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21. Teruhisa Noguchi : Studies on the Biosynthesis of Nucleic Acids. III[1]. A New Arylamine found during Biosynthesis of DNA.

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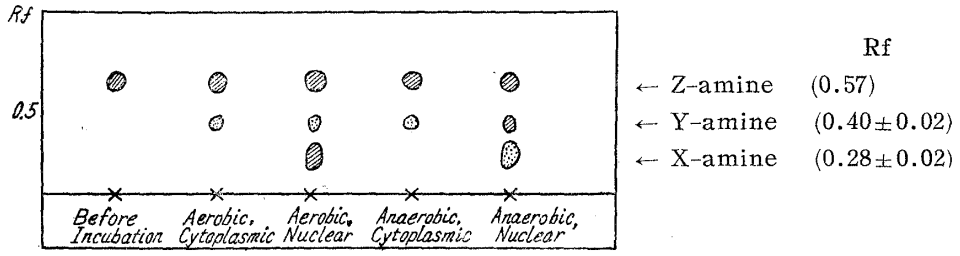
During the course of studies on the incorporation of 4-aminoimidazole[4-^{14}C]-5-carboxamide(AICA-^{14}C)into polynucleotide purines with subcellular fractions of pigeon liver,[2] the author noted the net incorporation of AICA-^{14}C into deoxyribonucleic acid (DNA) without adding deoxyribose in the medium which contained only ribose-5'-phosphate and fructose-1,6-diphosphate as sole sources of sugar. On the assumption that AICA-^{14}C should be bound with deoxyribose moiety, which would be derived from ribose-5'-phosphate or fructose-1,6-diphosphate before incorporation into polynucleotides, attempt was made to find these intermediates (e.g. "active" AICA deoxyriboside) by paper chromatography of the acid-soluble fraction of the incubation medium.

Actually, two or three spots were found in the acid-soluble fraction of each incubation. They were designated as X (Rf 0.28±0.02), Y (Rf 0.40±0.02), and Z (Rf 0.57) as demonstrated in Fig. 1.

Fig. 1. Paper Chromatogram of Acid-soluble Fraction after Incubation with Subcellular Fractions



As all these spots were positive to color reaction by the Tsuda reagent[3] or the Pauly reagent[4] on the paper chromatogram, it is postulated that these substances, X, Y, and Z, are arylamine derivatives.

In Table I and Fig. 1, it is demonstrated that the cytoplasmic fraction did not yield the X-amine spot but afforded only Y- and Z-amine spots (Pauly reaction : pale purple and blue, respectively). Under the same conditions, the incubation with nuclear fraction offered definite X-amine spot (Pauly reaction : yellow). It is of interest to note that, whereas formation of X-amine was stimulated in the aerobic glycolysis system, it was suppressed by anaerobic glycolysis incubation. However, formation of Y-amine increased in anaerobic glycolysis.

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1) Part II. T. Noguchi : This Bulletin, **4**, 97(1956).
2) T. Noguchi, Y. Miura : J. Biol. Chem.(1956), in press.
3) K. Tsuda, *et al.* : J. Pharm. Soc. Japan, **62**, 362(1942); **67**, 239(1947).
4) B. N. Ames, H. K. Mitchell : J. Am. Chem. Soc., **74**, 252(1952).

Table I. Detection of Each Spot after Incubation with Pigeon Liver Nuclear Fractions

Medium	Spots	Rf	Tsuda test	Pauly test	Bial test	Tryptophan test	Seliwanoff test
Before incubation	AICA	0.57	+++	Blue(+++)	−	−	−
After incubation in aerobic glycolysis	X	0.28±0.02	++	Yellow(+)	Brown(++)	−	++
	Y	0.40±0.02	±	Purple(±)	Green(±)	−	−
	Z	0.57	+++	Blue(+++)	−−	−	−
After incubation in anaerobic glycolysis	X	0.28±0.02	±	Yellow(±)	Brown(±)	−	+
	Y	0.40±0.02	+	Purple(+)	Green(+)	−	−
	Z	0.57	+++	Blue(+++)	−−	−	−

Sugar moiety of these spots was examined. X-Amine exhibited dark brick color with the Bial's orcinol-HCl test,[5] the Seliwanoff's resorcinol test[6] was positive, and diphenylamine reaction[7] and tryptophan test[8] were negative. Y-Amine exhibited green color with the Bial's test for a pentose moiety, and other reactions were negative. Z-Amine was negative to all sugar reactions. The detection of phosphoric acid moiety on the paper chromatogram by the Hans-Isherwood's procedure[9] failed.

Paper with these spots was cut into narrow strips, each of which was eluted with 0.01*N* HCl, 0.02*M* phosphate buffer (pH 7.4), or 0.03*N* NaOH. Then each solution was placed in a 1-cm. cell and the ultraviolet absorption of these spots was measured by the Beckman spectrophotometer. The absorbance ratios in 0.01*N* HCl were calculated. The results are listed in Table II. Maximum absorption of X-amine appeared at 261 mμ in 0.01*N* HCl, distinctly differing from those of Y- and Z-amines and of AICA.

