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REDUCTION BY A MODEL OF NAD(P)H. BIOMIMETIC RESPIRATORY CHAIN COUPLED WITH OXIDATIVE PHOSPHORYLATION

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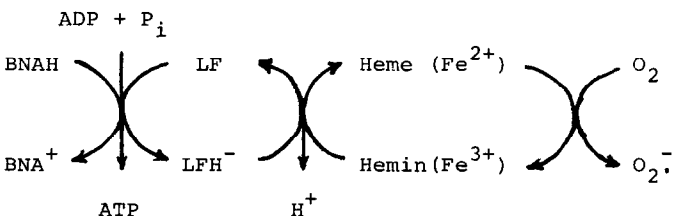
Nicotinamide adenine dinucleotides, $NAD(P)^+$ and NAD(P)H, play an important role in biological electron-transport systems (electron-bridges). Namely, respiratory chain starts from the reaction: NADH $\longrightarrow$ NAD^+, and photosynthetic electron transport ends at the reaction: $NADP^+$ $\longrightarrow$ NADPH.

In a previous paper of this series, we reported the constraction of electron-bridges, in which an electron originally located on N-benzyl-1,4-dihydronicotinamide (BNAH), a model of NAD(P)H, was transferred onto another organic molecule by the aid of a catalyst.[1)]

The vital role of biological electron transportation is, however, not only to oxidize or reduce a substrate but also to prepare an energy-rich compound, ATP. We now wish to report that one of our artificial electron-bridges *does* indeed conjugate with ATP-synthesis, then the system can be claimed as a biomimetic respiratory chain conjugated with oxidative phosphorylation.



Since the reaction is sensitive to light and oxygen, all solutions used were prepared in dark and flashed with argon sufficiently prior to the use. The uv cell was also filled with argon. To a pyridine solution of hemin (ferriprotoporphirin chloride), a model of cytochromes, in an uv cell equipped with an nonaero-silicon-rubber stopper, pyridine solution of BNAH was injected by means of a microsyringe. The intensities of the absorption spectrum at 526 and 558 nm, due to reduced hemin (heme),[2] increased gradually, and after 40 min at room temperature the spectrum changed from a to b as shown in Figure 1. On the other hand, when small amount of N-methylphenazium methosulfate (PMS) in pyridine was added to this solution, the intensity at 558 nm increased immediately as shown by the spectrum c in Figure 1. The spectrum c changed to d after the solution was exposed to air for 10 hr. These spectral change reveals tha an electron on BNAH is transferred to PMS, to hemin, then to oxygen *via* electron-bridges.[3]

The time-dependencies of the intensity under various conditions are represented by Figure 2, where lumiflavin (LF) is used in place of PMS: to a solution of hemin (7.4×10^{-5} mol) in pyridine (1.5 ml), pyridine solution (1.5 ml) of BNAH (5.2×10^{-4} mol) was added and a spectrum was recorded. After 10 min a pyridine solution (10^{-2} ml) of LF (7.4×10^{-10} mol) was injected, which resulted in complete reduction of hemin within 10 min. The amount of LF employed is not the lowest effective one, instead it is the lowest practically accessible one. This result invokes that LF turned over at least 10^5 cycles as an electron-bridge catalyst. Such a high efficiency is scarcely reported, if any. When the reaction was run under aerobic condition, the reduction of hemin took place quite slowly, probably because the reduced LF was directly oxidized by oxygen. Lumichrome (1.2×10^{-9} mol) and flavin monomucleotide (6.9×10^{-10} mol) can also be substituted for LF or PMS.

Bechara and Cilento have reported that phosphorylation of ADP (in 3% yield) takes place in conjugation with oxidation of BNAH by equivalent amount of N,N,N',N'-tetramethyl-*p*-phenylenediamine. In order to test the conjugation of oxidative phosphorylation with the present biomimetic electron-bridge, the reaction was run in the presence of ADP (tris-salt) and tetra(*n*-butyl)ammonium

Figure 1. Absorption spectra of a mixture of hemine (3.1×10^{-4}mol) and BNAH (2.8×10^{-4}mol) in pyridine. (a) Right after mixing. (b) After 40 min. (c) One minute after PMS (3.3×10^{-5}mol) was injected. (d) One day after the spectrum c was recorded and air was introduced to the system.

