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Light-Induced Free-Radical Reactions of Nucleic Acid Constituents. Effect of Sequence and Base–Base Interactions on the Reactivity of Purines and Pyrimidines in Ribonucleotides[†]

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ABSTRACT: The reaction with 2-propanol of purines and pyrimidines, induced photochemically with light of λ >300 nm and di-*tert*-butyl peroxide as an initiator, was applied to a variety of adenosine-, guanosine-, and uridine-containing ribonucleotides in order to determine the rules which govern the reactivity of the heterocyclic bases of nucleotides. The reactivity of the purine moieties was found to depend on the conformation of the appropriate nucleotide (anti or syn) and on the site of binding of the phosphate group to the ribose moiety. Adenosine moieties (assuming an anti conformation) blocked at their 3′-hydroxyl reacted faster than those blocked at their 5′-hydroxyl. The reactivity of the guanosine moieties (tending to assume a syn conformation) was independent of the site of binding of the phosphate. The uridine moieties of the various nucleotides exhibited a wide range of reactivity. A correlation between the reactivity of the uridines and their involvement in stacking interactions with next- and second-neighboring purines could be made. Thus, the uridine moieties of U-U-U, G-U, U-G, A-U-A, and A-U-G were reactive, while those of A-U and A-U-U were unreactive. The relative reactivity of uridine moieties of nucleotides can, therefore, be used as a measure of the extent of pyrimidine–purine stacking and vice versa.

Exposure of cells to ultraviolet irradiation leads to the induction of a variety of chemical changes in the genetic material. These changes are manifested in biological effects, such as mutations, aging, carcinogenesis, and lethality (see reviews by Setlow, 1968; Burr, 1968; Rahn, 1972; Varghese, 1972; Smith, 1976). The multiplicity of photoproducts interferes with the chemical characterization and the direct assignment of a given chemical change to the consequent biological effect. We have approached this problem through the development of selective photochemical reactions for the various moieties of nucleic acids (Frimer et al., 1976). Accordingly, some selective photochemical modifications of purine and pyrimidine moieties of DNA have been developed (Ben-Ishai et al., 1973; Salomon and Elad, 1974a; Lorberbaum et al., 1976). In a previous publication (Havron et al., 1976), we formulated some rules which govern this selectivity in the reactions of adenine and

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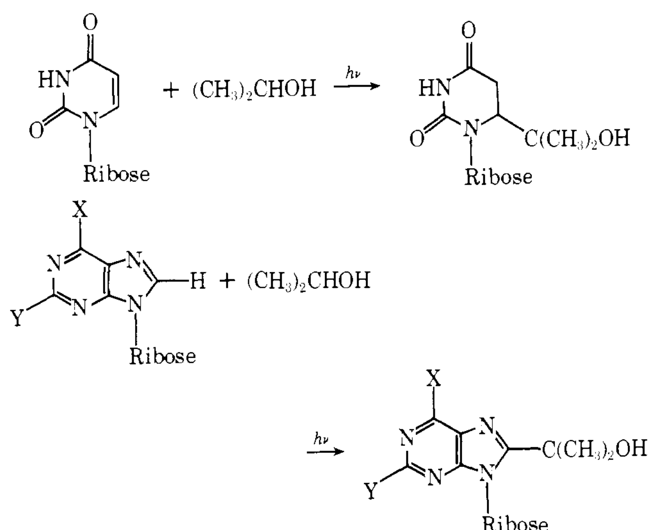
SCHEME I.



Adenosine (X = NH_2, Y = H); Guanosine (X = OH, Y = NH_2)

uridine moieties of dinucleoside monophosphates. However, the extension of these rules to other bases and their validity in the reactions of oligo- and polynucleotides required further verification. The derivation of such rules should make possible the identification of ultraviolet-sensitive regions in the nucleic acid molecule and the localization of the corresponding photoproducts. Furthermore, such studies will provide a means for the determination of the photochemical and chemical reactivity of the various bases as a function of their location in the polynucleotide chain and thereby serve as a probe for studying the spatial relationship of the bases in a given nucleic acid sequence.

