pH is the most compelling argument in support of such a mechanism, despite its improbability based on other considerations. Further study of the nature of any conformational changes between pH 6 and 3 should help to resolve this residual uncertainty.

Acknowledgments

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Ficellomycin and Feldamycin; Inhibitors of Bacterial Semiconservative DNA Replication†

Fritz Reusser

ABSTRACT: The two peptide-like antibiotics ficellomycin and feldamycin impair semiconservative DNA replication but not DNA repair synthesis in bacteria. Specifically both antibiotics cause the accumulation of a 34S DNA species in toluenized *Escherichia coli* cells which lacks the capability of being integrated into larger DNA pieces and eventually the complete bacterial chromosome. Novobiocin, a known inhibitor of replicative DNA synthesis, was investigated for comparative purposes. The action of this latter antibiotic differs from the ones exerted by ficellomycin and feldamycin in that novobiocin appears to block an event associated with the initiation of Okazaki fragments. The fact that novobiocin impairs DNA gyrase suggests that this enzyme plays an essential role during the initiation of Okazaki pieces.

The three antibiotics ficellomycin, feldamycin, and nojirimycin are produced by and were isolated from the culture broth of *Streptomyces ficellus* (Argoudelis et al., 1976b,c). Ficellomycin and feldamycin are new antibiotics; nojirimycin has been described previously and was shown to be 5-amino-5-deoxy-D-glucose (Ishida and Kumagi, 1967).

Chemically both ficellomycin and feldamycin represent dipeptide-like structures. Ficellomycin is a basic antibiotic and possesses a molecular formula of $C_{13}H_{24}N_6O_3$ corresponding to a formula weight of 312. The compound contains one valine residue per molecule and an unknown amino acid. Feldamycin is an amphoteric dipeptide composed of one residue of *N*-methylhistidine and a new histidine-like amino acid designated feldamycic acid (Argoudelis et al., 1976a) (Figure 1). The formula weight of feldamycin amounts to 407.

Both antibiotics inhibit a variety of bacteria in vitro. However, their specific activities assayed in vitro against whole bacterial cells are rather low. Accordingly, their minimal inhibitory concentrations (MIC) amount to >1000 μg/mL against most organisms when determined in broth. Nevertheless, ficellomycin is effective for the treatment of experimental *Staphylococcus aureus* infections in mice; feldamycin lacks any in vivo activity in these tests (Argoudelis et al., 1976b,c).

Investigations of the mode of action of ficellomycin and feldamycin have shown that these agents selectively impair semiconservative DNA replication when studied in *Escherichia coli* cells deficient in DNA polymerase I and rendered permeable to nucleotides by toluene treatment. Specifically these

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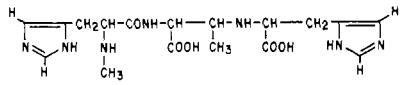

FELDAMYCIN

FIGURE 1: Structure of feldamycin.

antibiotics cause the accumulation of DNA molecules sedimenting at approximately 34 S.

Methods

Strain. The mutant strain *E. coli* H560 was used throughout these studies and was originally obtained from Dr. Hoffmann-Berling. This strain is F^+, *endoI*$^-$, *polA*$^-$, su_{am}^-, sm^r, $T6^R$, ϕX^S.

Growth and Toluene Treatment of Cells. Cells were grown in a medium containing per liter: tryptone (Difco), 10 g; NaCl, 5 g; thymine, 10 mg; thiamine, 200 μg. They were grown to an optical density of 0.6 OD_{570} unit, harvested by centrifugation, and washed once with 0.25 culture volume of 0.05 M potassium phosphate buffer (pH 7.5). The cells were then suspended in 0.1 vol of 0.05 M potassium phosphate buffer containing 1% toluene and stirred at room temperature for 10 min (Moses and Richardson, 1970; Moses, 1972). Following toluene treatment the cells were removed by centrifugation, the pellet and tube walls thoroughly rinsed with 0.05 M potassium phosphate buffer, and the pellet was resuspended with the same buffer in 0.1 vol of the original culture medium. This cell suspension was divided into small aliquots and stored in a liquid nitrogen atmosphere until used.