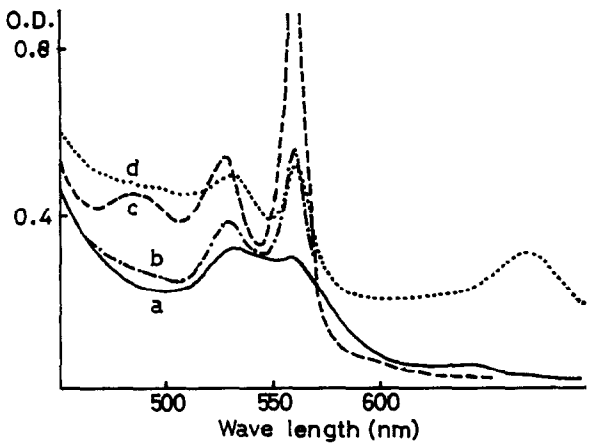


Figure 2. Time dependency of the optical density at 560 nm for a mixture of hemin (7.4×10^{-5}mol) and BNAH (5.6×10^{-4}mol) in pyridine. (a) Non-aerobic condition. (b) Aerobic condition. LF (7.4×10^{-8}mol) was added as indicated by an arrow.

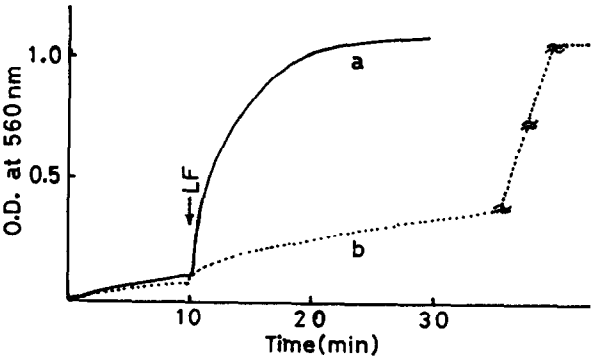


Figure 3. Separation of ATP from ADP with Dowex-1x2 ion-exchange resin. Aqueous HCl-KCl (0.01 N) was used for eluent.

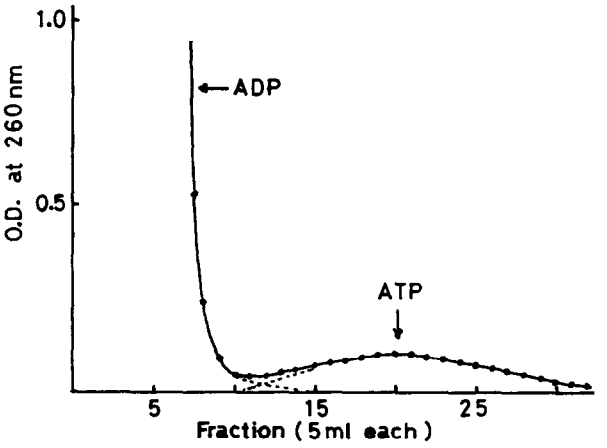


TABLE 1. Oxidative Phosphorylation Conjugated with an Electron-Bridge.[a]

BNAH, μmol	Hemin, μmol	LF, μmol x 10^5	ADP,[b] μmol	HPO_4^{--},[c] μmol	Time, min	ATP μmol	ATP Yield,%[d]
29.9	4.99	0	49.8	50.1	120	0.28	5.6
30.0	5.02	9.8	50.1	49.9	20	1.11	22.1
29.9	4.99	9.8	50.3	51.0	120	1.16	23.2

a) In pyridine at room temperature (~22°C). b) Tris-salt. c) $(n\text{-Bu})_4N^+$-salt d) Based on hemin used.

phosphate and, after treatment of the reaction mixture with Dowex-1x2 ion-exchange resin (200 - 400 mesh, 1.0 cm ϕ x 6 cm),[4] the optical density at 260 nm, due to adenine moiety, was measured for each 5 ml fraction (Figure 3). The spectrum in Figure 3 can be reproduced by an authentic mixture of ADP and ATP, from which the formation of ATP in about 20% yield (based on hemin used) can be confirmed. The reaction conditions and results are summarized in Table 1. It was also confirmed that none of BNAH, hemin, ADP, and the phosphate can be neglected for obtaining ATP in detectable amount within 2 hr. Thus, it is obvious that oxidative phosphorylation takes place as quickly as the reduction of hemin proceeds, that is, both reactions are conjugated. Kinetic studies are now in progress to confirm the mechanism.[4]

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3. Cf. (a) M. B. Dearden, C. R. E. Jefcoate, and J. R. L. Smith, "Oxidation of Organic Compounds," Amer. Chem. Soc., Washington, D. C., 1968; (b) R. Dessy, J. Dillard, and L. Taylor, "Bioorganic Chemistry," Amer. Chem. Soc., Washington, D. C., 1971.

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