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Comments on recent efforts to estimate the molecular weight of the $(Na^+ + K^+)$-ATPase

A recent paper from the laboratory of M. Nakao reports on efforts to approximate the molecular weight of the $(Na^+ + K^+)$-ATPase (ATP phosphohydrolase, EC 3.6.1.3) using an enzyme fraction solubilized from pig brain microsomes[1]. No attempt was made to place this estimate in perspective with prior work. There are a number of reports in the literature wherein techniques similar to those used by MIZUNO *et al.*[1] and NAKAO *et al.*[9] were applied to the problem of estimating the molecular weight of the $(Na^+ + K^+)$-ATPase[2–6]. Since the results from various groups differ, it may be useful to summarize the current situation, and to suggest some basis for evaluating available data.

Because the $(Na^+ + K^+)$-ATPase has not yet been isolated in a pure form, attempts to estimate the molecular weight have had to rely on impure enzyme fractions from red cell membranes and brain or kidney microsomes. One approach suited to such conditions is the target theory analysis of radiation inactivation data[7,8]. Usually, samples are irradiated in air or *in vacuo* and one reports the D_{37} (dose to reduce initial enzyme activity to 1/e = 37 %) which is related to the molecular weight by the equation: mol. wt. = constant/D_{37}. Either kind of data can be used to estimate the enzyme molecular weight, but different constants must be used since the D_{37}^{air}

will in general be less by a factor of about two than the $D_{37}^{in\ vacuo}$. The actual value to be used for the constant is open to argument at the present time. One can, however, avoid this by using a calibration curve based on D_{37} values of enzymes of known molecular weight that have been irradiated *in vacuo*[4].

The earliest report of a radiation study on ATPase presented data on samples irradiated in air[2]. Data for irradiations conducted *in vacuo* using Mg^{2+}- and $(Na^+ + K^+)$-ATPase from several different sources were published subsequently and interpreted using the calibration curve procedure[3,4]. From *in vacuo* data the average value found for the molecular weight of each ATPase was 250000. A report from NAKAO *et al.*[9] presented a value of 500000 for the $(Na^+ + K^+)$-ATPase molecular weight, but did not state whether the samples were irradiated in air or *in vacuo*. Since the data were interpreted using only the theory, it is not possible to say if the correct constant was used unless the conditions of irradiation are known.

A second approach, analysis by ultracentrifugation and gel sieving of preparations such as those mentioned previously, is sensitive to the presence of heterogeneity in the enzyme fraction. In a *pure* enzyme preparation, the molecular activity can be calculated from the product of the specific activity and the molecular weight, *i.e.*,

$$\text{Molecular activity} = \text{mol. wt.} \times \text{specific activity} \qquad (1)$$

Alternatively, in a *heterogenous* preparation, if the molecular activity and molecular weight are known, then the measured specific activity can be compared with that predicted by (1) in order to estimate the extent of impurity in the preparation.

Applying this rationale to the data of MIZUNO *et al.*[1], we take the first approach and assume their preparation was relatively pure. From ultracentrifugation and gel sieving data they obtained a value of 500000 for the molecular weight and they cite 159 μmoles P_i per mg protein per h as the highest specific activity of their pig brain microsomal $(Na^+ + K^+)$-ATPase. These figures yield a molecular activity of about $1.3 \cdot 10^3$ min^{-1}. These data can be compared to experimental estimates of the ATPase molecular activity of $1.2 \cdot 10^4$ min^{-1} (refs. 10 and 11) and a predicted value of 2800 μmoles P_i per mg per h for specific activity[4]. (Highly purified Mg^{2+}-ATPases from mitochondria[12,13] and bacteria[14] with molecular weights of about 300000 also give molecular activities of $2 \cdot 10^4$–$3 \cdot 10^4$ min^{-1} and specific activities of the order of 4500 μmoles P_i per mg protein per h.) Apparently either the isolation procedure has induced drastic changes in the enzyme so that its molecular activity has changed by an order of magnitude, or the preparation is far from pure.

Exploring the latter possibility, we find a molecular activity of $1.2 \cdot 10^4$ min^{-1} and a molecular weight of 500000 give a specific activity of 1400 μmoles P_i per mg protein per h. This leads to the estimate that 159/1400 or about 10% of the protein of the microsomal fraction is $(Na^+ + K^+)$-ATPase. Higher specific activities for ATPase from microsomes have in fact been observed (300 and 360 μmoles P_i per mg per h)[11,15]. Aggregation is a common occurrence in solubilized heterogenous protein preparations. This possibility must be accounted for in estimates of the molecular weight by ultracentrifugation or gel sieving, especially for an enzyme which may only make up 10% of the total.

Other workers who applied gel sieving to a brain microsomal ATPase fraction (solubilized by Lubrol) obtained values of 775000 for the molecular weight of the

$(Na^+ + K^+)$-ATPase and 265000 for the Mg^{2+}-ATPase[6]. This is in contrast to the value of 500000 obtained by MIZUNO *et al.*[1] with their deoxycholate solubilized preparation. The differing influence of the detergents makes it difficult to assess the reliability of such solubilized preparations for molecular weight estimations. BROWN *et al.*[5] reported a value of 150000 for the molecular weight of a Mg^{2+}-ATPase from the heart muscle based on ultracentrifugation analysis of a digitonin or butanol solubilized fraction.

Estimates of the molecular weight of the $(Na^+ + K^+)$-ATPase thus give values of the order of 10^5 and this degree of accuracy is adequate for giving guidance for purifying the enzyme. MIZUNO *et al.*[1] suggested that a rough idea of molecular weight may be helpful in speculation upon the active transport mechanism. A number of calculations bearing on this point have been recently published[4].

Addendum. At the recent New York Heart Association Symposium on Membrane Proteins, L. Hokin reported a revised estimate of 270000 for the molecular weight of $(Na^+ + K^+)$-ATPase.

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