Photochemical free-radical-type reactions of purines and pyrimidines with a variety of organic compounds have been described by us recently (Leonov and Elad, 1974a; Salomon and Elad, 1974b). With purines these reactions result in the substitution of the appropriate moiety for the H-8 atom of the purine system, with uracil the reactions resulted in addition across the 5,6 double bond, and with thymine there was either substitution at the C-5 methyl group or addition across the 5,6 double bond. These reactions could be induced either directly with ultraviolet light of $\lambda > 260$ nm or with light of $\lambda > 300$ nm in the presence of a photoinitiator, e.g., di-*tert*-butyl peroxide [$(t\text{-BuO})_2$].[1] The reactions of adenosine, guanosine, and uridine moieties with 2-propanol are typical examples of these reactions and are presented in Scheme I. Since a single product of either purine or pyrimidine with 2-propanol was obtained in reactions initiated with light of $\lambda > 300$ nm and the peroxide, these reactions were chosen to be used for the comparison of the reactivity of the heterocyclic bases in various systems.

We found that the reactions of uracil and uridine with 2-propanol were usually faster than those of adenine and adenosine. However, in mixtures of the bases or of the nucleosides the reactions were selective for the purines (Frimer et al., 1976; Leonov and Elad, 1974b). It was further found that the reactivity of adenosine moieties in monoribonucleotides and in diribonucleoside monophosphates depended on the site of binding of the phosphate group to the sugar ring (Havron et al., 1976). Thus, an adenosine blocked at its 3′-position was more reactive than a 5′-blocked adenosine. The uracil moieties of 3′- and of 5′-UMP were modified with nearly equal rates, as was the case for both uracil moieties of U-U. However, the uracil moiety of U-A was tenfold more reactive than that of A-U, indicating the sequence dependence of the reactivity of the uracil moieties. It was assumed that this difference in the reactivity resulted from an intramolecular base-stacking type association of the bases (Havron et al., 1976).

In the current investigation, selected mono-, di-, and triribonucleotides were reacted photochemically with 2-propanol in order to verify the rules governing the selectivity of the reaction with a wide range of nucleotides. Here we report the application of the reactions to adenosine-, guanosine-, and uridine-containing systems. It has been found that the difference in the reactivity of the guanosine moieties in the isomeric 3′- and 5′-blocked mononucleotides and dinucleoside monophosphates is smaller than that observed for the corresponding adenosine-containing systems; similarly, the difference in the reactivity of uridine in sequence isomers of uridine–guanosine dinucleoside monophosphates is much smaller than that observed for the corresponding uridine–adenosine systems. These and our previous results lead us to conclude that the reactivity of purine moieties depends on the conformation of the appropriate nucleotide (anti or syn) and on the site of binding of the phosphate group to the ribose in anti conformers. The uridine moieties of the various nucleotides exhibited a wide range of reactivity, depending on their involvement in stacking interactions with the next- and even the second-neighboring purine moiety.

Experimental Procedures

Materials. Mononucleosides, dinucleoside monophosphates, and trinucleoside diphosphates were purchased from Sigma. A-U-A was prepared according to Thach (1966). 2-Propanol and $(t\text{-BuO})_2$ (Merck-Schuchardt, Munich) were of analytical grade. Snake venom phosphodiesterase was purchased from Boehringer. Polynucleotide phosphorylase, primer dependent, from *Micrococcus luteus*, was from P-L Biochemicals. Authentic samples of 8-α-hydroxyisopropylpurines and their nucleosides and nucleotides were prepared according to Salomon and Elad (1973). 6-α-Hydroxyisopropyl-5,6-dihydrouridine and 6-α-hydroxyisopropyl-5,6-dihydrouridine monophosphate were prepared according to procedures described by Frimer et al. (1976).

