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# DYNAMICS OF ALKALINE HYDROLYSIS OF RNA OLIGOADENYLATES DURING HYDROLYSIS OF HOMOGENATES OF RAT CEREBRAL CORTEX AND LIVER

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Attention has been drawn in the literature to the exceptional resistance to alkaline hydrolysis of RNA fragments and of individual ribo-oligonucleotides, consisting of chains of adenyl nucleotides cross-linked by a 3′,5′-phosphodiester bond, namely 3′,5′-oligoadenylates (3′,5′-A), which is largely responsible for the effects of interplanar interaction between adenine bases [6]. Many such regions, consisting of 100-200 AMP molecules, also contain *m*RNAs and their precursors [10]. The functional role of the polyadenyl fragments of *m*RNA has not yet been finally settled, largely due to the absence of any simple methods of their quantitative determination [10].

It has been shown that 3′,5′-A can exhibit physiological activity similar to that of 2′,5′-oligoadenylates (2′,5′-A), which, together with *c*AMP, are mediators of the single regulatory system of the cells [2]. At the present time a search is being made among the 2′,5′-A for effective antiviral and antitumor therapeutic agents [4]. Considering also that 3′,5′-A can be converted into 2′,5′-A by the action of weakly basic ion-exchange resins [6], the results of the study of hydrolysis of RNA oligoadenylates during alkaline hydrolysis of tissue homogenates, which are not to be found in the literature, are not only of theoretical, but also of practical importance.

## EXPERIMENTAL METHOD

Alkaline hydrolysis products of RNA were obtained by the method of Schmidt and Thannhauser in Trudolyubova's modification [8]. Samples of tissue homogenates were incubated at 37°C for 1, 5, and 20 h in 0.3 M KOH [8]. After precipitation of DNA and proteins by perchloric acid, the resulting alkaline RNA digests were neutralized with ammonia and reprecipitated with $NH_4Cl$ saturated with isopropanol (1:20), and then filtered on four layers of "Filtrac-90" filter paper (East Germany), under a vacuum to remove the salts. After the filters had been washed with isopropanol and ether the alkaline hydrolysis products of RNA adsorbed on the paper were washed off with bidistilled water and evaporated to dryness in vacuo at 37°C. The

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TABLE 1. Dynamics of Hydrolysis to Yield of $UMP_n$, $GMP_n$, and AMP (in $\mu$moles/g tissue) and Changes in Calculated RNA Content (in $\mu$g/g tissue) according to Absorption of Hydrolysis Products of Homogenates of Rat Cerebral Cortex and Liver during Incubation of Tissues in 0.3 M KOH (37°C)

| Tissue | Duration of hydrolysis, h | RNA | $UMP_n$ | $GMP_n$ | CMP | AMP |
|---|---|---|---|---|---|---|
| Control | 1 | 1014±22,0 | 1,07±0,032 | 1,52±0,046 | 0,45±0,056 | 0,28±0,026 |
| | 5 | 1114±101,4 | 1,11±0,024 | 1,51±0,113 | 1,08±0,109* | 0,42±0,032 |
| | 20 | 1279±65,7* | 1,09±0,020 | 1,37±0,032 | 1,03±0,105 | 0,69±0,025** |
| Liver | 1 | 5658±541,9 | 6,31±0,092 | 9,13±1,157 | 3,60±0,475 | 1,21±0,189 |
| | 5 | 6445±263,0 | 5,31±0,195 | 9,95±0,483 | 8,04±0,574* | 1,54±0,202 |
| | 20 | 6590±366,8 | 5,05±0,308 | 9,52±0,587 | 8,41±0,696* | 2,22±0,139* |

