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Review

P/O ratios of mitochondrial oxidative phosphorylation

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Abstract

Mitochondrial mechanistic P/O ratios are still in question. The major studies since 1937 are summarized and various systematic errors are discussed. Values of about 2.5 with NADH-linked substrates and 1.5 with succinate are consistent with most reports after apparent contradictions are explained. Variability of coupling may occur under some conditions but is generally not significant. The fractional values result from the coupling ratios of proton transport. An additional revision of P/O ratios may be required because of a report of the structure of ATP synthase (D. Stock, A.G.W. Leslie, J.E. Walker, Science 286 (1999) 1700–1705) which suggests that the H+/ATP ratio is 10/3, rather than 3, consistent with P/O ratios of 2.3 with NADH and 1.4 with succinate, values that are also possible.

Keywords: Oxidation phosphorylation; P/O ratio; Proton leak; Slip; ATP synthase

1. Introduction

P/O ratios of oxidative phosphorylation (the ATP produced per oxygen atom reduced by the respiratory chain) were first studied in the 1940s and 1950s when the mechanistic values were assumed to be integers. Interest arose again in the 1980s because chemiosmotic theory allowed fractional values and studies of proton transport were not all consistent with integer P/O values. We proposed mechanistic P/O ratios of 2.5 and 1.5 with NADH-linked substrates or succinate, respectively, based on measurements by two methods [1–3] and compatible with H^+/O=10 or 6 with NADH or succinate, H^+/ATP=3 for ATP synthase and H^+/ATP=1 for ATP transport to the cytoplasm. Recent structural studies of ATP synthase [4] now suggest that even the H^+/ATP ratio may not be an integer, since it is the ratio of two molecular motors, and so P/O ratios should be reconsidered yet again. The current situation is that some textbooks give P/O's of 3 and 2, one saying they are "more established values" [5], others give 2.5 and 1.5, and a recent review said only that the values are variable [6].

The purpose of this review is to summarize old and new results relevant to P/O ratios and provide analyses of some results to help indicate what values are feasible.

2. Measurements of P/O ratios

A collection of P/O measurements is shown in Table 1. Most studies chosen were about P/O values rather than routine reports in papers about some other aspects of oxidative phosphorylation. Numbers have been rounded to two significant figures because, considering possible systematic errors, it is appropriate, and standard deviations are omitted because the degree of reproducibility does not reflect absolute accuracy. In some cases several numbers are reported in which case the range is listed. Most reports are with rat liver mitochondria and exceptions are noted in the text. Some comments on the values in Table 1 follow.

Abbreviations: BSA, bovine serum albumin; SMP, submitochondrial particles; Δp, the electrochemical proton gradient; state 3, respiration with ADP and P_i present; state 4, respiration without ADP; TMPD, *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine; RCR, respiratory control ratio

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Table 1
Uncorrected P/O and ADP/O measurements

Authors	*Method*	NADH-O_2	Succinate-O_2	Site 2	Site 3
Ochoa 1943 [9]	A	0.96–2.5			
Lehninger and Smith 1949 [10]	A	1.2–2.4			
Cross et al. 1949 [11]	A	0.7–3.3	0.88–1.6		
Copenhaver and Lardy 1952 [12]	A	2.1–3.2	1.2–1.9	0.6	
Chance and Williams 1955 [13]	B	2.3–3.0	1.5		
Greengard et al. 1959 [14]	A		1.3–2.2		
Jacobs and Sanadi 1960 [15]	A			0.45	0.95
Estabrook 1967 [16]	B		1.7		
Lee et al. 1967 [17]	C			0.99	
Chamalaun and Tager 1969 [18]	A				0.94
Klingenberg 1975 [19]	C		1.4		
Hinkle and Yu 1979 [1]	B, C	2.2	1.4		
Pozzan et al. 1979 [20]	B, C			0.44	
Azzone et al. 1979 [21]	C		1.9		1.3
Lemasters 1984 [22]	B	2.6	1.7		
Beavis and Lehninger 1986 [23]	D	2.7–2.9	1.6–1.8		
Jensen et al. 1986 [24]	C		1.4–1.8		
Stoner 1987 [25]	C	2.6	1.5	0.50	1.0
Hafner and Brand 1988 [26]	B		1.4		
Luvisetto and Azzone 1989 [27]	C		1.3–1.5		
Toth et al. 1990 [28]	B	3.3–3.5			
Hinkle et al. 1991 [3]	B	2.3	1.5	0.50	0.98
Davis and Davis-von Thienen 1991 [29]	B	2.5			
Lee et al. 1996 [30]	B, C	2.9	1.8		
Fontaine et al. 1997 [31]	C		1.3–1.5		
Devin et al. 1997 [32]	C	2.5			
Gnaiger et al. 2000 [33]	E		1.6		

