min at 26° using 0.01 *M* NaOH as the titrant. Protease effect was studied under similar conditions except that 1 mg protease was added to the mixtures instead of the phospholipase C. Alternatively the 1.0 mg protease was added to such mixtures which were previously digested for 40 min by phospholipase C. Lipid phosphorus content of the sarcoplasmic reticulum was assayed as described by Masoro (15).

Results. It may be seen from Table I that we were unable to detect any conspicious

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TABLE I. Amino Acid Composition of Sarcoplasmic Proteins Obtained from Various Sources.

	Amino acid composition (%)[a]												
	Normal chicken		Dystrophic chicken		Normal mice		Dystrophic mice		Rabbit skeletal		Dog heart		Average
Amino acid	(%)	Δ	(%)	Δ	(%)	Δ	(%)	Δ	(%)	Δ	(%)	Δ	(%)
Lysine	5.208	−0.181	5.846	+0.457	4.881	−0.508	4.618	−0.771	4.550	−0.839	7.228	+1.839	5.389
Histidine	2.238	+0.097	2.361	+0.220	2.078	−0.063	2.047	−0.094	1.605	−0.536	2.516	+0.375	2.141
Ammonia	9.123	+0.498	6.983	−0.642	7.439	−0.186	9.976	+2.351	5.627	−1.998	7.601	−0.024	7.625
Arginine	5.403	−0.071	5.762	+0.288	5.249	−0.225	5.844	+0.370	4.655	−0.819	5.933	+0.459	5.474
Aspartic acid	8.124	−0.766	8.818	−0.072	10.099	+1.209	9.998	+1.108	8.684	−0.206	7.619	−1.271	8.890
Threonine	4.561	−0.478	5.525	+0.486	5.634	+0.595	5.217	+0.178	4.779	−0.260	4.519	−0.520	5.039
Serine	4.298	−0.577	5.584	+0.709	5.368	+0.493	5.441	+0.566	4.193	−0.682	4.364	−0.511	4.875
Glutamic acid	9.543	−0.916	11.013	+0.554	11.123	+0.664	10.394	−0.065	11.325	+0.866	9.357	−1.102	10.459
Proline	3.804	−0.560	4.326	−0.038	4.241	−0.123	4.741	+0.377	4.385	+0.021	4.684	+0.320	4.364
Glycine	6.142	−0.762	7.163	+0.259	6.847	−0.057	7.160	+0.256	6.433	−0.471	7.678	+0.774	6.904
Alanine	7.670	−0.490	8.280	+0.120	8.544	+0.384	8.028	−0.132	7.638	−0.522	8.799	+0.639	8.160
Half cystine	trace	−0.076	0.456	+0.380	trace	−0.076	trace	−0.076	trace	−0.076	trace	−0.076	0.076
Valine	6.221	+0.015	5.613	−0.593	6.873	+0.667	6.346	+0.140	6.105	−0.101	6.078	−0.128	6.206
Methionine	2.033	+0.265	2.218	+0.450	1.413	−0.355	trace	−1.768	2.484	+0.716	2.461	+0.693	1.768
Isoleucine	4.914	−0.343	5.203	−0.054	5.435	+0.178	5.565	+0.308	5.245	−0.012	5.179	−0.078	5.257
Leucine	8.225	−0.760	8.654	−0.331	9.763	+0.778	9.355	+0.370	8.610	−0.375	9.301	+0.316	8.985
Tyrosine	2.185	−0.288	2.904	+0.431	2.219	−0.254	1.906	−0.567	2.322	−0.151	3.299	+0.826	2.473
Phenylalanine	2.826	−0.257	3.293	+0.210	2.793	−0.290	3.364	+0.281	2.839	−0.244	3.383	+0.300	3.083

[a] Since most probably a mixture of proteins rather than a specific protein was analyzed in the case of each preparation, the amino acid composition is given as a percentage of the total. The average yield was 700 moles of amino acids per 10^5 g of protein.

differences in amino acid composition of the sarcoplasmic proteins obtained from various normal and dystrophic muscles. According to the results compiled in Table II, the total phospholipid concentration of the sarcoplasmic reticulum in normal and dystrophic chickens was similar. On the other hand, after 40 min of phospholipase C digestion the dystrophic sarcoplasmic reticulum lost 45.68% of its phospholipid content while under similar conditions the phospholipid content of the normal controls was diminished only by 25.14%.

Figure 1 shows the effect of protease on the sarcoplasmic reticulum of normal and dystrophic chickens. It may be seen that this enzyme digested the normal and dystrophic sarcoplasmic reticulum more or less equally. After 40 min of phospholipase C digestion, however, the sarcoplasmic reticulum of the dystrophic chickens was hydrolyzed somewhat faster by proteinase than that of the similarly treated normal controls.

As Fig. 2 shows, the sarcoplasmic reticulum obtained from dystrophic chickens accumulated indeed less ^{45}Ca than its normal

TABLE II. Effect of Phospholipase C on Total Lipid Phosphate Content of the Sarcoplasmic Reticulum of Normal and Dystrophic Chickens.

