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The molecular size of the calcium-transport ATPase of sarcotubular vesicles estimated from radiation inactivation

Sarcotubular vesicles from muscle behave as a simple active-transport system; aside from a basic ATPase, the presence of Ca^{2+} induces an activated ATPase coupled with a massive inward flow of Ca^{2+} (*e.g.* HASSELBACH AND MAKINOSE[1], EBASHI AND LIPMANN[2]). In this work, we have investigated whether a molecular-size range could be established, with the method of radiation inactivation, of the structural entities responsible for (i) the basic ATPase, (ii) the Ca^{2+}-activated ATPase, and (iii) the transport function. In the first stage of the work (with Mr. C. CHAMBERLAIN, 1964–1965), we surveyed the problem and its methodology, with preliminary results on all three properties, in the second stage (with Mr. P. SPIEGLER, 1966–1967), means were found to improve the methods for the experiments under (i) and (ii), and these results will be reported here. With respect to (iii), the indications are that this function is much more susceptible to inactivation, indicative of a larger target volume. This does not, however, indicate a larger size of the transport unit; the results could also be explained by assuming that, once a vesicle has received a few hits, it becomes non-retentive and so does not allow the detection of its Ca^{2+} uptake. We believe this to be the most likely explanation, but the radiological methodology is not sufficiently precise to conduct accurate studies on this point, so that we have not further pursued question (iii).

Vesicles were prepared from rabbit muscle in the purest form obtainable (SERAYDARIAN AND MOMMAERTS[3]). From the final suspension in sucrose, they were washed twice by ultracentrifugation with 0.1 M KCl. Aliquots of 0.6 ml were pipetted into glass ampoules and dried *in vacuo* from the frozen state. Each ampoule was then evacuated, flushed twice for 20 min with pure N_2, evacuated, and sealed. This procedure leads to little change in the Ca^{2+}-ATPase per mg of protein, but reduces the basic ATPase to about 0.4 times the original activity.

The closed vials were placed, at room temperature, in the electron beam of the 6-MeV accelerator (Varian Associates, Palo Alto, Calif.; *cf.* HAIMSON AND KARZMARK[4]) of the Department of Radiology, UCLA Medical Center. The dose rate was about $2.5 \cdot 10^5$ rads$\cdot$min^{-1}, checked occasionally with a liquid dosimeter (FRICKE AND MORSE[5], SHALEK AND SINCLAIR[6]). Doses, from 1–4 Mrads, were varied by changing the exposure times. The tubes were then kept at $+2^\circ$ until assayed within 24 h; after opening, the original amounts of water were added back. For the assays (*cf.* ref. 3), there was 0.4 mg vesicle protein in 1 ml of medium, 0.1 M KCl, 5 mM potassium oxalate, 5 mM $MgCl_2$, 10 mM imidazole (pH 7.0), 5 mM ATP and 0.55 mM ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*′-tetraacetic acid. For the basic ATPase, phosphate analyses[3] were performed after 0, 4, 8 and 12 min of incubation at 25°. For the Ca^{2+}-ATPase, $CaCl_2$ was added to a final concentration of 0.5 mM, and this was the trigger for the assay; the reaction times were (0), 15, 30, 45, and 60 sec, and the basic rate was subtracted from the observed.

The results were evaluated according to simple target theory, as evolved in the writings of LEA and co-workers[7,8] and HUTCHINSON and POLLARD and their co-workers[9–13]. Assuming that an average of 75 eV are required per inactivating event,

we originated the formula $M = (D_{0.37})^{-1} \cdot 0.72 \cdot 10^{12}$, where $D_{0.37}$ is the dosage in rads at which the control activity is reduced to a fraction e^{-1}, and M is the molecular weight in Daltons. This theory has an extensive empirical foundation in the study of enzymes and viruses (*cf.* refs. 7–13) and, while this work was in progress, has been applied to the target sizes of ATPase in erythrocyte ghosts by KEPNER AND MACEY[14], and of an $(Na^+ + K^+)$-activated ATPase from brain microsomes by NAKAO *et al.*[15]; values of 10^6 and $0.5 \cdot 10^6$, respectively, were obtained for these cases, which have similarities to the point investigated here.

The experiments to be reported were done on four separate preparations from different rabbits. Each of these was made up into 20 samples, so that each control or irradiated data point was run in quadruplicate. The ATP-splitting by the basic ATPase was a rectilinear function of time; likewise for the Ca^{2+}-ATPase but for a non-zero intercept at zero time.

This indicates an initial burst, which may be related to the rapid initial Ca^{2+}-binding process studies by EBASHI AND YAMANOUCHI[16]. This initial burst was also inactivated with increased dose but the accuracy was not satisfactory. The linear reaction rates were plotted semilogarithmically as a function of dose, and regression lines drawn with the least-squares procedure; the results are stated with the 0.95 confidence intervals according to the Student's *t*-test.

Table I gives a breakdown of the calculated target sizes for each of the 4 series separately as well as for the aggregate.

TABLE I

RESULTS OF 4 SERIES OF EXPERIMENTS ON RADIATION INACTIVATION

Target sizes in Daltons, Confidence intervals, ± 0.95.

Expt.	*Basic ATPase*	*Ca^{2+}-activated ATPase*
1	210 000 ± 85 000	210 000 ± 90 000
2	180 000 ± 80 000	160 000 ± 50 000
3	100 000 ± 40 000	170 000 ± 45 000
4	140 000 ± 70 000	200 000 ± 34 000
Combined:	160 000 ± 45 000	190 000 ± 34 000

The target sizes for the basic and Ca^{2+}-activated ATPases, respectively, are not significantly different, and it cannot be decided whether these activities are seated in the same or different units; the preferred removal of basic ATPase by washing may be due to the selective elimination of a separate activity, and the remaining activities may reflect two manifestations, without and with Ca^{2+}, of the same active unit. This unit, or these units, of a mass of the order of 175000 Daltons, thus appear to be the entities responsible for ATP-splitting, and may also be the units of the Ca^{2+} transport. It is of interest to compare this size with the result of HASSELBACH AND SERAYDARIAN[17], according to which there is one critical sulfhydryl group, essential for transport, per 100000 g of protein. If our target volume includes lipid as well (of which there is about 0.35 g per g protein in KCl-washed preparations (ref. 3); *cf.* MARTONOSI[18]), these values would come closer, though clearly there are many

reasons why they do not have to be identical, in view of the heterogeneous protein composition of the vesicles (MOMMAERTS[19], MARTONOSI[20]).

While the absolute accuracy of the target-method is limited, and is often stated to allow an ambiguity by a factor of 2 (*cf.* refs. 6–12), its power lies in the fact of allowing the size-determination of a functionally defined unit regardless of its state of association. It is of interest that an activity basic to a transport process is found to reside within a lipo-protein unit of such small size as is indicated by our work.

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