Irradiation Procedure. Irradiations were carried out with a 450-W Hanovia high-pressure mercury vapor lamp, inserted in a water-cooled Pyrex jacket to cut light of wavelengths shorter than 300 nm. The lamp was covered with a copper network of 100 mesh to reduce light intensity. Reaction mixtures consisted of the nucleotides (0.25–0.40 mM) and $(t\text{-BuO})_2$ (0.5 M) in a mixture (3:2, v/v) of 2-propanol and sodium cacodylate (5 mM, pH 7). In reactions of GMP, G-U, and U-G no cacodylate was used. The reaction mixtures, in 3-mL spectrophotometric Pyrex cells, were flushed with nitrogen for 15 min and irradiated at 25 °C at a distance of 2 cm from the light source.

Light Intensity. The reaction described is photoinitiated by $(t\text{-BuO})_2$ and light of $\lambda > 300$ nm which is absorbed by $(t\text{-BuO})_2$ (end absorption up to ca. 350 nm). The efficient light intensity, i.e., the amount of photons at the region 300–350 nm which was absorbed by the reaction mixture, was determined by subtracting the intensity transmitted through the reaction mixture from the incident intensity. A benzophenone–benzhydrol actinometer was used according to Rosenthal and Bercovici (1976), and the efficient light intensity thus measured was $0.9\text{–}1.0 \times 10^{-8}$ einstein min^{-1} cm^{-2}.

Separation, Identification, and Quantitative Determination

[1] Abbreviation used: $(t\text{-BuO})_2$, di-*tert*-butyl peroxide. Abbreviations used for nucleotides are according to the IUPAC–IUB Commission on Biochemical Nomenclature (1971), *J. Mol. Biol. 55*, 299–310.

TABLE I: R_f Values of Nucleosides, Nucleotides, and Their Photoproducts.

Compound	Solvent system[a] A	B	C	D[b]	E
2′(3′)-AMP	0.18	0.33	0.29	0.15	0.53
8-α-Hydroxyisopropyl-2′(3′)-AMP	0.33	0.47	0.46	0.25	0.55
5′-AMP	0.20	0.28			
8-α-Hydroxyisopropyl-5′-AMP	0.33	0.38			
Adenosine	0.59	0.66		0.81	0.73
8-α-Hydroxyisopropyladenosine	0.72	0.80		1.03	0.83
2′(3′)-GMP	0.13	0.16		0.06	
8-α-Hydroxyisopropyl-2′(3′)-GMP	0.30	0.28		0.16	
5′-GMP	0.13	0.16			
8-α-Hydroxyisopropyl-5′-GMP	0.30	0.28			
Guanosine	0.55	0.45	0.39	0.68	0.40
8-α-Hydroxyisopropylguanosine	0.65	0.52	0.52	0.88	0.47
2′(3′)-UMP	0.19	0.31	0.29	0.24	0.26
Uridine	0.65	0.62		1	0.40

[a] Compositions of solvent systems are as follows: (A) ethanol-1 M ammonium acetate, pH 7.5 (7:3, v/v) (ascending); (B) 2-propanol-water-concentrated ammonium hydroxide (3:1:1, v/v) (descending); (C) 1-propanol-concentrated ammonium hydroxide-water (6:3:1, v/v) (descending); (D) ethanol-1 M ammonium hydroxide, pH 7.5 (85:15, v/v) (descending); (E) isobutyric acid-concentrated ammonium hydroxide-water (66:1:33 v/v) (ascending). [b] R_f values are relative to uridine taken as unity (the solvent was allowed to drip off the paper).

TABLE II: Chromatographic Fractionation of Alkaline Hydrolysates of Modified Trinucleoside Diphosphates.

