MOLECULAR WEIGHT ESTIMATION OF MEMBRANE BOUND ATPase BY IN VACUO RADIATION INACTIVATION*

GORDON R. KEPNER** and ROBERT I. MACEY

Department of Physiology-Anatomy, University of California

Berkeley, California

Received February 12, 1968

Radiation inactivation has been utilized to estimate the equivalent molecular weight of sodium-potassium stimulated adenosinetriphosphatase (Na-K-ATPase) in erythrocyte ghosts (Kepner and Macey, 1966) and more recently in NaI treated brain microsomes (Nakao et al. 1967). Since oxygen is known to influence the radiation sensitivity of enzymes, radiation target theory is most applicable to dried samples irradiated in vacuo. Accordingly, we have restudied the radiation sensitivity of membrane bound ATPase carrying out all irradiations in vacuo. We find the presence of air has a marked effect on the ATPase sensitivity and suggest that previous estimates of its molecular weight based on air irradiation must be re-evaluated. Our current data suggest a molecular weight of the order of 250,000 for the Na-K ATPase of human erythrocytes and guinea pig kidney cortex microsomes.

METHODS

Freeze dried human red cell ghost ATPase was prepared and assayed by methods described in our previous report (Kepner and Macey, 1966). Guinea pig kidney cortex microsomes prepared by the method of Post and Sen (1967)

---

*Supported by A.E.C. Contract AT (11-1) -34 Project No. 136-3.

**Present address: Institute of Animal Physiology, Babraham, Cambridgeshire, England.

were suspended in 1mM EDTA, 1mM NaCl (pH = 7.4) at a protein concentration of 10 mg/ml. Aliquots of approximately 0.1 mg/ml were taken for freeze drying and subsequent radiation. The microsomal ATPase was assayed at 37° in a solution (pH = 7.5) containing 20mM glycylglycine-HCl, 20mM imidazole, 0.5mM EDTA, 4mM $MgCl_2$, 3mM ATP, 150mM NaCl and 30mM KCl both in the presence and absence of 0.01 to 0.1mM ouabain. The activity when Na^+, K^+ and Mg^{++} are present is designated as the total activity. The total activity minus the activity when either ouabain is present or K^+ is absent is defined as the Na-K ATPase.

Samples were irradiated with 7.5 Mev electrons provided by the electron linear accelerator of the Lawrence Radiation Laboratory. Dosimetry was conducted with cobalt glass chips (Bausch and Lomb Optical Co.) calibrated against a Fricke dosimeter. The beam was continuously monitored by a transmission detector of the secondary emission foil type. Dose rates were typically of the order of 1 Mrad/min.

Radiation in vacuo was accomplished by connecting the enzyme containing flask to a vacuum pump and thermocouple gauge. Vacuum was applied for two minutes before irradiation began, and continued throughout the exposure.

Equivalent molecular weights were calculated by the following expression:

