Some Characteristics of and Structural Requirements for the Interaction of 24,25-Dihydrofusidic Acid with Ribosome · Elongation Factor G Complexes†

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ABSTRACT: Fusidic acid inhibits polypeptide chain elongation by binding to the ribosome · elongation factor-G · GDP complex and thereby preventing its dissociation. The experiments reported here quantitate the interaction of the antibiotic [^{3}H]-24,25-dihydrofusidic acid, an active analog of fusidic acid, with the ribosome · elongation factor-G · GDP complex. All components of the complex are essential for [^{3}H]-24,25-dihydrofusidic acid binding. The stoichiometry of the interaction is *ca.* 1:1, and the K_a apparent, as determined by equilibrium dialysis, is $2.6 \times 10^6\ M^{-1}$. It is further shown that GTP and GDP are equally effective in forming complexes to which the antibiotic may bind, whereas GMP and β,γ-methyleneguanosine triphosphate will not form complexes to which the antibiotic may bind. In order to examine the structural basis of the mode of antibiotic action shown by fusidic acid, we have considered two activities of 21 structural analogs of this antibiotic: ability to bind to the aforementioned ternary complex and ability to stabilize this complex. The comparative binding capability of the analogs were established through competition experiments with [^{3}H]-24,25-dihydrofusidic acid. The data obtained from these experiments can be summarized as follows. (1) The C_{17-20} double bond of fusidic acid appears to be critical for both binding and complex stabilization activities. (2) A carboxyl group in the vicinity of the C_{20} carbon is also essential for both activities. (3) Modifications of other functional groups in the molecule can lead to significantly decreased stabilization of the ternary ribosome complex and/or ability to compete with [^{3}H]-24,25-dihydrofusidic acid for binding to the complex, but do not demonstrate absolute structural requirements for either activity.

Elongation factor G (EF-G)[1] functions in the translocation step of protein synthesis to: (1) move the peptidyl-tRNA from the A to the P site; (2) move the ribosome along the mRNA so as to bring the next codon into juxtaposition with the empty A site, and (3) hydrolyze GTP (for a recent review, see Haselkorn and Rothman-Denes, 1973). During protein synthesis, GTP hydrolysis is coupled to reactions 1 and 2 above and presumably supplies the energy for them. Using purified components in an in vitro system, however, one can observe an uncoupled GTP hydrolysis in the absence of protein synthesis catalyzed by just EF-G and the ribosome (Nishizuka and Lipmann, 1966). This uncoupled reaction has provided a model system for the study of translocation, since both it and the overall process of translocation are apparently inhibited similarly (Tanaka et al., 1968).

Of the antibiotic inhibitors, fusidic acid has proven singularly useful in examining the mechanism of the translocation step in protein biosynthesis. Its inhibitory action derives from its ability to stabilize both prokaryotic (Bodley et al., 1969) and eukaryotic (Bodley et al., 1971) ribosome · translocation factor · GDP complexes and thus prevent growth of the polypeptide chain. The stoichiometry of the stabilized complex is *ca.* 1:1:1, and if GTP is used as substrate hydrolysis takes place even in the presence of the antibiotic, so that the same GDP-containing complex is isolated (Bodley et al., 1970a,b). Fusidic acid does not inhibit GTP hydrolysis per se, but prevents the reutilization of EF-G by stabilizing the ribosome · EF-G · GDP complex. The post-translocation ribosomal complex which occurs during protein synthesis is also stabilized by fusidic acid (Modolell and Davis, 1970). Thus fusidic acid appears to act in both systems by the unique process of delaying the dissociation of a product-containing complex which occurs in the normal course of the interaction of EF-G with the ribosome. Okura et al. (1971) have shown that in stabilizing the ribosome·EF-G · GDP complex, [^{3}H]fusidic acid binds to it. Unfortunately, the examination of this binding reaction has been limited by the relatively low specific radioactivity of [^{3}H]fusidic acid.

