Interactions of Periodate-Oxidized Guanine Nucleotides with *Escherichia coli* Elongation Factor G and the Ribosome†

James W. Bodley and Julian Gordon*

ABSTRACT: Both periodate-oxidized GDP (GDP^{ox}) and its KBH_4-reduction product (GDP^{ox-red}) bind to the ribosome and EF-G in the presence of fusidic acid. The complexes formed with these two nucleotides are only slightly less stable than that with GDP itself. However, the binding of both GDP^{ox} and GDP^{ox-red} as observed by Millipore filtration is more dependent on the presence of fusidic acid than is the binding of GDP. A similar pattern of binding was observed with the analogous nucleotides derived from GTP (GTP^{ox} and GTP^{ox-red}) except that the γ-phosphate was released on binding. However, in the usual catalytic hydrolysis reaction performed in the absence of fusidic acid, both GTP^{ox} and GTP^{ox-red} were poor substrates with rates of hydrolysis <0.3% that of GTP. The reduction of free GDP^{ox} to GDP^{ox-red} by KBH_4 is shown to involve the transient appearance of a previously undescribed intermediate which is presumed to be a monoalcohol derivative. The binding of GDP^{ox} to the ribosome and EF-G in the presence of fusidic acid reduces the rate of reduction of the bound nucleotide to about one-tenth of that of the free nucleotide. Reduction of the bound GDP^{ox} yields the same products as with the free nucleotide. Thus, although the binding of GDP^{ox} to the ribosome and EF-G partially protects the nucleotide from KBH_4 reduction, this binding does not appear to involve the formation of a Schiff's base with the macromolecular components of the complex.

Guanine nucleotides play a conspicuous role in protein synthesis. GTP hydrolysis is an obligatory requirement for the initiation, aminoacyl-tRNA-binding and translocation steps. In none of those cases, however, is the site or mechanism of hydrolysis clearly understood (for a recent review, see Haselkorn and Rothman-Denes, 1973).

In a recent preliminary report we observed that periodate-oxidized GDP and its subsequent borohydride reduction product were unable to bind to EF-Tu but were capable of binding to EF-G and the ribosome (Gordon and Bodley, 1974). This observation provided the first evidence of a difference in substrate specificity of the nucleotide-binding sites of these two systems. In addition, the fact that these nucleotides do interact with EF-G and the ribosome affords an opportunity to gain further insight into the nature and specificity of this nucleotide binding and hydrolytic site.

Here we report on the interaction of EF-G and the ribosomes with GDP^{ox}, GDP^{ox-red}, GTP^{ox}, and GTP^{ox-red}.[1] The interactions are compared with those of the parent compounds in terms of binding and hydrolytic reactions. In addition, the borohydride reducibility of bound GDP^{ox} is compared with that of the free nucleotide.

Materials and Methods

Materials. EF-G was prepared from *Escherichia coli* by an affinity purification technique described elsewhere (Rohrbach *et al.*, 1974). The preparation of *E. coli* high-salt washed ribosomes was also described previously (Bodley, 1969). [^{3}H]GDP and [^{3}H]GTP were purchased from Radiochemical Centre, Amersham, and had specific activities of 5.0 and 8.7 Ci per mmol, respectively. [α- and γ-^{32}P]GTP were obtained from both Amersham and the New England Nuclear Corp. [α-^{32}P]GDP was prepared by the hydrolysis of [α-^{32}P]GTP with EF-G and the ribosome as described earlier (Rohrbach *et al.*, 1974). Fusidic acid was a gift of Ms. Barbara Stearns of E. R. Squibb.

Preparation of Nucleotides. The preparation of the oxidized and oxidized-reduced guanine nucleotide derivatives was essentially that described by Ofengand and Chen (1972) for the preparation of the analogous derivatives of ATP. Nucleotide solutions were diluted to final concentrations of up to 1 mM in 0.1 M potassium borate buffer (pH 9.0) and the following additions were made per ml of this original nucleotide solution. Oxidation was accomplished by the addition of 0.1 ml of a freshly prepared 0.08 M $NaIO_4$ solution followed by incubation in the dark for 30 min at 30°. The oxidation reaction was terminated by the addition of 0.01 ml of a 10% glycerol solution. The oxidized nucleotides were either stored in this form or subsequently reduced. The reduction reaction was accomplished by the addition of 0.1 ml (per ml of the original nucleotide solution) of a 0.25 M KBH_4 solution which was prepared in water immediately before use. After incubation for 10 min at 30° the reaction was stopped by the addition of 0.01 ml of cyclohexanone. Nucleotide derivatives prepared in this way did not undergo significant degradation upon storage at −20°. Nonetheless, fresh preparations were made every few days.