Reaction Mixtures, DNA Synthesis. Reaction mixtures to assess ATP-dependent DNA replication contained in a total volume of 0.3 mL: Tris-HCl[1] buffer, pH 8.0, 50 mM; KCl, 0.1 M; $Mg(OAc)_2$, 10 mM; ATP, 2 mM; dCTP, dGTP, dATP, 0.5 mM each; [^{3}H]TTP, 0.02 mM containing 2.5 μCi/sample and approximately 2×10^8 cells/sample. Assay mixtures for the assessment of DNA repair synthesis were as above except that they lacked ATP but contained 0.15 μg of pancreatic DNase I (Worthington) per sample. Incubations were carried out at 37 °C for 30 min. The reaction was terminated by the addition of 3-mL portions of cold 10% trichloroacetic acid (Cl_3AcOH)–0.1 M sodium pyrophosphate. The precipitates were collected on 0.45 μm Millipore filters and washed extensively with cold 0.1 N HCl. Radioactivity was determined by conventional liquid scintillation spectrometry techniques.

Reaction Mixtures, RNA Synthesis. RNA synthesis in toluenized *E. coli* cells was assayed in mixtures of 0.3 mL total volume and contained: Tris-HCl, pH 8.0, 50 mM; KCl, 10 mM; $Mg(OAc)_2$, 10 mM; $MnCl_2$, 0.2 mM; ATP, CTP, GTP, 0.5 mM each; [^{14}C]UTP, 0.1 mM containing 0.05 μCi per sample, and approximately 2×10^8 cells per sample. Further processing of the samples was as described above for the DNA synthesis assay.

Isopycnic Centrifugations. Reaction mixtures of 1.2 mL volume were prepared as described above with [^{3}H]TTP replaced by 0.5 mM bromodeoxyuridine triphosphate and dCTP replaced by 0.02 mM [^{3}H]dCTP containing 15 μCi/mL sample. The reaction mixtures contained either ATP or pancreatic DNase and no ATP. Incorporation of label was terminated after 30 min by the addition of 1.2 mL of cold 0.1 M Na_2EDTA, the cells were centrifuged and washed once in 1 mL of cold Tris-EDTA-NaCl buffer containing 0.01 M Tris-HCl (pH 8.0), 0.01 M Na_2EDTA, 0.1 M NaCl. The pellets were then resuspended in 1 mL of cold Tris-EDTA-NaCl buffer defined above, 500 μg of lysozyme was added, and the suspensions were incubated at 37 °C for 30 min. The cells were then lysed by the addition of 0.1 mL of 5% sarcosyl (Ciba-Geigy) and the viscosity of the lysates was reduced by strongly stirring the samples on a vortex mixer. Aliquots of 0.9 mL of lysate were mixed with 8.1 mL of CsCl solution (the latter possessing a density of 1.732) in a polyallomer tube which was then filled with light mineral oil. The samples were centrifuged in a fixed angle 75-Ti Spinco rotor for 40 h at 50 000 rpm at 19 °C. After completion of the run, the tubes were pierced from the bottom and four-drop fractions were collected. Densities were determined by refractometry. Acid-insoluble materials were precipitated by the addition of 1 mL of cold 10% Cl_3AcOH per fraction; the precipitates were collected on Millipore filters, washed with 0.1 N HCl, and counted.

Velocity Sedimentation Analysis. Reaction mixtures of 1.2 mL total volume were prepared. The composition of these mixtures was as described above for the assessment of semiconservative replication. The cells were pulse labeled for 10 min with [^{3}H]TTP in the presence or absence of antibiotic. Appropriate control samples were removed at this time. Parallel samples were chilled and centrifuged for 5 min at 10 000*g* in a refrigerated Sorvall centrifuge and the pellets were washed with 1 mL of 0.05 M potassium phosphate buffer (pH 7.5) and then resuspended in the standard reaction mixture in which [^{3}H]TTP was replaced with 0.02 mM unlabeled TTP. The samples were reincubated for an additional 40 min to chase the radioactive label. The reaction was terminated by the addition of 1.2 mL of cold 0.1 M Na_2EDTA. The cells were recovered by centrifugation and lysed as described above in a total volume of 0.5 mL. DNA in the lysates was denatured by the addition of concentrated NaOH to 0.1 N and 0.4-mL sample aliquots were applied on top of an alkaline linear 5–20% sucrose gradient. The gradients had a total volume of 12 mL in a polyallomer tube and contained in addition to sucrose 0.1 N NaOH, 0.9 N NaCl, and 2 mM Na_2EDTA. The samples were centrifuged in an SW40Ti rotor for 3 h at 38 000 rpm and 19 °C. Tubes were pierced from the bottom and four-drop fractions were collected. One milliliter of a 10% cold Cl_3AcOH solution was added per fraction and the acid-precipitable material was collected on Millipore filters, washed, and counted.

Results

Effects of Ficellomycin and Feldamycin on DNA Replication and Repair Synthesis. Ficellomycin showed inhibition of semiconservative DNA synthesis in toluenized *E. coli* cells when present in relatively high concentrations (Table I). Inhibitions of approximately 50% resulted in the presence of 0.5 to 1 mM ficellomycin. DNA repair synthesis on the other hand was not impaired by ficellomycin.