By all criteria which have been examined, 24,25-dihydrofusidic acid (DHFA) is functionally equivalent to fusidic acid. Both compounds are equally effective in inhibiting bacterial growth (Godtfredsen, 1967), in inhibiting in vitro polyphenylalanine formation, and in stabilizing the ribosome · EF-G · GDP complex (Bodley and Godtfredsen, 1972). Of equal importance to the present work is the fact that [^{3}H]DHFA can be readily obtained with high specific radioactivity. Utilizing such material we demonstrate in the present report that [^{3}H]DHFA not only binds to the ribosome · EF-G · GDP complex, but exhibits an affinity which is essentially identical with that of fusidic acid.

Utilizing [^{3}H]DHFA and the techniques of Millipore filtration and equilibrium dialysis the experiments reported here are designed to examine the nature of the binding reaction between DHFA and the ribosome and EF-G as well as to define the structural features of the antibiotic which are required for this binding.

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[1] Abbreviations used are: EF-G, elongation factor G; FA, fusidic acid; DHFA, 24,25-dihydrofusidic acid; GDPCP, β,γ-methyleneguanosine triphosphate.

Table I: Requirements for [^{3}H] DHFA Binding as Determined by Millipore Filtration.[a]

System[b]	[^{3}H] DHFA Bound (pmol)
−R, −EF-G, −GTP	(0.02)[c]
Complete	1.40
−R	0.03
−EF-G	0.02
−GTP	0.04

[a] Binding reaction and Millipore filtration was performed as described in the Experimental Section. [b] The complete system, in addition to the usual components contained: 56 pmol of ribosomes, 33 units of EF-G, 5.0 nmol of GTP, and 12 pmol of (3.6×10^5 cpm) [^{3}H] DHFA. Omissions were as indicated. [c] Experimental results were corrected for this blank value.

Materials and Methods

The nucleotides GTP, GDP, and GMP were purchased from Sigma Chemical Corp. GDPCP was purchased from Miles Laboratories. [^{3}H]GTP (specific activity 5860 cpm/pmol) was obtained from New England Nuclear Corporation, and cellulose nitrate filters (HAWP) from the Millipore Corporation.

Fusidic acid was used as its sodium salt. The fusidic acid analogs were synthesized by procedures elsewhere described (Godtfredsen and Vangedal, 1962, 1966; Godtfredsen et al., 1966a,b; Godtfredsen, 1967; Janssen and Vanderhaeghe, 1967; Von Daehne et al. 1968) and checked for purity by thin-layer chromatography in several solvent systems. The analogs were dissolved in ethanol and then converted to the sodium salt by the addition of sodium bicarbonate. The ^{3}H-labeled 24,25-DHFA (specific activity 3×10^4 cpm/pmol) was prepared by the New England Nuclear Corporation from fusidic acid through a catalytic hydrogenation procedure based on that described by Godtfredsen and Vangedal (1962). Purity of the labeled product was examined by silica gel thin-layer chromatograph with ascending development in chloroform–acetic acid–cyclohexane–methanol, 32:4:4:1. Eighty-two percent of the radioactivity comigrated with DHFA.

Ribosomes were prepared from *Escherichia coli* B cells, as previously described (Bodley, 1969). EF-G from the same source was prepared (Rohrbach et al., 1974). Since the ribosomes and EF-G were stored in buffers containing 50% glycerol, these components of the reaction mixtures were dialyzed to remove the glycerol before use in the equilibrium dialysis experiments. Ribosome concentrations were calculated on the basis of their A_{260}, and EF-G was assayed by the isolation of the ribosome · EF-G · [^{3}H]GDP complex on Millipore filters. One unit of EF-G corresponds to 1 pmol of [^{3}H]GDP bound to the Millipore membrane under specified conditions (Highland et al., 1971).

Equilibrium Dialysis. These experiments were performed in the dark at 0–2° in a solution which contained: 10 m*M* Tris-Cl (pH 7.4), 10 m*M* NH_4Cl, 10 m*M* magnesium acetate, 1 m*M* dithiothreitol, 1% ethanol (v/v), 5×10^{-5} *M* GDP, and [^{3}H]DHFA (*ca.* 5×10^5 cpm/ml) plus sufficient unlabeled DMFA to yield a final concentration of 2×10^{-7}–1×10^{-3} *M*. Samples (0.1–0.5 ml) of ribosomes and/or EF-G in Viscane tubing (Fischer Corp.) were dialyzed against 5 ml of the above buffer. The internal and external fluids were periodically sampled and the dialysis was continued until equilibrium was reached in 6–9 days. Control experiments indicated that ribosomes and EF-G were stable under these conditions and that GDP degradation was insignificant.

