METHOD FOR SELECTIVE CHEMICAL DEGRADATION OF RNA. BASIC PRINCIPLE

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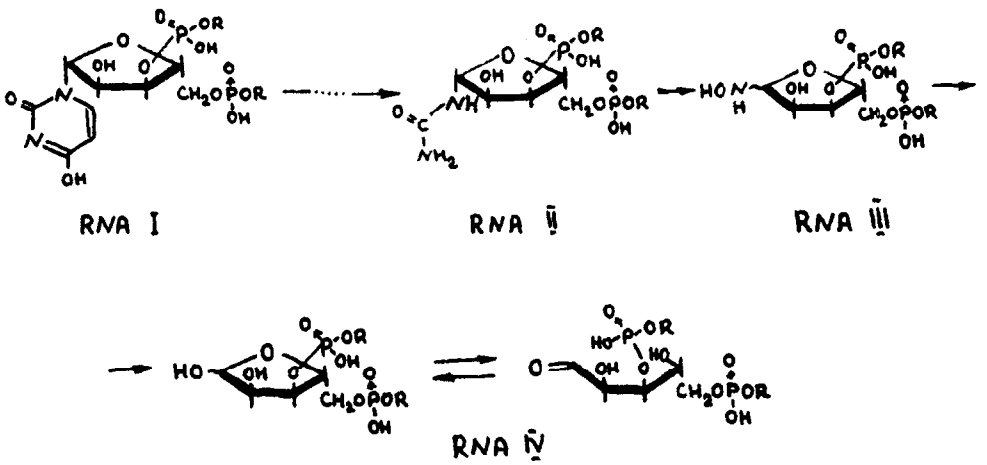
The present paper is concerned with the general idea and experimental verification of the principle of chemical modification of RNA for the purpose of selective degradation for sequence analysis.

Obviously, modification of base residue only in the polymer chain of RNA will not directly effect the stability of the phosphodiester bond. Weakening of the internucleotide bond can be achieved by appropriated interconversion of the ribose residue. One of the possibilities is cleavage of the N-glycosidic bond of appropriately modified nucleoside residue, as a result of this appeared the opportunity of tautomeric transformation of the cyclic form of the ribosyl residue to open-chain al-form.

Appearance of a carbonyl in the β-position at the 3'-phosphoester bond may direct the splitting by alkaline conditions to a way, involving β-elimination (Brown and Todd, 1955; Baron and Brown, 1955) instead of usual for RNA way - formation and splitting of cyclic esters.

This approach will be valid only if the rate of such β-elimination (V_β) is much greater, than the rate of usual alkaline hydrolysis (V_a). Presumably, a ratio $\frac{V_\beta}{V_a} \geqslant 10^2$ will be enough for selective cleavage at the modified units and its

practical uses for sequence analysis.



For verification of the approach proposed, we used the reaction of hydroxylamine with nucleosides and RNA, now studied in detail in this laboratory.

It is known, that at high pH values hydroxylamine reacts with RNA at 0° resulting in cleavage of uracyl and the formation of ribosylurea residues (Kochetkov, Budowsky et al., 1963) /I→II/. At elevated temperature or after prolonged treatment ribosylurea is transformed to ribosyloxime/I→II→III/ (cf. Schuster, 1961; Verwoerd et al., 1961, 1963).

As seen in Fig. 1, sRNA when treated with concentrated hydroxylamine at pH 10.0 and 37° *) released a theoretical amount of isoxazolidone (cf. Verwoerd et al., 1961) and urea in ca. 30 hours; after this, the amount of bound hydroxylamine remained constant, thus indicating complete substitution of uracyl by oxime groupings /I→II→III/. The modified polymer treated with cyclohexanone to remove oxime residues afforded the desired deuracil-RNA (IV) - model polynucleotide having potential al-form ribose residues in place of uracil residues of the starting RNA.

*) A detailed description of the procedure will be published elsewhere.