Feldamycin also inhibited DNA replication in the test system if present in relatively high concentrations while DNA repair synthesis was only moderately affected (Table I).

Neither of these antibiotics inhibited highly purified DNA polymerase I.

Effects of Ficellomycin and Feldamycin on RNA Synthesis in Toluenized E. coli Cells. Ficellomycin and feldamycin inhibited RNA synthesis in this system to a moderate extent only (Table II). In addition ficellomycin did not impair the function of highly purified *E. coli* RNA polymerase. Feldamycin on the other hand inhibited this enzyme moderately.

[1] Abbreviations used are: Tris, tris(hydroxymethyl)aminomethane; Cl_3AcOH, trichloroacetic acid; EDTA, ethylenediaminetetraacetic acid.

TABLE I: Effect of Ficellomycin and Feldamycin on DNA Replication and Repair in Toluenized *E. coli* Cells.[a]

Sample	[^{3}H]TMP incorpd (cpm/sample)	% inhibition
DNA replication		
Control	8240	0
Ficellomycin, 1 mM	4580	45
0.5 mM	4100	51
0.1 mM	6980	16
DNA repair		
Control	3140	0
Ficellomycin, 1 mM	3140	0
0.5 mM	3580	0
DNA replication		
Control	4980	0
Feldamycin, 1 mM	1012	80
0.1 mM	4060	19
DNA repair		
Control	3280	0
Feldamycin, 1 mM	2900	12
0.1 mM	2930	11

[a] Reaction mixtures (replication) contained in a total volume of 0.3 mL: Tris-HCl buffer, pH 8.0, 50 mM; KCl, 0.1 M; $Mg(OAc)_2$, 10 mM; ATP, 2 mM; dCTP, dGTP, dATP, 0.5 mM each; [^{3}H]TTP, 0.02 mM containing 2.5 μCi/sample and approximately 2×10^8 *E. coli* cells/sample. To assess DNA repair the samples contained no ATP but 0.15 μg of pancreatic DNase per sample. Incubation of the reaction mixtures was for 30 min at 37 °C.

TABLE II: Effect of Ficellomycin and Feldamycin on RNA Synthesis in Toluenized *E. coli* Cells.[a]

Sample	[^{14}C]UMP incorpd (cpm/sample)	% inhibition
Control	1160	0
Ficellomycin, 0.5 mM	1051	10
Feldamycin, 0.5 mM	950	18

[a] Reaction mixtures contained in a total volume of 0.3 mL: Tris-HCl, pH 8.0, 50 mM; KCl, 10 mM; $Mg(OAc)_2$, 10 mM; $MnCl_2$, 0.2 mM; ATP, GTP, CTP, 0.5 mM each; [^{14}C]UTP, 0.1 mM containing 0.05 μCi per sample and approximately 2×10^8 cells/sample. Incubation was at 37 °C for 30 min.

Effects of Ficellomycin and Feldamycin on Peptide Biosynthesis. Neither of the two antibiotics under study inhibited a Nirenberg-type cell-free amino acid incorporation system prepared as described by Reusser (1973) (Table III).

Isopycnic Analysis of DNA Synthesized. The products of DNA synthesis were also analyzed by density label experiments done in the presence and absence of the two antibiotics under study. In these experiments TTP was replaced by its analogue bromodeoxyuridine triphosphate, dCTP was replaced by [^{3}H]dCTP, and the products of de novo DNA synthesis were analyzed in neutral CsCl equilibrium gradients. Under conditions of semiconservative replication the radioactive label should band in the hybrid density region of the gradient; under conditions of DNA repair synthesis, the label should be confined to the light region of the gradient.

In the presence of ficellomycin it was found that replicative DNA synthesis was inhibited to some extent (Figure 2). DNA repair synthesis on the other hand was not impaired by this antibiotic (Figure 3).

TABLE III: Effect of Ficellomycin and Feldamycin on Peptide Synthesis.[a]

Sample	Incorpn (cpm/sample)	% of control
Control	3980	100
Ficellomycin, 1 mM	3890	97
0.1 mM	3620	90
Feldamycin, 1 mM	3700	93
0.1 mM	4060	101

[a] Reaction mixtures contained in a total volume of 0.25 mL: Tris-HCl buffer, pH 7.8, 100 mM; magnesium acetate, 14 mM; KCl, 60 mM; ATP, 1 mM; GTP, 0.03 mM; mercaptoethanol, 6 mM; phosphoenolpyruvate, potassium salt, 75 mM; pyruvate kinase (Calbiochem), 10 μg; ^{12}C-labeled amino acid mixture of 19 amino acids, 0.2 mM each; [^{14}C]phenylalanine, 1 mM containing 1.75 μCi per sample; polyuridylate, 15 μg; S-30 enzyme, 430 μg of protein. The samples were incubated at 37 °C for 30 min.