Chromatography of Nucleotides. Nucleotides were analyzed by thin-layer chromatography on 10- or 20-cm polyethyleneimine cellulose plates (Machrey-Nagel) as described by Cashel *et*

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[1] The abbreviations used here to designate the periodate-oxidized nucleotides and their subsequent borohydride reduction products follow the convention adopted by Ofengand and Chen (1972). Namely, GDP^{ox} and GTP^{ox} are the periodate oxidation products of GDP and GTp, respectively. GDP^{ox-red} and GTP^{ox-red} designate GDP and wgtp, respectively, which were first oxidized with periodate and then reduced with borohydride.

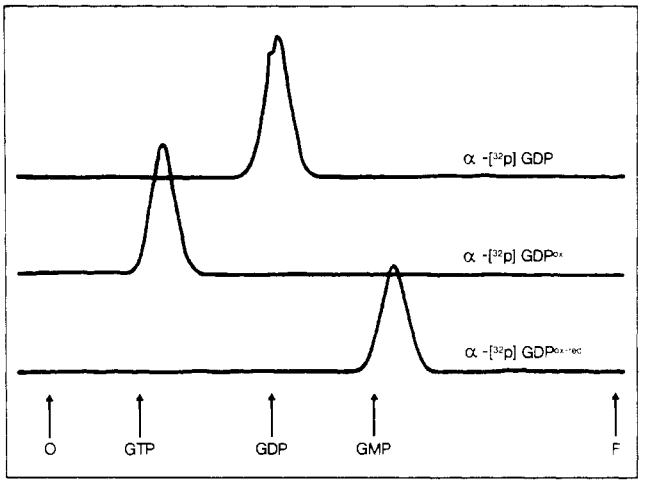

FIGURE 1: Chromatography of α-^{32}P-labeled guanine nucleotides. Thin-layer chromatograms were developed with 0.75 M potassium phosphate (pH 3.4) and scanned as described in Materials and Methods. The chromatographic origin, front and positions of marker nucleotides, as detected by their uv absorption, are indicated by the arrows.

al. (1969). Chromatograms were routinely developed with 0.75 M potassium phosphate (pH 3.4) but the slower moving triphosphate derivatives were also chromatographed with 1.5 M potassium phosphate (pH 3.4). A representative chromatogram is shown in Figure 1. Tritium-labeled nucleotides were located by scanning the chromatogram with a Berthold radiochromatogram scanner. ^{32}P-Labeled nucleotides were also located by radioautography. The nucleotides were quantitated by cutting out the chromatographic spot and counting it in a liquid scintillation counter. Generally, nucleotides prepared as described above chromatographed as a single spot contained >95% of the total radioactivity and in no case less than 90%.

The apparent chromatographic mobilities of the nucleotides discussed in this report are listed in Table I. GDP$^{ox\text{-}red}$ and GTP$^{ox\text{-}red}$ both migrated significantly faster than the parent compounds. GDP$^{ox\text{-}red*}$ and GTP$^{ox\text{-}red*}$ refer to the putative monoalcohol reduction intermediates discussed in Results. These two species exhibited mobilities only slightly different from their respective parent nucleotides.

It will be noted that the apparent mobility of GDP^{ox} and GTP^{ox} depended upon the position of the label. Tritium label present on the base ring remained at the origin, while ^{32}P originally present in the α position migrated entirely free of it (Table I). [α-^{32}P]- and [γ-^{32}P]GTP^{ox}, however, chromatographed identically. Periodate-oxidized nucleotides are well known to undergo condensation and subsequent phosphate elimination reactions with amines and imides (Whitfeld, 1954; Brown *et al.*, 1955) and such a reaction with the chromatographic medium employed here is presumed to account for this anomalous behavior. It is clear that fragmentation of this type was not significant prior to chromatography, since none of the putative fragmentation products remained after reduction of the nucleotide (*e.g.*, Figure 1).

