

Incorporation of Cytidylic Acids *a* and *b* Into Liver Pentose Nucleic Acid

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The existence of two isomers of cytidylic acid, both obtained from an alkaline hydrolysate of ribonucleic acid (PNA), has been demonstrated by Cohn¹ and by Loring *et al.*² The structure of these isomers, called cytidylic acids *a* and *b* respectively, has not been definitely established, but the evidence indicates that both isomers are derivatives of cytidine, one being the 2'-phosphate and the other the 3'-phosphate^{3,4}.

The biological significance of this type of isomerism, which occurs in all mononucleotides obtained from an alkaline hydrolysate of PNA, is not fully understood. Brown and Todd⁴ have suggested that for the most part only one of the isomers exists in the polynucleotide and that a mixture of both is obtained through the action of alkaline hydrolysis. Studies with labelled formate⁵ and adenine⁶ showed no difference in the incorporation of these precursors into the *a* and *b* isomers of adenylic acids obtained from liver PNA. Differences in incorporation were found, however, when labelled adenylic acids *a* and *b* were added to growing *Lactobacillus casei*⁷. The *b* nucleotide was utilized for the synthesis of polynucleotides to an appreciable extent, while the utilization of the *a* isomer was very small and could have been due to isomerization to the *b* nucleotide during the course of the experiment.

The present experiments were carried out to investigate the metabolic pathways of the two N¹⁵-labelled isomers of cytidylic acid in the rat. It was thought that the time period during which the animals received the isotopic substances should be as short as possible. In order to obtain a signifi-

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Table 1. Administration of N^{15} -cytidylic acids a and b to partially hepatectomized rats. Each animal received a total of 210 mg of mononucleotide per kg of body weight divided among three equal doses given 20, 22, and 24 hours after the operation.

Isolated	Cytidylic acid a		Cytidylic acid b	
	N^{15} atom per cent excess	Per cent N^{15} calculated on basis of 100 per cent N^{15} in cytidylic acid injected	N^{15} atom per cent excess	Per cent N^{15} calculated on basis of 100 per cent N^{15} in cytidylic acid injected
	Injected cytidylic acid			
	4.20	100	4.20	100
PNA Cytidine	0.097	2.3	0.123	2.9
	0.081	2.0	0.093	2.2
Uridine	0.080	2.0	0.091	2.2
	0.071	1.7	0.079	1.9

cant incorporation of the isotope into the PNA the experiments were carried out in a rapidly growing tissue, namely regenerating rat liver, at the height of nucleic acid synthesis⁸.

N^{15} -cytidylic acids a and b were obtained from biologically marked PNA and were injected subcutaneously into two groups of rats 20, 22 and 24 hours after the animals had been subjected to partial hepatectomy. Cytidine and uridine were isolated from the liver PNA in each group and analyzed for N^{15} . The results which are summarized in Table 1 indicate no significant difference in the utilization of the two isomers.

EXPERIMENTAL

Preparation of N^{15} -cytidylic acids. The starting material was biologically marked PNA, isolated from *E. Coli*, grown on an $N^{15}H_4Cl$ containing medium. The cultivation of the bacteria has been described earlier⁹. The extraction and preparation of PNA from the bacteria was carried out according to Hammarsten¹⁰. The free mononucleotides were prepared via the mercury salts¹¹ and chromatographically separated on a Dowex 50 (H^+) column using 0.1 N acetic acid as eluting agent¹². The cytidylic acid fractions were localized by their characteristic light absorption in the ultra violet, pooled and evaporated to dryness *in vacuo*. Mixed cytidylic acids, totaling 950 mg, were obtained.

Separation of cytidylic acid isomers. Resolution of the isomers was carried out as described by Loring *et al*¹³. Ion exchange chromatography on Dowex 2 (formate) was

used. A solution of 800 mg of mixed cytidylic acids in 20 ml of water was made with the aid of sodium hydroxide (final pH 8) and adsorbed on a Dowex 2 (formate) column, length 20 cm, diameter 4 cm. Elution was carried out with 0.05 *N* formic acid, which was run through the column at a rate of 0.5 ml/min. In contrast to the experiments of Loring *et al.*¹³ complete separation of the two isomers was obtained, probably because of the much smaller load on the column in the present experiment.

