ATP-ADP EXCHANGE CATALYZED BY COUPLING FACTOR II

Robert E. Beyer

Institute for Enzyme Research
University of Wisconsin
Madison, Wisconsin 53706

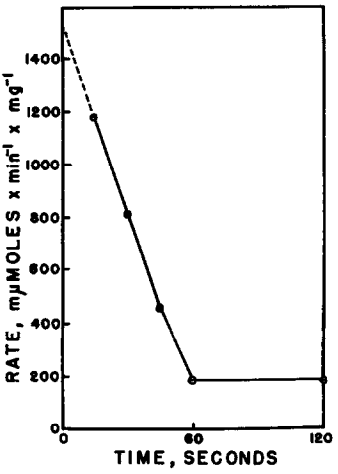
Received September 29, 1964

Coupling factor II (CF II) has been shown to restore the ability of submitochondrial particles to synthesize ATP coupled to electron flow in the segment of the mitochondrial electron transfer chain between reduced coenzyme Q and cytochrome c, i.e. that segment of the chain in which the complex is localized which has reduced coenzyme Q-cytochrome c reductase activity (Beyer, 1964a). (For terminology of coupling factors and phosphorylation sites see Green et al., 1963). Such a demonstration, however, does not demonstrate unequivocally that a coupling factor is involved directly in the sequence of reactions by which ATP is produced from the first high energy bond by successive displacement reactions. Three additional conditions have to be met to establish the participation of a coupling factor in the normal phosphorylation process. (1) The coupling factor must be capable of forming a high energy phosphorylated compound by interaction either with inorganic orthophosphate during electron transfer or with ATP in the absence of electron transfer. (2) The coupling protein must be capable of catalyzing the transfer of the terminal phosphate of ATP to the terminal phosphate of ADP. (3) The rate of the transfer should be no slower than the overall rate of oxidative phosphorylation at the site concerned. We have already adduced evidence in a previous communication (Beyer, 1964b) that CF II can form a phosphorylated intermediate either by oxidative esterification of inorganic phosphate or by interaction with ATP. The present communication deals with the ATP-ADP exchange reaction catalyzed by CF II and provides evidence that the rate of the exchange is sufficiently high to account for the overall rate of oxidative

phosphorylation at phosphorylation site II.

Rate of Exchange as a Function of Time.

The rate of the exchange reaction is not linear with time. It declines rapidly during the first 60 seconds and then levels off to a plateau value less than one seventh of the initial velocity estimated by extrapolation to zero time (cf. Figure 1). Once the plateau rate is reached no further change is observed for at least 15 minutes. Routine assays have extended over a period of 5-10 min; the rates for such long term assays have ranged between 185 and 245 mμmoles per min per mg of protein. The initial rate of the exchange re-



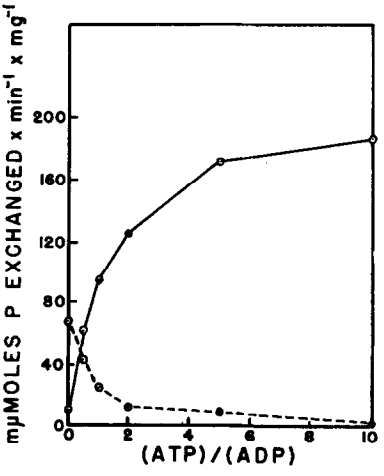


Fig. 1. (Left) The initial rate of the ATP-ADP exchange catalyzed by CF II. A solution (3.9 ml) containing 24 μmoles of ATP, 3.6 μmoles of ADP (labeled with ^{32}P in the α position), 18 μmoles of imidazole (pH 6.8), 30 μmoles of $MgCl_2$, was incubated at 30°. The reaction was initiated by the addition of 1.2 mg of CF II. Aliquots were removed at the times indicated and adenylate kinase and ATP-ADP exchange activities were calculated from the specific activities of the ^{32}P in the nucleotide fractions.