Nucleotide	Solvent system, moiety determined[a] (position in sequence[b]) First dimension	Second dimension	Third dimension
A-U-U	D, uridine (3′-t) or E, UMP (m); uridine (3′-t)	E, AMP (5′-t); AMP*[b] (5′-t); UMP (m) A, AMP (5′-t); AMP* (5′-t)	
A-U-A	E, UMP (m)	A, AMP (5′-t); AMP* (5′-t); adenosine (3′-t); adenosine* (3′-t)	
U-A-A	D, adenosine (3′-t); adenosine* (3′-t) or E, UMP (5′-t)	E, UMP (5′-t); AMP (m); AMP* (m) A, AMP (m); AMP* (m); adenosine (3′-t); adenosine* (3′-t)	
A-U-G	E, UMP (m)	A, AMP (5′-t), AMP* (5′-t)	C, guanosine (3′-t); guanosine* (3′-t)
		C, guanosine (3′-t); guanosine* (3′-t)	E, AMP (5′-t), AMP* (5′-t) UMP (m)

[a] Modified moieties are labeled by an asterisk. [b] 3′-t, 5′-t, and m denote 3′-terminal, 5′-terminal, and middle moieties, respectively.

of Products. Samples of the irradiated solutions were withdrawn periodically and freeze-dried. Mononucleotides and their photoproducts were dissolved in water (100 μL), applied directly to Whatman No. 1 paper, and analyzed chromatographically in solvent systems A and B (see Table I). Samples of di- and trinucleotides were subjected to alkaline or enzymatic hydrolysis prior to their chromatographic analysis, as described below. The detailed procedure for the quantitative determination of the photoproducts has been described elsewhere (Havron et al., 1976).

Dinucleoside Monophosphates. Freeze-dried samples of irradiated dinucleoside monophosphates were hydrolyzed in NaOH (0.3 N, 0.3 mL, at 37 °C for 18 h) to yield mixtures of nucleosides, nucleoside 2′(3′)-monophosphates, and their corresponding photoproducts. These were analyzed chromatographically in solvent systems A and B (Table I), using hydrolyzed nonirradiated dinucleoside monophosphates as controls. Guanosine and guanosine photoproducts, which were poorly resolved in either solvent system A or B, were eluted from the paper and rechromatographed in solvent system C (see Table I).

Homotrinucleoside Diphosphates. Freeze-dried irradiated samples were hydrolyzed with NaOH (0.3 N, 0.3 mL at 37 °C for 18 h) and with snake venom phosphodiesterase (7 μg in 0.6 mL of 30 mM ammonium bicarbonate, pH 8.8, 0.16 mM magnesium acetate at 37 °C for 4 h). The hydrolyzed samples were freeze-dried, taken up in water, loaded on Whatman No. 1 paper, and chromatographed in solvent system A.

The 3′- and 5′-terminal bases, modified and unmodified, were determined as nucleosides in the alkaline and the diesterase hydrolysates, respectively. The middle bases were determined by subtracting the amount of the 5′-end nucleoside (determined by the diesterase hydrolysis) from the sum of the 5′-end and the middle bases, which were determined as nucleoside 2′(3′)-monophosphates in the alkaline hydrolysis. Alternatively, the amount of the 3′-end nucleoside (determined by the alklaine hydrolysis) was subtracted from the sum of the 3′-end and the middle bases, which were determined as 5′-nucleoside monophosphates in the diesterase hydrolysis. Identical results were obtained in both ways.

Heterotrinucleoside Diphosphates. A freeze-dried sample of each of the irradiated trinucleotides was hydrolyzed with NaOH as above to give a mixture of nucleosides (resulting from 3′-terminal moieties) and nucleoside 2′(3′)-monophosphates (resulting from the 5′-terminal and middle moieties). Each mixture was then fractionated by two-dimensional paper chromatography (or three dimensional for A-U-G) as summarized in Table II. Unresolved spots were either eluted from the paper and reloaded on a new sheet of paper for separation in the second dimension or were cut off the paper, stitched to