**Legend.** Differences significant relative to data for 1-h (*$p < 0.05$) and 5-h (**$p < 0.05$) hydrolysis.

alkaline hydrolysis products of the tissue homogenates were then divided into fractions on "Dowex-50" cationic resin by the method described previously, with minor changes in the conditions of chromatography [1, 7]. Samples of RNA digests were applied to a "Dowex-50" column (0.8 × 12 cm) in 1 ml of 0.05 M HCl, with successive elution with 0.05 M HCl at the rate of 1 ml/min and collection of the UMF fraction; bidistilled water was added and the GMP fraction collected, after which 0.5 M HCl was added and the fraction of AMP and CMP together was collected. The fraction of oligonucleotides containing AMP and CMP residues was eluted from the column with 2 M HCl. Concentrations of $UMP_n$ and $GMP_n$ were calculated with the aid of molar coefficients of adsorption of UMP and GMP at 260 nm [4], and AMP and CMP in the combined fraction were determined by the corresponding molar coefficients of absorption at 260 and 280 nm, using the significant differences in their adsorption spectra at these wavelengths [4]. The RNA concentration were calculated by Spirin's method [8]. The purity of the corresponding chromatographic fractions was verified by differential spectral photometry in 0.1 M HCl and 0.1 M KOH [5].

In a series of experiments products of 1-h alkaline hydrolysis of cerebral cortical homogenates were hydrolyzed for 4 and 19 h in the presence of 0.3 M KOH, and also of 0.3 M KOH and salts 0.4 M $KClO_4$ and 0.3 M $NH_4ClO_4$, followed by neutralization by HCl, liberation from the salts, and fractionation on the cation-exchange resin Dowex-50, as described above.

Fractions of oligoadenylates obtained by fractionation of products of 5-h hydrolysis of cerebral cortical homogenates on Dowex-50 were collected, neutralized with ammonia, freed from salts as described above, and fractionated on a DEAE-Sephadex A-50 column (1 × 20 cm; from Pharmacia, Sweden) in 200 ml of a linear gradient of 0.0-0.3 M $NH_4Cl$, 0.02 M acetate buffer (pH 5.6), 7 M urea, at the rate of 1.5 ml/min according to the method described previously, with minor modifications of the conditions of chromatography [9, 10]. AMP, ADP, UMP, GMP, CMP, adenosine, and NADP (from "Reanal," Hungary), and the adenyl oligonucleotides 2′,5′-ApApA and A-5′-ppp-5′-A (from "Sigma," USA) were used as reference substances.

## EXPERIMENTAL RESULTS

Fractionation of alkaline hydrolysis products of RNA from rat cerebral cortex and liver on the cation-exchange resin Dowex-50 showed that during incubation of the samples for 1 h at 37°C in 0.3 M KOH, the total yield of nucleotide components of RNA containing $UMP_n$ and $GMP_n$ was obtained, but only an extremely low yield of AMP and CMP (Table 1). Fractionation of 5-h hydrolysis products of tissue RNA revealed a total yield of CMP from the RNA. In this case the dynamics of the yield of AMP from RNA of rat cerebral cortex and liver continued for 20 h of tissue hydrolysis and was more marked when hydrolysis of samples of the cerebral cortex was studied, possibly due to the high content of *m*RNA noted in the literature [10], which contains very long polyadenyl fragments, in nerve tissue structures (Table 1).

During incubation of products of 1-h hydrolysis of cerebral cortical homogenates for 19 h in medium containing 0.3 M KOH, and also 0.3 M $KClO_4$ 0.3 M $NH_4ClO_4$ present in RNA digests at the stage of their isolation, we found no increase in the yield of AMP and CMP compared with the results of the study of the nucleotide composition of 1-h hydrolysates of cerebral cortical homogenates (Fig. 1a, b). However, during hydrolysis of 1-h digests of cerebral cortical homogenates for 4 and 19 h in 0.3 M KOH, a yield of AMP and CMP was observed in agreement with results obtained during 5- and 20-h hydrolysis of cerebral cortical homogenates in 0.3 M KOH (Fig. 1a, c, d), evidence that alkaline digests of cerebral cortical homogenates contain oligonucleotides of low molecular weight, consisting of adenylic and cytidylic acids, and not precipitated by $HClO_4$, along with DNA and proteins, the hydrolysis of which in KOH depends on the ionic composition of the medium.

