ON THE EFFICIENCY OF OXIDATIVE PHOSPHORYLATION IN ISOLATED HEART MITOCHONDRIA

Gerald P. Brierley*

Department of Physiological Chemistry, College of Medicine,
Ohio State University, Columbus, Ohio 43210

Received April 1, 1965

Smith and Hansen (1964) have reported experiments which suggest that the efficiency of mitochondrial oxidative phosphorylation may be much greater than the classically accepted P/O ratios of 3 for DPN+-linked substrates and 2 for succinate. Under their conditions of measurement P/O ratios approaching 6 for pyruvate and 4 for succinate were obtained. These high P/O ratios have been confirmed by other investigators using manometric procedures similar to those of Smith and Hansen (W. S. Lynn and R. H. Brown, personal communication) (Lenaz and Beyer, 1965). Because of the fundamental importance of this observation and its relevance to proposed mechanisms for oxidative phosphorylation (Boyer, 1963) (Beiber, 1964) it was felt desirable to obtain verification of the high P/O ratios by independent procedures. In the present communication the P/O ratios of preparations of beef heart mitochondria as estimated by the method of Smith and Hansen (1964) and by two independent polarographic procedures are reported. Preparations which yield P/O ratios for pyruvate considerably in excess of 3 when examined by the method of Smith and Hansen invariably yield values very close to 3 when measured by the procedure of Chance and Williams (1955) and by direct phosphate analysis in conjunction with polarographic oxygen determination.

---

* Established Investigator American Heart Association.

The data presented in Table I confirm that beef heart mitochondria show P/O ratios in excess of 3 when pyruvate plus malate is oxidized under the conditions of Smith and Hansen (1964). In contrast to the findings of Smith and Hansen, however, no requirement for pre-incubation in the presence of substrate was noted. Data for three different preparations are tabulated (Table I). When these same preparations were analyzed

TABLE I

P/O RATIOS ESTIMATED BY THE PROCEDURE OF SMITH AND HANSEN (1964)

Preparation	Specific Activity*	O_2 Uptake (uatoms)	P_i Uptake (umoles)	P/O
HBHM No. 1	0.21	5.7 ± 0.4	24.7 ± 1.4	4.3 ± 0.5
Nagarse No. 1	0.14	4.0 ± 0.2	21.5 ± 2.3	5.4 ± 0.5
Nagarse No. 2	0.23	6.4 ± 0.6	23.4 ± 0.4	3.7 ± 0.4

* uatoms O_2 min^{-1} mg^{-1}

Oxygen and P_i uptake were determined in the Warburg apparatus as described by Smith and Hansen (1964). The flasks were incubated for 5 minutes at 30° with a shaking rate of 120 oscillations per minute. The reaction was started by the addition of ADP and hexokinase and was followed for 7 minutes. The values reported are the means of 4 determinations with the range of the determinations indicated. HBHM No. 1 represents beef heart mitochondria prepared by a modification of the method of Hatefi and Lester (1958). Nagarse No. 1 and No. 2 were beef heart mitochondria prepared as described by Hatefi et al (1961). All reagents and additions were identical to those specified by Smith and Hansen (1964).

by the procedure of Chance and Williams (1955) using the identical protein concentrations (but with the omission of hexokinase and ADP) two discrepancies became apparent. The studies shown in Table II establish that the rate of State 3 respiration (Chance and Williams, 1955) was considerably higher in the Clark electrode cell than in the Warburg apparatus. P/O ratios estimated by this method were also significantly lower than those reported in Table I. Although the ratio of the P_i taken up to the total O_2 consumed is probably the more valid estimate of the efficiency of phosphorylation

(and is more comparable to the oxygen uptake as measured by manometry) it should be noted that the P/O ratio based on "extra oxygen" is also considerably lower than that found in the manometric experiments. In a total of six

TABLE II

P/O RATIOS OF THE PREPARATIONS OF TABLE I AS ESTIMATED BY THE METHOD OF CHANCE AND WILLIAMS (1955)

Preparation	Specific Activity[1]	Respiratory Control[2]	Total O_2 (uatoms)	Extra O_2 (uatoms)	P/O	
					Total O_2	Extra O_2
HBHM No. 1	0.31	2.2	0.55	0.31	1.7 ± 0.2	3.0 ± 0.3
Nagarse No. 1	0.51	3.8	0.34	0.25	2.7 ± 0.1	3.6 ± 0.2
Nagarse No. 2	0.35	4.0	0.49	0.33	1.9 ± 0.1	2.8 ± 0.3

1 uatoms O_2 min^{-1} mg^{-1} (State 3)
2 Ratio of State 4 to State 3 respiration

The reactions were carried out in the closed cell of a modified Clark electrode (Lessler et al, 1965) using the identical concentrations of reagents and mitochondria as in Table I with the omission of hexokinase and ADP. ADP (0.91 umoles) was added with a micro buret and the transition from State 4 to State 3 and the return to State 4 were recorded (Chance and Williams, 1955). Oxygen uptake was calculated assuming a concentration of 0.47 uatoms per ml at 32° (Umbreit, 1957). Each value is the mean of at least four deflections. The P/O ratio was calculated from the total O_2 consumed in the presence of ADP (Total O_2) and also from the extra O_2 (total O_2 minus the State 4 uptake of O_2 for a comparable period).

preparations examined by this criterion, only one (Nagarse No. 1) showed a P/"extra oxygen" ratio which was in excess of 3. Large variations in the amount of ADP added and in the concentration of mitochondria present did not significantly affect the ratios reported in Table II. Since the manometric determinations were made after the polarographic studies, it did not appear that the lower efficiency noted in Table II was a result of deterioration of the preparations.

