oxidative phosphorylation has recently been detected (Cross *et al.*, 1970), it would appear that the second interpretation is not right.

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Effects of Dimethyl Sulfoxide on the Degradation of Ribonucleic Acid Catalyzed by Alkali*

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ABSTRACT: The degradation of RNA by alkali has been measured in media containing Me$_2$SO, a strong nucleic acid denaturant. At 37°, in 0.1 M NaOH, the degradation, measured as the disappearance of acid-precipitable material follows first-order kinetics with a half-life of 23 min (k = 0.03 min^{-1}). Increasing Me$_2$SO concentrations stimulate the degradation rate, until at 60% (v/v) Me$_2$SO the half-life is 1.1 min (k = 0.63 min^{-1}).

Similar kinetic effects are seen following the disappearance of material excluded from Sephadex G-25 (no Me$_2$SO, k = 0.14 min^{-1}; 60% Me$_2$SO, k = 1.39 min^{-1}). The rate enhancement produced by Me$_2$SO is seen between 0.01 and 0.3 M NaOH. Activation enthalpies in 0.1 M NaOH do not differ between 0 and 60% Me$_2$SO (21,200 and 20,700 cal mole^{-1}, respectively). Following the degradation kinetics by means of chromatography on DEAE-Sephadex in the presence of urea, it is seen that in 0.1 M NaOH–60% Me$_2$SO at 55°, in 1 hr more than 96% of RNA has been converted into mononucleotides. This is applicable to the determination of nucleotide composition, where results comparable to other established procedures are obtained. Me$_2$SO also increase the degradation of the dinucleotide UpA, but to a lesser extent than what is seen with polyribonucleotides (k = 0.24 and 0.58 hr^{-1}, for 0 and 60% Me$_2$SO, respectively).

Dimethyl sulfoxide, as well as other aprotic solvents such as formamide, is frequently used as a denaturing agent for nucleic acids (Helmkamp and Ts'o, 1961; Katz and Penman, 1966; Legault-Démare *et al.*, 1967). Although the exact manner by which it acts is not clear, it is generally assumed that its denaturing effectiveness is due to its ability to act as a rather strong acceptor for hydrogen bonding (Lindberg, 1961).

In a different experimental direction, it has been reported that a great number of base-catalyzed reactions, including ester saponification and nucleophilic displacements, are greatly accelerated when performed in media containing Me$_2$SO (Kingsbury, 1964; Roberts, 1965, 1966).

RNA is very sensitive to degradation in alkaline aqueous solutions, but there is no information concerning its sensitivity to alkali in Me$_2$SO–water mixtures. We now wish to report the marked changes in degradation kinetics that occur in such systems, as well as experiments that try to explain the mechanisms involved.

Materials and Methods

The progress of alkali-catalyzed degradation of RNA, measured as the disappearance of acid-precipitable material was as follows. Incubation mixtures, containing NaOH, NaCl when appropriate, and water or Me$_2$SO–water mixtures in a volume of 0.9 ml, were brought to the desired temperature, and the reaction was started by adding 0.1 ml of a solution containing 20 mg/ml of yeast RNA, also equilibrated to the desired temperature. Mixing was made in less than 10 sec. At appropriate intervals 0.1-ml aliquots were taken and added to test tubes containing 0.8 ml of ice-cold 10% perchloric acid–0.25% uranyl acetate; 0.2 ml of 10% beef serum albumin was added as a coprecipitant and the mixture was agitated. After 45 min at 5° the tube contents were filtered through Whatman No. 42 paper. Two-tenth milliliter of the filtrate was

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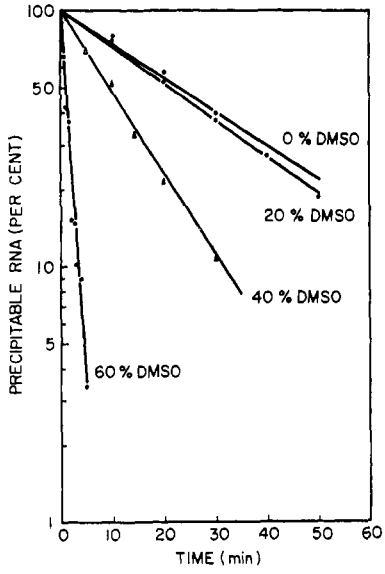

FIGURE 1: Kinetics of RNA degradation in 0.1 M NaOH. Incubation was at 37°. The ordinate indicates the remaining RNA precipitable by perchloric acid–uranyl acetate as described in Methods. Per cent Me_2SO is v/v.

