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PHOTOCATALYZED ANAEROBIC OXIDATION OF NICOTINAMIDE COENZYME DIMERS TO NAD^+ BY ADRIAMYCIN

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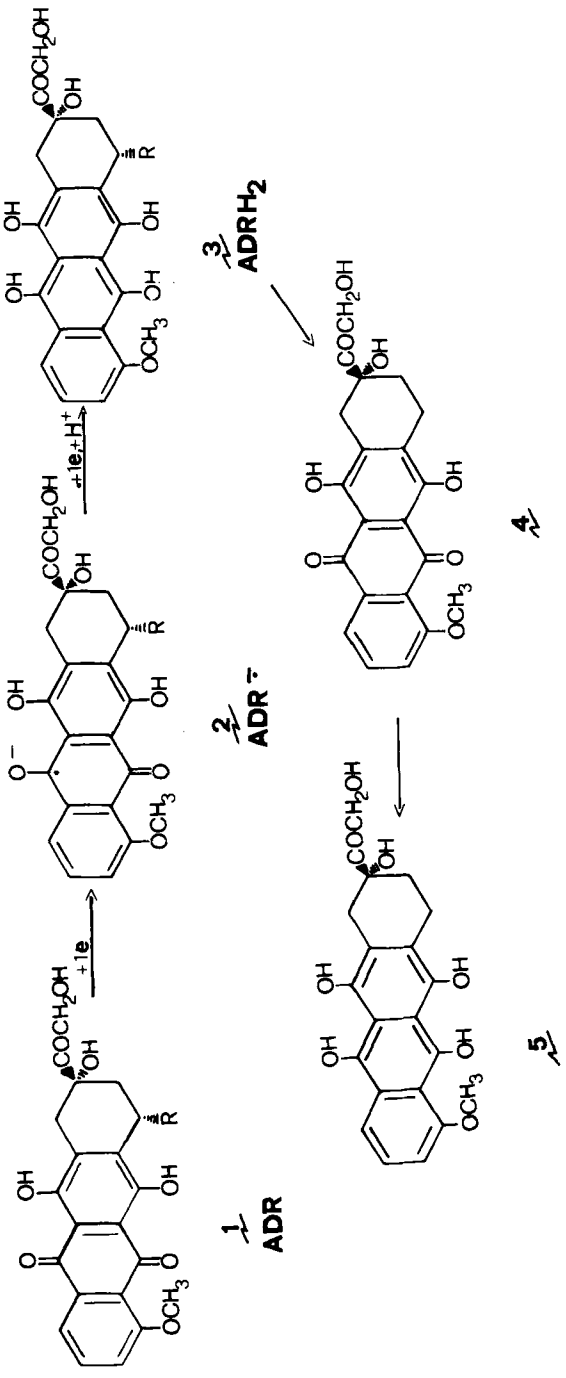
The nicotinamide adenine dinucleotide dimers $(NAD)_2$ obtained by electrochemical reduction of NAD^+ are oxidized by adriamycin in anaerobic photocatalyzed reaction yielding NAD^+ and 7-deoxyadriamycinone. Under the same conditions NADH is not oxidized.

KEY WORDS: Photocatalysis, NAD^+, adriamycin

ABBREVIATIONS: ADR, adriamycin; $ADR^{\cdot}$, adriamycin semiquinone; $ADRH_2$, adriamycin hydroquinone.

INTRODUCTION

Adriamycin is among the most useful of the anthracycline antitumor antibiotics. A well-known limitation to its clinical use is due to toxic side-effects, especially cardiotoxicity. Many biological effects of anthracyclines are related to their quinone moiety, which acts as an oxidant, being reduced first to the semiquinone anion radical. The subsequent chemical metabolism depends on the presence or absence of molecular oxygen.[1] If oxygen is present, rapid electron transfer from the semiquinone to oxygen occurs with regeneration of the quinone and the formation of the superoxide anion radical, $O^{\cdot}$. In the absence of oxygen, further reduction of semiquinone to the hydroquinone occurs. This results in the splitting off the C-7 sugar moiety.[2–5] The key step of chemical transformation of anthracycline antibiotics however appears to be the formation of one-electron reduced semiquinone intermediate. In fact it has been shown that the reductive cleavage of the anthracycline glucosides or the oxygen uptake, are catalyzed exclusively by enzymes operating *via* a single electron-transfer mechanism.[6] Since we have previously reported that the dimers $(NAD)_2$, obtained by electrochemical reduction of NAD^+, are able to participate in single electron transfer processes,[7] we have extended our investigations to the antibiotic adriamycin. In this context, it should be pointed out that radicals able to reduce molecular oxygen are generated by irradiation of $(NAD)_2$ with light of wavelength above 300 nm.[8] This investigation deals with the anaerobic photochemical reduction of adriamycin by $(NAD)_2$.