It appeared possible, however, that the lower P/O ratios observed by the method of Chance and Williams resulted from a much less efficient acceptor system (since hexokinase was not present and considerably less ADP was added than in the manometric assay.) Accordingly the P/O ratio

of several preparations was also estimated by the procedure described in Table III. This method combines a direct determination of P_i with polarographic recording of the rate of oxidation. This method has the advantage

TABLE III

P/O RATIOS OF HBHM No. 1 AS ESTIMATED BY P_i ANALYSIS AND POLAROGRAPHIC RECORDING OF O_2 UPTAKE

Mg Protein	Specific Activity*	Reaction Time (min)	O_2 Uptake (uatoms)	P_i Uptake (umoles)	P/O
8.6	0.29	0.84	2.07	5.1	2.5
4.3	0.30	1.50	1.93	5.5	2.8
2.15	0.27	3.51	2.00	5.3	2.7

* uatoms O_2 min^{-1} mg^{-1}

Five ml of a solution containing the reagents and at the concentrations specified by Smith and Hansen (1964) were equilibrated at 32° in the closed cell of the modified Clark electrode (Lessler et al, 1965). The reaction was started by the addition of the indicated amount of HBHM No. 1 in 0.2 ml or less of 0.25 M sucrose. The oxygen uptake was a linear function of time. As the cell approached anaerobiosis the reaction was stopped by the addition of 1.0 ml of 3N $HClO_4$ and the decrease in P_i was determined by the method of Lindberg and Ernster (1956). The oxygen consumption was calculated using 0.47 uatoms per ml as the solubility of oxygen at 32° (Umbreit, 1957).

that all of the reagents present in the manometer flasks are also present during this assay. It can be run for only short periods of time, however. The data of Table III establish that the P/O ratio of the preparation shown was between 2.5 and 2.8 when measured in this manner. This is to be compared with values of 4.3 by the manometric procedure and 3.0 by the method of Chance and Williams (based on extra oxygen). The specific activity corresponded well with the rate shown in Table II and was considerably higher than that obtained by manometry.

The explanation for the discrepancy between the P/O ratio as estimated from the manometric procedure and that found by the polarographic methods may be found in the following considerations. The rate of oxygen and P_i uptake observed in the Clark electrode was directly proportional to the pro-

tein concentration (Table III). This condition was not met in the manometric experiments which showed much lower specific activities for oxidation in the presence of 4 mg of protein (the amount specified by Smith and Hansen) than in the presence of 2 mg of protein. The observed manometric rate of O_2 uptake was also strongly dependent upon the shaking rate. Much higher P/O ratios were observed when the rate of shaking was lower than 120 oscillations per minute (the maximum available with our apparatus). One nagarse preparation, for example, which showed a P/O ratio of 3.5 ± 0.1 at 120 oscillations/min showed a ratio of 4.4 ± 0.4 at 80 per minute. If the State 3 respiration rates measured in the polarograph are indeed the true rates of respiration then it appears that the diffusion of O_2 into the liquid phase may be affecting the manometric rates observed under the conditions of Smith and Hansen. Umbreit (1957) states that, using 15 ml flasks and a shaking rate of 120 per minute, adequate O_2 diffusion will be obtained if less than 300 ul of O_2 per hour (0.44 uatoms per min) are taken up. Four mg of mitochondrial protein with a specific activity of 0.3 uatoms min^{-1} mg^{-1} represents an O_2 uptake considerably in excess of this amount. It also should be noted that about 3 uatoms of O_2 are dissolved in the 3 ml of solution at 0° which are added initially to the Warburg flasks in the manometric assay. This amount can be quite significant in manometric assays which record a total of only 3 to 6 uatoms of O_2 uptake and in which diffusion of O_2 into the medium may be a limiting factor.

These studies indicate that considerable disparity exists between the P/O ratio as estimated by the manometric method and that found in the two polarographic procedures. The concept of increased efficiency of phosphorylation advanced by Smith and Hansen (1964) is not disproved by these experiments, but it appears that considerable difficulty can be encountered with the manometric method under their conditions of measurement.

Acknowledgments

These studies were initiated in 1964 in collaboration with Dr. A. L.

Smith at the Institute for Enzyme Research, University of Wisconsin. Research at the present address was supported in part by National Institute of Health General Research Support Grant and by American Cancer Society Grant IN-16-G. The technical assistance of Miss Betty Shearman and David Purviance is gratefully acknowledged. I thank Dr. Milton Lessler and the Yellow Springs Instrument Co. for the use of the modified Clark electrode and cell.

References

Bieber, T.I., Biochem. Biophys. Research Communs., 16, 501 (1964).
Boyer, P. D., Science, 141, 1147 (1963).
Chance, B. and Williams, G. R., J. Biol. Chem. 217, 383 (1955).
Hatefi, Y. and Lester, R. L., Biochim. Biophys. Acta, 27, 83 (1958).
Hatefi, Y., Jurtshuk, P., and Haavik, A. G., Arch. Biochem. Biophys., 94, 148 (1961).
Lenaz, G. and Beyer, R. E., Federation Proc., 24, 363 (1965).
Lessler, M. A., Mulloy, E., and Schwab, C. M., Federation Proc., 24, 336 (1965).
Lindberg, O. and Ernster, L. in D. Glick (Editor) Methods of Biochemical Analysis, Vol. III, Academic Press, N. Y., 1956, p. 1.
Smith, A. L. and Hansen, M., Biochem. Biophys. Research Communs., 15, 431 (1964).
Umbreit, W. W. in W. W. Umbreit, R. H. Burris, and J. F. Stauffer, Manometric Techniques, Burgess, Minneapolis, 1957, p. 13.