[^{3}H]GDP Binding. In these experiments the ability of a particular fusidic acid analog to stabilize the ribosome · EF-G · GDP complex was compared to that of fusidic acid. The formation of this complex in the presence of fusidic acid, or an analog, was conducted as previously described (Highland et al., 1972). Each 50-μl reaction mixture contained reaction buffer (10 m*M* Tris-Cl (pH 7.4), 10 m*M* NH_4Cl, 10 m*M* magnesium acetate, and 1 m*M* dithiothreitol), 500 μg/ml of FA or FA analog, 13.4 pmol of [^{3}H]GTP, 46 pmol of ribosomes, and 6.9 units of EF-G. The reaction mixture was incubated at 0° for 5 min and then filtered with a wash buffer containing 10 m*M* Tris-Cl (pH 7.4), 10 m*M* NH_4Cl, and 10 m*M* magnesium acetate. The filters, after drying, were counted in a Beckman LS-100 liquid scintillation counter.

[^{3}H]DHFA Binding to Complexes Isolated on Millipore Membranes. [^{3}H]DHFA · ribosome · EF-G · GDP complexes were isolated on Millipore filters (type HP) essentially as described above for the isolation of the ribosome · EF-G · [^{3}H]GDP complex in the presence of fusidic acid. The binding mixtures contained the buffer employed in [^{3}H]GDP binding and ribosomes, EF-G, nucleotide, and antibiotic detailed for the specific experiments. Extra care was required to keep the filtration time short and constant. The inclusion of 10^{-5} *M* fusidic acid in the buffer used for presoaking the filters (also used for dilution and wash buffer) and prefiltration of [^{3}H]DHFA was found to reduce [^{3}H]DHFA binding in the absence of ribosomes and EF-G.

Fusidic Acid Analog Competition Binding Experiments. The experiments were based on the Millipore assay for DHFA binding to the EF-G · ribosome · GDP complex. Competition experiments consisted of first adding a constant amount of [^{3}H]DHFA and varying concentrations of fusidic acid analog to a reaction system containing the components of the ternary complex, and then quantitating the effectiveness of a particular analog in replacing the labeled DHFA. A 50-μl reaction mixture contained: the buffer employed in [^{3}H]GDP binding and 46 pmol of ribosomes, 7.6 pmol of [^{3}H]DHFA, and fusidic acid analog concentrations ranging from 0.05 to 1500 μg/ml. The reaction was initiated by the addition of 5 units of EF-G and the reaction mixtures were incubated for 10 min at 0° and then filtered. The concentration of analog necessary to reduce [^{3}H]DHFA binding by 50% was determined by graphic interpolation. The reproducibility of the system was tested in four separate experiments with fusidic acid where the range of values was found to be within 10% of the mean. [^{3}H]DHFA binding in the absence of analogs corresponded to approximately 1 pmol.

Results

The Requirements for and Kinetics of [^{3}H]DHFA Binding. The data shown in Table I demonstrate that [^{3}H]DHFA, like fusidic acid, binds to the ribosome · EF-G · GDP complex. Moreover, because [^{3}H]DHFA can be prepared with high specific radioactivity, the binding of this compound to the ribosome can be assessed by Millipore filtration. These data also demonstrate that this binding requires all three components of the complex, and at these low concentrations, DHFA does not appear to bind either to the ribosome or EF-G alone.[2]

[2] EF-G like the ribosome adsorbs to Millipore filters (L. Lin and J. W. Bodley, unpublished observations).