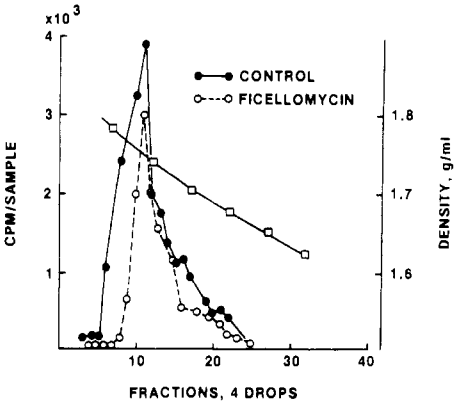

FIGURE 2: Isopycnic analysis of DNA replication products, effect of ficellomycin. Reaction mixtures and analysis were performed as described under Methods. Ficellomycin concentration was 1 mM.

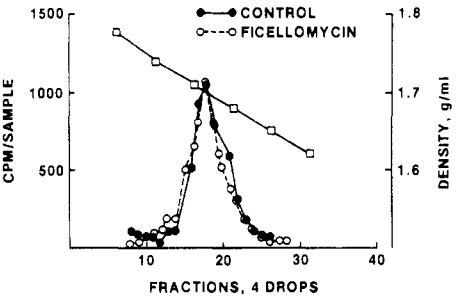

FIGURE 3: Isopycnic analysis of DNA repair synthesis products, effect of ficellomycin. Reaction mixtures and analysis were as described under Methods. Ficellomycin concentration was 1 mM.

Feldamycin also inhibited semiconservative DNA synthesis to a moderate extent (Figure 4). Repair DNA synthesis was not inhibited by feldamycin and appeared actually somewhat stimulated by this antibiotic (Figure 5).

The extent of inhibition of semiconservative DNA replication observed in these experiments appeared small and did not convincingly account for the antibacterial activities of these antibiotics. The possibility was thus considered that the antibiotics might not appreciably inhibit the de novo synthesis of small precursor DNA molecules such as Okazaki pieces but

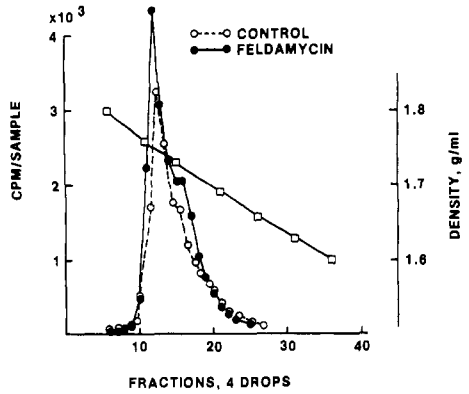

FIGURE 4: Isopycnic analysis of DNA replication products, effect of feldamycin. Feldamycin concentration was 1 mM.

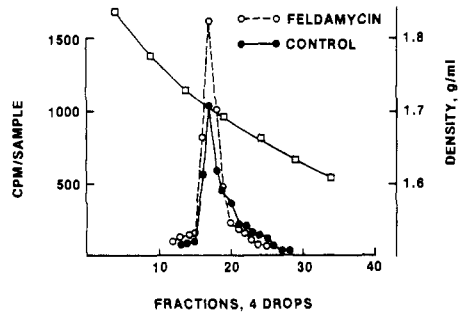

FIGURE 5: Isopycnic analysis of DNA repair synthesis products, effect of feldamycin. Feldamycin concentration was 1 mM.

might impair the further integration of the latter into larger DNA fragments and eventually the bacterial chromosome. For this reason pulse-chase experiments were done to test this hypothesis.

Pulse and Chase in the Presence of Ficellomycin. In these experiments the cells were labeled for 10 min with [^{3}H]TTP in the presence of drug. The label was then chased for 40 min with unlabeled TTP. The newly synthesized DNA was analyzed in alkaline sucrose gradients.