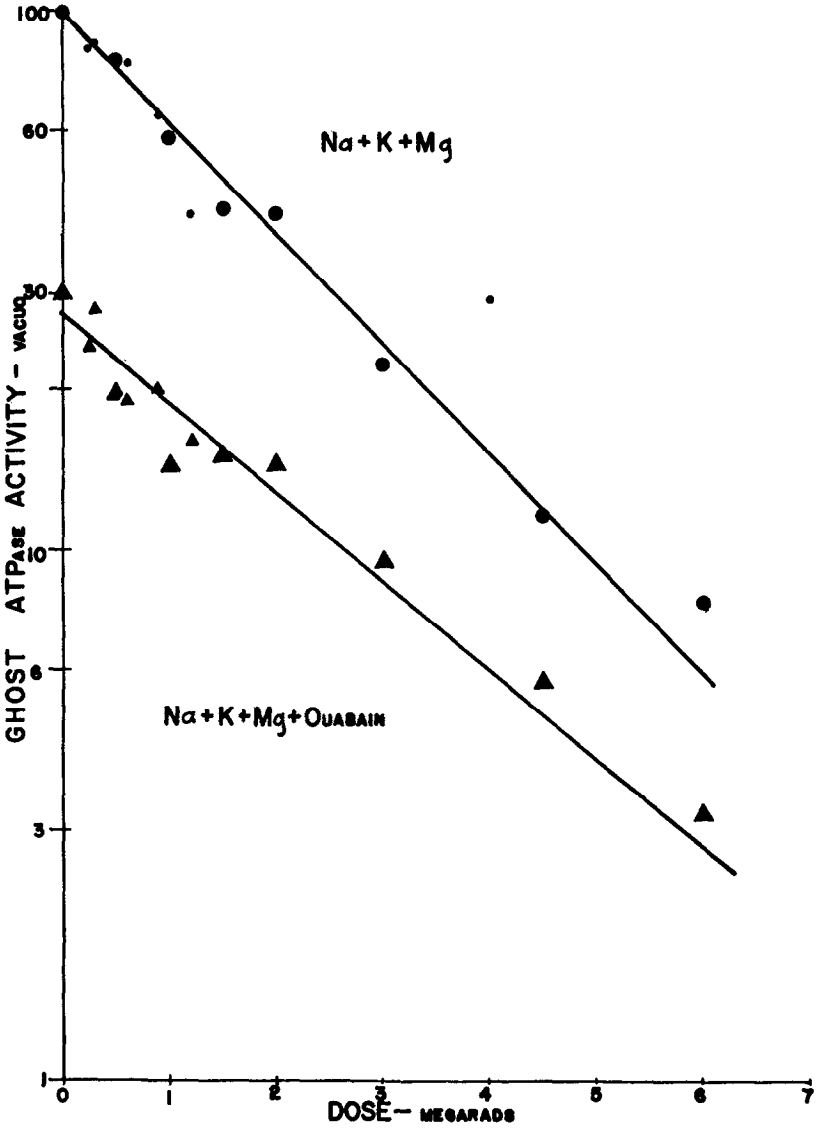
$$\text{mol. wt.} = 6.4 \times 10^{11}/D_{37}(\text{rads})$$

The factor 6.4×10^{11} was obtained from a calibration curve (mol. wt. vs. D_{37}) constructed from literature D_{37} values of enzymes with known molecular weights irradiated dry and in vacuo with low LET radiations (Kepner 1968, Kepner and Macey 1968). Although the factor is obtained independently of theoretical calculations it is of interest that it corresponds to a value of 66ev/inactivating event which differs only slightly from the range 70 - 100ev cited by Hutchinson (1965) and the figure 75ev utilized by Nakao et al. (1967).

RESULTS

In vacuo radiation curves for ATPase of red cell ghosts assayed in

the presence and absence of ouabain are illustrated in figure 1. This figure is a result of pooled data from 6 experimental runs, each "run" consisting of 1 to 4 samples at each of several doses. Individual dosage points were weighted according to the number of experiments run at that dose, and the weighted results fit by a least squares procedure to the anticipated exponential decay. The standard deviation of the resulting decay constants



were about 10% of the estimated value. The larger data point symbols in the figure simply indicate that more experiments were run at those points (i.e. those points have larger weights).

The upper curve has a D_{37} = 2.1 Mrad corresponding to the total activity which represents the sum of the ouabain insensitive ATPase and the Na-K ATPase. The lower curve shows the ouabain insensitive enzyme with an estimated D_{37} = 2.4 Mrad. Replotting the data by subtracting the ouabain insensitive component from the total activity results in a $D_{37} \approx 2.1$ Mrad for the ouabain sensitive component (Na-K ATPase) which does not differ from the total. This follows because: a) the D_{37}'s for ouabain insensitive and total activities are very similar, and b) the ouabain insensitive component only accounts for a small fraction (30%) of the total. These two factors also imply that possible variations in ouabain insensitive activity resulting from freeze dry procedures (Nakao et al. 1967) does not substantially affect our results.