P/O or ADP/O ratios were measured by the following methods: (A) Warburg manometer and phosphate uptake, (B) Chance and Williams oxygen electrode and ADP pulse (see text), (C) oxygen electrode and phosphate or ATP assay, (D) oxygen electrode and pH change, (E) oxygen electrode and injection of ADP at a known rate.

2.1. Studies with succinate or NADH-linked substrates

The earliest studies reported P/O ratios in minced tissue of about 2 [7,8]. A P/O ratio of 3 was first proposed by Ochoa in 1943, who corrected values of 0.96–2.5 (Table 1) to 2.4–4.0 (average 3.06) by measuring glycolysis in the same crude heart extract [9]. I discussed P/O ratios with Ochoa in 1987 and he was not surprised that they could be lower and was highly amused that they were still in question.

Lehninger and Smith [10] used a crude mitochondrial fraction with 3-hydroxybutyrate and AMP as substrates and reported P/O ratios that ranged from 1.5 to 2.44 at 4 min, and from 1.3 to 1.88 at 12 min. Studies with Warburg manometers usually give higher values at short times which have been shown to be overestimates (see later). They concluded that the ratio was "somewhat more than 2".

Cross et al. [11] used rabbit kidney and liver mitochondria (called "cyclophorase") and included hexokinase and glucose to trap the ATP synthesized, as have most groups since. The values show a wide range (Table 1). Assuming the variation was from random errors, the values are consistent with 2.5 or 1.5 with NADH or succinate, respectively.

Copenhaver and Lardy [12] reported a more narrow range of values (Table 1) that are also compatible with fractional ratios. The variation probably stemmed from the use of a Warburg manometer rather than the mitochondrial preparations because the preparations were essentially the same as were used later when there was high reproducibility using an oxygen electrode. However, people tended to attribute the lower values to bad mitochondria.

The first measurements of P/O ratios with an oxygen electrode were by Chance and Williams [13], who developed an excellent method that has been used in countless studies since. The amount of oxygen uptake stimulated by an addition of a known amount of ADP was used to calculate the P/O ratio, which is often called the ADP/O ratio when measured in this way. In that first study they used a vibrating oxygen electrode dipping into an optical cuvette, which gave much more diffusion of oxygen from the air than a modern closed cell does. They measured the diffusion into the cell as ". . .0.1 μM O_2 per second when half of the oxygen had been used up", but did not correct for it. With 3-hydroxybutyrate as substrate they gave three pulses of ADP and calculated ADP/O ratios of 2.3, 2.7, and 3.0, increasing as the experiment progressed, which they attributed to endogenous substrates lowering ratios of the first ADP pulses. The amounts of oxygen that diffused into the solution from the air can be calculated based on Fig. 2A of Ref. [13] and their estimate of the rate, which varies from zero at the top to 24 μM O/min at the bottom of the chart. Since the rate of diffusion from the air was less than 15% of the respiration rate, it is a reasonable approximation to use linear rates and calculate the amount of diffusion from the average rate during a state 3 burst and the length of time of