Fig. 2. (Right) The effect of the ATP:ADP ratio on adenylate kinase and ATP-ADP exchange activities of CF II. A solution (0.65 ml) containing 0.6 μmole of ADP (labeled with ^{32}P in the α-position), 3 μmoles of imidazole (pH 6.8), 5 μmoles of $MgCl_2$, and 0.2 mg of CF II was incubated for 10 min at 30°. The ATP concentration varied between zero and 6 μmoles. The solid line indicates ATP-ADP exchange and the broken line adenylate kinase activity.

---

action is roughly equivalent to the increased rate of oxidative phosphorylation at site II that obtains when exogenous coupling factor is present in the routine assay. For example, 60 μg of CF II per mg of particle protein in the assay system induces a doubling of the rate of phosphorylation (from

100 mμmoles to 200 mμmoles per min per mg of particle protein). Per mg of CF II protein, the phosphorylation rate per min is 1660 mμmoles. The exchange activity catalyzed by CF II is about 1500 mμmoles per min per mg during the initial rapid phase.

Effect of ATP:ADP Ratio.

The ATP:ADP molar ratio has a profound effect on the rate of the exchange reaction (Figure 2).. Initially, the rate is nearly proportional to the value of this ratio. Maximal exchange activity obtains only when the ratio is in excess of 10. The effect of varying the ATP:ADP ratio on exchange activity has also been examined by Glaze and Wadkins (1964) in their studies of a protein (Wadkins and Lehninger, 1960) which has properties in common with the coupling factor for site III (Webster, 1962; Wadkins and Lehninger, 1963).

Purified preparations of CF II contain adenylate kinase (EC 2.7.4.3, ATP:AMP phosphotransferase). Under the conditions used for the measurement of ATP-ADP exchange activity (high ATP:ADP ratio) adenylate kinase activity was negligible (cf. Figure 2). It cannot be excluded at present that the adenylate kinase activity of CF II is an expression of the catalytic capability of the coupling factor and not of a contaminant kinase.

Specificity of the Exchange Reaction.

We have studied the ability of CF II to catalyze the transfer of a phosphoryl group from a number of compounds to ADP. The triphosphates of inosine, guanosine, uridine, and cytosine can serve as phosphate donors for the exchange reaction but all are less effective than ATP (Table I).
The order of efficiency is the same as that reported by Löw et al. (1963) for the efficiency with which nucleoside diphosphates replace ADP as phosphate acceptor during oxidative phosphorylation catalyzed by submitochondrial particles derived from beef heart mitochondria. Since oxidative phosphorylation catalyzed by intact mitochondria is specific for ADP as a phosphate acceptor (Boyer et al., 1956; Boyer, 1958), the difference between the mitochondrion on the one hand and the submitochondrial particle and CF II on the other may possibly be explained in terms of the penetration of the inner mitochondrial

membranes by nucleoside diphosphates.

Table I

TRANSFER OF A PHOSPHORYL GROUP FROM VARIOUS POTENTIAL DONORS TO ADP IN THE PRESENCE OF CF II

	Phosphate Donor	Exchange rate, mμmoles x min^{-1} x mg^{-1}
1)	ATP	187
	UTP	81
	GTP	105
	CTP	55
	ITP	146
	DeoxyATP	163
	DeoxyUTP	47

The assay procedure was the same as that described in the legend of Fig. 2. Six μmoles of phosphate donor were added to each assay. The exchange rates have been corrected for adenylate kinase activity.

Specificity of Divalent Ions.

The ATP-ADP exchange reaction has an absolute requirement for a divalent ion (cf. Table II). Mg^{++} is the most effective metal ion but other divalent metal ions are able to replace Mg^{++} at least in part. It is of interest that the suppression of adenylate kinase activity by ATP applies only in the presence of a metal ion that is effective in the exchange reaction.