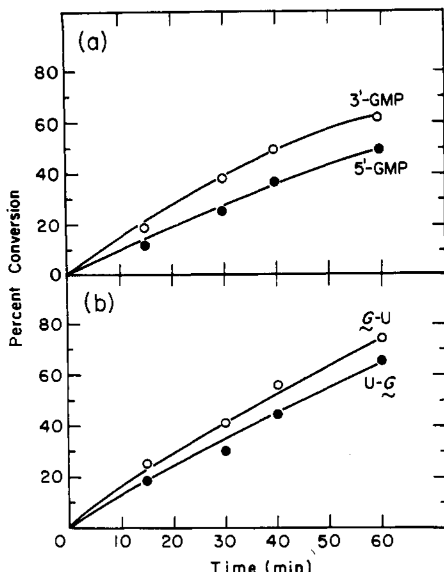


FIGURE 1: Time course of photoalkylation of guanosine moieties with 2-propanol in the presence of $(t\text{-BuO})_2$: (a) 3′-GMP (○), 5′-GMP (●); (b) *G*-U (○), U-*G* (●).

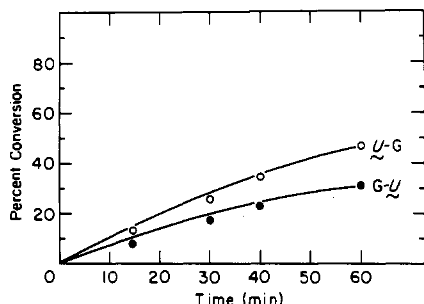


FIGURE 2: Time course of the UV-induced $(t\text{-BuO})_2$-initiated addition of 2-propanol to the uridine moieties of *U*-G (○) and G-*U* (●).

a new sheet, and chromatographed in the second dimension perpendicularly to the first one.

Results

Guanosine-Containing Monoribonucleotides and Diribonucleoside Monophosphates. It is seen from Figure 1 that 5′-GMP reacts nearly as fast as 3′-GMP; similarly, the guanosine moiety of U-G reacts nearly as fast as that of G-U. Concerning the uridine moieties of U-G and G-U, it is seen from Figure 2 that their reactivities were very close; the former reacted slightly faster.

Triribonucleoside Diphosphates. Figure 3 indicates that the 5′-terminal adenosine moiety of A-A-A, which is blocked at its 3′ position, was modified approximately twice as fast as the other two adenosine moieties. Similarly, Figure 4 indicates that the 5′-terminal adenosine moieties of heteronucleotides reacted twice as fast as any other adenosine in the molecule, irrespective of the nature of the other bases. The reactivities of the uridine moieties in the various nucleotides can be related to that of U-A-A, which is taken arbitrarily as unity, and are presented in Table III. In U-U-U, as Figure 5 and Table III indicate, all uridines exhibited similar reactivities—0.8 with respect to U-A-A. The two uridine moieties of A-U-U were relatively unreactive—0.14 with respect to U-A-A (Figure 4a and Table III). The uridine moiety of A-U-A and that of A-U-G exhibited intermediate reactivity—0.63 with respect to U-A-A (Figure 4b,c and Table III).

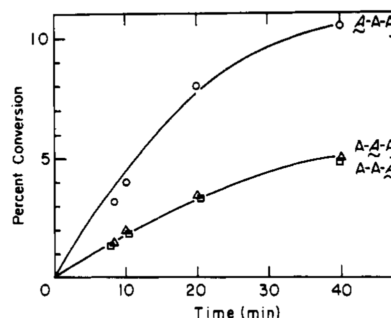


FIGURE 3: Time course of photoalkylation of A-A-A with 2-propanol in the presence of $(t\text{-BuO})_2$. *A*-A-A (○); A-*A*-A (△); A-A-*A* (□).

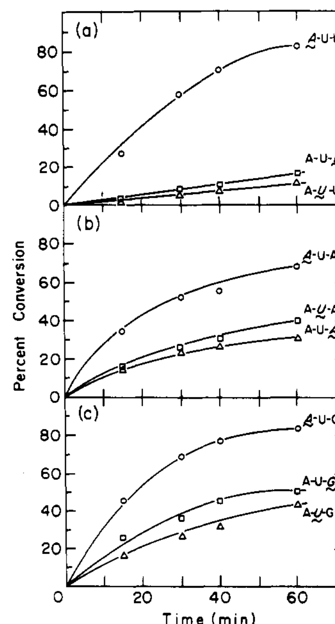


FIGURE 4: Time course of photoalkylation of heterotrinucleoside diphosphates with 2-propanol in the presence of $(t\text{-BuO})_2$: (a) *A*-U-U (○), A-*U*-U (△), A-U-*U* (□); (b) *A*-U-A (○), A-*U*-A (□), A-U-*A* (△); (c) *A*-U-G (○), A-*U*-G (△), A-U-*G* (□).