Nucleotide Binding. The binding of nucleotides to EF-G and the ribosome was conducted as described earlier (Highland *et al.*, 1971) except that potassium borate was substituted for Tris-HCl buffer. The binding reactions were conducted in a final volume of 50 μl which contained 10 mM potassium borate (pH 9.0), 20 mM magnesium acetate, 1 mM dithiothreitol, and 3 mM fusidic acid in addition to 600 μg of ribosomes and 50 units of EF-G. In some cases, as noted in the text, the reaction volume was scaled up without altering the concentrations of the components. The binding reaction was initiated by the addition of 10–15 pmol of labeled nucleotide. Under these conditions the concentration of EF-G and ribosomes exceeded that of nucleotide by at least sevenfold, thus maximizing nucleotide binding. After incubation for 5 min at 0° the reaction mixtures were filtered through Millipore filters (type HAWP 45 μ, Millipore Corp.). The filters were washed with a solution containing 10 mM potassium borate (pH 9.0), 20 mM magnesium acetate, and 10^{-5} M fusidic acid as described earlier. After drying the filters were counted in a toluene-based liquid scintillation medium.

TABLE I: Chromatographic Mobility of Guanine Nucleotide Derivatives.

Nucleotide	Mobility (R_F)	
	^{3}H	α-^{32}P
GDP	0.36	0.36
GDP^{ox}	0.00	0.19
GDP$^{ox-red*}$	0.37	0.37
GDP^{ox-red}	0.58	0.58
GTP	0.16	0.16
GTP^{ox}	0.00	0.05
GTP$^{ox-red*}$	0.06	0.06
GTP^{ox-red}	0.29	0.29

Nucleotide Hydrolysis. Hydrolysis of the GTP derivatives was conducted in a final volume of 100 μl which contained: 10 mM potassium borate (pH 9.0), 20 mM magnesium acetate, 100 mM KCl, 0.5 mg of ribosomes, EF-G, and the appropriate [α-^{32}P]nucleotide. When GTP was the substrate, 0.05 unit of EF-G was used and 5 units of EF-G was used when either GTP^{ox} or GTP$^{ox\ red}$ was the substrate. The nucleotide was added to initiate the reaction after a brief preincubation at 37°. Incubation was continued at this temperature and 5-μl aliquots of the reaction mixture were periodically spotted for chromatography. Reactions containing GTP or GTP$^{ox\text{-}red}$ were chromatographed with 0.75 M potassium phosphate, while reactions containing GTP^{ox} were chromatographed with 1.5 M potassium phosphate. Following chromatography and radioautography the spots were excised and counted.

Results

In an earlier report (Gordon and Bodley, 1974) we showed the ability of GDP^{ox} and GDP$^{ox\text{-}red}$ to substitute for GDP in the formation of the EF-G–ribosome–GDP complex, stabilized by fusidic acid. Here we examine this in more detail. We have extended the observation to examine the fusidic acid dependence of the binding; to determine whether the corresponding GTP derivatives are bound under the same conditions and, if so, whether the terminal phosphate is cleaved under these conditions, as is known for the parent nucleotides (Bodley *et al.*, 1970b). Table II shows the results of these experiments. The expected behavior of the parent compounds [^{3}H]GDP, [^{3}H]GTP, and [γ-^{32}P]GTP was found. The binding of the [^{3}H]nucleotides is maximal in the presence of fusidic acid, while the γ-^{32}P is released. Similarly, the ^{3}H-labeled nucleotide derivatives are also bound in the presence of fusidic acid. However, the binding of these derivatives is far more dependent on the addition of fusidic acid than is the binding of the parent compounds.