	Total lipid phosphate of the sarcoplasmic reticulum ($\mu g\ PO_4$/mg protein) Time of phospholipase C digestion in minutes		Loss of lipid phosphate after 40 min of phospholipase C digestion (%)
	0	40	
Normal chicken			
2	26.50	15.08	40.10
3	20.04	15.29	23.70
4	25.14	20.88	16.45
5	21.93	17.47	20.34
Average	23.40	17.18	25.14
Dystr. chicken			
2	26.22	14.10	46.22
3	25.74	13.78	46.46
4	23.23	14.12	39.22
5	21.21	10.43	50.83
Average	24.10	13.10	45.68

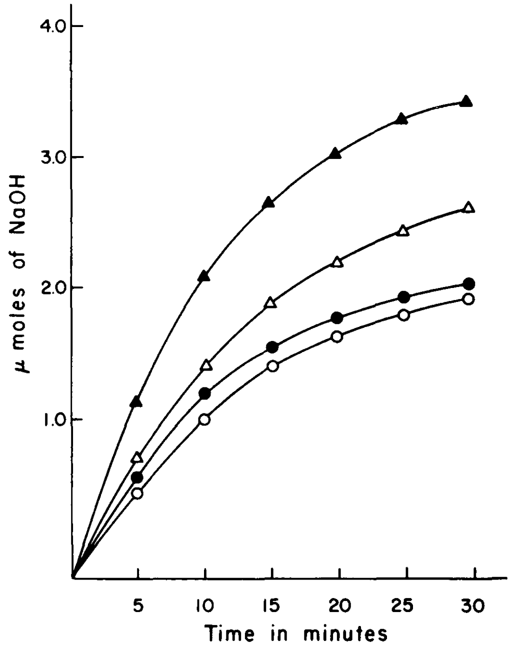

FIG. 1. Effect of protease before and after phospholipase C digestion on the sarcoplasmic reticulum of normal and dystrophic chickens. The amount of NaOH consumed by 4 mg sarcoplasmic protein during protease digestion is plotted as a function of the time of proteolysis. Each tube contained 4 mg sarcoplasmic protein, 0.1 mole KCL, AND 1.0 mg proteinase at pH 7.5. Total final volume was 4.0 ml. (For details see Materials and Methods). Normal (○); dystrophic (●); after 40 min of phospholipase C digestion: normal (△) dystrophic (▲).

control (8–10). A 1-min digestion with protease depressed the first minute uptake in case of both sarcoplasmic reticulum. If the digestion time was increased from 2–5 min (not shown in the figure) protease destroyed the ^{45}Ca uptake of both preparations more or less similarly. A marked difference was, however, observed between the ^{45}Ca uptake of the sarcoplasmic reticulum of normal and dystrophic chickens if these preparations were digested by phospholipase C for 40 min. This treatment depressed 64% of the total 5–15 min ^{45}Ca uptake of the dystrophic sarcoplasmic reticulum while the 5–15 min ^{45}Ca uptake of the normal controls was not at all inhibited.

Discussion. Several previous authors sug-

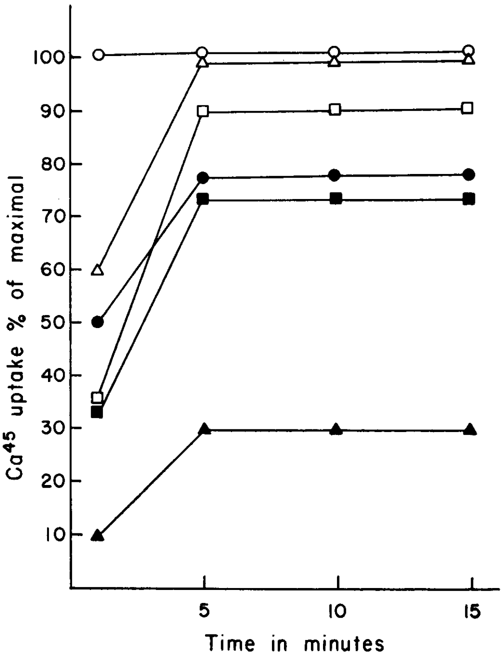

FIG. 2. Effect of phospholipase C and protease on the ^{45}Ca uptake of sarcoplasmic reticulum obtained from normal and dystrophic chickens. Each tube contained 0.05 mg/ml sarcoplasmic protein, 0.1 mmole ^{45}CaCl, 1.0 mmole ATP, 5.0 mmoles MgCl, 6.0 mmoles oxolate, 0.1 mole KCl, 0.02 mole NaCl and 0.05 mole pH 7.2 Tris buffer. Total final volume was 5.0 ml. Normal (○); dystrophic (●); after 40 min of phospholipase C digestion: normal (△); dystrophic (▲); after 1 min of protease digestion: normal (□); dystrophic (■). One hundred per cent of Ca uptake was 2.0 μmoles/mg protein.

gested that muscular dystrophy may involve a biochemical lesion of the phospholipid membranes (10, 16). The amino acid composition and the total phospholipid concentration of the sarcoplasmic reticulum was similar in normal and dystrophic chickens. The availability of sarcoplasmic proteins for protease digestion was also similar in the normal and dystrophic preparations.

On the other hand, after 40 min of phospholipase C digestion the sarcoplasmic reticulum of the dystrophic chickens compared to the normal controls lost a larger percentage of its total phospholipid content along with its ^{45}Ca-accumulating capacity. Also the effectiveness of protease to digest sarcoplasmic proteins became substantially increased in the case of the dystrophic animals.

These results would seem to suggest that perhaps the phospholipid structure of the sarcoplasmic reticulum was altered in case of the dystrophic chickens. In the dystrophic muscle cell the various structures are constantly exposed to an increased free lysosomal hydrolytic enzyme activity (1–4). Sawant *et al.* have shown that lysosomal enzymes are able to digest mitochondria and microsomes (17). Whether a prolonged *in vivo* exposure to lysosomal enzymes or a primary biochemical alteration of the phospholipid composition is responsible for the increased phospholipase C sensitivity of the dystrophic preparations can not be answered at this time. Further research will hopefully enable us to gain more insight into this problem.

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