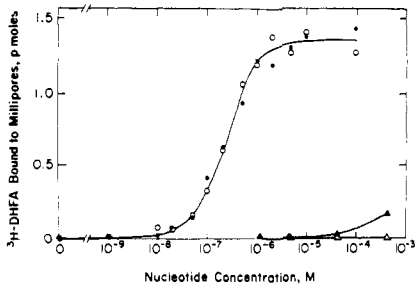

FIGURE 1: Guanine nucleotide requirement for the binding of [^{3}H]DHFA. The reaction mixture contained the components described in Table I plus the indicated level of nucleotide which was added to start the reaction: (●) GTP; (○) GDP; (▲) GMP; (△) GDPCP.

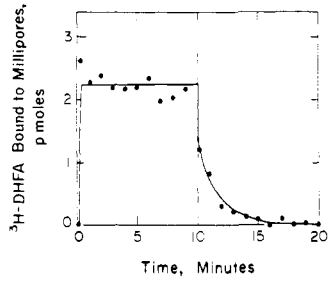

FIGURE 2: Kinetics of [^{3}H]DHFA binding and dissociation. The reaction mixture (0.6 ml) contained: 1200 pmol of ribosome, 1×10^{-4} *M* GDP, and 900 pmol of (9.6×10^6 cpm) [^{3}H]DHFA. The reaction was started by the addition of 180 units of EF-G, and 20-μl aliquots were periodically withdrawn, diluted, and filtered as described in the Experimental Section. After 10 min of incubation the concentration of DHFA was brought to 3.3×10^{-4} *M* by addition of 10 μl of 10^{-2} *M* unlabeled DHFA. A blank value of 0.22 pmol of [^{3}H]DHFA was subtracted from each point.

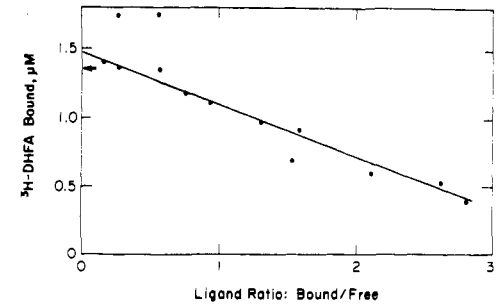

FIGURE 3: Scatchard plot of [^{3}H]DHFA binding to the ribosome · EF-G · GDP complex as determined by equilibrium dialysis. Each dialysis bag contained 1.32×10^{-5} *M* ribosomes and 1.36×10^{-6} *M* EF-G (arrow). GDP (5×10^{-5} *M*) was present in the dialysis fluid.

Table II: Requirements for [^{3}H]DHFA Binding as Determined by Equilibrium Dialysis.[a]

Components	Concn (*M*)	[^{3}H]DHFA Concn (*M*)	Ligand Bound/Free ± SD[b]
EF-G + GDP		1×10^{-5}	−0.04 ± 0.03
	1.6×10^{-5}	1×10^{-4}	+0.07 ± 0.05
	5×10^{-5}	1×10^{-3}	+0.01 ± 0.02
Ribosomes + GDP		1×10^{-5}	−0.03 ± 0.03
	3.1×10^{-5}	1×10^{-4}	−0.01 ± 0.03
	5×10^{-5}	1×10^{-3}	+0.01 ± 0.02
EF-G		2×10^{-7}	+0.11 ± 0.04
	2.4×10^{-6}	8×10^{-7}	+0.05 ± 0.04
	1.3×10^{-5}	4×10^{-6}	+0.10 ± 0.08

[a] The conditions were the same as in Figure 3 except that one component was omitted. [b] The data represent the averages of at least triplicate determinations of one or more experiments.

The nucleotide requirement for [^{3}H]DHFA binding is explored in more detail by the experiment shown in Figure 1. Maximal binding is achieved with only a slight molar excess of either GTP or GDP over the ribosome and EF-G and the two nucleotides are equally effective, while neither GMP nor GDPCP promotes significant binding. The small amount of binding induced by very high levels of GMP presumably represents trace contamination by either the di- or triphosphate.

The kinetics of [^{3}H]DHFA binding to the ribosome · EF-G · GDP complex at 0° are illustrated in Figure 2. The reaction is complete before the reaction can be measured (*ca.* 10 sec after the addition of [^{3}H]DHFA). Following the addition of a large excess of unlabeled DHFA, bound [^{3}H]DHFA dissociated with a half-time under these conditions of *ca.* 30 sec.