At the end of the pulse period the control sample yielded a broad single peak whose apex had a sedimentation value similar to λ DNA which was used as a marker and amounts to approximately 34 S (Figure 6). For the sake of simplicity we shall refer to this population of DNA molecules as 34S material throughout this paper. In the presence of ficellomycin a peak of nearly the same intensity and position on the gradient was observed. Following a chase of 40 min the gradient profile of the control sample revealed a much broader peak than observed at the end of the pulse period with a maximum located in the middle of the gradient and thus well above a sedimentation value of 34 S. This indicates that during the chase the label in the control sample was gradually integrated into a population of more heterogeneous and larger sized DNA fragments. On the other hand, if ficellomycin was present the 34S peak observed after the pulse did not shift and remained in the same position. Hence little or no active label could be chased into larger DNA fragments. This suggests that ficellomycin causes the accumulation of a DNA population with a mean size of 34 S which cannot be integrated further into DNA fragments of larger sizes. It is of interest to note that these 34 S fragments are larger than the Okazaki fragments which have a size of approximately 10 S.

Pulse in the Absence and Chase in the Presence of Ficellomycin. If the cells were pulse labeled in the absence of drug

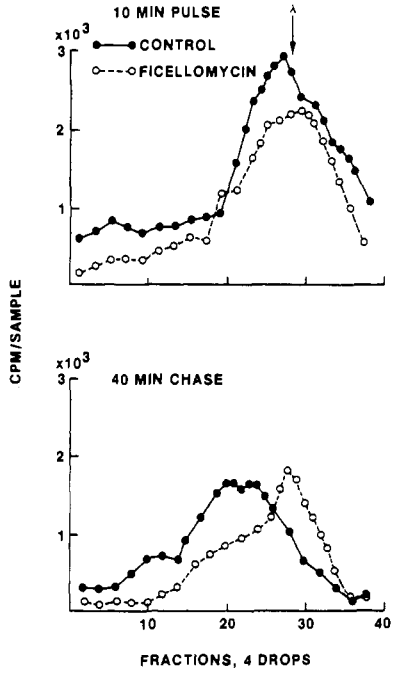

FIGURE 6: Pulse and chase in presence of ficellomycin (1 mM). Analysis on alkaline sucrose gradients. The reaction mixture for semiconservative DNA synthesis and preparation of the sucrose gradients are described under Methods. Direction of gradients is from right to left.

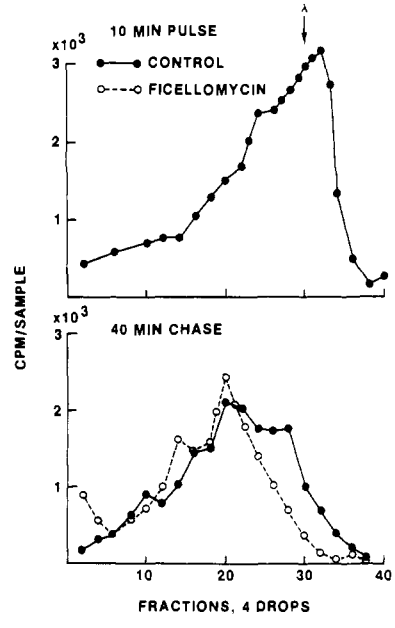

FIGURE 7: Pulse in the absence and chase in the presence of ficellomycin (1 mM). Analysis on alkaline sucrose gradients.

and then chased in the presence of ficellomycin the accumulated pulse-labeled material was now integrated during the chase into DNA fragments of a size larger than 34 S (Figure 7). Hence, if the pulse-labeled fragments are formed in the absence of ficellomycin the drug does not impair their further incorporation into larger DNA fragments.

This indicates that ficellomycin does not inhibit the initial steps of replication including the polymerases. However, the 34S fragments formed in the presence of the antibiotic must be deficient in some function and lack the ability of being further integrated into larger DNA fragments and eventually the whole bacterial chromosome.

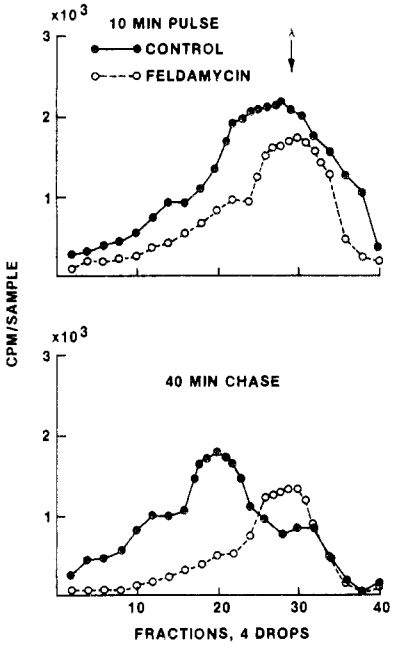

FIGURE 8: Pulse and chase in the presence of feldamycin (1 mM). Alkaline sucrose gradient analysis.