Conversions of the most reactive base moiety of each system usually ranged between 60 and 80%.

Discussion

The reactions described involve free-radical intermediates and proceed through photolysis of the peroxide to yield oxy radicals which abstract a hydrogen atom from the C-2 of 2-propanol to give ketyl radicals $\dot{C}(CH_3)_2OH$. These are subsequently scavenged by the purine or the pyrimidine to give the adduct. The reaction with the purines proceeds through an initial attack of the ketyl radical at C-8, and with uracil the initial attack is at C-6 (Frimer et al., 1976).

It has been previously pointed out that steric hindrance imposed by the phosphate group and base stacking type interactions determine the selectivity of the reactions for adenosine and uridine moieties in monoribonucleotides and in diribonucleoside monophosphates (Havron et al., 1976). The 8 position of the adenosine moiety of a 3′-blocked ribonucleotide is more exposed to an attack by a free radical than that of a 5′-blocked adenosine. This is due to the fact that the 5′-phosphate group of an adenosine ribonucleotide lies in close proximity to the 8 position of the purine (Ts'o, 1974a). Our previous data also indicated that the 5,6 double bonds of the uridine moieties of 3′- and 5′-UMP were nearly equally exposed to an

TABLE III: Relative Rates of Photoalkylation of Uridine Moieties of Trinucleoside Diphosphates at 25 °C.

Nucleotide	Rate[a]
U-A-A	1.0
U-U-U	0.8
U-*U*-U	0.8
U-U-*U*	0.8
A-*U*-G	0.63
A-*U*-A	0.63
A-*U*-U	0.14
A-U-*U*	0.14

[a] The relative rates, with respect to that of the uridine moiety of U-A-A, were determined from the slopes of the linear parts of the curves shown in Figure 5. The estimated experimental error is ±5%.

TABLE IV: Relative Rates of Photoalkylation of Purine Moieties of Nucleotides at 25 °C.

Nucleotides	Ratio
3′-AMP/5′-AMP	2.4
A-A/A-*A*	2.6
A-U/U-*A*	2.1
3′-GMP/5′-GMP	1.5
G-U/U-*G*	1.3

attack by free radicals, as was the case with both uridine moieties of U-U. On the other hand, the uridine moiety of U-A was more reactive than that of A-U. This difference in reactivity was explained (Havron et al., 1976) as resulting from an intramolecular base stacking type association, which is stronger in A-U than in U-A (Ezra et al., 1977). It should be noted that the reactions were carried out in very dilute solutions of the nucleotides (10^{-4}–10^{-5} M), thus minimizing intermolecular base stacking interactions (Ts'o, 1974a,b). The data presented in this paper will be discussed in terms of the above outlined effects.

Monoribonucleotides and Diribonucleoside Monophosphates. We found that the difference in the reactivity of the guanosine moieties of 3′- and 5′-GMP was much smaller than that observed for 3′- and 5′-AMP. A similar effect was observed for the guanosine moieties of G-U and U-G, where the difference in the reactivity between the two guanosine moieties was much smaller than that observed for the analogous adenosine moieties of A-U and U-A (see Table IV). We have previously proposed that the difference in reactivity between adenosine moieties in nucleotides results from steric hindrance imposed by the 5′-phosphate group on the 8 position of the purine. Assuming that the reactivity of guanosines is also influenced by the same effect, our present results indicate that steric hindrance is less effective in guanosine nucleotides than in adenosine nucleotides. This implies that the 8 position of the 5′-blocked guanosine moiety is less hindered than that of an analogous adenosine moiety. In this context, the conformation of the nucleotides must be considered. In purine nucleotides, assuming the anti conformation, the 5′-phosphate group is in close proximity to the 8 position of the purine ring (Ts'o, 1974a), thereby hindering the approach of the attacking free radical. On the other hand, in the syn conformation the 5′-phosphate points away from the 8 position of the purine; thus, the effect of steric hindrance imposed by the phosphate group is diminished. Therefore, it can be anticipated that in pairs of purine nucleotides, assuming the syn conformation, the difference in reactivity between the 3′ and 5′ isomers would be smaller than that between the analogous anti nucleotides. It