Reduction of adriamycin. [R = L- Daunosamine]

MATERIALS AND METHODS

Adriamycin hydrochloride (ADR) was a generous gift of Farmitalia-Carlo Erba (Italy). NAD^+, NADH (100% pure) and lyophilized alcohol dehydrogenase (ADH, 400 U/mg) were from Boehringer (FRG). NADH dehydrogenase (cytochrome *c* reductase, 1–2 U/mg) was from Sigma (USA).

$(NAD)_2$, prepared according to Carelli *et al.*,[9] was stored at $-20°C$ under vacuum and dissolved immediately before use. All other chemicals were of reagent grade.

An authentic sample of 7-deoxyadriamycinone **4** (Chart I) was obtained by adding dropwise a saturated solution of $Na_2S_2O_4 \cdot 2H_2O$ in 50 mM $NaHCO_3$ to a deoxygenated solution of ADR (180 mg in 280 ml of 50 mM $NaHCO_3$). The red precipitate was centrifuged, washed with 10 mM HCl, then with H_2O and dried (m.p. 258–260°C, dec., m/e 398 (M), 380 (M–H_2O), 339 (M – $COCH_2OH$), i.r.: 3460, 1725, 1615 cm^{-1}).[10]

The reaction between $(NAD)_2$ and ADR was performed using a Thunberg cuvette with a stopper and a latex cap. Oxygen was removed by five cycles of vacuum-refilling with 99.9% Ar or N_2 purified over an alkaline-dithionite trap. The irradiation was performed with a 125 W medium pressure Hg lamp (Applied Photophysics, U.K.) water cooled, the major emission lines ranging from 350 nm to 600 nm.

HPLC analysis was performed in a Perkin-Elmer Series 3 liquid-chromatograph equipped with an LC 55B spectrophotometric detector, an LC 55S digital scanner and a Hewlett-Packard 3390 A integrating recorder, using a Merck Hibar RP-18 RT-250-4-LiChrosorb 10 μm column under experimental conditions described in Ref. 8.

The NAD^+ was determined by its retention time in HPLC in comparison to that of an authentic sample and by an enzymic test based on ADH activity. Visible and UV spectra were recorded on a Perkin-Elmer 555. IR spectra were taken by Nujol or KBr dispersion with either a 257 or 281 Perkin Elmer IR spectrophotometer.

Mass spectra were determined by a Hewlett-Packard 5980 A quadrupole spectrometer.

Melting points were determined with a Tottoli instrument (Büchi) and were uncorrected.

RESULTS

No significant reaction was observed between $(NAD)_2$ and ADR under anaerobic conditions when the solution was incubated in the dark, whereas exposure for 4 hours to sunlight of a 130 μM solution of $(NAD)_2$ in the presence of 75 μM ADR caused a 90% decrease of the absorption maxima at 340 nm and 480 nm, typical of the 4,4' tetrahydrobipyridine and of the quinone chromophores respectively (Fig. 1). The formation of a red precipitate was also observed and the presence of the NAD^+ in the solution was detected. The red precipitate was found to be 7-deoxyadriamycinone **4** by comparison with an authentic sample (see Materials and Methods).

The reaction was much faster when the solution was irradiated with a 125 W Hg vapour lamp. Table I shows the amount of NAD^+ formed in 30 min at different $(NAD)_2$/ADR molar ratios. Remarkably, at high values of $(NAD)_2$/ADR ratios, no precipitate was found and a band at 420 nm became evident.

While NADH alone was unable to reduce adriamycin under the same conditions, the reductive glycosidic cleavage was readily detectable after addition of NADH dehydrogenase to the solution.[6]

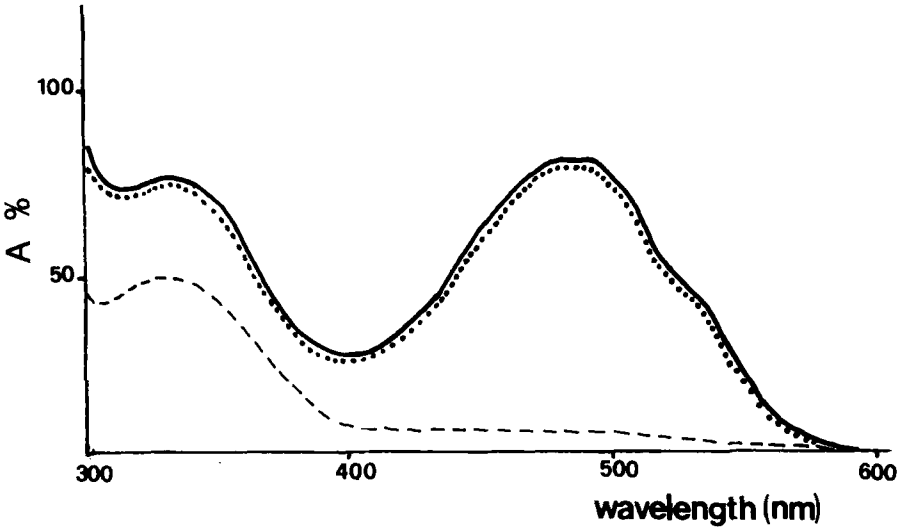


FIGURE 1 Absorption spectrum of an anaerobic solution of ADR (75 μM) and $(NAD)_2$ (130 μM) in 25 mM NH_4HCO_3 and 25 mM $AcONH_4$ as such (continuous line) and after exposure to light (dashed line). The dotted line represents the absorption spectrum of the same solution kept in the dark.