Table II. Absorption Spectra and Absorbance Ratio of Each Spot (λ_{max} mμ)

Medium	X	Y	Z	AICA
0.01*N* HCl	261	266	266	266
0.02*M* Phosphate buffer (pH 7.4)	264	267	267	267
0.03*N* NaOH	270	278	278	278

Absorbance Ratio (0.01 *N* HCl)

Amine	250/260	270/260	280/260
X-Amine	0.82	0.83	0.45
Y-Amine	0.74	1.00	0.51
Z-Amine	0.75	1.00	0.61
AICA	0.75	1.04	0.68

These spots were hydrolyzed with 0.1*N* or 1*N* HCl at 100° for 5 mins. The solution was again chromatographed on paper and the spots were detected by the Pauly reagent. As demonstrated in Table III, X-amine was resistant to boiling in water and hydrolysis with weak acids but was hydrolyzed with 1*N* HCl, while Y-amine was decomposed to liberate pentose from the parent compound by acid hydrolysis and other moiety moved to the same Rf as that of AICA and Z-amine.

It is, therefore, concluded that X-amine is a kind of arylamine associated with a ketose, which is found to be neither ribose nor deoxyribose. From the above results, Y-amine was identified as AICA riboside in other experiments,[10] and Z-amine agreed with AICA itself. Further research on X-amine, the structure of which is

5) M. Bial: Deut. Med. Wochschr. **29**, 253, 477(1903); W. Mejbaum: Z. physiol. Chem., **258**, 117 (1939).
6) T. Seliwanoff: Ber., **20**, 181(1887).
7) F. B. Seibert: J. Biol. Chem., **133**, 593(1940).
8) P. Thomas: Z. physiol. Chem., **199**, 10(1931).
9) C. S. Hanes, F. A. Isherwood: Nature, **164**, 1107(1949).
10) T. Noguchi, Y. Miura: Unpublished data.

not clear in this stage, should be of interest, if the amine is a new intermediate in the biosynthesis of DNA.

Table III. Paper Chromatogram of Each Spot after Hydrolysis

Amines	HCl added	Rf	Pauly test
AICA	None (H_2O)	0.57	Blue
AICA	0.1*N*	0.57	〃
AICA	1.0*N*	0.57	〃
X-amine	None (H_2O)	0.28	Yellw
X-amine	0.1*N*	0.28	〃
X-amine	1.0*N*	0.7	Pink
Y-amine	None (H_2O)	0.40	Pale purple
Y-amine	0.1*N*	(0.40) + 0.57	(Pale purple) + Blue
Y-amine	1.0*N*	0.57	Blue
Z-amine	None (H_2O)	0.57	〃
Z-amine	0.1 *N*	0.57	〃
Z-amine	1.0 *N*	0.57	〃

Hydrolysis : 100°, 5 mins. Solvent : BuOH:EtOH:H_2O

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Experimental

Preparation of Subcellular Fractions—For fractionation of subcellular components, the Hogeboom and Schneider's method[11] was used. Starting with 14~15 g. of pigeon liver, 20% liver homogenate was prepared in 0.25 *M* sucrose (Potter-Elvehjem type homogenizer was used). The nuclear fraction was obtained by three centrifugations at 700×*g* for 10 mins.; the supernatant obtained from the first centrifugation was used for the cytoplasmic fractions.

Incubation of Subcellular Fractions—The incubation of nuclear fraction or cytoplasmic fractions was carried out at 37° for 100 mins. under aerobic or anaerobic conditions. The composition of the medium for anaerobic glycolysis were K-phosphate buffer (pH 7.4) 0.0024*M*, $KHCO_3$ 0.025*M*, nicotinamide 0.04*M*, K-ATP 0.00033*M*, DPN 0.00022*M*, fructose-1,6-diphosphate 0.002*M*, glucose 0.01*M*, $MgCl_2$ 0.007*M*, K-pyruvate 0.005*M*, KF 0.01*M* and Solution F. The medium for aerobic glycolysis contained K-phosphate buffer (pH 7.4) 0.0033*M*, $KHCO_3$ 0.0033*M*, fructose-1,6-diphosphate 0.01*M*, DPN 0.00022*M*, $MgCl_2$ 0.0033*M*, KF 0.01*M* and Solution F. The composition of Solution F : AICA-^{14}C 0.06 *mM*, ribose-5′-phosphate 0.06 *mM*, Na-formate 0.06 *mM*, Ca leucovorin 50 γ (total volume : 15 cc.).

Paper Chromatography and Identification of Acid-soluble Fractions—The acid-soluble fractions were obtained by the addition of conc. $HClO_4$ to the medium (final concn., 4%). Then, the paper chromatography of this fraction was developed with BuOH:EtOH:H_2O (50:15:35)(Markham-Smith's solvent[12]), using acid-treated filter paper (Toyo Roshi No. 51-A). The ultraviolet light was used for the detection of spots.

Summary

During the course of studies on the incorporation of 4-aminoimidazole[4-^{14}C]-5-carboxamide into polynucleotide purines with subcellular fractions of pigeon liver, a new diazotizable amine was obtained by the paper chromatography of the acid-soluble fraction of the incubation medium. The amine is a kind of arylamine associated with a ketose, which is neither ribose nor deoxyribose.

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11) G. H. Hogedoom, W. C. Schneider : J. Biol. Chem., **197**, 611(1952).
12) R. Markham, J. D. Smith : Biochem. J. (London), **45**, 294(1949).