Volume 4 Number 11 November 1977 **Nucleic Acids Research**

A ^{13}C NMR study of poly(adenosine diphosphate ribose) and its monomers: evidence of α-(1"→2') ribofuranosyl ribofuranoside residue

Masanao Miwa, Hazime Saitô*, Harutake Sakura, Noriko Saikawa, Fumiko Watanabe**, Taijiro Matsushima** and Takashi Sugimura

Virology Division and Biophysics Division*, National Cancer Center Research Institute, Tsukiji, Chuo-ku, Tokyo 104, and Department of Molecular Oncology, Institute of Medical Science, University of Tokyo**, Shirokanedai, Minato-ku, Tokyo 108, Japan

Received 6 September 1977

ABSTRACT

The ^{13}C NMR spectra of poly(adenosine diphosphate ribose), ribosyl adenosine 5',5"-bis(phosphate) and related compounds were analyzed. The structure of the ribose-ribose linkage was determined as α-(1"→2')ribofuranosyl ribofuranoside, from the ^{13}C chemical shifts of methyl-α- and methyl-β-D-ribofuranosides, and from the downfield displacements of ^{13}C NMR signals by glycosidic bond formation.

INTRODUCTION

Nuclei of animal cells contain a unique enzyme which polymerizes NAD^+ to a nucleic acid-like biopolymer, poly(ADP-Rib), in which the ADP-Rib moieties of NAD^+ are polymerized through ribose-ribose glycosidic bonds with concomitant release of nicotinamide moieties.[1]. There is some evidence that synthesis of poly(ADP-Rib) is related to DNA synthesis,[2-4] mitosis[5] and cell differentiation.[6] Moreover, an antibody against this biopolymer is found in the sera of patients with systemic lupus erythematosus.[7] The studies reported here show that poly-(ADP-Rib) is a polymer of (1"→2')-α-D-ribofuranosyl-adenosine-5',5"-bis(phosphate).

MATERIALS AND METHODS

Poly(ADP-Rib) was synthesized using calf thymus nuclei and NAD^+.[1] It was purified by hydroxylapatite column chromatography essentially as described by Sugimura et al.[8] A sample of 51 mg of poly(ADP-Rib) was digested with 0.1 mg/ml of snake venom phosphodiesterase at 37°C for 2 hrs, and fractionated by Dowex 1 column chromatography as described previously.[9] The yield of Ado(P)-Rib-P was 41 mg. The mobilities of the compound on paper chromatography were as reported previously.[9] The λmax and λmin for Ado(P)-Rib-P at pH 2, 7 and 12 are the same as for 5'AMP. Ado(P)-Rib-P yielded 5'AMP, Rib5P and adenine on incubation in

0.5N HCl in absolute methanol at 25°C for 20 hrs.

ADP-Rib, 5'AMP and Rib5P were purchased from Sigma Chemical Co., St.Louis, Mo., USA. Rib was obtained from E. Merck, Darmstadt, Germany. Poly(A) was from Miles Laboratories, Kankakee, Ill., USA.

Sample was dissolved in deuterium oxide (40 mg/ml) and was contained in a tube (outer diameter = 10 mm) with a teflon vortex plug. ^{13}C NMR spectra were recorded by a JEOL PFT-100/EC-100 pulsed Fourier transform spectrometer operating at 25.03 MHz. Spectra were usually recorded using 8K data points and a spectral width of 4KHz. ^{13}C Chemical shifts were expressed in parts per million downfield from tetramethylsilane used as an external standard.

RESULTS

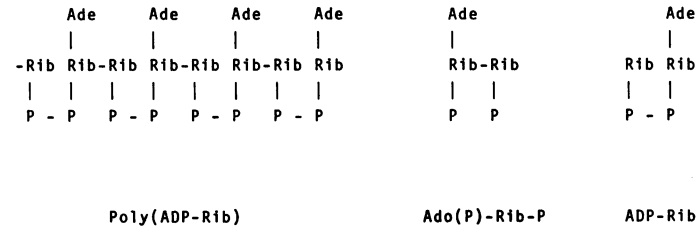
Assignment of ^{13}C NMR peaks of ADP-Rib, Rib and Rib5P