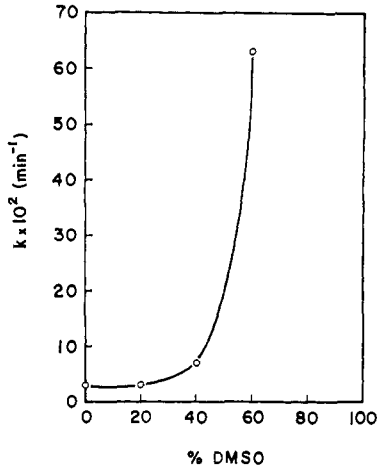

FIGURE 2: Apparent first-order rate constants at different Me_2SO concentrations. Rate constants were obtained from the data of Figure 1, using the formula $k = 0.693/t_{1/2}$.

diluted tenfold with 10% perchloric acid and its absorbance was measured at 260 mμ.

Zero per cent degradation was estimated by adding one-tenth volume of each component of the incubation mixture directly to cold perchloric acid–uranyl acetate. One-hundred per cent degradation was obtained by incubating RNA (2 mg/ml) in 0.1 N NaOH for 18 hr at 37°, and processing it as indicated.

Chromatography on DEAE-Sephadex was essentially according to Ishikura *et al.* (1966), with the following exceptions. The column (1 × 32 cm) was equilibrated with 0.1 M NaCl, 7 M urea, and 0.02 M Tris-HCl (pH 7.45). Samples (0.2 ml) from the incubation mixtures were neutralized with HCl and applied to the column. Elution was with a linear 0.1–0.3 M NaCl gradient, at a flow of 0.5 ml/min. Fractions (1.6 ml) were collected and their optical density at 260 mμ was measured. The column was calibrated with cytidine, all four 2′- and 3′-mononucleotides, and ADP before use. NaCl molarities were measured with a Radiometer CDM2 conductivity meter.

Gel filtration was done on a 1 × 53 cm column of Sephadex G-25 (20–80-μ beads) equilibrated with 0.1 M NaCl–0.02 M Tris-HCl (pH 7.45). Flow rate was 0.3 ml/min and 1-ml fractions were collected.

The cleavage of UpA[1] was measured as follows. Test tubes containing, in a volume of 1 ml, NaOH (100 μmoles), UpA (0.2 mg), and, when appropriate, Me_2SO (0.6 ml), were incubated at 37°. At the intervals indicated in Figure 8, 0.2-ml aliquots were withdrawn and added to sufficient HCl to give a final pH of 3.2. This was quantitatively applied to a 0.9 × 3 cm column of Dowex 1-X8 (200–400 mesh) equilibrated with 0.001 N HCl. Elution was at a flow rate of 0.4 ml/min with 0.001 N HCl up to an elution volume of 25 ml, then 1 N HCl was passed through the column until the total eluted volume was 60 ml. All of the cleaved adenosine is eluted in the first 17 ml, intact UpA, 2′,3′-cyclic UMP, and 2′- or 3′-UMP are eluted with 1 N HCl. The percentage of dinucleotide cleaved was calculated measuring the quantity of free adenosine in each experimental point as well as that produced under conditions of complete dinucleotide cleavage.

All the first-order rate constants reported are apparent, and were calculated from $k_{obsd} = 0.693/t_{1/2}$.

The separation and estimation of ribonucleotides was done by the procedure of Katz and Comb (1963).

Rat liver ribosomes were obtained following Palade and Siekevitz (1956). *E. coli* and wheat-germ ribosomes were obtained by the methods of Nathans and Lipmann (1961) and Allende (1967), respectively, and were a gift of Dr. Jorge E. Allende. RNAs were obtained from them by the phenol procedure of Kirby (1956). Yeast RNA was also purified by the Kirby method.

