Table 2. The effect of fusidic acid on the guanosine triphosphatase activity of ribosomes, factor T_2 alone, and combined. The assay mix contained, in a total volume of 0.125 ml: tris-HCl (*p*H 7.4), 6.25 μmole; KCl, 10 μmole; $MgCl_2$, 1.25 μmole; DTT, 2.0 μmole; GTP-γ-P^{32} (31,000 count/min per nanomole), 2.0 nmole; and ribosomes, T_2 factor, and fusidic acid, as indicated. Incubation was for 15 minutes at 37°C. The liberated P_i^{32} was determined as by Felicetti and Lipmann (*2*).

Ribosomes (μg)	T_2 (μg)	Fusidic acid (m*M*)	P_i^{32} released (count/min)	Inhibition (%)
119			4,801	
119		1.6	1,172	75
	7.5		3,003	
	7.5	1.6	136	95
119	7.5		24,176	
119	7.5	1.6	3,177	86
119	7.5	0.16	10,950	53
119	7.5	0.016	15,760	32

is desirable to strengthen the argument for general analogy (*2*).

A new opportunity for comparison seemed to be offered by the study of Tanaka *et al.* (*8*) on inhibition of bacterial protein synthesis by the steroid antibiotic fusidic acid, which has a lanostane-like skeleton (*9*). They report it to inhibit specifically the bacterial elongation factor G. They found an inhibition of amino acid polymerization parallel with that of G-linked guanosine triphosphatase (*4*, *8*). Haenni and Lucas-Lenard (*10*) have confirmed the inhibition specificity of G by showing that after transpeptidation fusidic acid prevents a translocation of newly formed peptidyl-tRNA.

We report here on inhibition of reticulocyte protein synthesis by fusidic acid, which we find is specific for T_2, the mammalian analog of G. In an assay with a suspension of intact rabbit reticulocytes (*11*), 0.8 m*M* fusidic acid causes a 96 percent inhibition of the incorporation of C^{14}-valine into hemoglobin, which shows permeability of the cell membrane to fusidic acid. The cell-free system responds similarly, with the use of poly U–dependent phenylalanine polymerization (Table 1). Judging from its activity at a concentration of 0.016 m*M*, which causes 37 percent inhibition, fusidic acid seems to be slightly more active with the reticulocytes than with the preparation of *Escherichia coli* (*8*).

To explore specificity of action, the fusidic acid effect was tested on the T_1-dependent binding of phenylalanyl-tRNA and on the ribosome-linked guanosine triphosphatase of T_2. The T_1-linked binding was not affected by the antibiotic (see column marked "Binding" in Table 1). However, T_2-linked guanosine triphosphatase (Table 2) was inhibited to an extent similar to that of polymerization. The ribosome and T_2-fraction alone caused less of a breakdown of GTP, which is also sensitive to fusidic acid. The ribosome-linked reaction, which exceeds about threefold that of the separate fractions, was strongly inhibited. In Table 3, hydrolysis of ATP and GTP are compared with the combined system. It shows that the preparation was contaminated with a nonspecific nucleotide triphosphatase, since ATP was hydrolyzed only slightly less than GTP. Hydrolysis of GTP, however, was more strongly inhibited by fusidic acid; ATP hydrolysis responded with slight inhibition, which may indicate a more general effect of fusidic acid on this type of reaction. It seems pertinent to add that there may be differences in the active centers of T_2 and of G; in contrast to fusidic acid, diphtheria toxin–diphosphopyridine nucleotide inhibits T_2 specifically (*12*), but does not inhibit G (*13*).

Table 3. The effect of fusidic acid on the breakdown of H^3-GTP and H^3-ATP. The reaction mixture was the same as in Table 2, with ribosomes and T_2 combined, except that H^3-GTP and H^3-ATP (0.25 μc) were used. After 15-minute incubation at 37°C, 20-μl samples were removed from the incubation mix and applied to strips of diethylaminoethyl-cellulose paper (Whatman DE-81) for ascending chromatography in 0.25*M* triethylamine in 16 percent acetic acid (adenine nucleotides), or in 0.5*M* triethylamine in 32 percent acetic acid (guanine nucleotides). After they had dried, the nucleotides were identified under ultraviolet light; the areas were cut out, heated for 10 minutes at 100°C, placed in vials containing 5 ml of Bray's solution (*15*), and counted in a Nuclear-Chicago scintillation counter. The percentage of breakdown of the nucleotide refers to the amount of radioactivity remaining as the triphosphate after incubation with and without fusidic acid.

Sample	Triphosphate	Breakdown (%)	Inhibition (%)
Control	GTP	65	
Fusidic acid (0.16 m*M*)	GTP	25	62
Control	ATP	55	
Fusidic acid (0.16 m*M*)	ATP	40	27

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Cellulose: Refutation of a Folded-Chain Structure

Abstract. *Calculations of modulus of elasticity for extended- and folded-chain configurations have been compared with the experimental observations of mechanical properties of native cellulosic fibers. A recent folded-chain proposal is incompatible with the experimental evidence.*

The proposal of a folded-chain configuration for crystalline native cellulose (*1*) has led to a great deal of controversy concerning interpretation of evidence to favor such structure or the older extended-chain structure. We offer a simple calculation to show that the modulus of elasticity for the axial (microfibrillar) direction of the proposed folded chain would be several orders of magnitude lower than values obtained from tests on native cellulosic fibers.

Muggli has shown that determinations of molecular weight of carbanilated native (ramie) cellulose are consistent only with an extended-chain conformation (*2*). The molecular weights correspond closely with length

of chain molecules that would be predicted if these chains extended from cut surface to cut surface in severed segments of ramie fibers.

