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REDUCTION BY A MODEL OF NAD(P)H. 31. SYNTHESES AND REACTIONS OF KERATIN-BOUND COENZYME-MODELS

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Summary: NAD(P)H-models which are covalently bound to keratin are synthesized and reacted with N-methylacridinium iodide and α,α,α-trifluoroacetophenone.

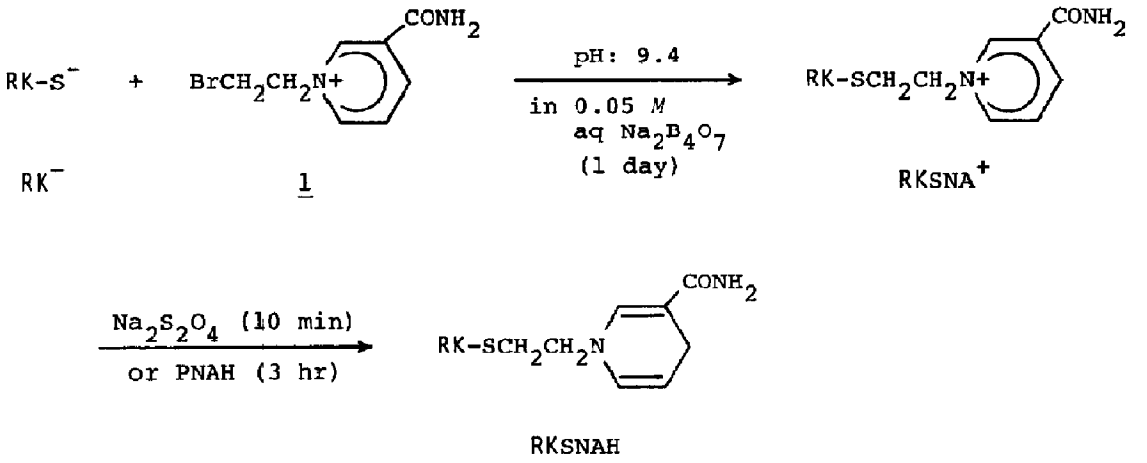
NADH and NADPH are coenzymes that are widely distributed in biological redox systems. Although tremendous amounts of studies on their model compounds have been done within the last decade,[1-5] the model reactions so far reported are fairly inferior to the enzymic reactions in views of versatility, reactivity, and stereospecificity. The superiority of *in vivo* reactions comes from special environment created by an enzyme at a reaction site. On the other hand, it has been reported that reduced keratin from human hair catalyzes the reduction of NAD^+ to NADH and the conjugate oxidation of glyceraldehyde to glyceric acid.[6] The fact that non-enzymic protein behaves like an oxidoreductase encourages us to expect some assistance of a protein even for the reaction of model compounds. In order to test the idea, we synthesized two 1,4-dihydropyridine derivatives covalently bound to reduced keratin.

The reduced keratin (RK) was prepared from human hair by reductive solubilization by using sodium thioglycolate in aqueous sodium sulfite solution.[6b] After the dialysis against 0.05 *M* sodium tetraborate buffer (pH = 9.4), the protein was subjected to the succeeding reactions.

In *Method A* synthesis, 3 mg (excess) of 1-(2-bromoethyl)-3-carbamoylpyridinium bromide (1) was added to 5 ml of 0.5 wt.% aqueous solution of RK. The whole solution was allowed to stand for a day until no mercapto group could be

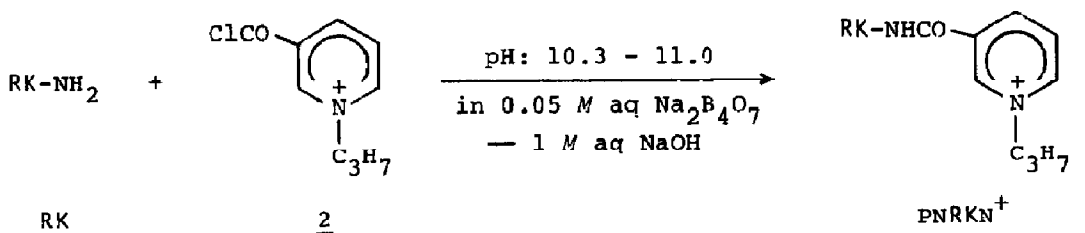
detected on the Ellman's test. Thus obtained pyridinium salt ($RKSNA^+$)[7] was reduced to a 1,4-dihydropyridine derivative (RKSNAH) by the reaction with sodium dithionite or 1-propyl-1,4-dihydronicotinamide (PNAH) (Scheme 1). The modified RK was purified by gel filtration on a column of Bio-gel P-6. UV-visible absorption spectrum of RKSNAH (Figure 1) has a maximum at 355 nm and is virtually identical with that of PNAH. The concentration of RKSNAH employed for further reaction was 1.6×10^{-4} *M*.[8]