Figure 1 shows the ^{13}C NMR spectra of poly(ADP-Rib), Ado(P)-Rib-P, ADP-Rib and poly(A). The ^{13}C peaks of ADP-Rib were assigned by comparing them with those of 5'AMP,[10] and also by the fact that there are two sets of peaks for the carbons of the ribose due to the presence of α- and β-anomeric species (Table I). Rib in aqueous solution is known to consist of four tautomeric forms: 56% β-ribopyranose, 20% α-ribopyranose, 18% β-ribofuranose and 6% α-ribofuranose.[11] Thus the ^{13}C signals of α- and β-ribofuranoses can be assigned on the basis of their relative intensities (Table I and footnote h. For the major tautomeric forms, α- and β-ribopyranoses, see references 12 and 13.). The C-5' signals of ADP-Rib and Rib5P are displaced downfield by 2.5 ppm due to the presence of a phosphate group.[10]

Assignment of ^{13}C NMR peaks of poly(ADP-Rib) and Ado(P)-Rib-P

As expected, ten peaks appear for the two kinds of ribose moieties in poly-

(INSET)



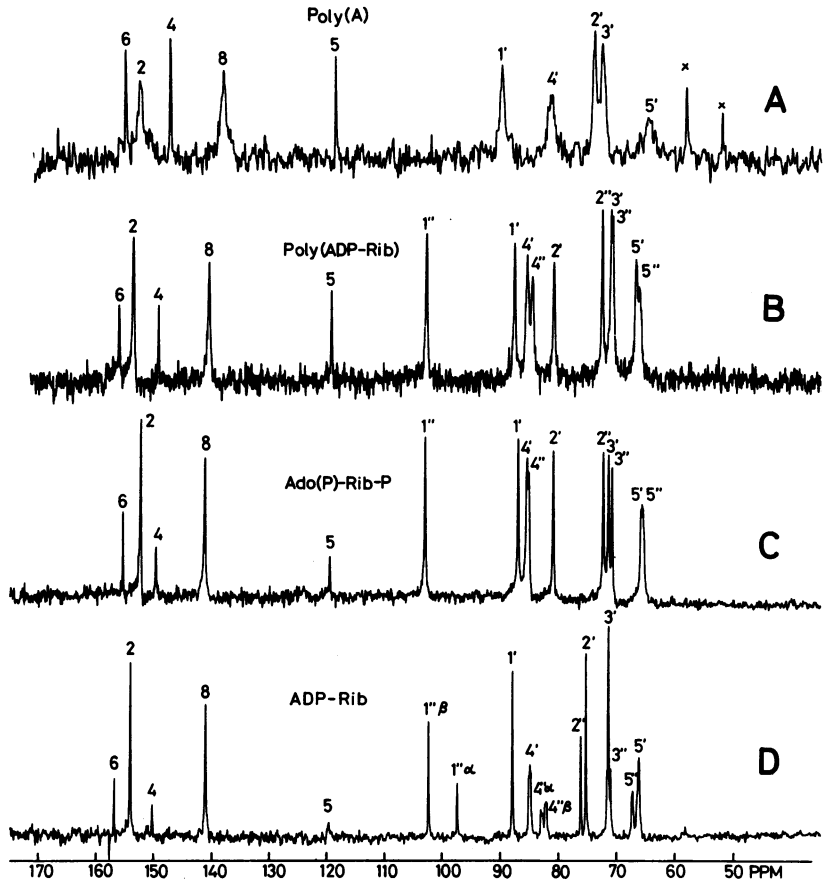


Figure 1. ^{13}C NMR spectra of poly(ADP-Rib), Ado(P)-Rib-P, ADP-Rib and poly(A)(40 mg/ml in $^{2}H_2O$, pD 7). Spectral width, 4 KHz, 8K data points(repetition time 1.1 sec). A. Poly(A), 90° pulse, 26,000 transients. Two peaks with cross sign(x) are due to impurities. B. Poly(ADP-Rib), 90° pulse, 41,749 transients. C. Ado(P)-Rib-P, 45° pulse, 50,473 transients. D. ADP-Rib, 45° pulse, 40,982 transients.