Because the primary bonds between monomeric residues in cellulose occur only in the chain direction, maximum stiffness of crystalline cellulose must be associated with this direction. Furthermore, because natural fibers used in experiments will not have all their cellulose chains aligned with the direction of loading and also will generally contain other substances of less stiffness than extended-chain cellulose, any experimentally determined modulus must be less than the true modulus of pure native cellulose in the chain axis direction if indeed the chains are extended. Conversely, an experimental modulus higher than one can calculate from a folded-configuration model refutes that model effectively.

The simplest basis for this calculation is to treat Manley's folded chain as an isotropic homogeneous helical spring of rectangular cross section (Fig. 1). For a close-coiled spring of this type the following solution is given by Wahl (*3*). The spring extension δ is

$$\delta = 2\pi n \; PR^3/\beta ba^3 \; G \qquad (1)$$

Here P is the load, R the initial mean coil radius, and a and b are the cross-sectional dimensions as shown in Fig. 1; n is the number of turns in the helix, β is a geometrical factor depending upon the ratio of b to a, and G is the shear modulus of the material. Corresponding to this spring deflection the axial strain ϵ is

$$\epsilon = \delta/nh \qquad (2)$$

where h is the helical pitch (Fig. 1), found by

$$h = 2\pi nR \tan \alpha \qquad (3)$$

where α is the pitch angle. For Manley's model, $\alpha = 20°$.

To calculate an average axial stress in the cellulose, it is necessary to know the packing density of the helically coiled elementary fibrils (protofibrils) within the microfibril. With reasonable accuracy, and favoring a higher modulus, we take the area associated with each protofibril as πR^2. Then the axial stress σ is

$$\sigma = P/\pi R^2 \qquad (4)$$

The elastic modulus E in the axial direction is simply the ratio of axial stress to axial strain for this condition of loading. Thus, from Eqs. 2 and 4, we have

$$E = (\beta hba^3/2\pi^2R^5) \; G \qquad (5)$$

For the numerical values listed in Fig. 1, $b/a = 4.45$; $\beta = 0.286$ (*3*). Thus we determine from Eq. 5 that $E = 0.0165G$. Values for supermolecular shear moduli for cellulose have been calculated (*4*). The highest value obtained from any of these calculations is 0.66×10^{11} dyne cm^{-2}. Substitution of this value gives as Young's modulus $E = 0.0109 \times 10^{11}$ dyne cm^{-2}. Because experimental values have been observed for E as large as 13.43×10^{11} dyne cm^{-2} (*5*), it is obvious that the folded-chain structure proposed by Manley is incompatible with the observed mechanical properties of native cellulosic fibers.

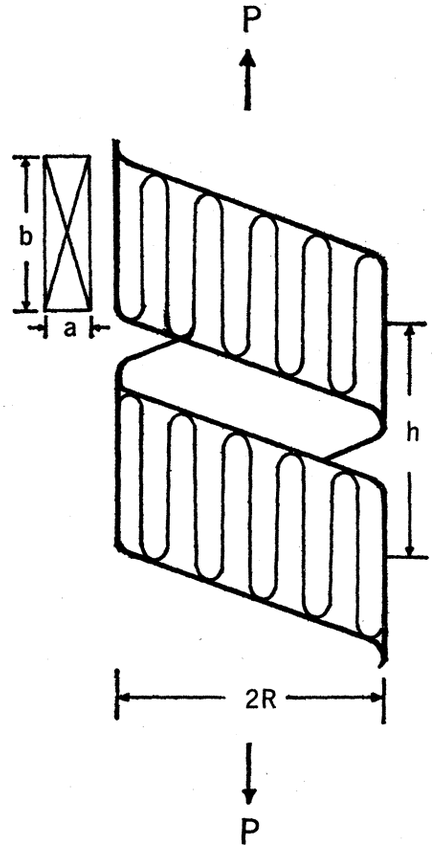

Fig. 1. The basic protofibril of native cellulose as conceived by Manley and considered here as a helical spring of diameter $2R = 35.0$ Å, pitch $h = 40$ Å, and rectangular cross section: $a = 7.86$ Å, $b = 35$ Å.

Two objections might be raised against the foregoing analysis. First, the homogeneous isotropic approximations may not fully describe the details of folded-chain behavior. We agree; in a more rigorous analysis (*6*) the elastic modulus of the folded-chain configuration was found to be even lower than the value calculated here. However, the present value is certainly low enough to make our point, and the underlying analysis is somewhat more easily understood.

Second, we neglected secondary bonds except to the extent that these bonds influence the shear modulus G. Intrachain hydrogen bonds have been shown (*4, 7*) to affect axial stiffness in the extended-chain structure of cellulose, but the magnitude of this effect is not significant enough to even begin to reconcile the discrepancy encountered here. At the same time our experience with such calculations gives us no reason to believe that any feature of the helical configuration would greatly enhance this effect for the proposed folded-chain structure. Intrachain hydrogen bonds within one fold-length would have almost no effect acting in parallel with primary bonds. On the other hand, if they were to bridge the gap between adjacent twists of the ribbon with a substantial regularity and significant stiffness, then the folded-chain structure would be tubular rather than helical. Although Manley's original drawings show the form illustrated in Fig. 1, his verbal description refers to the elementary fibrils as being in the form of "a tightly wound helix." In such a situation, the resultant tubular or cylindrical folded configuration would be mechanically similar to the fibrillar model proposed by Marx-Figini and Schulz (*8*).

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