Cleavage of the glycosidic linkage of pyrimidine ribonucleosides by the bisulfite-oxygen system

Naomi Kitamura and Hikoya Hayatsu

Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku,
Tokyo, Japan

Received 5 November 1973

ABSTRACT

When a solution containing 2 mM uridine, 20 mM sodium bisulfite, 0.1 mM MnCl_2 , and 100 mM sodium phosphate buffer of pH 7.0 was incubated aerobically at 37° or 0°, partial cleavage of the glycosidic linkage of uridine took place. About 20% of the uridine was converted to uracil by the incubation for 4 hrs. Cytosine was produced from cytidine by similar treatment with bisulfite. These reactions were caused by free radicals generated by Mn^{2+} -catalyzed autoxidation of bisulfite. Glycosidic bond cleavage by the bisulfite-oxygen system was not detected for adenosine, AMP, guanosine, GMP, thymidine, TMP, deoxyuridine, dCMP, dAMP, and dGMP. When poly(U) and poly(C) were treated with 20 mM sodium bisulfite in the same manner, chain fission of the polymer occurred as judged by the elution-pattern change in gel filtration through Sephadex columns. No change in the elution pattern was observed for bisulfite-treated poly(A), poly(U)·poly(A) or tRNA.

INTRODUCTION

Since the discovery of a bisulfite reaction with 4-thiouridine¹, a number of reactions between bisulfite and nucleic acid constituents has been disclosed. Noticeably, the reversible saturation of the 5,6-double bond of cytosine and uracil by bisulfite ion to give 5,6-dihydropyrimidine-6-sulfonate makes the 4-amino group of cytosine susceptible to hydrolysis²⁻⁵ as well as to transamination^{6,7} and causes the hydrogen-isotope exchange at position 5 of the pyrimidine rings^{8,9}. Because of the mildness of the reaction conditions and the selectivity for specific bases, the bisulfite reaction has been extensively utilized in chemical modification of nucleic acids¹⁰⁻²¹, and employed in synthetic procedures of nucleotides and nucleosides²²⁻²⁴.

Apart from the ionic processes which take place more readily at higher bisulfite concentrations (usually 1-3 M bisulfite solutions are used), bisulfite brings about free radical reactions with nucleic acids and their components at low concentrations (around 10^{-2} M). The radical reactions require the presence of oxygen and are apparently associated with the oxidation of bisulfite to sulfate. The radicals cause DNA chain cleavage²⁵

and inactivation of Bacillus subtilis transforming DNA²⁶. It is well-characterized that the sulfite free radical $\cdot\text{SO}_3^-$ adds to the sulfur atom of 4-thiouridine to give the corresponding thiosulfonate which readily undergoes nucleophilic substitution with sulfite ion producing uridine-4-sulfonate^{1, 27}. This reaction has been employed as a key procedure to transform a 4-thiouracil derivative into the cytosine derivative²⁸. Similar free radical-dependent transformation of 2-thiouracil to uracil was also reported²⁹. It is known that the sulfite radical adds to olefinic double bonds in the anti-Markownikoff sense³⁰ and such a reaction does occur to the side chain of 6-isopentenyladenosine^{10, 11, 31}. We now wish to report that under certain conditions the bisulfite-oxygen system cleaves with considerable velocity the glycosidic bond of uridine and cytidine, and that it causes chain fission of poly(U) and poly(C).

MATERIALS AND METHODS

Nucleosides and nucleotides were commercial products used without further purification. Uridyl(3'-5')adenosine was prepared synthetically³². Homopolymers of ribonucleotide including double-stranded poly(U) · poly(A) (1:1) were obtained from Miles. tRNA (a mixture, from Brewer's yeast) was a product of Boehringer. The bisulfite solutions were prepared freshly before use. Other agents used were reagent grade chemicals.