Table II

THE EFFECT OF DIVALENT IONS ON THE ATP-ADP EXCHANGE REACTION

Ion added	Rate, mμmoles x min^{-1} x mg^{-1}
None	0
Mg^{++}	239
Co^{++}	206
Mn^{++}	188
Ni^{++}	143
Fe^{++}	99
Cd^{++}	66
Ca^{++}	60
Cu^{++}	0

Experimental conditions were those described in the legend of Fig. 2, except that Mg^{++} was omitted when other divalent ions were added. All metal ions were added in the form of the chlorides except for iron which was added as ferrous ammonium sulfate. The data have been corrected for adenylate kinase activity.

Inhibition by NH_2OH

Hydroxylamine at a concentration of 0.25 M inhibits the exchange reaction and the transfer of a phosphoryl group from the phosphorylated form of CF II to ADP (Beyer, 1964b). The mechanism of these inhibitions is under investigation.

Discussion.

In oxidative phosphorylation two separate sets of reactions are now recognizable: (1) the generation of the first high energy intermediate by coupled electron flow; (2) the "conversion" of this intermediate by successive replacement reactions to ATP. The coupling factor for site II is intimately concerned with the second series of reactions. We have now demonstrated that all of the steps from the esterification of CF II by inorganic orthophosphate to the synthesis of ATP are catalyzed by a single protein and that the ATP-ADP exchange is an intrinsic part of this catalysis. What remains to be demonstrated for the second phosphorylation site is the nature of the linkage between CF II and P_i, the nature of the initial high energy intermediate involving an oxidation-reduction component of the electron transfer sequence, and the relationship between this initial high energy intermediate and CF II.

Since it has now been recognized that CF II functions in a series of reactions in which the high energy bond is conserved, while each of the original partners to the bond is successively replaced by another partner, it is perhaps no longer appropriate to employ the term coupling factor II because the coupling process precedes the intervention of the factor. Since the enzyme appears to catalyze a general type of reaction characteristic of a "synthetase"

$$A + B + ATP \rightleftharpoons AB + ADP + P_i$$

we propose the trivial name of ATP synthetase for the coupling factor and we shall distinguish this particular synthetase from the two acting at other sites by specifying that it is linked to phosphorylation at site II. ATP synthetase II would thus serve as the designation for CF II.

ACKNOWLEDGEMENTS

I am indebted to Dr. David E. Green for counsel and encouragement during this investigation. I also thank numerous colleagues for many valuable discussions, Dr. Mary Buell for helpful criticism, and Mr. Earl Fronk for skilled technical assistance. Dr. D. R. Sanadi contributed valuable suggestions concerning the chromatography of CF II. The author is the recipient of a Research Career Development Award (K3-GM-4862) from the Division of General Medical Sciences of the National Institutes of Health. This research was supported by research grant AM-06751-03 from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health. Meat by-products were generously supplied by Oscar Mayer and Co., Madison.

REFERENCES

Beyer, R. E., Biochem. Biophys. Res. Communs., 16, 460 (1964a).
Beyer, R. E., Biochem. Biophys. Res. Communs., 17, 184 (1964b).
Boyer, P. D., in Proceedings of the International Symposium on Enzyme Chemistry, Maruzen, Tokyo, 1958, p. 301.
Boyer, P. D., Ernster, L., and Lindberg, O., Federation Proc., 15, 223 (1956).
Glaze, R. P., and Wadkins, C. L., Biochem. Biophys. Res. Communs., 15, 194 (1964).
Green, D. E., Beyer, R. E., Hansen, M., Smith, A. L., and Webster, G., Federation Proc., 22, 1460 (1963).
Löw, H., Vallin, I., and Alm, B., in B. Chance (Editor), Energy-Linked Functions of Mitochondria, Academic Press, New York, 1963, p. 5.
Wadkins, C. L., and Lehninger, A. L., Proc. Natl. Acad. Sci., U.S. 46, 1576 (1960).
Wadkins, C. L., and Lehninger, A. L., Federation Proc., 22, 1092 (1963).
Webster, G., Biochem. Biophys. Res. Communs., 7, 245 (1962).