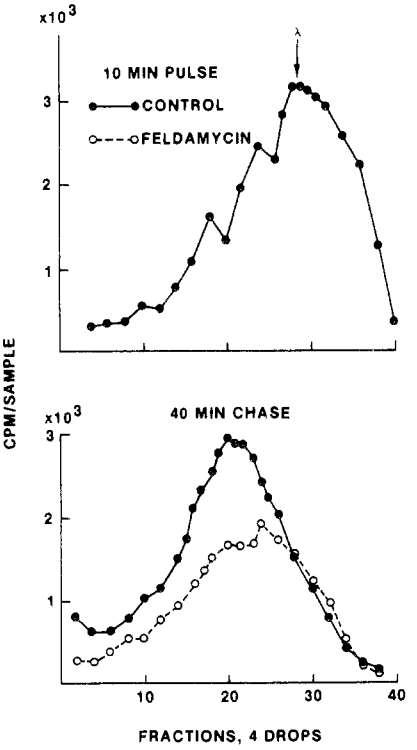

FIGURE 9: Pulse in the absence and chase in the presence of feldamycin (1 mM). Alkaline sucrose gradient analysis.

Pulse and Chase in the Presence of Feldamycin. If feldamycin was present during the pulse period, 34S DNA fragments accumulated which had a mean size marginally smaller than observed in the control sample (Figure 8). In addition less label was present in the sample containing feldamycin as compared to the control sample.

After a chase of 40 min the label in the control sample was integrated into larger DNA fragments as already discussed above in the experiments with ficellomycin. The labeled material accumulated in the presence of feldamycin was unable to integrate into fragments of larger sizes during the chase period and remained accumulated as 34S material.

It should be pointed out that some label was lost during the chase period in both the control and the drug containing samples which probably stems from some nuclease activity during the chase period. It is also possible that small DNA fragments escape from these toluenized cells during the chase period.

Pulse in the Absence and Chase in the Presence of Feldamycin. If the cells were pulsed in the absence of feldamycin and the drug was added at the start of the chase period the labeled 34S DNA fragments were now integrated to some extent into larger fragments (Figure 9). However, less labeled DNA was recovered from the sample chased in the presence of feldamycin as compared to the control sample.

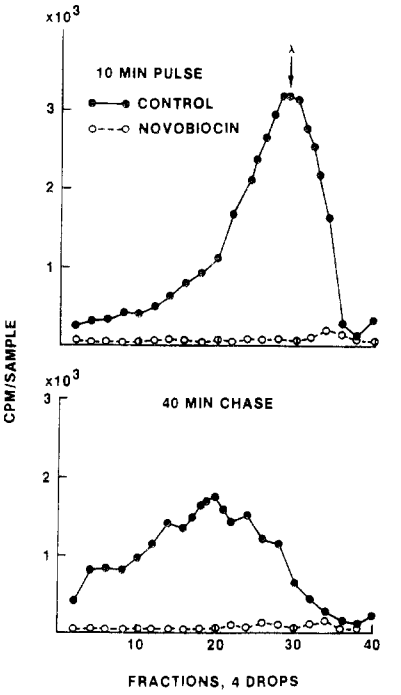

FIGURE 10: Pulse and chase in the presence of novobiocin (0.1 mM). Alkaline sucrose gradient analysis.

Pulse and Chase in the Presence of Novobiocin. Novobiocin is a known inhibitor of DNA replication in bacterial cells (Staudenbauer, 1975). Under our experimental conditions no [^{3}H]TTP incorporation occurred if novobiocin was present during the pulse and chase period (Figure 10).

Pulse in the Absence and Chase in the Presence of Novobiocin. If novobiocin was absent during the pulse period but added at the beginning of the chase period, the labeled material accumulated in the absence of novobiocin was now actively incorporated into DNA fragments of larger sizes (Figure 11). In other words, once small fragments (34 S) somewhat larger than Okazaki pieces are formed, novobiocin does not impair their further processing into larger DNA fragments. This indicates that novobiocin inhibits some function occurring during the early initiation of Okazaki pieces.