Reaction of nucleosides and nucleotides with bisulfite-oxygen A standard reaction mixture contained, in a total volume of 1.0 ml, 2 mM nucleoside or nucleotide, 100 mM sodium phosphate buffer of pH 7.0, 0.1 mM MnCl_2 , and 20 mM bisulfite (a 1:3, mole/mole, mixture of NaHSO_3 and Na_2SO_3). Test tubes (1 x 8 cm) were used as the reaction vessel and were covered lightly with aluminum foil during the incubation. The reaction temperature was 37° unless otherwise noted. Aliquots (100 μl) were withdrawn at intervals and added to 1 N NaOH (1.9 ml) and the resulting solutions were subjected to UV recording on a Beckman DU spectrophotometer. Formation of uracil from uridine (or cytosine from cytidine) was determined by calculation from the absorbancies at 260 nm and 282 nm (or 260 nm and 290 nm for cytosine and cytidine) using the following molar absorption coefficients: uridine, 7.7×10^3 at 260 nm and 2.3×10^3 at 282 nm; uracil, 4.4×10^3 at 260 nm and 5.7×10^3 at 282 nm; cytidine, 7.5×10^3 at 260 nm and 3.2×10^3 at 290 nm; cytosine, 2.4×10^3 at 260 nm and 5.2×10^3 at 290 nm. For identification of the reaction products, the reaction mixture was directly streaked on Toyo filter paper

No. 53 and chromatographed ascendingly using the following solvent systems; (i) n-butanol-water (86:14, v/v), (ii) isopropanol-conc. ammonia-water (7:1:2, by vol.), (iii) isobutyric acid-0.5 N ammonia (5:3, v/v), and (iv) ethylacetate-formic acid-water (60:5:35, by vol., upper phase). Solvent iv was used for the deoxyuridine reaction since uracil was separated well from deoxyuridine with this solvent.

Reaction of polynucleotides with bisulfite-oxygen A standard reaction mixture contained, in a total volume of 0.5 ml, 2 OD_{260 nm} units of polymer, 100 mM sodium phosphate buffer, pH 7.0, 0.1 mM MnCl₂, and 20 mM NaHSO₃-Na₂SO₃ (1:3, mole/mole). After incubation at 37°, the reaction mixture was directly loaded on a column of Sephadex and the column was eluted with 50 mM sodium phosphate buffer, pH 7.2. Fractions (1 ml) were collected at a flow rate of 5 ml/hr, and the absorbance at 260 nm was recorded. When poly(U) was treated with 1 M sodium bisulfite, the fractions were adjusted to pH 10.5 by addition of 0.3 ml of 0.1 N NaOH and after incubation at 37° for 30 min to decompose the dihydrouracil-6-sulfonate residues into uracil⁴ the optical densities were recorded. For the column chromatography at high temperatures, the column was fitted into a circulation glass tube and warm ethylene glycol of constant temperature was circulated using Haake thermostat-FSc. The sample submitted to the high temperature chromatography was freed of bisulfite by prior gel filtration through Sephadex. Control experiments were run by incubating the polynucleotide in the same manner as above in the absence of sodium bisulfite.

Identification of uracil liberated from poly(U) by treatment with bisulfite-oxygen was carried out as follows. Eight OD_{260 nm} units of poly(U) was incubated in a 2-ml solution containing 20 mM sodium bisulfite, 0.1 mM MnCl₂ and 100 mM sodium phosphate buffer, pH 7.0, at 37° for 4 hrs. The entire solution was then loaded on a sheet of Toyo filter paper No. 53 in a band of 8 cm width. The loading was done under drying with air stream at room temperature, taking about 3 hrs. The chromatography was run with solvent i. The UV absorbing zone having an R_F value (0.30) the same as uracil was eluted with 2 ml of 200 mM sodium phosphate buffer, pH 7.0, and the UV spectrum recorded. The spectra in the buffer as well as at pH 14 were identical with those of uracil. Polymers remained at the origin of the paper and no UV absorbing material other than uracil was observed on the chromatogram.