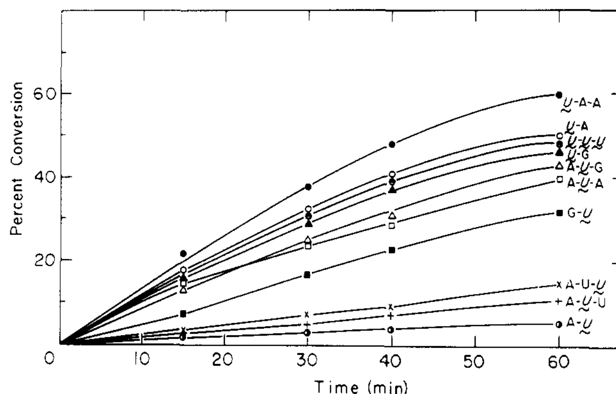


FIGURE 5: Time course of the UV-induced $(t\text{-BuO})_2$ initiated addition of 2-propanol to the uridine moieties of di- and triribonucleotides: A-*U* (◑), A-*U*-U (+), A-U-*U* (X), G-*U* (■), A-*U*-A (□), A-*U*-G (△), *U*-G (▲), *U*-*U*-*U* (⊖), *U*-A (○), *U*-A-A (●).

should be pointed out that our observation is consistent with conclusions based on spectral studies which state that adenosine nucleotides tend to display the anti conformation, while guanosine nucleotides tend to assume the syn conformation in solution (Ts'o, 1974a,b; Olson, 1973).

Concerning the uridine moieties of heterodinucleoside monophosphates, we have already shown that the uridine moiety of U-A reacted at least ten times faster than that of A-U (Figure 5). In contrast to this observation, the reactivity of the uridine moiety of G-U was not suppressed and was only slightly lower than that of the uridine of U-G (relative rates ca. 1:1.5). It has been argued previously (Havron et al., 1976) that an intramolecular base-stacking-type association, which is stronger in A-U as compared with U-A (Ezra et al., 1977), was responsible for the suppressed reactivity of the uridine moiety of A-U. Such stacking interactions are known to be rather weak in G-U, as well as in U-G, due to the syn conformation which guanosine moieties tend to assume (Brahms et al., 1969). Thus, since the uridine moieties in the unstacked conformation of G-U and U-G are exposed, they could be expected to have similar reactivities toward the attacking free radicals.

Triribonucleoside Diphosphates. Our results concerning the purine moieties of trinucleoside diphosphates showed that the 5′-terminal adenosine moieties of *A*-A-A, *A*-U-A, *A*-U-U, and *A*-U-G reacted twice as fast as those of U-*A*-*A*, the middle and the 3′-terminal adenosines of A-*A*-*A*, and the 3′-terminal adenosine of A-U-*A*. This could be expected from our previous observations for 3′-blocked and for 5′-blocked adenosines, respectively. The guanosine moiety of A-U-G was as reactive as the guanosines of mononucleotides and dinucleoside monophosphates. We, therefore, currently conclude that the reactivity of purine moieties of trinucleoside diphosphates could be inferred from the behavior of the analogous moieties in mono- and dinucleotides, as it obeyed the following tentative rule: the reactivity of adenosines depends exclusively on the presence or absence of a phosphate group at the 5′-hydroxyl of the ribose, while that of guanosine moieties is less dependent on the location of the phosphate group.