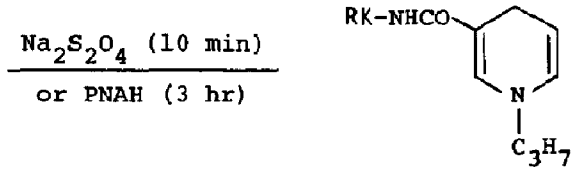
Scheme 1



In *Method B* synthesis, 30 - 40 mg of 1-propyl-3-chloroformylpyridinium bromide (2) was added in small portions to 5 ml of 0.5 wt.% aqueous solution of RK. The pH of reaction solution was kept at 10.3 - 11.0 by dropwise addition of 1 *M* aqueous sodium hydroxide to the borate-buffered reaction solution. The reaction proceeded immediately. The pyridinium salt ($PNRKN^+$) was reduced to PNRKNH and purified as described above (Scheme 2). UV-visible absorption spectrum of PNRKNH (Figure 2) again has a maximum at 360 nm.

Scheme 2





PNRKNH

RKSNAH and PNRKNH were oxidized by N-methylacridinium iodide immediately and were regenerated by sodium dithionite quantitatively, which were confirmed by observing the disappearance and appearance of an absorption at 355 or 360 nm. Pseudo-first-order kinetics was followed for the reduction of α,α,α-trifluoroacetophenone with RKSNAH or PNRKNH by observing the decrease in the intensity at 355 or 360 nm in 25% methanol—75% 0.05 *M* aqueous sodium tetraborate at 50°C and the second-order rate constants were calculated to be 6.1 x 10^{-2} M^{-1} min^{-1} for RKSNAH and 3.4 x 10^{-1} M^{-1} min^{-1} for PNRKNH, respectively.[9] Thus, the reduction with RKSNAH appeared about one order of magnitude slower than that with PNAH (k = 4.1 x 10^{-1} M^{-1} min^{-1}),whereas the reduction with PNRKNH proceeds comparably to that with PNAH. Further studies on the versatility and stereochemistry with the present coenzyme models are in progress in our laboratory.

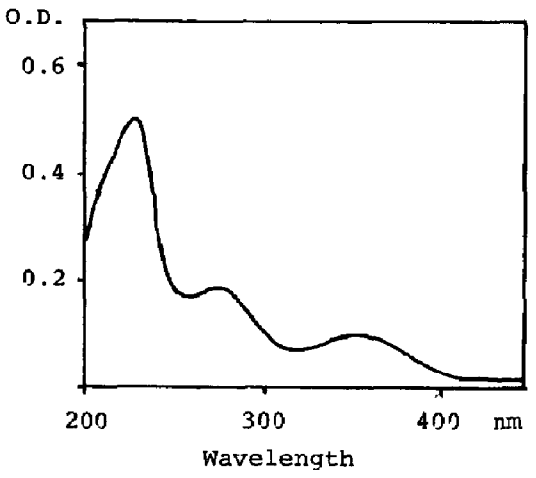


Figure 1. UV-visible absorption spectrum of RKSNAH in water (pH = 9.4).

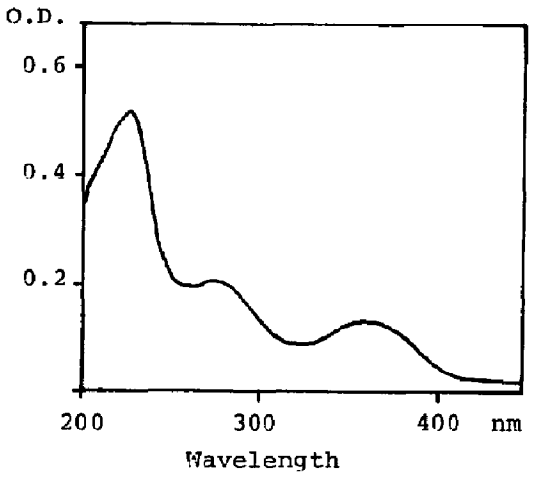


Figure 2. UV-visible absorption spectrum of PNRKNH in water (pH = 9.4).

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7. Following evidences eliminate the possibility of the addition of mercapto group to the 4-position of pyridinium; UV-absorption attributed to the adduct was not observed in the reaction and 1 reacted also with L-cystein under a similar condition to produce the corresponding sulfide : 1-[2-[(2-amino-2-carboxyethyl)thio]ethyl]-3-carbamoylpyridinium salt.
8. Determined by titration with N-methylacridinium iodide. The ε_{max} was calculated to be 5,500.
9. The rate constants for auto-decomposition of RKSNAH and PNRKNH were 1.9 x 10^{-3} min^{-1} and 3.4 x 10^{-3} min^{-1}, respectively. Hydration to α,α,α-trifluoroacetophenone was not taken into account.

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