(ADP-Rib) or Ado(P)-Rib-P. The ^{13}C resonance peaks from poly(ADP-Rib) and Ado-(P)-Rib-P are very similar to each other. From the spectra produced by improved digital resolution, the pairs (C-4'/C-4", C-5'/C-5", and C-3'/C-3") are easily differentiated by taking account of the $^{31}p-^{13}C$ spin-spin couplings.[10] As these pairs of peaks are found to coincide with each other, presumably due to dissociation of base-base stacking at elevated temperature (Figure 2A and Table I), the

Table I ^{13}C Chemical Shifts of Poly(ADP-Rib) and Ado(P)-Rib-P and Related Compounds(in ^2H_2O)[a]

							Reference compounds			
							α-anomer	β-anomers		Hexa-N-
	Poly(ADP-Rib)	Ado(P)-Rib-P	ADP-Rib	Rib5P	Rib[b]	5'AMP[c]	Methyl-α-D-ribofurano-side[d]	Methyl-β-D-ribofurano-side[d]	Ribo-stamy-cin[e]	acetyl-neomycin B[f]
C-6	156.0 (N.O.)[g]	154.4	156.5			156.0				
C-2	153.6 (153.8)	152.3	153.8			153.4				
C-4	149.1 (149.7)	149.7	150.0			149.6				
C-8	140.4 (140.8)	141.3	140.7			140.8				
C-5	119.2 (N.O.)	119.5	119.5			118.8				
C-1"	102.7 (102.7)	103.0	β 102.1[h] α 97.3[h]	102.0[h] 97.2[h]	101.8[h] 97.1[h]		104.2	109.0	109.1	109.3
C-1'	87.5 (87.3)	87.0	87.8			88.1				
C-4'	85.2 (84.8)	85.5	84.9			85.0				
C-4"	84.4 (84.4)	85.3	β 83.0 α 82.1	83.5 82.9	83.4 83.9		85.5	83.9	83.4	82.4
C-2'	80.7 (80.4)	81.0	75.3			75.4				
C-2"	72.4 (72.5)	72.4	76.2	76.3	76.1		72.1	75.3	75.7	74.5
C-3'	70.9 (70.6)	71.5	71.4			71.4				
C-3"	70.6 (70.6)	70.9	α 71.7 β 71.1	71.6 71.2	71.3 70.9		70.8	70.8	70.5	77.2[i]
C-5'	66.7 (66.4)	65.9	67.4			65.3				
C-5"	66.0 (66.0)	65.7	α 66.6 β 66.2	65.4 64.6	63.4 62.2		62.6	63.9	62.6	62.2

a: ppm from external TMS. Carbons of ribose linked to an adenine moiety are designated as prime. Other ribose carbons are shown as double-prime("). b: Only α- and β-ribofuranose tautomers are shown. c: Assignment based on Ref.10. d: Ref.14. e: Ref.18. f: Ref.19. g: at 78°C. N.O.: not observed. h: The ratio of the β-anomer to the α-anomer: 2.2(ADP-Rib), 3.0(Rib5P), and 1.5(Rib). i: Glycosidic bond at this carbon.

peaks which exhibit temperature dependence are ascribed to the ribose moiety directly linked to the adenine (C-3', C-4' and C-5'). The remaining resonances (C-1', C-1", C-2' and C-2") can be assigned unambiguously by reference to the data of ADP-Rib, 5'AMP, methyl-α-D- and methyl-β-D-ribofuranosides.[14,15]

The ^{13}C resonances of ribose linked to the adenine are very similar to those of 5'AMP except for C-2'. Downfield displacement (ca. 5ppm) with respect to 5'AMP and ADP-Rib of the C-2' signals of poly(ADP-Rib) and Ado(P)-Rib-P, together with slight upfield shifts[14] of the adjacent C-1' and C-3' signals, shows that the glycosidic bond is formed at the C-2' position of the AMP moiety. These data are consistent with the conclusion of Doly and Petek from methylation analysis that poly(ADP-Rib) has 1"→2' ribose-ribose bonds.[16] The involvement of a ribofuranose-type sugar structure is further supported by ^1H NMR data: the proton spin-spin coupling constants of the AMP moiety of Ado(P)-Rib-P are found to be $J_{1',2'}$ 5.4 Hz, $J_{2',3'}$ 4.6 Hz, and $J_{3',4'}$ 3.7 Hz, which are very similar to those of 5'AMP.[17]

α-Anomeric configuration of the ribofuranose-ribofuranose linkage

Methyl-α-D- and methyl-β-D-ribofuranosides are excellent reference compounds for determination of the anomeric configuration, since the ^{13}C chemical shifts

