Photoswitching of NAD+-Mediated Enzyme Reaction through Photoreversible Antigen–Antibody Reaction

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Received September 17, 1993
Revised Manuscript Received November 15, 1993

Switching of biological reactions by external signals, such as light irradiation, has been attracting the interest of a number of researchers.[1-5] On/off switching of enzymic reactions will have a large effect on the yield of final products because of the catalytic reactions of the enzymes. For this idea to be realized, however, it is crucial to attain absolute zero reactivity in the "off" state, since even minor reactivity in the "off" state will be amplified to cause significant total reactivity.

In the past, chemical modification of inhibitors, cofactors, or enzymes to attach photochromic groups, such as azobenzene or spiropyran, has been reported.[1-8] In these examples, one of the photoisomers shows higher activity than the other, resulting in the photocontrol of biological reactions. In most cases, however, the difference of the activities of the two photochromic states is not large enough, except for a few cases.[8] To achieve on/off switching, one should design a system in which a large effect is induced from a relatively small change in the molecular shape of photochromic groups. Another factor that may suppress the activity ratio in a system with azobenzene is that a pure cis state cannot be attained under UV irradiation. Therefore, the system should be "on" in the cis form and "off" in the trans form to achieve zero activity in the "off" state.

In this communication, we report a general approach to switching biological reactions using an azobenzene group combined with an antibody against a *trans*-azobenzene group. A monoclonal antibody against a nonnatural amino acid carrying an azobenzene group, L-*p*-(phenylazo)phenylalanine,[9] has been prepared by our group.[10] The antibody binds an azobenzene group when it is in the trans form, but releases it when the latter is photoisomerized to the cis form. The binding and release could be repeated many times. If one can attach an azobenzene group to some key substances, such as enzymes, substrates, ligands, receptors, etc., the biological activity of these substances can be photocontrolled in the presence of the antibody.[11] One of the important features of the azobenzene/antibody system is that the antibody binds and inactivates the trans form and can provide the "off" state.

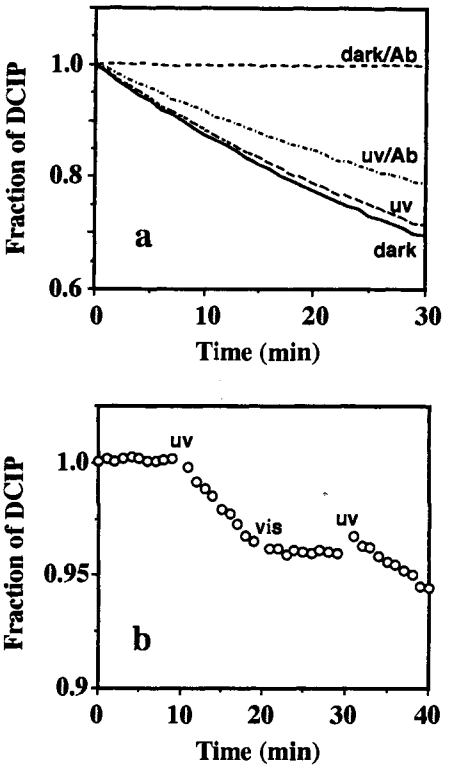

Figure 1. (a) Reduction of DCIP by the coupled enzyme system mediated with azoAla-NAD+: (—) in the dark without antibody; (- - -) after UV irradiation without antibody; (– – –) in the dark with antibody; (—) after UV irradiation with antibody. (b) A profile of on/off switching of the coupled enzymic reaction.

We have chosen NAD+ as the key compound and modified it with an azobenzene group. NAD+ acts as a common coenzyme for a large number of oxidoreductases in the respiration system. The control of NAD+ activity by the binding of antibody has been reported by Carrico et al.[12] They showed that DNP-bound NAD+ was inactivated by the addition of anti-DNP antibody.