Effect of post incubation with p-anisidine on the bisulfite-treated poly(U)

was investigated as follows. Poly(U) was treated with 20 mM sodium bisulfite as described above for 3 hrs, and filtered through Sephadex G-50 to remove salts (elution with water). Fractions containing UV-absorbing materials were collected and lyophilized. The residue was dissolved in 0.5 ml of 200 mM p-anisidine previously adjusted to pH 5 with 1 N HCl. The final pH was 5. The solution was incubated at 37° for 3 hrs and then subjected to the gel filtration through Sephadex G-50 (elution with 50 mM sodium phosphate buffer, pH 7.2).

RESULTS

Nucleosides and nucleotides Ribo- and deoxyribo-nucleosides and -nucleotides were treated with 20 mM sodium bisulfite at pH 7.0 and 37° under aerobic conditions. Analysis by paper chromatography showed that uracil was produced from uridine and uridine 5'-phosphate, and cytosine from cytidine and cytidine 5'-phosphate. No base liberation was detected for adenosine, adenosine 5'-phosphate, guanosine, guanosine 5'-phosphate, thymidine*, thymidine 5'-phosphate*, deoxyuridine, deoxycytidine 5'-phosphate, deoxyadenosine 5'-phosphate, and deoxyguanosine 5'-phosphate. Therefore the reaction is specific for pyrimidine ribonucleosides and -nucleotides. Uracil formed from uridine, and cytosine from cytidine were identified by comparing R_F values with standard samples using chromatographic solvents i, ii and iii, and by UV spectra taken at pH 1, 7,

Figure 1 Glycosidic bond cleavage of pyrimidine ribo-nucleosides and -nucleotides by the bisulfite-oxygen system.

and 14. The progress of the glycosidic bond cleavage was followed spectrophotometrically and the results are presented in Figure 1. It is seen that the liberation of uracil was faster than that of cytosine and the nucleosides were better substrates than the nucleoside 5'-phosphates. Evidence that the liberation of uracil from uridine was dependent on the free radicals produced by the Mn^{2+} -catalyzed autoxidation of bisulfite is presented in Table I. Thus, the reaction was completely stopped by

Table I Requirements for the liberation of uracil from uridine	
Content of the reaction mixture	Uracil formed by 4 hr-incubation (%)
Complete	25
+ Hydroquinone (5 mM)	0
in N ₂ atmosphere	0
- MnCl ₂	5
- Bisulfite	0

hydroquinone, a free radical scavenger, and by replacing the air with nitrogen, and the absence of MnCl₂ resulted in a significant retardation of the reaction. The glycosidic bond cleavage of cytidine was also completely inhibited by 5 mM hydroquinone. The fact that the reactions did not proceed beyond 10-25% can be accounted for in terms of the consumption of bisulfite by oxidation. Actually, the uracil liberation proceeded further when fresh bisulfite (each equivalent to 20 mM) was added to the uridine reaction-mixture at each 1 hr reaction period. Thus, after four such additions and incubation for a total of 5 hrs, the reaction mixture was made alkaline with ammonia in order to decompose any uridine- (or uracil-) bisulfite adduct formed⁴ and submitted to paper chromatographic analysis using solvent i : the uridine/uracil ratio on the chromatogram was 39/61, mole/mole. However, it was difficult to complete the reaction in this way since the uridine-bisulfite adduct was resistant to the free radical attack (see below).

Effect of varying the conditions of the uridine-bisulfite-oxygen reaction was investigated. Change in the concentration of uridine, while other conditions were the same as the original, greatly affected the rate

* Loss of UV absorption was noted for these compounds. Details of this chemistry will be reported elsewhere.

Figure 2 Effect of concentration of uridine on the liberation of uracil from uridine.