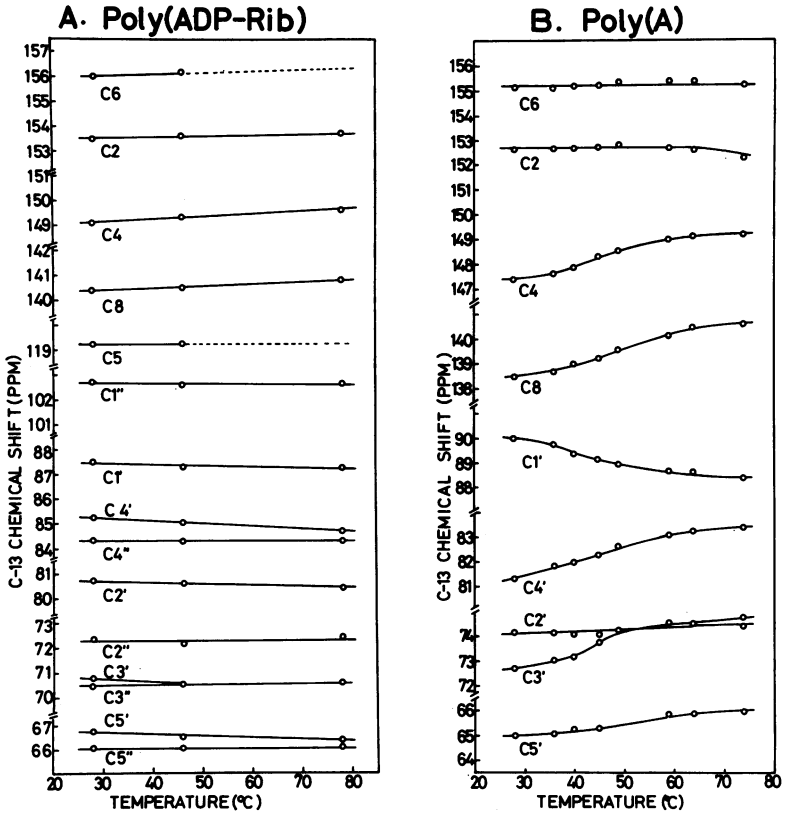


Figure 2. Temperature dependence of ^{13}C chemical shifts. A. Poly(ADP-Rib). B. Poly(A).

of methyl-β-D-ribofuranoside are very similar to those of naturally occurring ribofuranosyl components in ribostamycin[18] and hexa-N-acetyl neomycin B[19] (Table I), both of which contain a β-linked ribofuranosyl moiety.[20] In poly-(ADP-Rib) and Ado(P)-Rib-P, it was found that the positions of C-1", C-2", C-3", C-4" and also C-5" (in view of the effect of 5"-phosphate described above) were very close (less than 1.5 ppm) to those of methyl-α-D-ribofuranoside (not to those of methyl-β-D-ribofuranoside).

Furthermore, the C-1" peaks of poly(ADP-Rib) and Ado(P)-Rib-P appear downfield with respect to that of ADP-Rib due to glycosidic bond formation, the differences being 0.6-0.9 ppm (from the β-anomer of ADP-Rib) and 5.4-5.7 ppm (from the α-anomer). Generally, a downfield displacement in the range of 5-7 ppm due to glycosidic bond formation or O-isopropylation (a better model for the glycosidic bond than O-methylation) is noted for various pentoses and

hexoses.[21,22] Therefore, on the basis of the ^{13}C shift of ADP-Rib, the lowest peaks of sugar moieties at 102.7 and 103.0 ppm (5.4-5.7 ppm downfield shift) can be assigned with certainty to the C-1" of the α-linked ribofuranoside moiety of poly(ADP-Rib) and Ado(P)-Rib-P, respectively.

Thus, the glycosidic bond between the two ribofuranosyl residues is concluded to be an α-(1"→2')-linkage (Figure 3). The finding of $[\alpha]_D$= +37° for ribosyl adenosine, which is the hydrolysis product of Ado(P)-Rib-P by E. coli alkaline phosphatase, is also consistent with the presence of an α-linkage of the ribose-ribose bonds.

