PROPERTIES OF SELENONICOTINAMIDE-ADENINE DINUCLEOTIDE PHOSPHATE, AN ANALOGUE OF NADP

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1. Introduction

Since the discovery of the NAD(P) glycohydrolase enzyme, which catalyses the exchange of free and NAD(P)-linked nicotinamide [1], a great number of NAD analogues have been synthesized with alterations to the pyridine, to the sugar, and to the adenine parts of the molecule [2–5]. In the nicotinamide moiety the carboxamide oxygen atom has been replaced with a sulphur atom [6]. Thio-NAD(P) can be reduced and behaves like NAD(P) in various dehydrogenase systems [7]. It therefore was of interest to synthesize and determine the properties of the selenium-substituted analogues. To our knowledge, such a synthesis has not been reported to date.

2. Materials and methods

NADP and yeast D-glucose-6-phosphate:NADP oxidoreductase (E.C. 1.1.1.49) were purchased from Boehringer Soehne, Mannheim. DEAE-cellulose-TLC was obtained from Serva-Entwicklungslabor, Heidelberg.

Absorption spectra were measured with a Beckman model DK 2 A recording spectrophotometer as well as with a Zeiss model PMQ II spectrophotometer. Emission properties were examined with a Zeiss model ZFM 4 C spectrofluorimeter.

Synthesis of selenonicotinamide from 3-cyanopyridine and hydrogen selenide was carried out by the method described by Karrer and Schukri [8] for the synthesis of thionicotinamide. The reaction product was recrystallized from hot water. Long ochre-yellow needles were obtained and dried under vacuum. Melting point: 228–229° with decomposition (sealed tube). Upon exposure to air selenonicotinamide decomposes within a few hours yielding elemental grey selenium. Selenonicotinamide is stable for several months at room temperature in a sealed tube, provided light and moisture are excluded.

Selenonicotinamide so prepared was chromatographically pure (TLC on silica gel HF_{254} Merck, solvent benzene/acetone/methanol/ammonia (25%), 40:40:10:5 v/v). The absorption spectra of the material in water, acid, and alkali are shown in fig. 1. In alkali selenonicotinamide is obviously reconverted into 3-cyanopyridine, because the spectra of these two substances are identical in these particular conditions (maximum absorption at 264 mμ, ϵ = 2.9 cm^2 $\mu mole^{-1}$).

3. Results

The NADP analogue of selenonicotinamide was obtained using NAD(P) glycohydrolase (E.C. 3.2.2.6) from rat spleen microsomes. The reaction mixture contained 13.8×10^{-3} M selenonicotinamide and 6.3×10^{-4} M NADP in 1/15 M phosphate buffer, pH 6.6. After incubation at 37°, the protein was precipitated with $HClO_4$. The orange-coloured clear supernatant was neutralized with KOH and freeze-dried. Excess selenonicotinamide was then extracted with ethylacetate and the material desalted and completely freed from selenonicotinamide by chromatography on Sephadex G-10 using water or 0.05 M ammonium formate as solvent. The purified

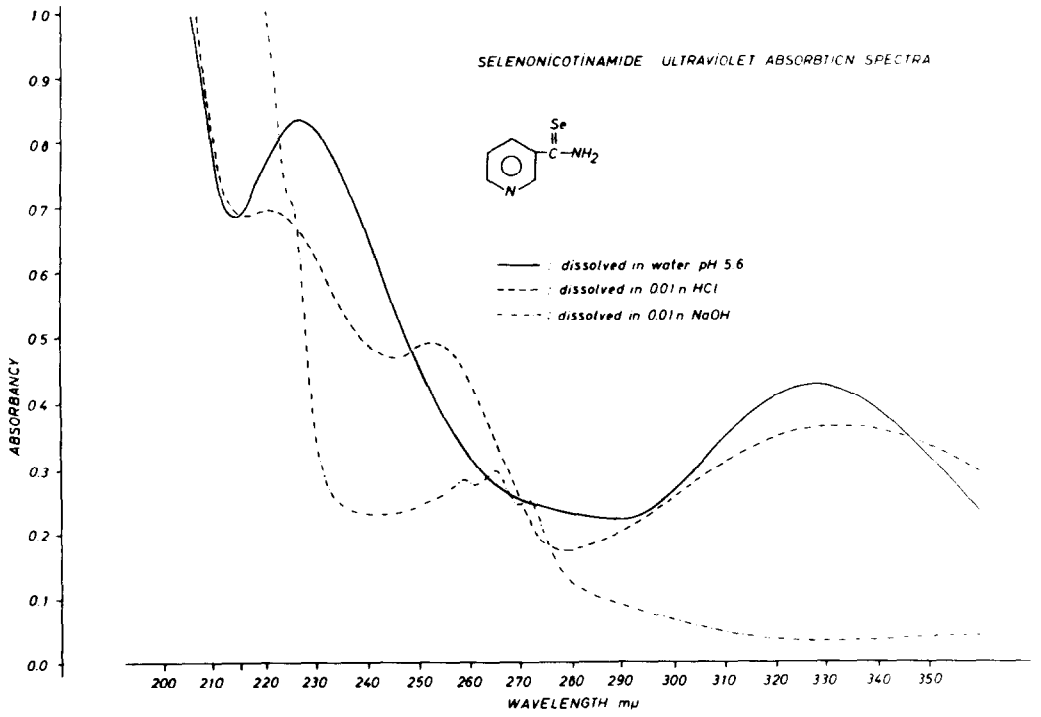
SELENONICOTINAMIDE ULTRAVIOLET ABSORBTION SPECTRA
Se
C–NH2
N
dissolved in water pH 5.6
dissolved in 0.01 n HCl
dissolved in 0.01 n NaOH
ABSORBANCY
WAVELENGTH mμ