Discussion

The dipeptide antibiotics ficellomycin and feldamycin both contain new unusual amino acids. Feldamycin contains two histidine-like entities. Ficellomycin contains valine and an unknown amino acid. Both ficellomycin and feldamycin do not stabilize double-stranded natural DNA against heat dena-

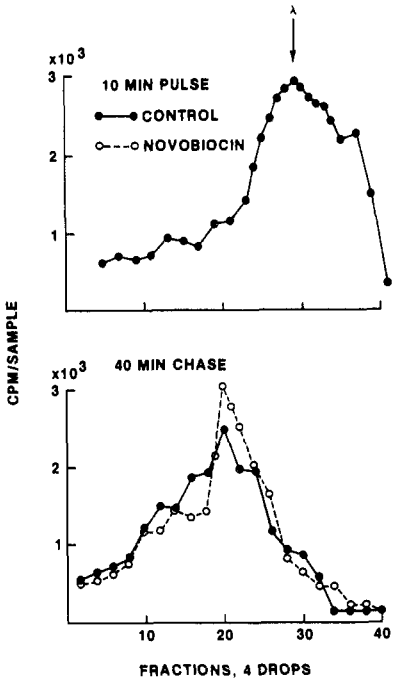

FIGURE 11: Pulse in the absence and chase in the presence of novobiocin (0.1 mM). Alkaline sucrose gradient analysis.

turation but they increase the melting temperature of the synthetic polydeoxynucleotide poly[d(A-T)] by 1–3 °C. No evidence of any perturbation of the base pairs in native DNA was found when analyzed by ultraviolet (UV) difference spectroscopy or circular dichroism (CD) (the latter done by Dr. Krueger). In terms of their structure, ficellomycin and feldamycin bear some resemblance to the histidyl, tyrosyl, and tryptophanyl di- and oligopeptides synthesized by Novak and Dohnal (1974a,b). These authors found that such peptides stabilize native DNA to some extent and induce snapback of denatured DNA. The extent of snapback exerted by ficellomycin and feldamycin was, however, found to be minimal when measured by thermal denaturation–renaturation kinetics and by the amount of double strandedness of denatured DNA with *Neurospora crassa* nuclease (results not shown).

Neither of the two antibiotics under study inhibits the action of highly purified DNA polymerase I, but feldamycin inhibits highly purified *E. coli* RNA polymerase to some extent.

Pulse-chase experiments have shown that ficellomycin and feldamycin cause the accumulation of a 34S DNA species during active semiconservative DNA replication in toluenized *E. coli* cells. If these 34S DNA fragments are preformed in the absence of the antibiotics and are then exposed to the drugs during the chase period only, ficellomycin does not impair the further integration of these fragments into DNA species larger than 34 S. Feldamycin does not suppress this latter process when present during the chase only but inhibits it to some extent and differs thus in its specific action from ficellomycin.

However, neither antibiotic inhibits steps occurring during the early initiation of replication and they do not inhibit the polymerases per se but instead cause the formation of deficient 34S DNA fragments. These fragments are functionally deficient in that they lack the capability to be further integrated into larger DNA fragments during the maturation and assembly process of the bacterial chromosome effected by the replication machinery. It is a consequence of this deficiency that these 34S DNA species accumulate and not due to an interference with an event occurring during the further processing of these 34S DNA species.

Novobiocin, which was included in these studies for comparative purposes, is a known inhibitor of DNA replication although it was unknown at which step inhibition occurs during replication. The results presented in this paper indicate that novobiocin must inhibit some event associated with the initiation of the replication process. This can be deduced from the observation that small DNA fragments formed in the absence of novobiocin can effectively be chased into larger DNA fragments despite the presence of the drug during the chase period. Gellert et al. (1976) showed very recently that novobiocin inhibits DNA supercoiling by DNA gyrase. In conjunction with our results this indicates that DNA gyrase is involved in DNA replication and exerts its action prior to or at the onset of initiation of Okazaki pieces.

It has also been shown that nalidixic acid causes the accumulation of DNA fragments sedimenting at 30–40 S during semiconservative DNA replication (Pisetsky et al., 1972; Crumplin and Smith, 1976) and thus appears to exert a similar if not identical effect as found for ficellomycin and feldamycin. Ficellomycin, novobiocin, and nalidixic acid have all shown therapeutic usefulness for the treatment of bacterial infections to various degrees. This indicates that the replicative processes in procaryotes and eucaryotes differ somewhat from each other and that these differences might suffice to be used for therapeutic purposes. An analogous situation pertains to pro- and eucaryotic ribosomes.

Both ficellomycin and feldamycin are relatively weak antibiotics when assayed in vitro against whole bacterial cells and show minimal inhibitory concentrations in excess of 1 mg/mL including *E. coli*. In analogy to this observation we now find that their inhibitory effect on DNA replication in toluenized cells also requires relatively high concentrations of these antibiotics. However, the concentrations required to effectively inhibit DNA replication in toluenized cells (100–200 μg/mL) are significantly lower than the ones required to inhibit the growth of intact cells and support our conclusion that the DNA replication process is the primary target for these antibiotics.