Just as in the case of GTP, the terminal phosphate is released from GTP^{ox} and GTP$^{ox\text{-}red}$ when these nucleotides are bound in the presence of fusidic acid (Table II). The reaction

TABLE II: Binding of Guanine Nucleotide Derivatives to the Ribosome and EF-G.[a]

Nucleotide	Binding (pmol)	
	− Fusidic Acid	+ Fusidic Acid
[^{3}H]GDP	4.26	8.72
[^{3}H]GDP^{ox}	0.36	3.53
[^{3}H]GDP^{ox-red}	0.14	3.86
[^{3}H]GTP	3.36	8.12
[^{3}H]GTP^{ox}	0.39	3.98
[^{3}H]GTP^{ox-red}	0.05	2.59
[γ-^{32}P]GTP	0.01	0.05
[γ-^{32}P]GTP^{ox}	0.03	0.01
[γ-^{32}P]GTP^{ox-red}	0.00	0.00

[a] In each case 13.5 pmol of nucleotide was present in each 50-μl reaction. The values for nucleotide binding are the averages of duplicate determinations which were corrected for a small blank observed in the absence of EF-G.

products have been confirmed as GDP^{ox} and GDP^{ox-red} and P_i by chromatography with α- and γ-labeled derivatives, under the reaction conditions of Table I (data not shown). However, in the usual hydrolytic reaction with catalytic levels of EF-G, substrate levels of nucleotide and without fusidic acid, both GTP^{ox} and GTP^{ox-red} are poor substrates. Under these conditions (Table III) they were hydrolyzed at <0.3% the rate of GTP.

In addition to the enhanced requirement for fusidic acid in the binding of GDP^{ox} and GDP^{ox-red} compared with GDP (Table II), their binding is less stable than that of the parent compound, even in the presence of fusidic acid. This is demonstrated by the experiment shown in Figure 2. Both modified nucleotides yield complexes which are less stable to chase by an excess of unlabeled GDP in the order GDP^{ox}, GDP^{ox-red}.

In addition to the reduced stability to chase by added cold GDP, the binding of the GDP^{ox} and GDP^{ox-red} in the presence of fusidic acid is inherently more variable in our hands than the binding of GDP (*cf.* zero time point in Figure 2, Table II and data in Gordon and Bodley, 1974). The binding reported in Table II is the lowest we have observed. With GDP itself, only about half of the bound nucleotide is retained by Millipore filters due to dissociation of the complex under the filtration conditions (Bodley *et al.*, 1970a). The variability observed here presumably reflects an even greater sensitivity of the complex with GDP^{ox} and GDP^{ox-red} to the filtration conditions.

In Situ Reduction of the Ribosome-EF-GDP^{ox} Complex. Before examining the reactivity of bound GDP^{ox} it was necessary as a point of reference to establish the kinetics of reduction of the free nucleotide. This is shown in Figure 3A. Reduction is rapid under these conditions, but surprisingly the production of GDP^{ox-red} was preceded by the transient and almost immediate appearance of a new chromatographic species which is designated as GDP$^{ox-red*}$. The timing of the disappearance of GDP^{ox}, the transient occurrence of GDP$^{ox-red*}$ and the production of GDP^{ox-red} are consistent with a precursor-product relationship. To our knowledge, no such intermediate during the borohydride reduction of periodate-oxidized nucleotides has been previously reported.

An exactly parallel situation was observed upon the reduction of GTP^{ox}, except of course that the mobility of each of the components was reduced by the presence of the additional phosphate. We have made no further attempt to characterize GDP$^{ox-red*}$ or GTP$^{ox-red*}$, but it seems likely that they repre-

TABLE III: Hydrolysis of Guanine Nucleotide Derivatives by the Ribosome and EF-G.

Substrate	Rates of Nucleotide Hydrolysis (mol of Nucleotide/mol of EF-G per min)	
	10^{-5} M	10^{-4} M
GTP	63	330
GTP^{ox}	<0.1	<0.5
GTP^{ox-red}	0.13	0.5

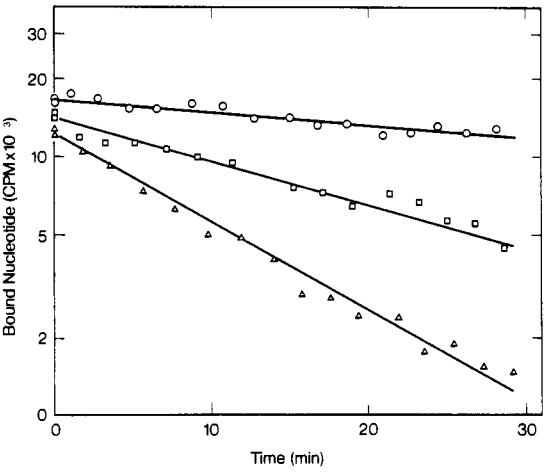

FIGURE 2: Stability of nucleotide complexes. Nucleotide binding was conducted as described in Table II, except that the temperature was 37° and the reaction volume was scaled up to 1 ml. At the conclusion of the binding reaction the chase of bound nucleotide was begun by the addition of 20 μl of a 50 mM GDP and periodically 50-μl samples were withdrawn for filtration in the usual way: GDP (○); GDP^{ox} (□); GDP^{ox-red} (△).