DISCUSSION

The disappearance of $(NAD)_2$ and ADR from irradiated solutions in anaerobic conditions, and the concomitant formation of NAD^+ and 7-deoxyadriamycinone clearly indicate the occurence of a redox reaction.

The results obtained agree with the general behaviour of adriamycin and daunomycin toward one-electron reducing agents[1–6] and indicate the following reaction pathway. The anaerobic reduction starts with the transfer of one electron from $(NAD)_2$ to ADR **1** to give the semiquinone anion radical **2**. The semiquinone undergoes a further one-electron reduction to the transient hydroquinone **3**, which is converted to 7-deoxyadriamycinone **4** *via* the intramolecular elimination of the C-7 sugar (Chart I).

The data in table I indicate that when the ADR/$(NAD)_2$ ratio ranges from 1:1 to 1:4 the amount of NAD^+ formed is constant and corresponds to 2 moles of NAD^+

TABLE I
Photocatalyzed anaerobic oxidation of $(NAD)_2$ by ADR[a]

ADR mM	$(NAD)_2$ mM	NAD^+ formed mM[b]	NAD^+/ADR
0.5	0.5	0.95	1.90
1.0	2.0	1.80	1.80
0.8	2.0	1.50	1.88
0.5	2.0	0.98	1.96
0.25	2.0	0.85	3.4
0.10	2.0	0.39	3.9

[a] Irradiation for 30 min. in a Thunberg cuvette with a 125 W medium pressure Hg lamp.
[b] Enzymatically determined.

per mole of ADR. This implies that ADR is reduced to $ADRH_2$ by $(NAD)_2$ to a stoichiometry consistent with the reaction pathway reported in Scheme 1. The data of Table I also show that the amount of NAD^+ formed at higher $(NAD)_2$/ADR ratios exceeds that expected from the above described reaction pathway. This finding can be explained by the ability of $(NAD)_2$, when present in large excess, to further reduce **4** to **5**, the formation of which is proved by the absorption band at 420 nm.[11]

$$1)\ 1/2\ (NAD)_2 + ADR \longrightarrow ADR^{\cdot-} + NAD^+$$

$$2)\ 1/2\ (NAD)_2 + ADR^{\cdot-} \longrightarrow ADRH_2 + NAD^+$$

$$3)\ ADRH_2 \longrightarrow \mathbf{4}$$

$$4)\ (NAD)_2 + \mathbf{4} \longrightarrow \mathbf{5} + 2\ NAD^+$$

SCHEME 1

The role of light in the reaction between $(NAD)_2$ and ADR deserves a comment. As stated above, the reaction does not occur in the dark and is clearly photocatalysed. Taking into account the emission range of the lamp used, two different photochemical effects are possible, namely the homolysis of $(NAD)_2$ to give NAD radicals or the formation of an excited state of ADR. Both photoinduced species may be able to iniziate the redox process according to the reactions 5), 6), 7), 8) (Scheme 2).

$$\text{light}$$

$$5)\ (NAD)_2 \longrightarrow 2\ NAD^{\cdot}$$

$$6)\ NAD^{\cdot} + ADR \longrightarrow NAD^+ + ADR^{\cdot-}$$

$$\text{light}$$

$$7)\ ADR \longrightarrow ADR^*$$

$$8)\ ADR^* + (NAD)_2 \longrightarrow ADR^{\cdot-} + NAD^{\cdot} + NAD^+$$

$$9)\ ADR^{\cdot-} + NAD^{\cdot} \longrightarrow ADRH_2 + NAD^+$$

$$10)\ ADR^{\cdot-} + (NAD)_2 \longrightarrow ADRH_2 + NAD^{\cdot} + NAD^+$$

SCHEME 2

The further reduction of $ADR^{\cdot-}$ to hydroquinone **3** might be operated by the NAD radical or by $(NAD)_2$ according to 9) and 10).

The reductive glycosidic cleavage observed in the reaction between $(NAD)_2$ and ADR confirms the ability of $(NAD)_2$ to participate in one-electron redox processes. However, the inability of NADH to reduce ADR, unless an enzyme like NADH dehydrogenase is present further stresses the different redox behaviour of the two reduced nicotinamide nucleotides, NADH and $(NAD)_2$.

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