Fig. 1

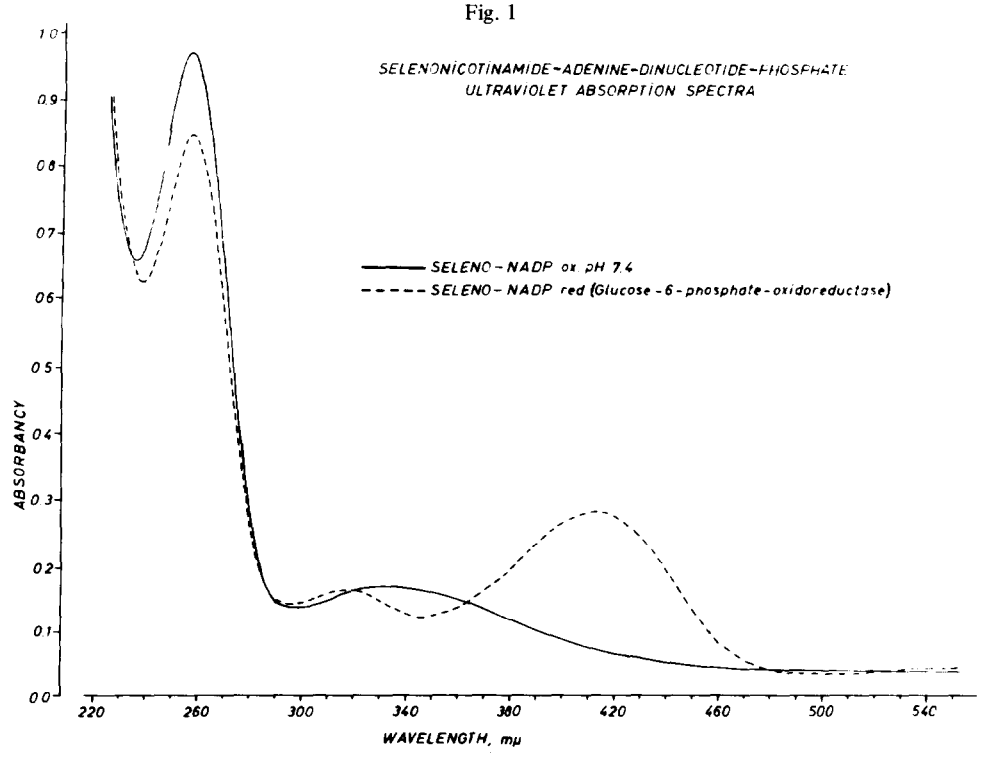
SELENONICOTINAMIDE-ADENINE-DINUCLEOTIDE-PHOSPHATE
ULTRAVIOLET ABSORPTION SPECTRA
SELENO-NADP ox. pH 7.4
SELENO-NADP red (Glucose-6-phosphate-oxidoreductase)
ABSORBANCY
WAVELENGTH, mμ

Fig. 2

material was again freeze-dried. The light orange-coloured seleno-NADP was completely free of NADP, although it still contained inorganic phosphate.

It was possible to obtain seleno-NADP pure enough for subsequent analysis by ion-exchange thin-layer chromatography on DEAE-cellulose (layer thickness about 250 μ) in 0.03 N HCl [9]. The R_F values for NADP and seleno-NADP were 0.44 and 0.26, respectively.

The seleno-NADP contained 2.2 mole ribose and 3.37 mole phosphate per mole of adenine.

It was not possible to further purify the NADP analogue by ion-exchange chromatography, paper chromatography, and high-voltage electrophoresis [10,11], as the selenium-containing compounds are relatively unstable and prone to rapid decomposition.

4. Spectral properties

Fluorescence emission of seleno-NADP in 0.2 M tris buffer, pH 7.5, has a maximum at 484 mμ at room temperature using an exciting wavelength of 391 mμ. Neither selenonicotinamide nor 3-cyanopyridine possessed exciting or emission maxima at these wavelengths. Ultraviolet absorption spectra of oxidized and reduced seleno-NADP are shown in fig. 2. The maximum at 333 mμ is probably due to selenonicotinamide (cf. fig. 1).

Following enzymic reduction of seleno-NADP with D-glucose-6-phosphate:NADP oxidoreductase, the colour of its solution changes from slightly yellow to lemon-yellow. The absorption spectrum changes and shows a maximum at 417 mμ (fig. 2, dashed curve).

The bathochromic shift in the ultraviolet spectra on going from thionicotinamide and thio-NADP to the corresponding seleno-compounds is in good agreement with the results of Mautner and Bersgon [12], according to whom a bathochromic shift occurs when sulphur is replaced by selenium in –NH–CS–C= or –NH–CS–N= groups.

A summary of the absorption maxima and the corresponding extinction coefficients for oxidized and reduced seleno-NADP are given in table 1.

Table 1
Spectrophotometric properties of seleno-NADP.
pH 7.5, 0.2 M tris buffer.

	λ_{max} (mμ)	ϵ
Oxidized	258	18.5
	333	3.04
Reduced	258	16.1
	317	2.93
	417	5.71–5.79

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