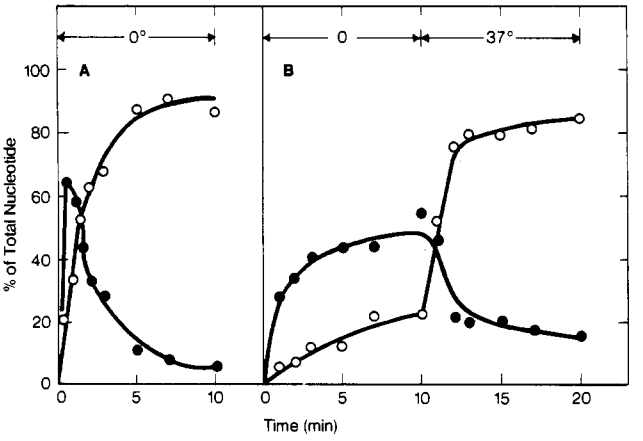

FIGURE 3: The kinetics of borohydride reduction of free and bound [α-^{32}P]GDP^{ox}. In the first phase of the experiment two [α-^{32}P]GDP^{ox} binding mixtures (200 μl), as described in Materials and Methods, were incubated for 5 min at 0°. These two solutions were identical except that one did not contain EF-G (panel A), while the other did contain EF-G (panel B). At the conclusion of this incubation, 20 μl of 0.25 M KBH_4 was added. Incubation was continued after this addition and periodically 10-μl samples were withdrawn and added to 1 μl of cyclohexanone to stop reduction. Portions (5 μl) of these solutions were subsequently chromatographed and the percentage of total [^{32}P]nucleotide present as either GDP$^{ox-red*}$ (closed circles) or GDP^{ox-red} (open circles) was determined as described in Materials and Methods. After 10-min incubation the reaction temperature was increased to 37° as indicated in panel B.

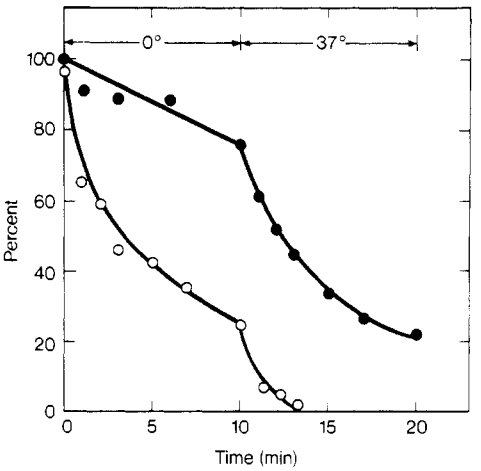

FIGURE 4: Stability of bound [α-^{32}P]nucleotide during the reduction of [α-^{32}P]GDP^{ox}. The data shown by the open circles were taken from the experiment described in part B and represents the rate of disappearance of [α-^{32}P]GDP^{ox} during the reduction of bound nucleotide. The experiment shown by the closed circles was performed in exact parallel to this except that the simultaneous reduction and chase of bound nucleotide was begun by the addition of 40 μl of a solution containing 0.125 M KBH_4 and 0.025 M GDP. After this addition, 15-μl aliquots were periodically removed and analyzed directly for bound [^{32}P]nucleotide by Millipore filtration as described in Materials and Methods.

sent hybrid molecules in which only one aldehyde group has been reduced.