of the reaction. As Figure 2 shows, the reaction became slower as the concentration of uridine was increased from 1 mM to 20 mM; almost no reaction occurred with 20 mM uridine. This suggests that uridine, the reaction substrate itself, is an inhibitor of the reaction. The inhibitory effect was not accounted for only by the loss of bisulfite due to the formation of the uridine-bisulfite adduct, because even with 20 mM uridine most of the 20 mM bisulfite remained unbound: uridine/5,6-dihydrouridine-6-sulfonate (mole/mole) was 9/1 after the incubation for 1 hr. Lowering of the MnCl_2 concentration from 0.1 mM to 0.05 mM did not affect the rate of the reaction. Remarkably, lowering or raising the reaction temperature had little effect on the extent of the uracil liberation by 4 hr-reaction: Thus, the reaction extents were 22.5% at 0°, 25.2% at 15°, 28.2% at 37°, and 25.0% at 50°. The independence on the temperature is analogous to the one observed for the inactivation of *B. subtilis* DNA with bisulfite-oxygen²⁶. Effect of pH was examined by buffering the reaction mixture with 100 mM sodium phosphate of pH 6.0, 6.5, 7.0, 7.5 or 8.0. The reaction extents were; at pH 6.0, 8% (2 hr) and 14% (27 hr); at pH 6.5, 8.5% (2 hr) and 18% (27 hr); at pH 7.0, 14% (2 hr) and 25% (27 hr); at pH 7.5, 11% (2 hr) and 24% (27 hr); at pH 8.0, 8.5% (2 hr) and 25% (27 hr). It is seen that the reaction can proceed in this range of pH, and pH around 7 appears to be the optimum. When tris-HCl buffer was used in place of the phosphate

in the pH 7.5-reaction, the glycosidic bond cleavage was inhibited. Thus, the reaction extents at 4 hr-period were; in 100 mM tris-HCl, 9.2%; in 200 mM tris-HCl, 0.8%; in 100 mM sodium phosphate, 24%. Another inhibitor found was adenosine 5'-phosphate, whose presence in the uridine-bisulfite-oxygen reaction reduced the reaction extent: uracil liberated at 4 hr was 25% with 0 mM AMP, 14.3% (43% inhibition) with 2 mM AMP, 10.3% (59% inhibition) with 5 mM AMP, and 8.2% (68% inhibition) with 20 mM AMP. The inhibitions by uridine, tris-HCl, and adenosine 5'-phosphate may be due to the polyhydroxylic nature of these compounds because mannitol is known to inhibit the oxidation of methionine with the bisulfite-oxygen system³³.

In order to investigate whether the glycosidic bond cleavage occurs through the 5, 6-dihydrouridine-6-sulfonate formation, the latter compound⁴ was subjected to the same treatment as uridine. Rather a slower cleavage occurred: Thus, 5% cleavage was observed after 1 hr treatment while for uridine 15% reaction took place under the same conditions. Since 5, 6-dihydrouridine-6-sulfonate slowly regenerates uridine under the conditions employed, the glycosidic bond cleavage appears to take place not through the uridine-bisulfite adduct but for uridine itself. No glycosidic bond cleavage of uridine was observed in 1 M sodium bisulfite solution at pH 7.0 confirming the results previously reported⁴. In this solution most of the uridine was in the form of the bisulfite adduct. Further, in such a solution of high bisulfite concentration, the free radical reactions will generally be inhibited^{26, 27}.

Polynucleotides Poly(U) was treated with 20 mM sodium bisulfite at pH 7.0 (100 mM phosphate) and 37° in the presence of 0.1 mM MnCl₂ for 4 hrs, and analyzed by means of gel filtration through Sephadex. As Figure 3 shows, a considerable change in the elution pattern resulted by the bisulfite treatment, indicating an extensive cleavage of the internucleotide linkages. No further change in the pattern was found by reaction for 24 hrs. This chain fission was again dependent on the free radicals generated by autoxidation of bisulfite, since the elution pattern did not differ from the control (a) when 5 mM hydroquinone was present in the reaction mixture, (b) when MnCl₂ was absent, or (c) when the polymer was treated with 1 M sodium bisulfite instead of 20 mM bisulfite. Paper chromatographic analysis revealed that 0.15 OD_{260 nm} units of uracil was produced from 8 OD_{260 nm} units of poly(U) by the 4 hr-reaction. Post-incubation of the bisulfite-treated poly(U) with p-anisidine was carried out in order to investigate the nature of the internucleotide bond fission, because p-

Figure 3 Gel filtration of poly(U) treated with bisulfite-oxygen. —○—, After incubation in the bisulfite-oxygen system. - - -○- - -, Control; after incubation in the absence of bisulfite. —●—, After incubation in the bisulfite-oxygen system in the presence of 5 mM hydroquinone, - - -●- - -, After post-incubation with *p*-anisidine.

anisidine is known to catalyze the β -elimination leading to the cleavage of the phosphodiester bonds³⁴. The result given in Figure 3 indicates that there was no great change in the elution pattern before and after the *p*-anisidine treatment.