Stoichiometry and Affinity of [^{3}H]DHFA Binding. The number of [^{3}H]DHFA binding sites on the ribosome · EF-G · GDP complex and the apparent binding constant for this interaction were determined by equilibrium dialysis. Figure 3 shows a Scatchard plot of these data. Ribosomes and GDP were present in molar excess so that the concentration of complex was limited by the concentration of EF-G. Using the relationship: 1 unit = 2 pmol of EF-G (Highland et al., 1971), the concentration of complex in the experiment shown in Figure 3 was 1.36 μM, indicating that 1.09 mol of [^{3}H]DHFA was bound/mol of complex. The slope of the Scatchard plot yielded an apparent binding constant of 2.6×10^6 M^{-1}. In all experiments a single slope was obtained except at exceedingly high DHFA concentrations where measurements became unreliable.

Equilibrium dialysis also offers an opportunity to determine if [^{3}H]DHFA binding occurred with a reduced affinity to either the ribosome or EF-G alone. Representative data from experiments designed to detect such binding are given in Table II. Within the limitations of these measurements we observed no significant binding in the presence of GDP to either the ribosome or EF-G alone. Assuming that we could have detected a bound to free ligand ratio as low as 0.06, then if DHFA binds to these individual components it must do so with a binding constant less than 4×10^3 M^{-1}. Similarly no significant binding to EF-G and ribosomes was detected in the absence of guanine nucleotide.

Complex Stabilization and Competition Binding Experiments with Fusidic Acid Analogs. Fusidic acid analogs were tested for their ability to compete with [^{3}H]DHFA in binding to the ribosome · EF-G · GDP complex. Figure 4 presents the data obtained with a representative group of analogs. Similar curves were constructed for each of the 23 compounds studied. The concentration of analog necessary to achieve 50% inhibition of [^{3}H]DHFA binding was determined graphically from these curves, and the results are summarized in Table III.

Additionally, the 21 analogs and helvolic acid were compared to fusidic acid with regard to their capacity to stabilize the ribosome · EF-G · GDP complex. Retention of labeled guanine nucleotide on Millipore filters, a measure of complex stabilization, was determined as described under Materials and Methods and the results of these experiments are also tabulated in Table III. The 23 compounds, on the basis of complex stabilization, have been classified into three groups: those possessing activity greater than or equal

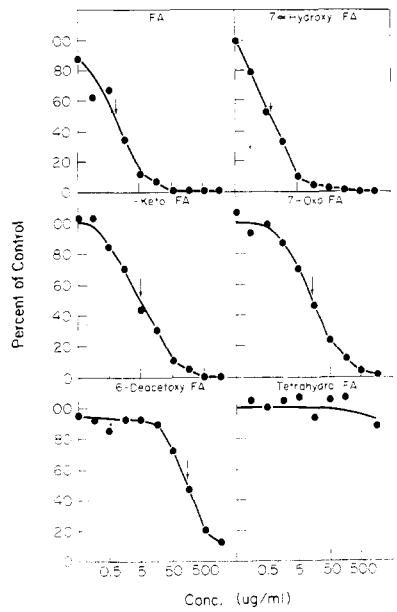

FIGURE 4: The concentration dependence of the competition between various fusidic acid analogs and [^{3}H]DHFA in binding to the ribosome · EF-G · GDP complex. The arrows indicate the concentration of analog which resulted in a 50% reduction of [^{3}H]DHFA (final concentration 6.6 × 10^{-7} M) binding.

to fusidic acid, intermediate activity, and marginal or no detectable activity.

With few exceptions, these analogs can be assigned to the same three categories on the basis of capacity to compete with [^{3}H]DHFA for binding to the ternary complex. However, the group III analogs can now be subdivided further. Under the experimental conditions employed, there are five analogs which exhibited some ability to compete with [^{3}H]DHFA but were unable to stabilize the ternary complex to any detectable extent. Three analogs, 16-epifusidic acid 3-acetate, tetrahydrofusidic acid, and lumifusidic acid, showed marginal or no activity with respect to both binding to and stabilization of the ribosome · EF-G · GDP complex. Similarly, among group II analogs, some modifications selectively reduced complex stabilization, without abolishing it altogether. 6β-Hydroxy-7-oxofusidic acid competes with [^{3}H]DHFA for binding 100-fold better than 3-keto-11α,32-oxido-24,25-dihydrofusidic acid yet gives a lower complex yield (31 and 47%, respectively).

