CHEMISTRY LETTERS, pp. 805-808, 1983.

ENZYMIC SYNTHESIS AND CHARACTERIZATION OF SOME NOVEL INDAZOLE NUCLEOTIDES

Shuichi TONO-OKA,* Isao SEKIKAWA, and Ichiro AZUMA
Section of Chemistry, Institute of Immunological Science,
Hokkaido University, Kita-ku, Sapporo 060

NADase-catalysed enzymic reaction was successfully applied to the synthesis of some novel β-indazole dinucleotides. Their structures were determined on the basis of UV- and ^{1}H-NMR spectral data. Chemical properties thereof are also described.

Porcine-brain NADase is known as an enzyme to catalyse a base exchange reaction between β-NAD and structurally related pyridine compound,[1] in addition to the hydrolytic cleavage of nicotinamide-ribose glycosidic linkage. We have previously reported[2,3] the preparation of some new types of NAD analogs containing 3,4-disubstituted pyridine derivatives. These NAD analogs can be useful intermediates to convert to corresponding pyridine nucleosides,[2] and interestingly some of the analogs show notable immunological activities.[4]

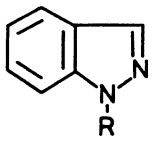
Now from view point of applying the enzymic synthesis to other heterocycle nucleotides, our attention has initially been focused on readily available indazole bases. The present communication describes the first application of the enzymic reaction to some indazole bases and characterization of the resulting novel dinucleotides.

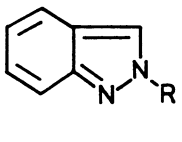
As previously reported,[3] no exchange reaction occurs when a replacing base has a large basicity (pKa $\gtrsim$ 9). Indazole bases[5] now to be used are relatively weak in basicity (pKa ~ 5),[6] so the occurrence of the reaction was enough expected. A problem in performing the reaction was poor solubility in water of indazole compounds. However, it was overcome by the addition of DMSO (10 - 15 %) to the reaction system, thereby no remarkable drop in NADase activity was observed.

Each of indazole bases and NAD were incubated at 37 °C with NADase[3] in 0.1 M (1 M = 1 mol dm^{-3}) Tris-HCl (pH 7.2) until the spot of NAD disappeared on TLC.[7] At the end of incubation, the resulting mixture showed three major spots on TLC, which were due to the unreacted indazole base, desired product, and ADP-ribose. Their isolation and purification were made by our previously reported method.[8] When 5- and 6-acetamido-indazoles as well as indazole were used as replacing bases, single dinucleotides (6, 7, and 3) were yielded without detectable amount of other product. In case of 5- and 6-aminoindazoles, the minor products (4b and 5b) were also obtained in addition to the major ones (4a and 5a). The ratios of major to minor product were ca. 7 : 3 for 4 and 8 : 2 for 5. The yields and spectroscopic

properties of thus obtained dinucleotides[9] are summarized in Table 1.

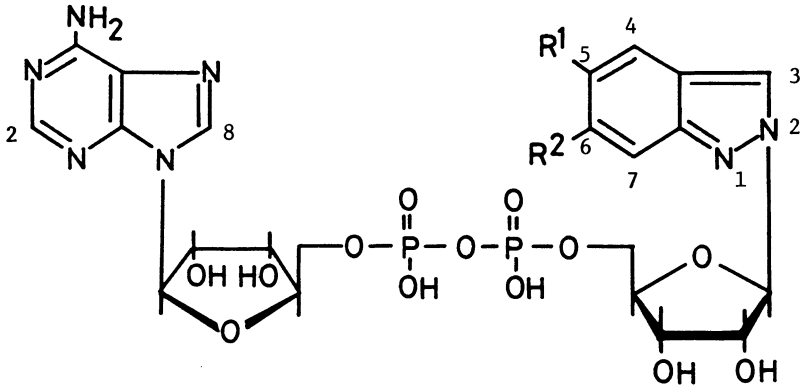
In order to clarify the site of ribosylation in the nucleotides, the UV spectra of indazole mononucleotides, which were given by treatment of dinucleotides with phosphodiesterase, were examined comparing with those of the related indazole compounds.





1a	R = H	1b	R = H
2a	R = CH_3	2b	R = CH_3

Indazole mononucleotide (8) showed absorption maxima at 276 and 295 nm (log ε 3.85, 3.78). The absorption pattern of 8 was quite similar to that of 2-methylindazole



3	$R^1 = R^2 = H$
4a	$R^1 = NH_2$, $R^2 = H$
5a	$R^1 = H$, $R^2 = NH_2$
6	$R^1 = NHCOCH_3$, $R^2 = H$
7	$R^1 = H$, $R^2 = NHCOCH_3$

which exhibited the maxima at 275 and 295 nm (log ε 3.80, 3.78), but markedly different from that of 1-methylindazole which exhibited the maxima at 254 and 292 nm (log ε 3.53, 3.72). From these observations, the site of ribosylation was ascertained to be N^2-atom, indicating the quinoid structure of 3 as well as 8. Such was similarly found to be the cases for 4a, 5a, 6, and 7. On the other hand, 4b and 5b were proved to exist as N^1-ribosylated benzenoid structure: each UV spectrum of mononucleotides obtained from 4b and 5b was similar to that of corresponding aminoindazole, predominantly existing benzenoid form (1a) of which was established previously.[10] The formation of minor 4b and 5b could be arised from a quinoid-type tautomer (1b) of 5- and 6-aminoindazoles, which would come to occur to a small extent on account of possible influence of the amino substituent.