The chain of poly(C) was also cleaved by treatment with 20 mM bisulfite for 20 hrs as illustrated in Figure 4a. The elution pattern of poly(A) after similar treatment with bisulfite (16 hrs) was identical with that of the control (Fig. 4b). This is consistent with the observation that adenosine was unaffected by the bisulfite-oxygen system. Interestingly, poly(U) molecule in the form of poly(U)·poly(A) duplex was protected from the attack of the bisulfite-oxygen free radicals. (Figure 4c. Reaction time, 16 hrs). In this analysis the Sephadex column was run at 80° in order to melt the duplex. The inhibition of the free radical attack on poly(U) may be a result of the hydrogen bonding between uracil and adenine or merely a result of the presence of a free radical scavenger poly(A) in the vicinity of the poly(U) molecule. In this connection it is interesting to note that uridylyl(3'-5')adenosine was not affected by treatment with 20 mM bisulfite at pH 7.0 and 37° for 4 hrs (checked by paper chromatography): in this case the simplest explanation for the inertness of the molecule is the presence of the free radical scavenging adenylate moiety close to the reactive site. When tRNA from Brewer's yeast was subjected to the bisulfite-oxygen

Figure 4 Sephadex column chromatography of polymers treated with bisulfite-oxygen. (a) —, Poly(C) treated with bisulfite-oxygen; ----, Control, i.e., poly(C) incubated in the absence of bisulfite. (b) —, Poly(A) treated with bisulfite-oxygen; ----, Untreated poly(A). (c), —, Poly(U)·poly(A) treated with bisulfite-oxygen, filtered once through Sephadex G-50 at room temperature, and then rechromatographed at 80°; ----, Poly(U)·poly(A) incubated in the absence of bisulfite and chromatographed at room temperature. (d), —, tRNA treated with bisulfite-oxygen, filtered once through Sephadex G-100, and rechromatographed at 70°; ----, Untreated tRNA eluted at 70° (elution at room temperature gave an identical pattern). The arrows indicate the void volume.

treatment (18 hrs) followed by Sephadex column chromatography, there was no difference in the pattern between the treated and the untreated tRNA's. Rechromatography of the tRNA fraction through the same column heated at 70° again gave the same elution pattern (Figure 4d). If the tRNA molecule had been split, for example, at the uracil residue commonly present next to the 5'-end of anticodon, half molecules that will result should have been eluted later than the intact molecules under these conditions³⁵

DISCUSSION

The transition metal-catalyzed autoxidation of bisulfite has been recognized as a free radical chain reaction³³. Among the free radicals involved in the process, the sulfite radical $\cdot\text{SO}_3^-$ has been detected by ESR³⁶ and has been assigned to be the reactive species in a number of organic reactions as mentioned above³⁰. In addition, the hydroperoxide radical $\cdot\text{O}_2\text{H}$ has been considered to be participating in the oxidation of methionine with the bisulfite-oxygen system^{33, 37}. We have presented evidence that the pyrimidine-ribonucleoside-specific cleavage of the glycosidic linkage in dilute bisulfite solutions is also caused by the free radical generated during the autoxidation of bisulfite. However, the mechanism of the glycosidic bond cleavage is obscure at this stage of the study. Attempts to elucidate the fate of the sugar moiety of the nucleosides in the reaction have so far been unsuccessful. As judged by the substrate specificity of the reaction, a possible pathway is the initial attack of a free radical on the C(2')-OH group which causes labilization of the glycosidic bond; and an alternative pathway is a direct attack of a free radical on the glycosidic linkage which is somehow assisted by the presence of the 2'-OH group.