Me_2SO was obtained from Mann Research and from Recalcine Laboratories. The same results were obtained with these two preparations as well as with Me_2SO distilled at reduced pressure. Yeast RNA (type XI) and UpA were from Sigma Chemical Co. Sephadex and DEAE-Sephadex were from Pharmacia, Uppsala.

Results

The degradation of RNA in 0.1 M NaOH, measured by the disappearance of acid-precipitable material, follows first-order kinetics (Figure 1), with a half-life at 37° of 23 min. Increasing Me_2SO concentrations progressively reduce the half-life, until at 60% (v/v) it becomes 1.1 min. This rate increase is a non-linear function of the concentrations of Me_2SO (Figure 2). Due to poor solubility of RNA, no satisfactory kinetic data are available at higher Me_2SO concentrations. Similar kinetic effects are obtained by measuring the rate of disappearance of the RNA that is excluded from Sephadex G-25 (see Methods) (0% Me_2SO, $k = 0.14$ min^{-1}; 60% Me_2SO, $k = 1.39$ min^{-1}). Although both methods measure somewhat different reaction products, namely, Sephadex G-25 excludes and perchloric acid–uranyl acetate precipitates nucleotides of approximate

[1] Abbreviation used is: UpA, uridilyl-3′,5′-adenosine.

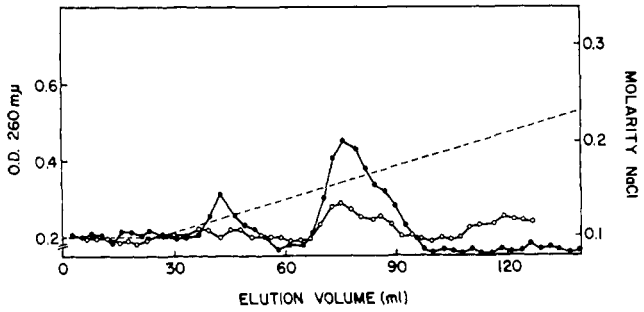

FIGURE 3: DEAE-Sephadex chromatography of RNA degradation products. The column was equilibrated with 7 M urea–0.1 M NaCl–0.02 M Tris-HCl (pH 7.45) as described in Methods. Samples were incubated for 30 min in 0.1 M NaOH at 37°, neutralized, and run separately on the same column. (O-O-O) incubated without Me$_2$SO, (●-●-●) with 60% Me$_2$SO, and (- - -) NaCl concentration. Nucleosides were eluted between 15 and 25 ml, 2′,3′-cyclic nucleotides between 35 and 65 ml; 2′- and 3′-nucleotides between 65 and 95 ml, and ADP (three negative charges) between 100 and 120 ml.

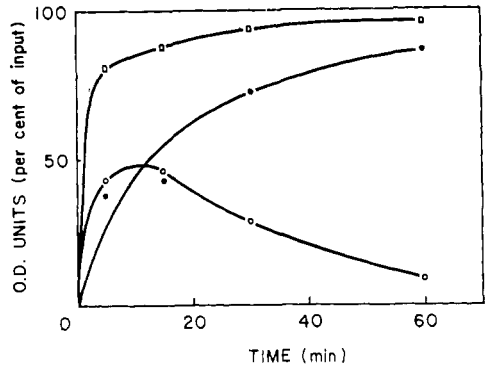

FIGURE 4: Time course of mononucleotide appearance during RNA degradation in 60% Me$_2$SO. Yeast RNA was incubated in 0.1 M NaOH–60% Me$_2$SO at 55°. Samples were withdrawn and neutralized at the times indicated, and then chromatographed on DEAE-Sephadex with 7 M urea (see Methods). Total optical density units at 260 mμ applied to the column, were obtained by hydrolyzing an amount of RNA equivalent to the one present in each sample, in 0.1 M NaOH at 37° for 18 hr. (O-O-O) Cyclic nucleotide peak, (●-●-●) 2′- and 3′-nucleotides, and (□-□-□) sum of both peaks.

chain length 15 and 4, or larger, respectively, the results obtained are in reasonable agreement.

