IODOHISTIDINE AS A POSSIBLE INTERMEDIATE IN OXIDATIVE PHOSPHORYLATION: ITS IDENTIFICATION AND ACTIVITY

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According to Boyer et. al. (1962), Peter and Boyer (1963), and Boyer (1963) a protein-bound phosphohistidine is formed in mitochondria. Since histidine itself is inactive in phosphorylation, it has been assumed that an "activated" form of histidine or of a derivative of histidine must be involved. From organic chemical and biochemical considerations, a derivative most likely to possess such activity is monoiodohistidine (MIH)*. For this reason it was decided to investigate monoiodohistidine as a possible intermediate in oxidative phosphorylation.

Monoiodohistidine was prepared according to the method of Brunnings (1947). Oxidative phosphorylation was measured by the method of Lardy and Wellman (1952) as modified by Gladte and Liss (1958) using the glucose-hexokinase ATP trap. Phosphorylating beef heart mitochondria were prepared by a modification of the procedure of Schneider (1948).

The effect on the P:O ratio of preincubation of MIH with mitochondria is shown in Table 1. In all cases, the increased P:O ratio is due to two effects: an increase in phosphorylation and a decrease in respiration. Thus, at concentrations of about 10^{-7} molar, MIH acts as

*MIH was identified in the thyroid gland by Roche et. al. (1952).

Table 1

Effect of preincubation with iodohistidine on the P:O ratio

Each preincubation vessel contained 5 umole/ml Mg^{++}, 2 umole/ml P_i, 6 ml mitochondrial suspension (0.6 mg N/ml) in 0.25 M sucrose + 0.002 M EDTA, 1 ml 0.01 M tris buffer pH 7.4, and MIH as indicated. Total volume was 7 ml. The mitochondria were preincubated for 1/2 hour at 25°C, then removed and resuspended in fresh sucrose-EDTA medium, for the assay. Each experiment had it's own control, in duplicate, without MIH.

MIH conc. molar	O_2 uptake % control	P_i uptake % control	P:O ratio actual	P:O ratio % control
5×10^{-7}	86	102	2.35	121
3.6×10^{-7}	83	106	2.29	127
2.5×10^{-7}	90	111	2.26	125
1×10^{-7}	83	110	2.40	133
average	86	107	2.33	127

a coupling agent. This affect was destroyed by addition of dinitrophenol to the substrate.

The presence of both Mg^{++} and inorganic phosphate in the preincubation mixture was required for activity.

The addition of as much as 5×10^{-5} M MIH without preincubation in the absence of ADP as a phosphate acceptor decreased oxygen uptake by more than 50%, i.e., the respiratory control was markedly increased. Furthermore, no inhibition of respiration by MIH occurred in the presence of ADP under the same conditions.

Histidine, iodine and KI at concentrations equal to those above had no activating effect.

Intact beef heart mitochondria incorporate P_i^{32} during respiration in the absence of ADP. P^{32} labeled mitochondria were extracted with alkaline 95% ethanol (pH 12.7 to 13.3 with KOH) and an acid-labile, base-stable, P^{32}-containing compound was separated from inorganic P^{32} by paper chromatography. An acid-labile, base-stable iodine-containing compound with the same R_f values as the unknown phosphate was also detected in three different chromatographic solvent systems. Acid

hydrolysis of the alcohol extract produced inorganic P^{32} and a new iodine-containing peak which had R_f values identical with synthetic iodohistidine in five different solvent systems (Table 2).

Table 2

R_f values of an acid-liberated iodine compound on paper chromatograms

20 ml of a mitochondrial suspension (0.6 mg N/ml) in 0.25 M sucrose + 0.002 M EDTA, pH 7.4, was incubated in 20 ml of the following substrate: α-ketoglutarate 30 umole/ml, malonate 30 umole/ml, $MgCl_2$ 15 umole/ml, P_i 6 umole/ml, 1 mcurie P^{32}, trace of ATP, pH 7.4; plus 20 ml 0.01 M tris buffer, pH 7.4 for ½ hour with occasional shaking. The mitochondria were removed by centrifugation and extracted with 20 ml 95% ethanol pH 12.7 to 13.3. After evaporation to 1 ml, half the extract was acidified with a drop of conc. HCl. The extracts were chromatographed on paper, with synthetic MIH and KI as standards. Iodinated compounds were detected by the F.F.C.A. method of Postmes (1963) after irradiation with U.V. light for ½ hour. The papers were scanned in a Spinco Analytrol densitometer equipped with a 600 mu filter.

Solvent systems	Iodide peak, acidified extract	R_f x 100 MIH standard	KI standard
2BuOH, NH_3 (4:1)	17-21	16-21	40
95% EtOH, H_2O (63:37)	52-57	56	82
$CHCl_3$, MeOH, 17% NH_3 (2:2:1)	79-83	81	88
nBuOH, HOAc, H_2O (78:5:17)	7.9	7.9	35
nBuOH sat. with 2N NH_4OH	6-14	7.8-15.5	24

Figure 1 is a composite of the densitometer tracings of the F.F.C.A. iodide stains before and after acid hydrolysis of the alcohol extract. The solid bars indicate the positions of the MIH and KI standards, the open bar shows the position of the acid-labile P^{32} peak. The possibility of a labeled phospholipid being responsible for the observed P^{32} peak was eliminated by subjecting the extract to alkaline hydrolysis. Garbus et. al. (1962) report that the rapidly labeled P^{32} phospholipid is acid-stable and base-labile. We found some loss of P^{32}, but the correspondance of the radioactive peak and the iodide peak remained.