Hydrogen peroxide and the hydroxylamine-oxygen system are known to cause a slow glycosidic bond cleavage of deoxyribonucleosides^{38, 39}. In this reaction, purine nucleosides as well as pyrimidine nucleosides were susceptible to the action of the free radicals generated from the reagents, and the cleavage is believed to occur by the action of the $\cdot\text{OH}$ radical on carbon-1'³⁸. The action of the bisulfite-oxygen on nucleosides are therefore clearly different from that of hydrogen peroxide or of the hydroxylamine-oxygen system. It is recalled that the inactivating effect of the bisulfite-oxygen on transforming DNA was not affected by the presence of catalase and therefore was thought to be different from the action of hydrogen peroxide²⁶.

The cleavage of the internucleotide linkages with the bisulfite-oxygen system was studied by means of gel filtration through Sephadex. This assay method will allow detection of only extensive chain fissions. Chain fissions that produce only large molecules may give the same elution pattern as the starting material. Indeed, when the poly(C) treated with bisulfite-oxygen was examined by use of Sephadex G-50 instead of G-100, there was little difference in the elution pattern between the treated and the untreated polymers (data not shown). Therefore, the possibility remains that poly(A) underwent a small number of chain cleavage but the cleavage escaped detection by this method.

The cleavage of internucleotide bonds of poly(U) and poly(C) with the bisulfite-oxygen is thought to be an event that occurs subsequent to the glycosidic bond cleavage of the polymer constituents. The susceptibility of the internucleotide bonds of poly(U) and poly(C) but not of poly(A) to the bisulfite-oxygen is parallel to the substrate specificity of the bisulfite-caused glycosidic bond cleavage. It is possible that some mechanism different from the β -elimination⁴⁰ is operating in this process, because the internucleotide bond cleavage by the β -elimination mechanism is unlikely to occur at pH 7^{34, 41}. In support of this argument, there was no great change in the gel filtration pattern of poly(U) treated with bisulfite-oxygen after post-incubation with *p*-anisidine which is known to be the most effective agent to accelerate the internucleotide bond fission by the β -elimination mechanism³⁴.

Anyone who uses bisulfite to chemically modify nucleic acids and their constituents is urged to be aware of this type of radical reaction that occurs under certain conditions such as those reported here.

REFERENCES

- 1 Hayatsu, H. (1969) J. Amer. Chem. Soc. 91, 5693-5694
- 2 Shapiro, R., Servis, R.E. and Welcher, M. (1970) J. Amer. Chem. Soc. 92, 422-424
- 3 Hayatsu, H., Wataya, Y. and Kai, K. (1970) J. Amer. Chem. Soc. 92, 724-726
- 4 Hayatsu, H., Wataya, Y., Kai, K. and Iida, S. (1970) Biochemistry 9, 2858-2865
- 5 Sono, M., Wataya, Y. and Hayatsu, H. (1973) J. Amer. Chem. Soc. 95, 4745-4749
- 6 Shapiro, R. and Weisgras, J. M. (1970) Biochem. Biophys. Res. Commun. 40, 839-843
- 7 Shapiro, R., Law, D.C.F. and Weisgras, J.M. (1972) Biochem. Biophys. Res. Commun. 49, 358-363
- 8 Kai, K., Wataya, Y. and Hayatsu, H. (1971) J. Amer. Chem. Soc. 93, 2089-2090
- 9 Wataya, Y. and Hayatsu, H. (1972) Biochemistry 11, 3583-3588
- 10 Furuichi, Y., Wataya, Y., Hayatsu, H. and Ukita, T. (1970) Biochem. Biophys. Res. Commun. 41, 1185-1191
- 11 Kučan, Z., Freude, K.A., Kučan, I. and Chambers, R.W. (1971) Nature New Biol. 232, 177-179
- 12 Singhal, R.P. (1971) J. Biol. Chem. 246, 5848-5851
- 13 Goddard, J.P. and Schulman, L.H. (1972) J. Biol. Chem. 247, 3864-3867
- 14 Schulman, L.H. and Goddard, J.P. (1973) J. Biol. Chem. 248, 1341-1345
- 15 Chambers, R.W., Aoyagi, S., Furukawa, Y., Zawadzka, H. and Bhanot, O.S. (1973) J. Biol. Chem. 248, 5549-5551
- 16 Shapiro, R., Cohen, B.I. and Servis, R.E. (1970) Nature 227, 1047-1048
- 17 Shapiro, R. and Braverman, B. (1972) Biochem. Biophys. Res. Commun. 47, 544-550