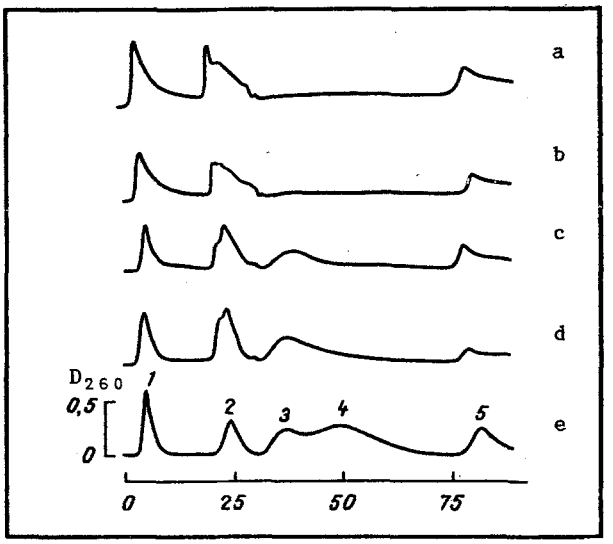


Fig. 1. Fractionation of DNA hydrolysis products on column of cation-exchange resin Dowex-50, obtained on incubation of rat cerebral cortical homogenates in 0.3 M KOH before (a) and after their 19-h hydrolysis in the presence of 0.3 M KOH, 0.3 M $KClO_4$, and 0.3 M $NH_4ClO_4$ (b), and also 4-h (c) and 19-h (d) hydrolysis in 0.3 M KOH. Abscissa, volume of eluate (in ml); ordinate, optical density units at 260 nm ($D_{260}$). e) Control fractionation: 1) UMP, 2) GMP, 3) CMP, 4) AMP, 5) oligoadenylate ApApA.

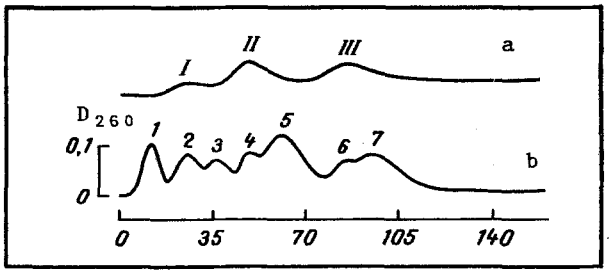


Fig. 2. Fractionation of RNA oligoadenylates of 5-h digest of rat cerebral cortical homogenates on DEAE-Sephadex A-50 column (a). Abscissa, volume of eluate (in ml); ordinate, their optical density units at 260 nm. b) Control fractionation: 1) adenosine, 2) oligoadenylate Ap-Ap-A, 3) $AMP^{2-}$, 4) oligoadenylate Ap-p-p-A, 5) $ADP^{2-}$, 6) $NADP^{4-}$, 7) $ATP^{4-}$.

On separation of fractions of oligoadenylates of 5-h digests of cerebral cortical homogenates depending on the number of negatively charged phosphate groups and of positively charged bases on the DEAE-Sephadex column, we found three mobilities (Fig. 2) which, considering that during alkaline hydrolysis of RNA only nucleotides and oligonucleotides with a phosphate group at the 2- or 3-end of the molecule are formed, and that their removal can also be catalyzed by metal ions under alkaline conditions [6], correspond to the oligoadenylates: I) Ap-Ap-A; II) Ap-$Ap^{2-}$, Ap-Ap-Ap-A, and IIII Ap-Ap-$Ap^{2-}$; Ap-Ap-Ap-Ap-A (Fig. 2).

The results are in agreement with data in the literature on the increase in resistance of oligoadenylates to alkaline hydrolysis with reduction of their molecular weight [6] and they are important in connection with the development of economic methods of obtaining drugs affecting the 2',5'-A system, and also in the use of various techniques used to study the content and composition of RNA in tissues.

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