# THE OCCURRENCE OF DIHYDRO RIBOTHYMIDINE IN CHROMOSOMAL RNA[1]

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Dihydrouridine (diHU) was the first naturally occurring reduced pyrimidine to be discovered (Madison and Holley, 1965). It occurs universally in transfer RNAs. It has also been found in substantial amounts in chromosomal RNA (cRNA) of peas (Huang and Bonner, 1965) and of chicken embryos (Huang, 1967; Huang et al., 1968). It might reasonably have been assumed that all cRNAs would contain this pyrimidine.

Magrath and Shaw (1967) have described a specific chemical conversion of diHU to β-alanine, a method which provides unequivocal proof of the presence of diHU in RNA. Rat ascites tumor cRNA does not produce any β-alanine under these conditions, even though it yields a positive test (Cline et al., 1957; Ceriotti and Spandrio, 1963) for ureido groups, as does diHU. Thus,the presence of a different saturated pyrimidine in rat ascites tumor cRNA is indicated. It is unlikely that this nucleotide could be diHC, since this readily deaminates spontaneously to diHU (Janion and Shugar, 1960). The conversion reaction of Magrath and Shaw when applied to rat ascites tumor cRNA yields an amino acid which elutes from the auto-analyzer in the region of β-amino-isobutyric acid, the conversion analog of dihydro ribothymidine (diHT).

This paper describes experiments which prove the existence of diHT

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as a component of the cRNA of rat ascites tumor.

## Methods

Preparation of authentic dihydro ribothymidine: Ribothymidine was reduced to diHT using rhodium or alumina catalyst (Cohn and Doherty, 1956).

Isolation of diHT from cRNA: Chromatin was prepared from ascites tumor cells by the method of Dahmus (1968) and from calf thymus by the method of Huang et al. (1964). cRNA linked to its binding protein (cRNA ∿ BP, Bonner and Huang, 1966) was dissociated from the chromatin by salt or was isolated directly from the nuclear material which is separated from chromatin during sucrose gradient purification of the latter. These preparations are described in detail elsewhere (Jacobson, 1968). The cRNA ∿ BP was chromatographed on DEAE-cellulose, recovered and digested with a mixture of $T_1$ and pancreatic RNase or hydrolyzed with NaOH. Chromatography on Biogel P-30 yielded the binding protein attached to a residual saturated nucleotide. The covalent bond between nucleotide and protein was hydrolyzed by 0.1 N HCl and the phosphate removed with alkaline phosphatase.

Chromatography of diHT and diHU: Open and closed ring forms of both nucleosides were chromatographed on thin layer plates of polyethanolinine-impregnated-cellulose using 0.1 N formic acid as eluent (Huang et al., 1968). They were also separated on S&S blackribbon paper (#589) by ascending chromatography using the 3 different solvent systems described by Cline et al. (1959) as well as 0.1 N formic acid.

The standards were identified by spraying with 0.5 N NaOH followed by P-dimethyl amino benzaldehyde solution (Cline et al., 1959). For maximum sensitivity, nucleosides from cRNA were eluted by 0.1 N HCl and their presence determined using the more sensitive color reaction of Ceriotti and Spandrio (1963).

Conversion to β amino acids: Nucleosides were converted to their respective β amino acids by the method of Magrath and Shaw (1967) and the products separated on an amino acid analyzer. For maximum separation, elution with

pH 3.25 citrate buffer was continued until β alanine was eluted. β Amino isobutyric acid was then eluted by pH 4.2 citrate buffer. Ammonia is also present as a byproduct of the conversion.

## Results

Table 1 shows the Rf values for the nucleosides in the various solvent systems. Since the differences were minimal for the open ring ribosides, the nucleosides of the cRNAs were analyzed only in the closed ring form. In the native state of cRNA they exist in the open ring, ureido positive form, but the acid incubation, essential to cleave the RNA-protein bond, reanneals the pyrimidine to the closed ring (Janion and Shugar, 1960).