The reduction reaction shown in Figure 3A was conducted in the presence of ribosomes in molar excess over GDP^{ox} and fusidic acid. An essentially identical reaction rate, including of course, the transient appearance of GDP$^{ox\text{-}red*}$, was observed when ribosomes and fusidic acid were omitted. Under conditions of complex formation, however, the rate of reduction of the bound nucleotide is considerably reduced (Figure 3B). This differed from the conditions of Figure 3A only in the addition of EF-G. It was necessary to elevate the reaction temperature to complete the reaction in a reasonable time. But the experiment shows that despite the substantial decrease in the rate of reduction, only GDP$^{ox\text{-}red*}$ (transiently) and GDP$^{ox\text{-}red}$ were observed as products. In a comparable experiment in which [α-^{32}P]GTP^{ox} was employed, the nucleotide has hydrolyzed during the binding phase of the experiment but the subsequent reduction was similar to that shown in Figure 3B.

In order to determine if reduction had actually occurred *in situ*, we compared the rate of GDP^{ox} reduction with its rate of dissociation from the complex. This was necessary because of the substantial protection of GDP^{ox} afforded by binding and because the reduction conditions might promote dissociation. Such a comparison is provided in Figure 4. The rate of reduction of GDP^{ox} clearly exceeds the rate of chase of bound GDP^{ox} by an excess of unlabeled GDP under identical conditions. It is therefore evident that reduction of GDP^{ox} has occurred while it is bound in complex.

Discussion

Periodate cleavage of the ribose ring of GDP, with or without subsequent borohydride reduction of the dialdehyde generated by this cleavage, does not substantially reduce the ability of the nucleotide to bind to a molar excess of EF-G and ribosomes in the presence of fusidic acid. In fact it would appear that under these conditions, with micromolar levels of the nucleotide, both GDP^{ox} and GDP$^{ox\text{-}red}$ are nearly quantitatively bound. This view is based on the finding that the retention of both modified nucleotides on Millipore filters, although variable and generally lower, none the less approaches that of GDP which is known to bind essentially quantitatively (Bodley *et al.*, 1970b). Moreover, GDP^{ox} when bound in this way is substantially protected from borohydride reduction. On the other hand, it is clear that GDP^{ox} and GDP$^{ox\text{-}red}$ are less stably bound than GDP, since both modified nucleotides are more rapidly chased from complex than is GDP. In addition, very little binding of either GDP^{ox} or GDP$^{ox\text{-}red}$ is observed in the absence of fusidic acid, although the binding of GDP is substantial under these conditions.

An essentially identical pattern of binding was observed with the analogous nucleotides derived from GTP, namely GTP^{ox} and GTP$^{ox\text{-}red}$, and both nucleotides were hydrolyzed upon binding. These results therefore indicate that neither the 2′- or 3′-hydroxyl nor an intact ribose ring are essential to either the binding or hydrolytic function of EF-G and the ribosome. However, both GTP^{ox} and GTP$^{ox\text{-}red}$ are extremely poor substrates in the usual hydrolytic reaction conducted in the absence of fusidic acid. This correlates with the poor binding of the ^{3}H derivatives in the absence of fusidic acid.

Periodate-oxidized nucleotides are well known to form Schiff's bases with a variety of amines including these found on proteins and these adducts are rendered stable by borohydride reduction (Erlanger and Beiser, 1964). We have established conditions where GDP^{ox} is efficiently bound to EF-G and the ribosome but borohydride reduction of this complex does not lead to the stable association of the nucleotide with a macromolecule. The bound nucleotide appears to be partially shielded and is reduced at a slower rate than the free nucleotide. However, an intermediate and a final product are formed during the reduction of bound GDP^{ox} which are chromatographically indistinguishable from those observed upon reduction of the free nucleotide. Moreover, all of our attempts to trichloroacetic acid precipitate nucleotide with the macromolecules of the complex following reduction of bound [^{3}H]GDP^{ox} or [α-^{32}P]GDP^{ox} have failed (Gordon and Bodley, 1974; Bodley and Gordon, unpublished observations). This suggests that there is not an amino group at or near the nucleotide binding site.

E. coli DNA-dependent RNA polymerase is known to possess an essential amino group in the vicinity of its nucleotide binding site (Ishihama and Hurwitz, 1969) and this amino group can form Schiff's base (Nixon *et al.*, 1972; Venegas *et al.*, 1973). It is therefore possible that periodate-oxidized nucleoside triphosphates would be useful in labeling the active site of this enzyme.

We conclude that neither the nucleotide binding nor hydrolytic functions of the ribosome and EF-G demand the integrity of the ribose ring or the presence of a 2′- or 3′-hydroxyl. When bound to the ribosome and EF-G, GDP^{ox} is substantially shielded from reduction by borohydride. This nucleotide, however, can be reduced while bound so that it apparently does not form a Schiff's base with the macromolecular components of the complex.