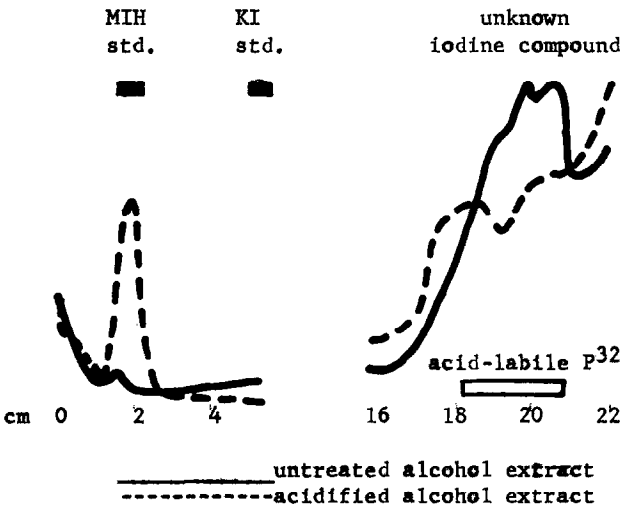


Figure 1
Appearance of MIH and disappearance of unknown iodine-containing compound on acidification.

The conditions are described in Table 2. Developing solvent: n-butanol, acetic acid, water (78:5:17)

Thus, acid hydrolysis of the unknown iodine-P^{32} compound yields inorganic P^{32} and iodohistidine.

In another experiment, ADP instead of acid was added to the extract. The extracts with and without ADP were chromatographed on paper with isopropanol-HCl as the developing solvent. Treatment with acid was unnecessary because of the acidity of the solvent mixture. The radioactive peaks of the chromatograms are compared in Figure 2. The ATP^{32} peak of the ADP-treated extract had 335 cpm, while the acid-labile P^{32} peak of the untreated extract had 425 cpm, (as in Figure 1), a ratio of 0.8 to 1.0. Therefore, most of the bound P^{32} of the acid-labile material was transfered to ADP to form ATP^{32}.

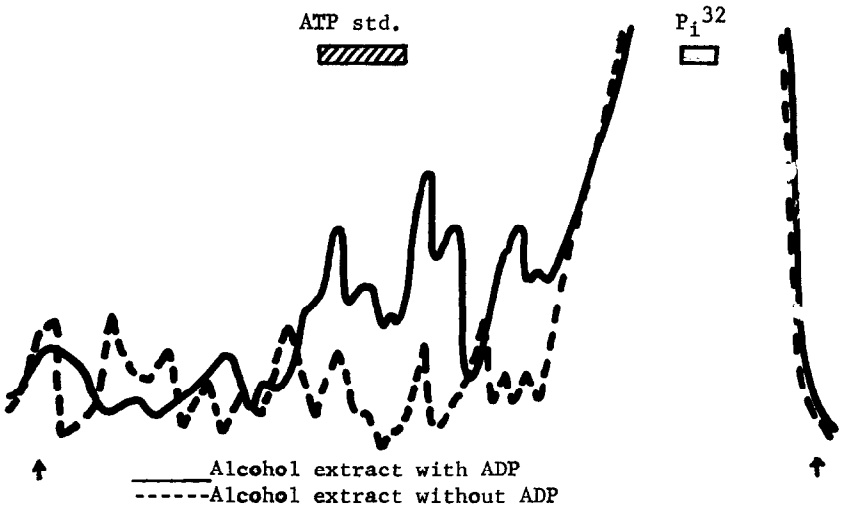
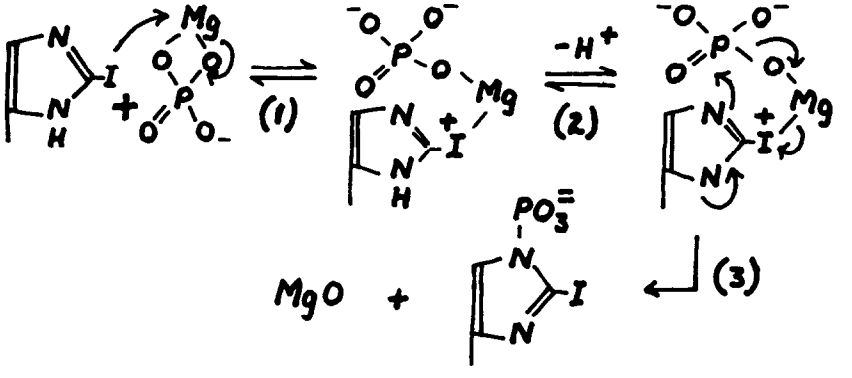


Figure 2

Appearance of ATP^{32} upon addition of ADP to the iodine-P^{32} acid-labile intermediate of Figure 1.

Incubation and extraction procedure as described in Table 2. 0.1 umole (Na_2) ADP was added to 1/2 the extract. ATP was located on paper by it's absorbtion of UV light. Solvent system: isopropanol, water, conc. HCl (65:18.4:16.6).

A possible mechanism for the reaction of iodohistidine with inorganic phosphate in the lipid matrix of the mitochondrion is outlined below:



Reaction (2) may be the energy requiring step; ferric and ferrous ions may be involved in the formation of the imidazolide ion. In reaction (3), the zwitterion is discharged through a six membered transition state, resulting in the formation of phosphoryliodohistidine and MgO.

Summary and Conclusions:

Synthetic monoiodohistidine increases the P:O ratio and respiratory control of isolated beef heart mitochondria. A possible high energy intermediate of oxidative phosphorylation has been isolated on paper chromatograms. This intermediate reacts with ADP to form ATP^{32}, and yields P_i^{32} and iodohistidine on acidification.

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