To determine more precisely the nature of the products formed, samples were withdrawn from reaction mixtures, with no or 60% Me$_2$SO, and chromatographed on DEAE-Sephadex in the presence of urea. After 30-min incubation there is a larger amount of material appearing in the region of two negative charges (2′- and 3′-nucleoside monophosphates), with those samples containing Me$_2$SO (Figure 3). In these samples there is also a peak in the region of one negative charge, corresponding to 2′,3′-cyclic nucleotides. A plot of the appearance of these Me$_2$SO-related fractions against time indicates (Figure 4), aside from the known precursor–product relationship between cyclic and open mononucleotides, that at 60-min incubation, with 60% Me$_2$SO 96% of the RNA is in the form of mononucleotides mainly in the open form, and that after 5-min incubation, the earliest time point examined, there were already no detectable oligonucleotides in the column fractions. These results can be explained by assuming that, in 60% Me$_2$SO, the transesterification of the internucleotidic phosphoryl group from the 5′- to the 2′-hydroxyl, which is the basic mechanism of RNA degradation in alkali, proceeds at a very fast rate, while the hydrolysis of the cyclic nucleoside 2′,3′-phosphodiester, proceeds at a much slower, rate-limiting, pace.

The degradation of RNA in 60% Me$_2$SO at 56° for 1 hr conserves the base structure as well as do conventional hydrolytic procedures (Table I).

Rate increases are produced by Me$_2$SO at NaOH concentrations ranging from 0.01 to 0.1 M, at constant ionic strength (Figure 5). Even at the lower concentration tested, there is an eightfold difference in rate constants. Increasing the hydroxide concentration to 0.3 M increases the degradation rate only slightly (about 20%).

Because of its implications concerning the mechanism of the Me$_2$SO effect, activation enthalpies were determined from Arrhenius activation energies, by the procedure outlined by Daniels and Alberty (1961) (Figure 6). Values for activation enthalpies did not differ significantly between 0 and 60% Me$_2$SO (21,200 and 20,700 cal mole^{-1}, respectively).

When the dinucleotide UpA was used as a model compound, 60% Me$_2$SO also enhances the degradation rate (Figure 7) (0% Me$_2$SO, k = 0.24 hr^{-1}, 60% Me$_2$SO, k = 0.58 hr^{-1}), but to a much lesser extent than in the case of polyribonucleotide degradation.

TABLE I: Nucleotide Composition of Different RNAs after Degradation in Me$_2$SO.[a]

		0% Me$_2$SO, 18 hr, 37°		60% Me$_2$SO, 1 hr, 55°	
	Nucleotide	μmoles/μmole of RNA	Std Dev	μmoles/μmole of RNA	Std Dev
Rat liver	AMP	16.0	±1.01	16.8	±0.68
	UMP	20.4	±0.14	21.0	±0.46
	GMP	33.6	±1.20	33.1	±0.43
	CMP	29.9	±0.28	29.1	±0.55
Wheat-germ rRNA	AMP	18.5	±1.00	19.3	±1.30
	UMP	24.2	±1.25	24.8	±0.56
	GMP	30.1	±1.72	29.3	±1.32
	CMP	27.3	±0.38	26.6	±0.46
E. coli rRNA	AMP	22.8	±1.04	21.1	±0.37
	UMP	20.5	±0.65	22.8	±0.37
	GMP	32.2	±1.15	32.5	±0.57
	CMP	24.6	±0.75	23.6	±0.53
Yeast RNA	AMP	20.4	±0.73	21.5	±0.82
	UMP	28.5	±0.98	29.0	±1.45
	GMP	29.0	±0.68	28.3	±0.28
	CMP	21.9	±0.94	21.6	±0.49

[a] Estimation of nucleotide composition was done by the procedure of Katz and Comb (1963). All values reported are mean plus and minus standard deviation of four determinations.