Table 1

Rf values for diHU and diHT
in both the closed and open ring form

| | diHU (authentic and calf thymus) | | diHT (authentic and rat ascites tumor) | |
|---|---|---|---|---|
| Ring structure | closed | open | closed | open |
| Solvent system | | | | |
| tert-BuOH, MEK, $NH_4OH$ | .78 | .19 | .62 | .25 |
| tert-BuOH, MEK, HCl | .73 | .88 | .78 | .86 |
| sec-BuOH, $H_2O$ | .24 | 0 | .125 | 0 |
| HCOOH on paper | .97 | .81 | .89 | .92 |
| HCOOH on TLC | .80 | .90 | .71 | .92 |

Figure 1 shows the elution pattern of β alanine and β amino isobutyric acid from the auto analyzer. If the elution with the first buffer is not extended until β alanine is eluted, both amino acids elute with the second buffer making the difference obscure.

The results indicate that the saturated nucleoside from ascites tumor cRNA is diHT while that from calf thymus is diHU, as shown by the Rf values

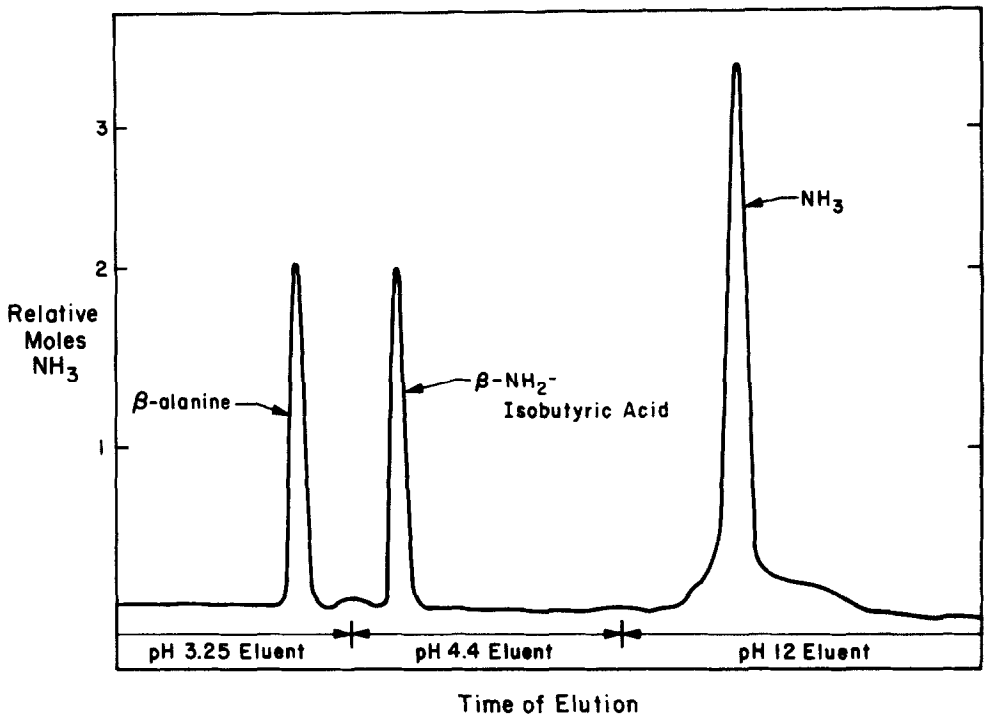


**Figure 1. A portion of an amino acid elution profile showing relative positions of β-alanine, β-$NH_2$ isobutyric acid and ammonia (a byproduct of the cleavage of dihydro pyrimidines by acid).**

Table 2

Nature and proportion of dihydro pyrimidines contained in chromosomal RNAs of varied organisms

| cRNA | Pyrimidine | % | Conversion Product |
|---|---|---|---|
| pea bud | diHU | 8.5[1] | β alanine |
| chicken embryo[2] | diHU | 9.6 | β alanine |
| calf thymus[3] | diHU | 8.5 | β alanine |
| ascites tumor | diHT | 8.1 | β amino isobutyric acid |

[1]Huang and Bonner (1965) report a higher figure. The present value is based on the more accurate analytical methods now available.

[2]Huang (1967).

[3]Shih (1967).

listed in Table 1 and the amino acid analysis. Table 2 summarizes the nature and amount of saturated pyrimidine ribosides contained in all studied cRNAs.

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