- 18 Shapiro, R., Braverman, B., Louis, J. B. and Servis, R. E. (1973) J. Biol. Chem. 248, 4060-4064
- 19 Budowsky, E. I., Sverdlov, E. D. and Monastyrskaya, G. S. (1972) FEBS Letters 25, 201-204
- 20 Sverdlov, E. D., Monastyrskaya, G. S., Budowsky, E. I. and Grachev, M. A. (1972) FEBS Letters 28, 231-235
- 21 Sverdlov, E. D., Monastyrskaya, G. S. and Budowsky, E. I. (1972) FEBS Letters 28, 236-238
- 22 Scherberg, N. H. and Weiss, S. B. (1972) Proc. Nat. Acad. Sci. USA 69, 1114-1118
- 23 Kuśmierk, J. T., Giziewicz, J. and Shugar, D. (1973) Biochemistry 12, 194-200
- 24 Boni, I. V. and Budowsky, E. I. (1973) J. Biochem. (Tokyo) 73, 821-830
- 25 Hayatsu, H. and Miller Jr., R. C. (1972) Biochem. Biophys. Res. Commun. 46, 120-124
- 26 Inoue, M., Hayatsu, H. and Tanooka, H. (1972) Chem. - Biol. Interactions 5, 85-95
- 27 Hayatsu, H. and Inoue, M. (1971) J. Amer. Chem. Soc. 93, 2301-2306
- 28 Hobbs, J., Sternbach, H., Sprinzl, M. and Eckstein, F. (1972) Biochemistry 11, 4336-4344
- 29 Sono, M. and Hayatsu, H. (1973) Chem. Pharm. Bull. (Tokyo) 21, 995-1000
- 30 Stacey, F. W. and Harris, Jr., J. F. (1963) in Organic Reactions, Vol. 13, pp. 196-200, Wiley, New York
- 31 Hayatsu, H., Wataya, Y., Furuichi, Y. and Kawazoe, Y. (1972) Chemosphere 1, 75-78
- 32 Ukita, T., Irie, M., Imazawa, M., Furuichi, Y., Nishimura, H. and Sekiya, T. (1968) Seikagaku 40, 363-371 (in Japanese)
- 33 Yang, S. F. (1970) Biochemistry 9, 5008-5014
- 34 Turchinskii, M. F., Guškova, L. I., Khazai, I., Budovskii, E. I. and Kochetkov, N. K. (1970) Molecular Biology 4, 343-348
- 35 Imura, N., Schwam, H. and Chambers, R. W. (1969) Proc. Nat. Acad. Sci. USA 62, 1203-1209
- 36 Ozawa, T., Setaka, M. and Kwan, T. (1971) Bull. Chem. Soc. (Japan) 44, 3473-3474
- 37 Inoue, M. and Hayatsu, H. (1971) Chem. Pharm. Bull. (Tokyo) 19, 1286-1289
- 38 Rhaese, H. and Freese, E. (1968) Biochim. Biophys. Acta 155, 476-490
- 39 Rhaese, H., Freese, E. and Melzer, M. S. (1968) Biochim. Biophys. Acta 155, 491-504
- 40 Kochetkov, N. K. and Budovskii (1972) Organic Chemistry of Nucleic Acids pp. 514-517, Plenum Press, London
- 41 Seifert, W. and Zillig, W. (1967) Hoppe-Seyler's Z. Physiol. Chem. 348, 1017-1026