The uridine moieties of the various trinucleoside diphosphates exhibited a wide range of reactivities, as seen from Figure 5 and Table III. These results can be interpreted on the basis of our previously proposed assumption that the reactivity of uridine moieties does not depend on the site of binding of the phosphate to the ribose but on their involvement in intramolecular stacking with purines. Uridine moieties which are strongly stacked with purines are unreactive, while those which

are unstacked or very slightly stacked are relatively reactive. In accordance with this assumption, the uridine moieties of U-U-U were all equally reactive, as were those of U-U. The uridine moiety of U-A-A, which is unstacked as being part of a U-A sequence (Ezra et al., 1977), was equally reactive. Similarly, the uridine moieties of A-U-A and A-U-G, which are known from CD measurements to be unstacked (Gray et al., 1972; Bloomfield et al., 1974), were also found to be reactive. On the other hand, the reactivity of both uridines of A-U-U was suppressed, presumably due to their involvement in stacking interactions with the adenosine moiety.

The middle uridine of A-*U*-U should be unreactive as part of the dinucleotidic A-U sequence; however, as part of a U-U sequence it could be expected to be reactive. The terminal uridine, being a part of a U-U dinucleotidic sequence, could also be expected to be reactive. The observed low reactivity of the middle uridine can be explained as resulting from strong stacking with its neighboring adenosine, and is therefore a nearest-neighbor effect. The low reactivity of the terminal uridine, as compared to that of the 3′-terminal uridine of U-U, was rather unexpected, since in a U-U sequence there is no suppression of the reactivity of any of the uridine moieties involved. Therefore, it must be concluded that the low reactivity of the terminal uridine of A-U-U results from its association with the adenosine moiety. This indicates that association is operative at such a distance and presents a case of a second-neighbor effect. It should be noted that Kan et al. (1973) indicated the existence of distant neighbor-shielding effect exerted by a terminal purine on a terminal pyrimidine moiety of deoxytrinucleoside diphosphates.

The uridine moieties of A-U-A and A-U-G exhibited intermediate reactivity (see Table III), indicating that substitution of a purine for the terminal uridine of A-U-U results in a dramatic enhancement of the reactivity of the remaining uridine. As part of the dinucleotide sequence A-U, such a uridine moiety could be expected to be unreactive; on the other hand, as part of a U-A sequence it should be rather reactive. Our data (Figure 5 and Table III) indicate that the uridine moieties of both A-U-A and A-U-G behave as if they were part of U-A or U-G sequences, respectively, in which they are unstacked, rather than that of A-U sequence, where they are strongly stacked. It should be emphasized that our results are in complete accordance with the conclusion based on CD and ORD measurements, which states that the uracil of a Pu-U-Pu sequence stacks very slightly (Gray et al., 1972; Kan et al., 1973; Bloomfield et al., 1974). The high reactivity of such a uridine moiety in our reactions can be explained by assuming that it swings out of the stack, thereby becoming exposed to an attack by the free radicals.

In summary, the reactivity of uridine moieties of the various nucleotides can be directly related to the degree of their involvement in stacking interactions with neighboring or next-neighboring purine moieties.

Conclusion

Our current experimental data concerning purines, summarized in Table IV, indicate that the reactivity of purine moieties of ribonucleotides in light-induced free-radical reactions depends on the conformation of the appropriate nucleoside (anti or syn) and on the presence or absence of a phosphate group at the 5′ position of the ribose. Hence, our results imply that the reactivity of purine moieties of triribonucleotides, and possibly those of single-stranded polynucleotides, can be inferred from that of the analogous moieties of mono- and dinucleotides.

The reactivity of uridine moieties, as Figure 5 and Table III indicate, is not dependent on the site of binding of the phosphate group to the ribose but strongly depends on the nature of the next and second neighbors and on the sequence of the nucleotide. This dependence is related to the involvement of the uridines in stacking interactions with purines—the stronger the stacking the lower the reactivity. The relative reactivity of uridine moieties of the various nucleotidic sequences can, thus, be used as a measure of the extent of pyrimidine-purine stacking in nucleotides and vice versa.

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