Table III: Complex Stabilization and Inhibition of DHFA Binding by Fusidic Acid Analogs.

Group	Compound	Ribosome·EF-G·[^{3}H]-GDP Relative Complex Yield[a]	50% Inhibition of [^{3}H]DHFA Binding[b] (μg/ml)
I	Helvolic acid	178	0.15
	Fusidic acid	100	0.75
	24,25-Dihydrofusidic acid	91	0.75
	7α-Hydroxyfusidic acid	85	0.75
II	3-Keto-11α,32-oxido-24,25-dihydrofusidic acid	47	200
	Fusidyl glucuronide	41	35
	6β-Hydroxy-7-oxofusidic acid	31	2
	3-Epifusidic acid	30	100
	7-Oxofusidic acid	25	12
	11-Ketofusidic acid	24	5
	Methyl fusidate 3-hemisuccinate	20	>1500
	25,26,27-Trinorfusidic acid	19	*c*
	Fusidic acid 3-acetate	16	15
	11-Epifusidic acid	14	500
	16-Epidesacetylfusidic acid	11	15
IIIA	3-Deoxy-3-dehydro-24,25-dihydrofusidic acid	2	12
	16-Desacetylfusidic acid	5	50
	3,11-Diketofusidic acid	4	75
	16-Deacetoxy-15-dehydrofusidic acid	−1	200
	16-Deacetoxy-24,25-dihydrofusidic acid	−1	250
IIIB	16-Epifusidic acid 3-acetate	7	≫1500
	Tetrahydrofusidic acid	1	>1500
	Lumifusidic acid	3	≫1500

[a] The data are expressed as percent [^{3}H] relative to that obtained with fusidic acid. (Each antibiotic or analog was present at 500 μg/ml.) Under these experimental conditions, the binding of the ternary complex to Millipore filters in the absence of antibiotic was 10% that in the presence of fusidic acid. [b] Inhibition of [^{3}H]DHFA binding through competion with unlabeled analog was conducted as described in Figure 4. [c] Not determined.

Discussion

On the basis of evidence presented here and earlier evidence discussed in the introduction, it is clear that DHFA is a suitable model with which to examine the functional characteristics of the fusidic acid like antibiotics. [^{3}H]DHFA can be readily prepared in highly radioactive form and as a consequence its interaction with ribosomal complexes can be easily assessed by a variety of means, including Millipore filtration. We have found that [^{3}H]DHFA binds to the ribosome · EF-G · GDP complex with a stoichiometry of *ca.* 1:1 and an apparent association constant of 2.6 × 10^6 M^{-1}. These results are similar to those reported by Okura et al. (1971) and are in reasonable agreement with the concentration dependence of complex stabilization and inhibition of GTP hydrolysis by fusidic acid (Bodley et al., 1970a). Thus the results presented here support the conclusion of Okura et al. (1971) that fusidic acid (and related antibiotics) stabilizes the ribosome · EF-G · GDP complex (and hence inhibit EF-G function) by binding to it.

In defining the mechanism of fusidic acid stabilization of the ribosome · EF-G · GDP complex it is important to determine the site of its binding. Earlier studies have shown that of the bacterial mutants thus far examined which demonstrate in vitro resistance to fusidic acid all have an altered EF-G (Tanaka et al., 1968; Tocchini-Valentini et al., 1969; Bernardi and Leder, 1970). These observations have lead to the conclusion that the antibiotic interacts largely if not entirely with the factor rather than the ribosome. Indeed, Okura et al. (1971) presented evidence for a weak interaction between EF-G and fusidic acid; however, the numbers were small so that the conclusions were not compelling. We have not been able to obtain any evidence for binding of DHFA to either the ribosome or EF-G alone or in combination with GDP. If such interactions occur, the apparent K_a must be <4 × 10^3 M^{-1}. Therefore, the evidence for EF-G as the site of fusidic acid binding is still indirect.

