THE OCCURRENCE AND SOURCE OF β-ALANINE IN ALKALINE HYDROLYSATES OF sRNA : A SENSITIVE METHOD FOR THE DETECTION AND ASSAY OF 5,6-DIHYDROURACIL RESIDUES IN RNA.

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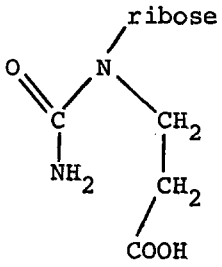
In a study of amino acids released from yeast sRNA by alkaline hydrolysis, a ninhydrin-reacting substance other than a protein-forming amino acid was detected. The substance emerged from a Beckman Model 120B Amino Acid Analyser after phenylalanine, in the general region of galactosamine, but its behaviour on paper chromatography did not correspond with this compound and suggested rather that it was an amino acid. Treatment with ninhydrin, followed by copper nitrate, resulted in a colour different from that typical of α-amino acids, suggesting that the compound might be a β- or γ-amino acid. When subjected to paper electrophoresis at pH's 1.9, 4.7 and 8.9, the compound behaved identically with β-alanine and was clearly differentiated from α- and γ-amino-n-butyric acids and α- and β-amino*iso*butyric acids. It also behaved as β-alanine both on paper chromatography in several solvent systems and on the amino acid analyser. Final proof of the identity of the two compounds was provided by a comparison of their N.M.R. spectra in D_2O and in N DCl.

The amount of β-alanine released when samples of yeast sRNA prepared according to Holley (1963) were heated in 0.2 N sodium hydroxide at 100° for 3 hours was ca. 2.5 μmole per 100 μmole mononucleotide (2.5 mole %). Heating for a further 2 hours resulted in only a marginal increase (to ca. 2.8 mole %). A 30 min. hydrolysis produced 0.9 mole %. Control experiments showed that β-alanine was neither present free in the nucleic acid samples nor derived from a contaminant originating in any of the reagents used in their preparation. Alkaline hydrolysis of two commercial samples of total yeast RNA also yielded β-alanine but in much smaller amounts.

These observations are consistent with the β-alanine being derived from sRNA chains but in view of the amount liberated and the rate of its release, not from the usual amino acid-accepting position at the 2' or 3' hydroxyl group of the terminal adenosine residue. Rather, it seemed likely that it arose by alkaline degradation of 5,6-dihydrouracil residues, recently shown to occur in yeast sRNA by Madison and Holley (1965). Such residues, whether in the form of the free base, the nucleoside or the nucleotide, are known to undergo facile ring-opening under mildly alkaline conditions to give β-ureidopropionic acid or derivatives (Batt et al., 1954; Green and Cohen, 1957; Cohn and Doherty, 1956). Further alkaline treatment could be expected to lead ultimately to the production of β-alanine.

When heated at 100° in 0.2 N sodium hydroxide for 3 hours, 5,6-dihydrouracil gave a ninhydrin-reacting material behaving as β-alanine on paper electrophoresis and chromatography and on the amino acid analyser column, but in only 22% yield. Even after 24 hours heating the hydrolysate still

gave a yellow colour with the p-dimethylaminobenzaldehyde reagent of Fink et al. (1956), indicating that the conversion of the intermediate ureido compound (β-ureidopropionic acid) to β-alanine was incomplete. However, 5,6-dihydrouridine gave a 68% yield of β-alanine after 3 hours hydrolysis; after a further 2 hours the yield had increased to 78%. Evidently, the presence of the sugar residue facilitates the cleavage of the ureido grouping in the intermediate compound, I, the sugar moiety subsequently being lost to give β-alanine.



I.

Presumably, the corresponding 2'- and 3'-phosphates of I, which would be rapidly formed from RNA under the conditions of hydrolysis employed, would show a similar lability towards alkali.

Hydrolysis of yeast sRNA in N HCl for 2 hours at 100° yielded only a trace of β-alanine, again indicating that the latter was derived from a source other than the usual amino acid-accepting position. If the acid hydrolysis was followed by a 3-hour alkaline hydrolysis β-alanine was liberated, but in an amount only 26% of that released by alkaline hydrolysis alone. This result would be expected if the β-alanine is formed by the degradation of dihydrouracil residues in the RNA chain. It is known that the glycosidic bond in dihydrouracil-riboside and -ribotide is readily cleaved by acid

(Cohn and Doherty, 1956), and so during the acid hydrolysis, free dihydrouracil would be rapidly liberated and would be relatively stable to further change. On subsequent alkaline hydrolysis, however, it would be rapidly degraded to β-ureidopropionic acid which would then break down only slowly to give β-alanine.

These results, therefore, indicate that the β-alanine found in alkaline hydrolysates of yeast sRNA originates from dihydrouracil residues present in the polynucleotide chain. Further, the estimation of β-alanine in such hydrolysates by the above procedure would seem to offer a convenient method for detecting and assaying dihydrouracil residues in RNA samples. Amounts of β-alanine down to ca. 0.01 μmole, ≡ ca. 0.15 mg of the yeast sRNA preparations used in the present work, can be successfully detected by this procedure in conjunction with an expanded scale attachment to the recorder of the amino acid analyser. Further work is in progress regarding the quantitative aspects of the procedure. Assuming similar rates of breakdown of compound I and the corresponding 2'- and 3'-phosphates to β-alanine, results so far indicate that the amount of β-alanine found in RNA hydrolysates prepared as described above (0.2 N NaOH, 3 hours, 100°) represents a yield of ca. 70% per dihydrouracil residue. Hence the procedure seems capable of providing an estimate of the number of such residues in an RNA sample. For the samples of yeast sRNA used in the present work, the 2.5 mole % of β-alanine found therefore indicates a dihydrouracil content of ca. 3.6 mole %. This may be compared with recent values found for individual yeast transfer RNA species. A value of two residues of dihydrouracil per chain (2.6 mole %) has been

found by Holley et al. (1965) for a yeast alanine transfer RNA and by Zachau et al. (1966) for two yeast serine transfer RNA's (2.4 mole %), and of six residues per chain (7.7 mole %) by Madison et al. (1966) for a yeast tyrosine transfer RNA.

Investigation of the dihydrouracil content of RNA's other than yeast sRNA by this method is in progress. A sample of E. coli sRNA obtained from Schwarz BioResearch, Inc., gave 1.5 mole % β-alanine, indicating a dihydrouracil content of ca. 2.2 mole %, whereas samples of rRNA prepared from isolated E. coli and yeast ribosomes gave only minimal values (ca. 0.02 - 0.04 mole % β-alanine). E. coli and yeast rRNA's prepared by fractionating the corresponding total RNA on powdered cellulose (Barber, 1966), also gave rise to only traces of β-alanine on alkaline hydrolysis. The formation of such low levels of β-alanine could well be due to the presence of small amounts (ca. 0.1 %) of contaminating sRNA in these preparations. If this be so, and the presence of dihydrouracil residues is characteristic of soluble but not ribosomal RNA's (but c.f. Carr and Grisolia, 1964), the detection of β-alanine in alkaline hydrolysates might, in certain cases, provide a useful supplement to analytical sucrose gradient centrifugation or methylated serum albumin chromatography in demonstrating the presence of sRNA in RNA preparations.

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