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Heat-Induced Deamination of Cytosine Residues in Deoxyribonucleic Acid†

Tomas Lindahl and Barbro Nyberg

ABSTRACT: The rate of deamination of cytosine residues in single-stranded and double-stranded *Escherichia coli* DNA, in the polynucleotides poly(dC) and poly(dG)·poly(dC), and in dCMP was investigated as a function of temperature, pH, and buffer composition. For this purpose, nucleic acids and polydeoxynucleotides specifically radioactively labeled in the cytosine residues were prepared. After heat treatment, the polymers were enzymatically degraded to mononucleotides or nucleosides, cytosine and uracil derivatives were separated by paper chromatography, and their radioactivity was determined. Cytosine in single-stranded DNA, poly(dC), or dCMP is similarly susceptible to hydrolytic deamination at pH 7.4, and the reaction proceeds at a rate of $k = 2 \times 10^{-7}$ sec^{-1} at 95°. From measurements at several temperatures it is estimated that the reaction is associated with an activation energy of 29 kcal/mol. These data indicate that a significant amount of conversion of cytosine to uracil occurs during heat denaturation of DNA by standard procedures. The cytosine residues in native DNA are well protected, and are deaminated at <1% of the rate observed with dCMP or poly(dC). In contrast, the cytosine residues in poly(dG)·poly(dC) were deaminated at 75% of the rate of those in poly(dC). The *in vivo* rate of deamination of cytosine residues in DNA is discussed.

If neutral aqueous solutions of DNA are incubated at high temperatures, changes in the covalent structure slowly accumulate. This heat-induced degradation is not indiscriminate. Instead, it is primarily due to hydrolytic attack at a small number of sensitive sites, in particular at the *N*-glycosyl bonds. Free purines and pyrimidines are consequently released at a slow rate from both single-stranded and double-stranded DNA at neutral pH (Greer and Zamenhof, 1962; Lindahl and Nyberg, 1972; Lindahl and Karlström, 1973). The deoxyribose residue at an apurinic or apyrimidinic site in DNA is no longer locked in the furanose form, and will be present in an equilibrium between this form and a reactive free aldehyde form. Several different types of secondary lesions therefore appear at such sites in DNA, including chain breaks (Tamm *et al.,* 1953; Lindahl and Andersson, 1972) and cross-links (Freese and Cashel, 1964; Burnotte and Verly, 1972).

Heat treatment of DNA might also lead to structural changes in base residues, in addition to the loss of occasional bases from DNA. Of the four common bases in DNA, cytosine should be most heat labile, as it is the base most sensitive to hydrolysis in either acid, neutral, or alkaline solution. Shapiro and Klein (1966) found that free cytosine and cytidine are relatively rapidly deaminated by heating in weakly acidic buffers, while there is no detectable deamination of adenosine or guanosine under such conditions, and they proposed that heat-induced cytosine deamination in DNA might have mutagenic implications. When the four common ribonucleotides (which have more stable glycosidic bonds than the deoxyribonucleotides) are incubated separately at 90° in neutral buffers of physiological ionic strength, deamination of CMP to UMP is the prevalent degradative reaction (Lindahl, 1967). Cytosine deamination is also the dominant mode of degradation for DNA in alkaline solution, as it takes place at an approximately tenfold faster rate than other alkali-catalyzed changes in the covalent structure of DNA, *e.g.,* alkali-catalyzed depurination and chain breakage, and imidazole ring opening in adenine residues (Hurst and Kuksis, 1958; Ullman and McCarthy, 1973; Garrett and Mehta, 1972). Further, transition mutations that accumulate spontaneously on prolonged storage of phage T4 suspensions, or on incubation of single-stranded T4 DNA in neutral solution, are apparently due to deamination of hydroxymethylcytosine residues (Drake, 1966; Baltz, 1973). In the present investigation, the rate of deamination of cytosine in DNA at pH values close to neutrality has been followed as a function of DNA conformation, temperature, pH, and buffer composition.

Experimental Section

Materials. Uniformly labeled [^{14}C]dCTP (432 Ci/mol) was

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