The combined fractions for each isomer were neutralized to pH 7 with *N* NaOH, and each solution was adsorbed on a Dowex 2 (formate) column, length 3 cm, diameter 1 cm. On elution with 0.5 *N* formic acid each isomer was eluted in a small volume and could be crystallized directly in good yield by the addition of two volumes of alcohol. Yields of 210 mg of cytidylic acid *a* ($[\alpha]_D = +48^\circ$, *c* 0.5 per cent, at pH 11) and 290 mg of cytidylic acid *b* ($[\alpha]_D = -8^\circ$, *c* 0.5 per cent, at pH 11) were obtained.

Administration of isotope — Eight rats, each weighing about 200 g were subjected to partial hepatectomy by a previously described technique¹⁴. The rats were divided into four groups each consisting of two animals. Twenty hours after the operation groups 1 and 2 received a subcutaneous injection of cytidylic acid *a*, groups 3 and 4 of cytidylic acid *b*. The injections were repeated 22 and again 24 hours after the operation. Each rat received a total of 210 mg of mononucleotide per kg of body weight equally divided among the three injections. The rats were killed 26 hours after the operation, and the livers of each group were pooled.

Preparation of pyrimidine nucleosides. Polynucleotides were prepared from the livers of each group according to Hammarsten¹⁰. The pyrimidine nucleosides from PNA were prepared as described earlier¹⁵.

DISCUSSION

The present results do not show any significant difference in the incorporation of the two isomers of cytidylic acid into pyrimidines of PNA. They differ in this respect from the results obtained by Balis *et al.*⁷ with *Lactobacillus casei*, in which the *b* nucleotide of adenylic acid was utilized to a far greater extent than was the *a* isomer*.

It has been shown earlier¹⁶ that N¹⁵-cytidine is an effective precursor of the pyrimidines of PNA. The present findings can therefore be explained either by the assumption that both isomers of cytidylic acid are utilized by the rat for the synthesis of PNA to about the same extent, or that each of them is first dephosphorylated to cytidine and then utilized.

SUMMARY

N¹⁵-cytidylic acids *a* and *b* were prepared from biologically marked PNA. After subcutaneous administration of each isomer to partially hepatectomized rats it was found that both mononucleotides were utilized to about the same extent for the synthesis of ribonucleic acid pyrimidines in regenerating rat liver.

* Brown and collaborators have found, however, that a difference in utilization of the two adenylic acids does not exist in the rat (personal communication from Dr. Brown).

REFERENCES

1. Cohn, W. E. *J. Am. Chem. Soc.* **72** (1950) 2811.
2. Loring, H. S., Luthy, N. G., Bortner, H. W., and Levy, L. W. *J. Am. Chem. Soc.* **72** (1950) 2811.
3. Loring, H. S., Hammell, M. L., Levy, L. W., and Bortner, H. W. *J. Biol. Chem.* **196** (1952) 821.
4. Brown, D. M., and Todd, A. R. *J. Chem. Soc.* **1952** 44.
5. Totter, J. R., Volkin, E., and Carter, C. E. *J. Am. Chem. Soc.* **73** (1951) 1521.
6. Marrian, D. H., Spicer, V. L., Balis, M. E., and Brown, G. B. *J. Biol. Chem.* **189** (1951) 533.
7. Balis, M. E., and Elion, G. B. *Federation Proc.* **11** (1952) 183.
8. Eliasson, N. A., Hammarsten, E., Reichard, P., Åqvist, S., Thorell, B., and Ehrensvärd, G. *Acta Chem. Scand.* **5** (1951) 431.
9. Reichard, P., and Estborn, B. *J. Biol. Chem.* **188** (1951) 839.
10. Hammarsten, E. *Acta Med. Scand. Suppl* **196** (1947) 634.
11. Reichard, P. *J. Biol. Chem.* **179** (1949) 763.
12. Cohn, W. E. *Science* **109** (1949) 377.
13. Loring, H. S., Bortner, H. W., Levy, L. W., and Hammell, M. L. *J. Biol. Chem.* **196** (1952) 807.
14. Bergstrand, A., Eliasson, N. A., Hammarsten, E., Norberg, B., Reichard, P., and von Ubisch, H. *Cold Spring Harbor Symposia Quant. Biol.* **13** (1948) 22.
15. Reichard, P. *J. Biol. Chem.* **197** (1952) 391.
16. Hammarsten, E., Reichard, P., and Saluste, E. *J. Biol. Chem.* **183** (1950) 105.

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