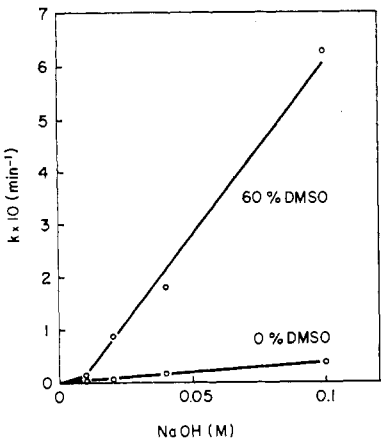

FIGURE 5: Degradation kinetics at different hydroxide concentrations. Incubations were at 37° and ionic strength was adjusted to 0.1 with NaCl. Rate constants were measured following the disappearance of acid-precipitable material, as described in Methods.

Discussion

The present findings reveal a new biochemical effect of Me_2SO, that has to be added to the rather broad spectrum of its biological and chemical actions (Weyer, 1967). Because of the magnitude of its influence on the stability of RNA in alkaline solutions, it should be used as a RNA denaturant with due precaution. On the other hand, this effect is such, that it allows a very fast degradation of RNA to mononucleotides, which has analytical applications, especially for designing a completely automated procedure for determining nucleotide compositions of RNA. Under the conditions used in Table I (1 hr at 55°), virtually all the RNA was as mononucleotides, but still 9% of these were cyclic. This is unimportant for the cation-exchange nucleotide separation employed, but will be significant when separations are done on anion-exchange columns or by electrophoresis. In these cases longer degradation times should be employed.

Of the many known physical and chemical properties of Me_2SO (MacGregor, 1967), several appear more likely involved in the effect described. Thus its dielectric constant,

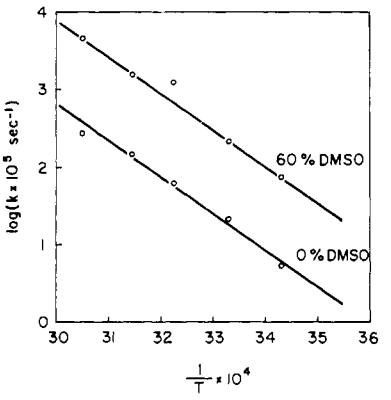

FIGURE 6: Arrhenius plot of RNA degradation kinetics. Incubations were in 0.1 M NaOH, and rate constants estimated by measuring the disappearance of RNA precipitable by perchloric acid–uranyl acetate.

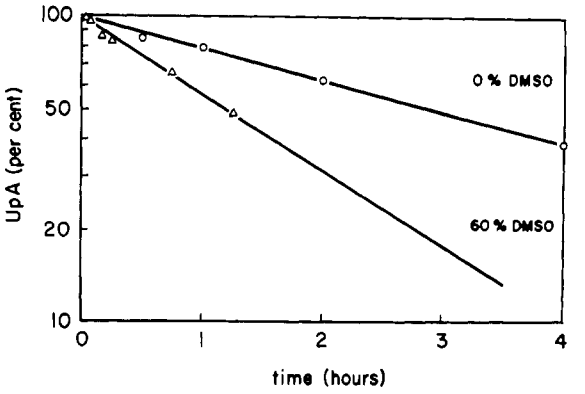

FIGURE 7: UpA Degradation at 0 and 60 % Me_2SO. UpA (0.2 mg/ml) was incubated in 0.1 M NaOH at 37°. Dinucleotide breakdown was followed as indicated under Methods.

lower than that of water (46.7 and 78.5, respectively), has been shown to account for the rate enhancements produced by it in the saponification of branched-chain esters (Roberts, 1965). In the case of RNA degradation, the mechanism by which the ribose 2′-hydroxyl approaches the phosphorus atom, as seen in molecular models, is through the rotation around the internucleotidic carbon–oxygen and oxygen–phosphorus bonds. A decrease in medium dielectric constant will increase the repulsion between the charged phosphates, and lead to an extension of the polynucleotide chain (Jordan, 1960). This will allow a freer rotation around the internucleotidic bonds and increase the probability of bond formation between the 2′-hydroxyl and the phosphorus. On the other hand, a decrease in dielectric constant will lead to a greater repulsion between the polarized (or charged) 2′-oxygen and the negatively charged phosphate oxygens, as well as to a larger attraction between the same oxygen and the polarized phosphorus atom. These multiple interactions are mostly in favor of a faster degradation, but no simple correlation between dielectric constant and reaction rate is obtained, as has been done in simpler systems (Glasstone *et al.*, 1941).