Similarly, DHFA was not observed to bind to the ribo-

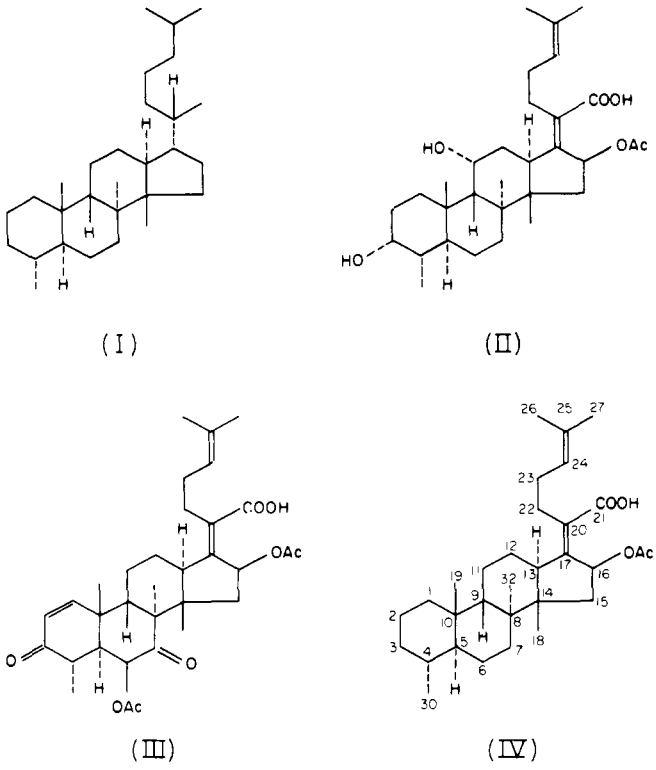

FIGURE 5: Structure of the fusidane-type antibiotics. The hypothetical hydrocarbon fusidane (I), fusidic acid (II), helvolic acid (III), and the structural elements common to all known fusidane-type antibiotics (IV).

some and EF-G in the absence of a guanine nucleotide. Either GDP or GTP could satisfy the nucleotide requirement and, as shown previously (Bodley et al., 1970b) when GTP is employed hydrolysis immediately ensues resulting in bound GDP. GDPCP also binds effectively to the ribosome and EF-G but this binding is not enhanced by fusidic acid (Brot et al., 1971; Bodley, 1972). As reported here, we have observed that the resulting ribosome · EF-G · GDPCP complex is also unable to bind DHFA. It seems likely that the hydrolysis of nucleotide (or the binding of the hydrolysis product itself) generates or uncovers the fusidic acid binding site. Whatever the case, since fusidic acid stabilizes translocational complexes from all sources which have been studied, it is reasonable to assume that the generation of this binding site is related in some fundamental but as yet unknown way to the mechanism of translocation.

One means of defining the nature of the fusidic acid binding site is through the study of its interactions with fusidic acid analogs and derivatives. The structural features of the fusidic acid like antibiotics are summarized in Figure 5. In a preliminary report (Bodley and Godtfredsen, 1972) we examined the ability of some derivatives to stabilize the ribosome · EF-G · GDP complex and these studies have been extended here. These results can be summarized in the following way. Two modifications, including the formation of DHFA, have little effect on this activity of the molecule. A number of modifications (group II compounds of Table III) reduce without abolishing this activity. Most noteable among these compounds are 25,26,27-trinorfusidic acid, fusidyl glucuronide, and methyl fusidate 3-hemisuccinate. Thus, it appears that the three terminal carbon atoms of the side chain are not essential to this function nor is the exact location of the carboxyl group crucial. Several modifications (group III compounds, Table III) reduce this activity below the detectable level. Two of these modifications involve the 17–20 double bond and several involve the 16-acetyl group.