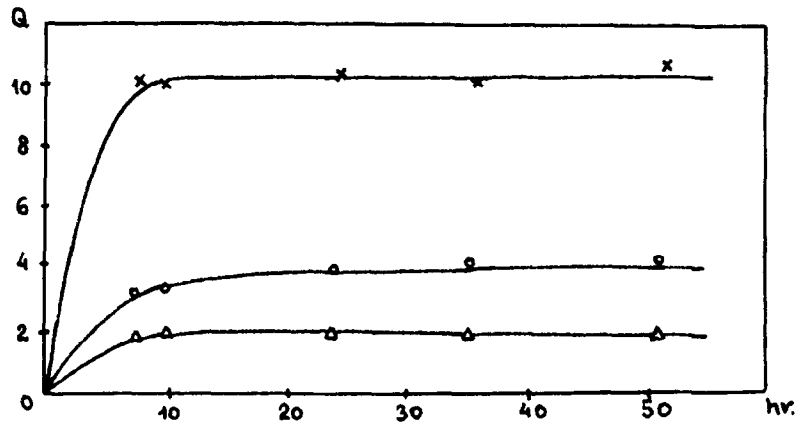
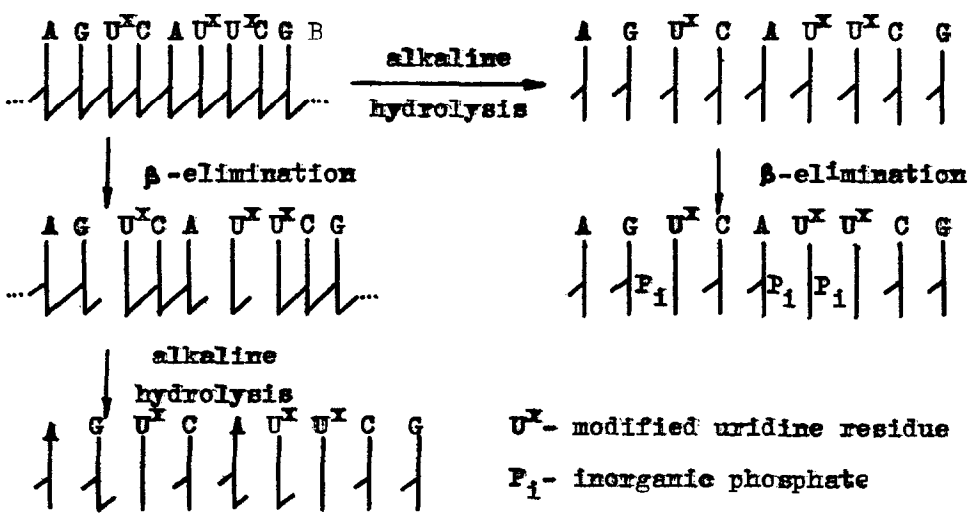


Fig. 1. The reaction of yeast sRNA with hydroxylamine.

—x—x— the isoxazolidone RNA ratio as revealed by optical densities of the polymer and monomer fractions, obtained by separation of the reaction mixture on Sephadex G-25 (%); —o—o— concentration of free urea in the reaction mixture (γ/l OU_{260}); —Δ—Δ— bound hydroxylamine (γ/l OU) in polymer fraction of the reaction mixture (Sephadex G-25), determined after hydrolysis (1N HCl, 100°, 1 hr).

Deuridylic-RNA (IV) could be cleaved by alkaline hydrolysis according to two mechanisms (i) involving β-elimination, leading to cleavage of the 3'-phosphodiester bonds at the modified residues and (ii) the usual one, leading to cleavage of the 5'-phosphodiester bonds. In order to evaluate the possibility of selective cleavage, the ratio of the rates of these reactions was to be determined.



If β-elimination is completed practically before any marked alkaline hydrolysis ($\frac{V_\beta}{V_a} \geq 100$), the content of nucleoside-2'(3')-5'-diphosphates in the hydrolysate will be equal to that of uridine in the starting RNA (18% the optical density of alkaline hydrolysate of RNA at 260mμ)*. On the other hand, if the rate of alkaline hydrolysis was of the same order or greater than that of β-elimination the amount of nucleosidediphosphates would drop down to zero; amount of inorganic phosphorous would rise up to ca 18% as limit.

As revealed by chromatography of the alkaline hydrolysate of deuridylic sRNA (sRNA IV) according to Bell et al., (1963) (Fig. 2) 18% of the optical density appeared in fact in the nucleoside diphosphate fraction; we did not find in this alkaline hydrolysate any detectable amounts of inorga-

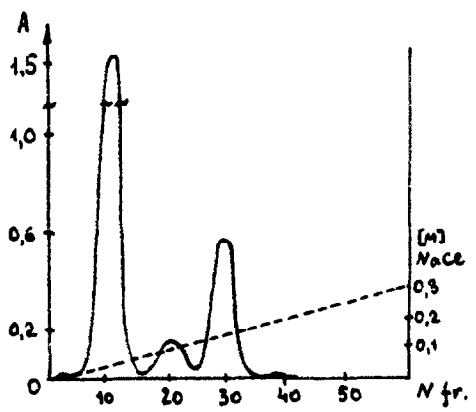


Fig. 2. The separation of alkaline hydrolysate of modified sRNA (IV) according to Bell et al.

120 OU of RNA (IV) alkaline hydrolysate (1N KOH, 37°C, 20 hrs) are separated on a column of Sephadex A-25 (1 x 10 cm) with a linear gradient of NaCl in 7 N urea; 250 ml gradient vessels.

* The content of nucleoside diphosphates was calculated on basis of the following RNA (yeast "soluble" RNA) composition: A - 19.2%, G - 25.6%, U - 18.2%, C - 26.6%, Ψ U - 4.1%, others 6.3%; molar extinction coefficients were assumed 14.5, 11.9, 9.9, 6.8, 8.6 and 10, respectively.

nic phosphorous, thus indicating the rate of β-elimination in alkaline medium to be at least 10^2-time higher than that of routine alkaline splitting of internucleotide bonds.

The above experimental evidence demonstrated the removal of base residue to be enough for effecting selective chemical degradation of RNA by β-elimination and this approach to be very useful for selective degradation and sequence analysis of RNA.

It will be mentioned also that nucleoside diphosphates formed after alkaline hydrolysis of modified RNA (IV) originate from nucleotides, neighbouring to the modified bases. This simple and convenient method for the analysis of frequency of nucleotide pairs (cf. M. Singer et al., 1959) may prove useful for studying template synthesis of RNA and afford some additional information for structural analysis of sRNA.

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