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Amino Acid Sequence of the V_H Region of a Human Myeloma Immunoglobulin (IgG New)†

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ABSTRACT: The amino acid sequence of the heavy-chain variable region of the human immunoglobulin. New has been determined. Since the amino terminus of the heavy chain was blocked, the sequence of residues 1-69 was established by digesting the appropriate CNBr fragment separately with trypsin, chymotrypsin, and thermolysin and sequencing the resulting peptides. The region from residues 70 to 120 was present in another CNBr fragment which was submitted directly to automatic Edman degradation. The result of this experiment extended the sequence to residue 100. The primary structure of the remaining portion of the V_H region was determined by automatic Edman degradation of a lysine-blocked tryptic peptide derived from this region which included residues 98-214. The sequence of the V_H region of New corresponds most closely to V_H sequences of proteins in the V_H II subgroup. This primary structure makes it possible to construct a model from the high-resolution electron-density map of protein New.

The three-dimensional structure of the Fab′ fragment of the human immunoglobulin New (IgG New) has been determined to a nominal resolution of 2 Å (Poljak et al., 1974). The light (L, λ) chain of IgG New has been sequenced and reported (Chen and Poljak, 1974). The study of the amino acid sequence of the heavy (H) chain was undertaken to obtain the necessary information for a complete interpretation of the 2.0-Å Fourier map of Fab′ New.

The amino acid sequence of IgG immunoglobulin H chains is usually divided into four homology regions, V_H, C_H1, C_H2, and C_H3, consisting of approximately 110 to 115 amino acids (Gally and Edelman, 1972). With the exception of well-characterized allotypic variants, human γ chains show sequence identity in their C_H regions as well as partial identity and strong homology in their variable V_H regions (Capra and Kehoe, 1975).

Two approaches were used to obtain peptides from the V_H region of New. In one case, Fab′ fragments were cleaved with CNBr and separated by gel filtration. This gave a fragment comprising residues 1-69. A combination of enzymatic and chemical degradation provided the required information to specify its sequence. The region from 70 to 120 was obtained via CNBr cleavage of partially reduced, alkylated heavy chain followed by separation of the fragments using gel filtration. Automatic sequenator runs on a CRBr fragment (residues 70-249) and on a tryptic peptide derived from this fragment (residues 98-214) gave results which allowed us to formulate a sequence for the entire heavy-chain variable region of protein New. This paper represents the evidence for the proposed sequence, gives a comparison of this sequence with other V_HII sequences, and discusses the relationship of this sequence with the electron-density map of protein New.

Experimental Section

Materials. Human myeloma immunoglobulin New (IgG_1, Gm(1+ 3− 4− 5−), λ) was purified from serum which had been kept frozen at −20 °C. Diethylaminoethylcellulose (0.9 mequiv/g) and carboxymethylcellulose (0.8 mequiv/g) were purchased from Serva. Sephadex G-100 and G-25 were obtained from Pharmacia. Tos-PheCH_2Cl trypsin, chymotrypsin, carboxypeptidase A (treated with DEF), carboxypeptidase B, and pepsin were purchased from Worthington Biochemical Corp. Thermolysin was obtained from Calbiochem. Dithiothreitiol, ethylenimine, cyanogen bromide, iodoacetic acid, dansyl chloride, and sequencing reagents were obtained either from Pierce Chemical Co. or from Beckman and were used fresh without further purification. Polyamide thin-layer sheets were purchased from Gallard-Schlesinger. Dansyl amino acid standards were prepared as described by Gray (1967a).

Preparation of H Chain from Protein New. IgG New was purified by precipitation with sodium sulfate followed by chromatography on diethylaminoethylcellulose and carboxymethylcellulose as described before (Rossi and Nisonoff, 1968). Mildly reduced and alkylated H chain was prepared by reducing a 20 mg/mL solution of IgG New in 0.2 M Tris-HCl (pH 8.5)-0.005 M EDTA-0.02 M dithiothreitol for 2 h at

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[1]Abbreviations used for immunoglobulins, their chains, and fragments are as recommended in *Bull. W.H.O. 30*, 447 (1964). Other abbreviations used are: dansyl, 1-dimethylaminonaphthalene-5-sulfonyl; DFP, diisopropyl fluorophosphate; Na_2EDTA, disodium ethylenedinitrilotetraacetate; PhNCS, phenyl isothiocyanate; Pca, pyrrolidonecarboxylic acid; Tos-PheCH_2Cl, 1-tosylamido-2-phenylethyl chloromethyl ketone; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; EDTA, (ethylenedinitrilo)-tetraacetic acid.