Using a D_{37} value of 2.1 Mrad we calculate an equivalent molecular weight of 300,000 for human red cell Na-K ATPase. The D_{37} for the ouabain insensitive component does not differ by more than 15% and as such cannot be distinguished from the ouabain sensitive fraction. Preliminary experiments with guinea pig kidney cortex microsomes yield a D_{37} of 3.4 and 3.5 Mrad for

TABLE I

ATPase Source	D_{37} (Mrad) in vacuo	in air
Erythrocytes		
Na-K	2.1	0.95
ouabain insensitive	2.4	0.95
Kidney Microsomes		
Na-K	3.4	2.8
ouabain insensitive	3.5	2.5

the ouabain sensitive and ouabain insensitive fractions; these correspond to molecular weights of 190,000 and 180,000 respectively. Although NaI pretreatment of the microsomal preparation increased the ratio of Na-K ATPase to ouabain insensitive ATPase (Nakao et al. 1967) it did not alter the inactivation curves.

When the same preparations are irradiated in air, they become more sensitive. The D_{37} data is summarized in Table I.

DISCUSSION

Target theory calculations of molecular weights assume that irradiations are carried out dry and in vacuo. In an earlier report (Kepner and Macey, 1966) we attempted to estimate molecular weights and sizes of membrane bound ATPases from radiation data obtained in air. This approach seemed reasonable in view of the findings of Hutchinson (1960, 1961) suggesting that high dose rates applied to large samples of high molecular weight enzymes would obviate the O_2 effect. The data reported here indicates that this conclusion is not applicable to our system; the D_{37} in air is roughly 1/2 the corresponding D_{37} in vacuo.

Radiation inactivation estimates of molecular weights of enzymes in heterogenous systems cannot be expected to give precise results. The difference in our estimates for red cell and microsomal ATPase are not large enough to imply that these enzymes are different in the two preparations. In fact, if the enzyme in both preparations had a true molecular weight of 250,000 then our estimates would be in error by only $\pm$ 25%. A molecular weight of 250,000 together with an assumed density of 1.3 corresponds to a spherical particle with a diameter of 85 A°.

The large discrepancy between our current estimate of 250,000 and our earlier estimate ($\approx 10^6$) is largely due to the fact that the radiations are now conducted in vacuo, and to a much lesser extent due to the fact that we currently use an empirical factor for relating D_{37} to molecular

weight. On the other hand, the newer in vacuo data is consistent with our conclusion that the sizes and molecular weights of ouabain sensitive and ouabain insensitive ATPases are the same.

Nakao et. al. (1967) recently estimated a molecular weight of 500,000 from their data on radiation inactivation of NaI treated brain microsomes. They do not indicate whether the irradiation was carried out in vacuo or in air. If the irradiations were performed in air, then their data would be consistent with our finding that radiation inactivation of the ATPase system is enhanced by air, lowering the D_{37} by about 50%. In other words, differences between in air, and in vacuo radiation may account for the major portion of the discrepancy of the two estimates.

ACKNOWLEDGEMENTS

We are indebted to Mr. Doug Pounds for his assistance with the radiations and to Dr. C. A. Tobias for providing accelerator time.

REFERENCES

Hutchinson, F., Rad. Res. Supp. 2, 49, (1960).
Hutchinson, F., Science 134, 533, (1961).
Hutchinson, F., in 18th Symp. Fund. Can. Res. Anderson Hosp. & Tumor Inst., Cellular Radiation Biology, Williams & Wilkins Co., Baltimore, (1965).
Kepner, G. R., Membrane Enzyme Systems: Molecular Sizes from Target Theory Analysis of Radiation Inactivation Data, Ph.D. Thesis, University of California, Berkeley (1968).
Kepner, G. R. and R. I. Macey, Biochem. Biophys. Res. Commun., 23, 202 (1966).
Kepner, G. R. and R. I. Macey, in preparation (1968).
Nakao, M., Nagano, K., Nakao, T., Mizuno, N., Tashima, Y., Fujita, M., Maeda, H., and H. Matsudaira, Biochem. Biophys. Res. Commun., 29, 588 (1967).
Post, R. and A. Sen, in Methods in Enzymology, X, Acad. Press, New York (1967).