Conformation of poly(ADP-Rib) and poly(A)

Poly(ADP-Rib) shows 10% hypochromicity of the UV spectrum indicating some base-base stacking, but base-base stacking of poly(ADP-Rib) is probably less than that of poly(A), which shows around 30% hypochromicity.[23] Poly(A) exists as a single-stranded helix at ambient temperature (pD 7) and undergoes conformational transition to random coil at a temperature around 50°C.[24] At ambient temperature, the ^{13}C signals of carbons directly linked to hydrogen (C-2, C-8, and ribose carbons) of poly(A) are very broad compared to those of poly(ADP-Rib), reflecting the slow molecular motion of the polymer chain due to the helical conformation. Figure 2B shows the temperature dependence of ^{13}C signals of poly(A) (The same result was obtained by Dr. I. C. P. Smith and Dr. P. Colson. Personal communication.). The downfield displacements of C-4 and C-8 of the

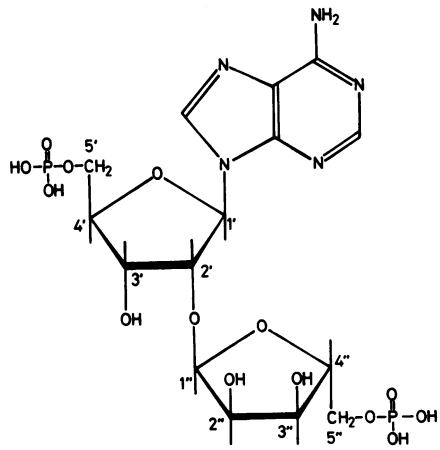


Figure 3. Structure of Ado(P)-Rib-P, the monomer form of poly(ADP-Rib) (see INSET).

purine moiety of poly(A) at elevated temperature (Figure 2B) are explained by the dissociation of base-base stacking as a result of conformational transition. Similar shifts of C-4 and C-8 are noted for poly(ADP-Rib) (Figure 2A), but the magnitudes are one third to one forth of those of poly(A). Furthermore, the peak positions of C-4 and C-8 of poly(A) at 78°C are very close to those of poly(ADP-Rib). Accordingly it might be concluded that poly(ADP-Rib) does not adopt such an ordered conformation as poly(A), but essentially a random-coil conformation.

DISCUSSION

As far as we know only the following compounds are known to have ribose-ribose linkages: polyribophosphate possessing a ribose-ribose β-(1→1) linkage in addition to 3→5 phosphodiester bonds[25]; ribosyl adenosine from yeast tRNA[26]; and ADP-ribosyl NAD^+ in which the ribose in the Rib5P moiety of ADP-Rib is bound to the ribose in the NMN moiety of NAD^+.[27] Recently, Suhadolnik *et al.* reported a ribose to 2'-deoxyribose 1"→3' glycosidic linkage.[28] Nothing is known about the anomeric forms of these three compounds.

This is the first report of an α-linkage of a ribose moiety to a sugar. This clearly requires anomerization of ADP-Rib during cleavage of the nicotinamide-ribose bond of β-NAD^+ during formation of poly(ADP-Rib) by the chromatin-associated enzyme. This possibility is consistent with the findings that α-NAD^+ inhibits the enzymatic synthesis of poly(ADP-Rib).[29]

Poly(ADP-Rib) glycohydrolase,[30,31] an enzyme that specifically cleaves this ribose-ribose linkage in poly(ADP-Rib), is also found in the nuclei and this enzyme is shown by this work to be an α-(1"→2') glycohydrolase of poly(ADP-Rib).

ACKNOWLEDGEMENTS

We wish to thank Dr. Hamao Umezawa, Dr. Tomio Takeuchi and Dr. Shinichi Kondo of the Institute of Microbial Chemistry for valuable suggestions. We also thank Dr. I. C. P. Smith and Dr. P. Colson of National Research Council of Canada for communicating their unpublished work. This work was partly supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

ABBREVIATIONS[1,32]

Poly(ADP-Rib), poly(adenosine diphosphate ribose); ADP-Rib, adenosine diphos-

phate ribose; Ado(P)-Rib-P, ribosyl adenosine 5',5"-bis(phosphate); Rib5P, ribose 5-phosphate; Rib, ribose; NMR, nuclear magnetic resonance; NMN, nicotinamide mononucleotide.

