was centrifuged, and the aqueous layer, representing one of the time periods of hydrolysis shown in Table 1, was injected intraperitoneally into adult male white mice. All doses contained the total nucleic acid present in one-half LD_{50} (mouse intraperitoneal) of polymerized DNA, as judged by ultraviolet absorption.

The results in Table 1 show increasing toxicity of mice as time of enzymatic hydrolysis increased, and a similar relationship of toxicity to time of hydrolysis by heat and acid up to 10 min. The cause of the low toxicity of the 20-min hydrolysis by heat and acid is unknown, but is presumed to be due to a heat or acid lability, or both, of the toxic component.

The toxicity of the slime layer of *P. aeruginosa* is thus felt to be due either to split products of the highly polymerized DNA or to non-nucleic acid substances liberated or unmasked by DNA breakdown. The probable presence of enzymes in the mouse capable of hydrolysis of injected polymerized DNA would explain the "toxicity" of the native DNA, and indeed such enzymes may well have skewed the results obtained here by virtue of further degradation of the products injected.

SOME PROPERTIES OF *METHANOBACTERIUM OMELIANSKII* FERREDOXIN

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Ferredoxin is a very electro-negative electron carrier (E'_0 = −420 mv at pH 7) present in anaerobic heterotrophic bacteria, photosynthetic bacteria, algae, and green plants (Mortenson et al., Biochem. Biophys. Res. Commun. **7**:448, 1962; San Pietro and Lang, J. Biol. Chem. **231**:211, 1958; Tagawa and Arnon, Nature **195**:537, 1962; Buchanan et al., Proc. Natl. Acad. Sci. U.S. **49**:345, 1963). The most extensively characterized ferredoxins have been isolated from fermentative clostridia and from spinach chloroplasts. Recent reports from this laboratory (Buchanan et al., Proc. Natl. Acad. Sci. U.S. **49**:345, 1963; Lovenberg et al., J. Biol. Chem. **238**:3899, 1963) have shown that the ferredoxins of five metabolically different clostridial species all have a molecular weight of about 6,000 and, on a dry weight basis, contain seven atoms each of iron and inorganic sulfide per mole. The proteins from these sources were found to differ, however, with respect to their crystalline appearance, absorption spectra, enzymatic activity in the *Clostridium pasteurianum* "clastic" assay system, and amino acid composition. The striking physiological characteristics as well as the relatively high ferredoxin content of *Methanobacterium omelianskii* prompted us to examine more closely the properties of the ferredoxin isolated from this organism.

M. omelianskii was grown on a medium of ethanol, sodium carbonate, and other constituents, essentially as given by Pine and Barker (J. Bacteriol. **68**:589, 1954); ferredoxin was isolated and crystallized, and the iron, inorganic sulfide, protein concentration, and amino acid composition were determined as reported previously (Lovenberg et al., J. Biol. Chem. **238**:3899, 1963).

The ferredoxin of *M. omelianskii*, like that of the clostridial species, is brown in color, and, like that of *C. cylindrosporum* and *C. butyricum*, forms small, round crystals in ammonium sulfate solutions. The absorption spectrum of crystalline *M. omelianskii* ferredoxin is shown in Fig. 1. The protein shows a single absorption peak in the visible region at 390 mμ and a peak in the ultraviolet region at 280 mμ, with a pronounced shoulder at about 300 mμ. Similar spectra were obtained with the clostridial ferredoxins.

Crystalline methanobacterium ferredoxin contains 0.48 μatoms of iron and 0.46 μatoms of inorganic sulfide per mg (phenol reagent method of protein determination). These values are

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lower than the values of 0.7 to 0.9 μatoms per mg found for the clostridial ferredoxins. The molecular weight of *M. omelianskii* ferredoxin has not been determined; but, on the basis of the molecular weight of 6,000 found for all clostridial ferredoxins examined, these analytical values for *M. omelianskii* ferredoxin correspond to 4 or 5 atoms each of iron and inorganic sulfide per mole. The specific enzymatic activity of *M. omelianskii* ferredoxin (29 in the *C. pasteurianum* "clastic" assay system) is also significantly lower than the value obtained with the clostridial ferredoxins.

The amino acid composition of *M. omelianskii* ferredoxin is shown in Table 1. Like all clostridial ferredoxins examined, this ferredoxin is devoid of methionine, histidine, and tryptophan. It is also free of arginine, phenylalanine, and leucine, as are certain of the clostridial ferredoxins. It is the only bacterial ferredoxin so far examined that is free of isoleucine. *M. omelianskii* ferredoxin is thus characterized by the presence of only 11 amino acids (a minimum of 12 was found previously with *C. butyricum* ferredoxin) and by its high alanine content. Alanine, in fact, accounts for about 25% of the total weight of this ferredoxin. This value is approximately twice the value observed with clostridial ferredoxins. Insofar as we know, such a striking abundance of alanine has not been reported for any other protein.

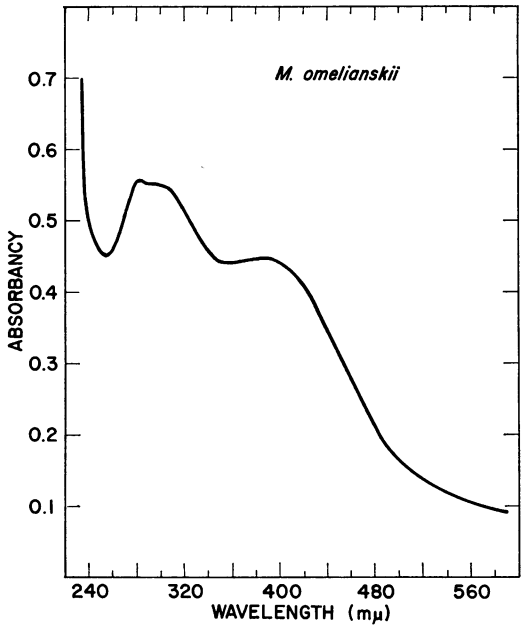

FIG. 1. *Absorption spectrum of crystalline ferredoxin from Methanobacterium omelianskii. The cuvette contained 0.14 mg of ferredoxin per ml in 0.07 M tris(hydroxymethyl)aminomethane-hydrochloric acid buffer at pH 7.3. The spectrum was measured with a Cary 14 M recording spectrophotometer.*

TABLE 1. *Amino acid composition of ferredoxin from Methanobacterium omelianskii**

Amino acid residue	Residues per mole	Amino acid residue	Residues per mole
Methionine	0	Lysine	2
Tryptophan	0	Tyrosine	2
Histidine	0	½ Cystine†	5
Arginine	0	Valine	5
Leucine	0	Aspartic Acid	3
Phenylalanine	0	Threonine	1
Isoleucine	0	Serine	2
Alanine	14	Glutamic acid	5
Glycine	6	Proline	2

* These values are based on a single determination with a recrystallized preparation and on an assumed molecular weight of 6,000; values were calculated as described by Lovenberg et al. (J. Biol. Chem. **239**:3899, 1963). They are therefore subject to further correction when a molecular weight determination is available.

† Determined on the untreated standard hydrolysate.

The ferredoxin of the facultative chemoautotrophic bacterium, *M. omelianskii*, is, therefore, similar to certain ferredoxins of the fermentative clostridia with respect to crystalline appearance and absorption spectrum. However, its low content of iron and inorganic sulfide and its amino acid composition distinguish it from the clostridial ferredoxins that have so far been examined.

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