It also seems possible that, due to the large dipole moment of Me_2SO (Schläfer and Schaffernicht, 1960), it may induce a polarization of the 2′-ribose hydroxyl, allowing it to make a more effective attack on the internucleotidic phosphorus. It is also known that Me_2SO forms with water hydrogen bonds that are 1.3 times stronger than those between water molecules themselves (Cowie and Toporowski, 1961). This makes Me_2SO an "order-destroying" agent for water structure (Kingsbury, 1964), a property that, due to the highly organized water that surrounds biopolymers, could be especially significant in explaining why the Me_2SO effect is greater on RNA than on a dinucleotide.

At mole fractions of 0.5 or larger, Me_2SO displaces water from the hydration shell of hydroxide ions, without formation of an equivalent Me_2SO shell. This raises considerably the activity of this ion (Kingsbury, 1964). However, in the effect on RNA degradation, rate enhancements appear at concentrations of Me_2SO (40% = 0.18 mole fraction), that are far too low to be explained by such a mechanism. Similarly, a decrease in activation enthalpy, which is the mechanism by which Me_2SO enhances nucleophilic displacement, is clearly not operative in the present effect (Figure 6). Finally, because

at pH 12 or higher, nucleic acids are completely denatured (Mathieson and Matty, 1957), it seems unlikely that the denaturing effect of Me_2SO is playing a role in the enhanced RNA degradation.

Acknowledgment

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Binding Properties of Dye-Tagged Polylysine Complexed to Deoxyribonucleic Acid*

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ABSTRACT: Emission properties of dansyl-labeled polylysine and dansyl-labeled polylysine complexed to DNA have been studied. Perrin plots of the fluorescence of the complex show three regions. The first, which occurs at high viscosity and low temperatures, is the same as a portion of the Perrin plot of the free dansyl-polylysine. The second region is of constant polarization, in which the anisotropy is appreciably above that of the free dansyl-polylysine. The third is a region concave upward, characteristic of a thermally activated rotation about one or more single bonds. In the dissociation of the complex by most salts, the fraction of polylysine bound is shown to be the same function of the anisotropy though salts of widely differing dissociating power are used. In general the turbidity is not proportional to the amount of complex present. It depends on the type of salt in the solution and on the salt concentration. It decreases with increasing excess DNA. The scattering unit, however, is well defined. Excess DNA solubilizes the complex and lowers the turbidity.

The interaction of polylysine with DNA has been studied extensively by a variety of techniques and the following properties are known. (1) At low salt concentrations, polylysine–DNA complex formation is irreversible, in the sense that DNA added, after the complex is formed, will not exchange with the DNA in the complex in times of the order of 1 day (Tsuboi *et al.*, 1966). At 1 M NaCl exchange is rapid (Leng and Felsenfeld, 1966; Shapiro *et al.*, 1969). (2) The complex has a definite stoichiometry of one lysine to one DNA phosphate (Olins *et al.*, 1967). (3) Polylysine stabilizes the DNA against melting (Raukas, 1965; Ohba, 1966; Tsuboi *et al.*, 1966; Olins *et al.*, 1967, 1968). (4) The binding is cooperative (Raukas, 1965; Tsuboi *et al.*, 1966; Leng and Felsenfeld, 1966; Olins *et al.*, 1967; Shapiro *et al.*, 1969). (5) In 1 M salt, the interaction is selective for (A-T)-rich DNAs (Leng and Felsenfeld, 1966; Shapiro *et al.*, 1969). (6.) The optical rotatory properties of solutions of complex are markedly different than those of DNA solutions (Cohen and Kidson, 1968; Inoue and Ando, 1968, 1970; Shapiro *et al.*, 1969).

Our laboratory has recently published a study of the interaction of DNA and polylysine, using the technique of polar-

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