A second measure of the activity of these analogs involves their ability to bind to the ribosome · EF-G · GDP complex. This we examined indirectly by assessing their ability to compete with [^{3}H]DHFA in binding. The compounds previously assigned to groups I and II showed normal and reduced but significant ability to compete, respectively. An exception was methyl fusidate-3-hemisuccinate which for presently inexplicable reasons showed negligible ability to compete with [^{3}H]DHFA for binding. Compounds which did not show detectable complex stabilization activity (group III) were further subdivided into those which exhibited significant ability to prevent [^{3}H]DHFA binding and those which did not. The latter category (IIIb) includes tetrahydrofusidic acid and lumifusidic acid (the 17–20 epimer of fusidic acid) which neither bind to nor stabilize the ribosome · EF-G · GDP complex.

Thus we can conclude that there is a rough parallel between the ability of a fusidic acid analog to stabilize the ribosome · EF-G · GDP complex and its ability to bind to it and that modifications of the 17–20 double bond appear to abolish both of these activities. Some modifications appear to selectively reduce complex stabilizing activity. No clear structural basis is apparent, however, which would distinguish the features of these compounds necessary for complex stabilization from those merely required in order to bind to the ribosome EF-G · GDP complex.

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The F3–F2a1 Complex as a Unit in the Self-Assembly of Nucleoproteins†

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ABSTRACT: A specific and stable interaction between histones f3 and f2a1 was demonstrated to take place in the absence of DNA. When a mixture of these histones was subjected to velocity sedimentation under conditions in which the separate histones are aggregated and precipitate, the mixture of f3 and f2a1 remained soluble and these histones appeared to cotransport through the gradient, indicating the establishment of an isolatable, stable f3-f2a1 complex. This isolated complex subsequently binds to DNA quantitatively to form nucleohistone. Stoichiometry data strongly suggest that histones f3 and f2a1 bind to DNA as a unit; this is the only type of f2a1 binding to DNA that can take place under mild conditions. Histone f1 can act as a modifier of the f3-f2a1-DNA interactions by augmenting the formation of the f3-f2a1 complex and consequently enhancing the overall binding of these histones to DNA. No significant interactions of histones f2b and f2a2 with other histones could be demonstrated. Because of the findings reported here and the known affinity characteristics of the arginine-rich histones to DNA in native chromatin (in particular their simultaneous extraction from chromatin by salt), we suggest that the (f3 + f2a1)-DNA complex is a structural component of native chromatin. We would also like to propose that, in vivo, histones may possess a considerable amount of quaternary structure, which would greatly increase the specificity of their role as potential regulators of the structure and function of the eucaryotic chromosomes.

The role of histones in the structure and function of chromatin continues to be the subject of intensive investigation. The difficulties involved in unraveling the complexities of chromatin have been by-passed by exploiting various model systems; these studies have resulted in a considerable body of information on the characteristics of complexes between histones and DNA. A generalization applicable to most of this work is that complexes between DNA and each class of histones have significant structural features specific to that histone, although functional differences among the various histone–DNA complexes have not been as pronounced.

The significance of these findings, however, is difficult to assess. One problem is the inability to demonstrate specific native chromatin structures which are comparable to those observed for DNA–histone complexes. A less technical but more serious shortcoming of model systems is that the types of structures formed by individual histone–DNA complexes may be markedly altered by the presence of other histones (or non-histone proteins) normally associated with chromatin. There may not be enough physical-chemical information in individual histone–DNA complexes to model the natural nucleoprotein.

It is to the latter problem that the present paper is directed. In a previous publication (Rubin and Moudrianakis, 1972) we demonstrated that the histones do interact during binding to DNA. The present paper contains evidence for a specific interaction between histones f3 and f2a1, on the role of the f3-f2a1 complex in histone binding to DNA, and evidence that f1 can enhance the formation of the f3–f2a1 complex. The mild procedures used here to demonstrate the binding of this f3-f2a1 complex to DNA, and the pattern of extraction of histones from chromatin by salt (Ohlenbusch et al., 1967), which suggest that these histones interact in chromatin, support our conclusion that *the complex between f3 and f2a1 may be a native structural component of chromatin.*

Since the completion of this work (Rubin, 1973), similar inferences have been made in studies in which histone interactions were monitored indirectly after chemical cross-linking (Kornberg and Thomas, 1974). More direct support for histone–histone interactions has been provided by D'Anna and Isenberg's studies of changes in the optical properties of

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