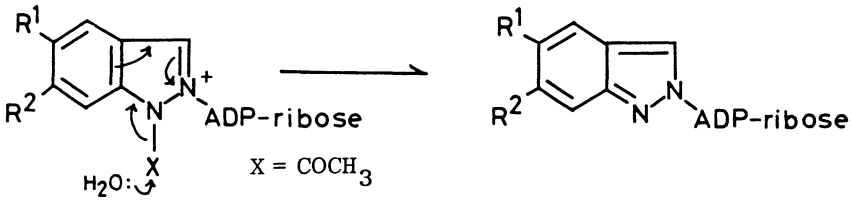
Compounds 3, 6, and 7 were also formed involving deacetylation of N^1-$COCH_3$ form the corresponding 1-acetylindazole bases, although the bases themselves remained unchanged, unless NAD was present, even on incubation with NADase. This facile deacetylation could be explained in terms of the strong electron-withdrawing effect of quaternary N^2-atom in once formed transient intermediate (Scheme 1).

Except 4a and 5a, all the ^{1}H-NMR spectra (400 MHz, D_2O) of compounds described herein were well compatible with the assigned structures (Table 1).
As for 4a and 5a, there appeared no signal due to an aromatic proton (H_4 or H_7) adjacent to the amino group, although the indazole bases themselves exhibited the corresponding signal. In determining in DMSO-d_6, however, it appeared as a singlet at δ 6.70 (H_4) or 6.49 (H_7). Such a deuterium exchange in D_2O was not observed for N^1-ribosylated 4b and 5b.

Table 1. The Yields and Spectroscopic Data of Indazole Dinucleotides.

Compd	Yield (%)[a]	UV/nm (log ε)[b]	^{1}H-NMR (400 MHz, D_2O, δ)[c] Indazole[d] H_3 (H_6)	H_4 (H_7)	H_5	Adenine H_2	H_8	Anomeric[e]	Acetyl
3	54	264 (4.33) 297 (3.78)	8.29 s (6.93 dd)	7.53 d (7.40 d)	7.19 dd	7.97 s	8.24 s	5.99 d 5.87 d	-
4a	35	261 (4.27) 330 (3.65)	8.17 s (6.89 d)	- (7.32 d)	-	7.95 s	8.10 s	5.94 d 5.86 d	-
4b	19	244 (4.31) 316 (3.55)	8.26 s (7.10 d)	7.20 s (7.35 d)	-	7.96 s	8.16 s	5.98 d 5.84 d	-
5a	42	263 (4.27) 296 (3.94)	8.21 s (-)	7.42 d (-)	6.62 d	7.97 s	8.16 s	5.91 d 5.87 d	-
5b	10	232 (4.32)† 260 (4.21)† 296 (3.88)	8.29 s (-)	7.47 d (7.05 s)	6.76 d	7.99 s	8.20 s	6.00 d 5.86 d	-
6	75	240 (4.35) 263 (4.13)† 311 (3.56)	8.21 s (7.00 d)	7.53 s (7.31 d)	-	7.90 s	8.12 s	5.98 d 5.79 d	2.16 s
7	47	239 (4.31) 265 (4.13)† 297 (3.84)	8.24 s (-)	7.41 d (7.51 s)	6.70 d	7.90 s	8.06 s	5.98 d 5.79 d	2.19 s

† Shoulder a) Based on NAD. b) Measured at pH 7. c) Chemical shifts of the ribose protons fell within δ 4.2 - 4.8. d) J_{vic} = 8.8 - 9.3 Hz. e) J = 4.0 - 5.0 Hz.



Scheme 1.

Herein we described the first application of the enzymic base exchange reaction to the synthesis of some novel β-indazole nucleotides. In case of necessity, they could readily be converted into the corresponding nucleosides.[2] Moreover, these compounds may have some possible biological activity. In any case, it is of interest that the reaction has been found to be available for other different heterocyclic bases than pyridines. Further investigations are now in progress.

References

1) P. Walter and N.O. Kaplan, J. Biol. Chem., 238, 2823 (1963), and references cited therein.

2) a) S. Tono-oka, A. Sasaki, H. Shirahama, T. Matsumoto, and S. Kakimoto, Chem. Lett., 1977, 1449. b) S. Tono-oka, Y. Sasahara, A. Sasaki, H. Shirahama, T. Matsumoto, and S. Kakimoto, Bull. Chem. Soc. Jpn., 54, 212 (1981).
3) S. Tono-oka, Bull. Chem. Soc. Jpn., 55, 1531 (1982).
4) I. Saiki, S. Tono-oka, and I. Azuma, Int. Vitam. Nutr. Res., 51, 239 (1981).
5) Indazole, 5- and 6-amino-, 5- and 6-acetamido-indazoles, and their 1-acetyl derivatives were used as replacing bases.
6) A.R. Katritzky "Physical Methods in Heterocyclic Chemistry," Academic Press, New York (1963), Vol. I, p. 98.
7) This reaction was carried out using 0.7 - 1.0 mmol of β-NAD (500 - 700 mg) under the following conditions: The base/NAD molar ratio was ca. 4 : 1, and base concentration was adjusted to more than 25 mM by the addition of DMSO.
8) S. Tono-oka, A. Sasaki, and S. Kakimoto, Bull. Inst. Immun. Sci., Hokkaido Univ., 38, 46 (1978).
9) Each of these new compounds showed a single spot on TLC (silica gel $60F_{254}$; 2-propanol — 0.2 % aq ammonia, 7 : 3 v/v), and gave a satisfactory elemental analysis as an ammonium salt.
10) J. Elguero, C. Marzin, A.R. Katritzky, and P. Linda "The Tautomerism of Heterocycles," Supplement 1, Advances in Heterocyclic Chemistry, Academic Press, New York (1976), p. 291.

(Received February 23, 1983)