REFERENCES

1 Sugimura, T. (1973) in Progress in Nucleic Acid Research and Molecular Biology, Vol. XIII, pp. 127-151. Academic Press. New York
2 Burzio, L., and Koide, S. S. (1970) Biochem. Biophys. Res. Commun. 40, 1013-1020
3 Smulson, M., Henriksen, O. and Rideau, C. (1971) Biochem. Biophys. Res. Commun. 43, 1266-1273
4 Colyer, R. A., Burdette, K. E. and Kidwell, W. R. (1973) Biochem. Biophys. Res. Commun. 53, 960-966
5 Miwa, M., Sugimura, T., Inui, N. and Takayama, S. (1973) Cancer Res. 33, 1306-1309
6 Caplan, A. I. and Rosenberg, M. J. (1975) Proc. Natl. Acad. Sci. USA 72, 1852-1857
7 Kanai, Y., Kawaminami, Y., Miwa, M., Matsushima, T., Sugimura, T., Moroi, Y. and Yokohari, R. (1977) Nature 265, 175-177
8 Sugimura, T., Yoshimura, N., Miwa, M., Nagai, H. and Nagao, M. (1971) Arch. Biochem. Biophys. 147, 660-665
9 Shima, T., Hasegawa, S., Fujimura, S., Matsubara, H. and Sugimura, T. (1969) J. Biol. Chem. 244, 6632-6635
10 Smith, I. C. P., Mantsch, H. H., Lapper, R. D., Deslauriers, R. and Schleich, T. (1973) in Conformation of Biological Molecules and Polymers, pp. 381-402 (ed. by B. Pullman and E. D. Bergmann) Academic Press. New York
11 Lemieux, R. U. and Stevens, J. D. (1966) Can. J. Chem. 44, 249-262
12 Perlin, A. S., Casu, B. and Koch, H. J. (1970) Can. J. Chem. 48, 2596-2606
13 Dorman, D. E. and Roberts, J. D. (1970) J. Amer. Chem. Soc. 92, 1355-1361
14 Gorin, P. A. J. and Mazurek, M. (1975) Can. J. Chem. 53, 1212-1223
15 Ritchie, R. G. S., Cyr, N., Korsch, B., Koch, H. J. and Perlin, A. S. (1975) Can. J. Chem. 53, 1424-1433
16 Doly, J. and Petek, F. (1966) C. R. Acad. Sci. Paris, Series D, 263, 1341-1344
17 Evans, F. E., Lee, C.-H. and Sarma, R. H. (1975) Biochem. Biophys. Res. Commun. 63, 106-114
18 Omoto, S., Inouye, S., Kojima, M. and Niida, T. (1973) J. Antibiot. 26, 717-724
19 Rinehart, Jr., K. L., Marik, J. M., Nystrom, R. S., Stroshane, R. M., Truitt, S. T., Taniguchi, M., Rolls, J. P., Haak, W. J. and Ruff, B. A. (1974) J. Amer. Chem. Soc. 96, 2263-2265
20 Akita, E., Tsuruoka, T., Ezaki, N. and Niida, T. (1970) J. Antibiot. 23, 173-183
21 Usui, T., Yamaoka, N., Matsuda, K., Tuzimura , K., Sugiyama, H. and Seto, S. (1973) J. C. S. Perkin 1, 2425-2432
22 Gorin, P. A. J. and Mazurek, M. (1976) Carbohydr. Res. 48, 171-186
23 Bobst, A. M., Rottman, F. and Cerutti, P. A. (1969) J. Mol. Biol. 46, 221-234
24 Leng, M. and Felsenfeld, G. (1966) J. Mol. Biol. 15, 455-466
25 Rosenberg, E. and Zamenhof, S. (1962) J. Biol. Chem. 237, 1040-1042
26 Hall, R. H. (1965) Biochemistry 4, 661-670
27 Imai, T., Okuda, S. and Suzuki, S. (1969) J. Biol. Chem. 244, 4547-4554
28 Suhadolnik, R. J., Baur, R., Lichtenwalner, D. M., Uematsu, T., Roberts, J. H., Sudhakar, S. and Smulson, M. (1977) J. Biol. Chem. 252, 4134-4144
29 Preiss, J., Schlaeger, R. and Hilz, H. (1971) FEBS Lett. 19, 244-246

30 Miwa, M. and Sugimura, T. (1971) J. Biol. Chem. 246, 6362-6364
31 Miwa, M., Tanaka, M., Matsushima, T. and Sugimura, T. (1974) J. Biol. Chem. 249, 3475-3482
32 IUPAC-IUB Commission on Biochemical Nomenclature (1977) Proc. Natl. Acad. Sci. USA 74, 2222-2230