As an azobenzene-bound NAD+ derivative, a conjugate of L-*p*-(phenylazo)phenylalanine (azoAla) methyl ester and N^6-carboxymethyl-substituted NAD+[13] (azoAla-NAD+, **1**) was designed.[14]

X-ray crystallographic analysis of several enzyme–NAD+ complexes revealed that the amino group of the adenine ring is exposed to the solvent,[15] suggesting that substitution of this amino group will not severely reduce its activity. In fact, N^6-substituted NAD+ has been shown to retain high activity.[12,13,16,17] The azobenzene-bound NAD+ was added to a coupled enzyme system

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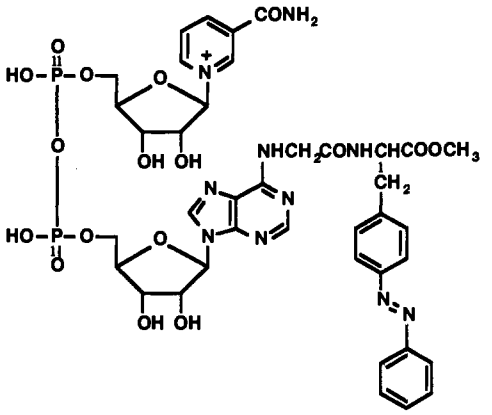


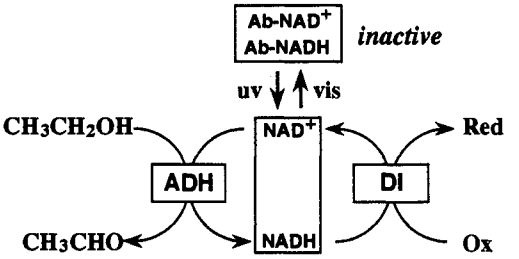
consisting of alcohol dehydrogenase, which reduces NAD^+ to NADH, and diaphorase, which oxidizes NADH to NAD^+ with an appropriate electron acceptor. As the latter acceptor, we have chosen 2,6-dichlorophenol-indophenol sodium salt (DCIP), because the reduction process can be followed by the fading of blue color.

As shown in Figure 1, the reduction of DCIP in the coupled enzyme system[18] is mediated by the azoAla-NAD^+ either in the trans state or in the cis state, with a little smaller rate in the latter state.[19] When the antibody was added to the enzyme system in the dark, the reduction of DCIP was completely stopped. The mediation activity recovered to about 72% of the inherent activity of azoAla-NAD^+ by the UV irradiation.[20] The incomplete recovery may be due to incomplete photoisomerization to the cis state. Addition of a large amount of azoAla methyl ester to the system in the presence of the antibody in the dark also recovered the mediation activity. This is interpreted in terms of the inhibitory effect of the azoAla methyl ester that competes with the azoAla-NAD^+ for the binding sites of the antibody.

Visible light irradiation of the UV-irradiated mixture again suppressed the mediation effect, and the photocontrol could be repeated many times under alternating photoirradiation with UV (on) and visible (off) light. This is expected since the azobenzene–antibody binding has been shown to be photoreversible.[10]

To conclude, the azoAla-NAD^+-mediated enzyme reaction could be photoswitched reversibly in the presence of the antibody against the *trans*-azobenzene group. The mechanism of the photoswitching may be illustrated as depicted in Scheme 1. There are several advantages in the present system. First, a complete "off" state was attained by the blocking of the *trans*-azobenzene group with the antibody. Second, since NAD^+ is a commonly occurring mediator in a variety of oxidoreductases, this principle can be easily applied to other systems. Furthermore, since the antibody against the azobenzene group may undergo little cross reactions toward other proteins, the azobenzene/antibody couple can be utilized in a wide variety of biological systems, if one can attach an azobenzene group to some key substances, such as enzymes, coenzymes, inhibitors, etc.

Scheme 1. Schematic Illustration of Photoreversible NAD^+-Mediated Coupled Enzyme Reaction. ADH = alcohol dehydrogenase; DI = diaphorase



Acknowledgment. The authors wish to thank Professor K. Ichimura of the Tokyo Institute of Technology for helpful discussion. They also appreciate Mr. M. Harada for preparing the antibody against *trans*-azobenzene.

(18) The reaction mixture contained 50 mM Tris-HCl (pH 7.5), 100 mM ethanol, 0.02 mM DCIP sodium salt, 1.25 units/mL yeast alcohol dehydrogenase, and 2.5 units/mL *Clostridium kluyveri* diaphorase. The reaction was initiated by adding azoAla-NAD^+ (total 0.5 μM) with or without the antibody (total 0.25 μm). The reaction was carried out at 25 °C and followed by the absorbance of DCIP at 600 nm.

(19) A Hg–Xe lamp with a bandpass filter (350 ± 20 nm) was used for UV irradiation of azoAla-NAD^+. A cutoff filter (>420 nm) was used for visible light irradiation.

(20) NADH is also photoexcited by the UV irradiation, and the excited NADH would reduce DCIP directly. The possible nonenzymatic reduction of DCIP, however, could not be detected in a control experiment in the absence of diaphorase and the antibody.