Chemical Identity of Iron in Wheat by Mössbauer Spectroscopy

Leopold May, Eugene R. Morris,* and Rex Ellis

The iron combined with phytate in aqueous salt extracts of wheat bran has previously been characterized as monoferric phytate. The nature of the endogenous iron in the bran and its relationship to isolated monoferric phytate were examined by using ^{57}Fe Mössbauer spectroscopy. The spectra of seeds and bran from wheat grown in an ^{57}Fe-enriched culture medium were compared with the spectra of both monoferric and diferric phytates. The spectra of the iron in seeds and bran are the same as the spectrum of solid monoferric phytate, indicating that most of the iron in wheat is combined in this chemical form. The Mössbauer parameters indicated that the iron is in the high-spin form.

In recent investigations more than 60% of the iron in wheat bran was extracted in association with phytic acid. Isolation of iron from wheat bran by use of aqueous solutions and gel chromatography yielded monoferric phytate (Morris and Ellis, 1976). Bioavailability to animals did not differ significantly between the isolated product and synthetic monoferric phytate (Morris and Ellis, 1976; Lipschitz et al., 1979). The synthetic product is miscible with the non-heme iron pool of meals consumed by human subjects, but wheat bran reduced absorption of nonheme iron (Simpson et al., 1980). Clarification of the chemical nature in situ of the iron in wheat bran might aid in explaining the action of bran on iron absorption.

We examined the nature of the endogenous iron and its relationship to the monoferric phytate to determine whether the isolated product represented an artifact of the isolation procedure. Mössbauer spectroscopy provides a probe of the iron atom that is sensitive to changes in oxidation and spin state and the configuration of the ligands around the iron (May, 1971). The Mössbauer spectra were compared between seeds and bran of wheat and iron phytates to determine the nature of the iron in the seeds and its relationship to isolated iron phytates.

EXPERIMENTAL SECTION

Preparation of the Wheat Seeds and Bran. Since the ^{57}Fe isotope is the Mössbauer nuclide, the wheat was grown in a culture medium in which the iron was enriched with 95% ^{57}Fe (Oak Ridge National Laboratories). A modification of the hydroponic medium of Johnson et al. (1957) was used. The enriched ^{57}Fe was dissolved in HCl and converted to ferric EDTA for use in the medium instead of ferrous sulfate. The wheat (*Triticum aestivum*) was the Sheridan variety of soft spring wheat. The seeds were harvested and examined directly by Mössbauer spectroscopy. The bran was hand dissected from the remainder of the seed.

Preparation of the Ferric Phytates. Monoferric phytate was prepared with normal iron as described by Lipschitz et al. (1979). Diferric phytate was prepared by reacting the monoferric phytate in 2.0 M NaCl solution (Ellis and Morris, 1979).

Mössbauer Spectroscopy. The Mössbauer spectrometer was of the constant acceleration type with moving source geometry with a $KrCO_2$ proportional counter (Reuter-Stokes) (Nassif et al., 1976). The source was maintained at room temperature. The samples were mounted in plastic holders. The crysostat and computer program were the same as those used previously (Nassif et al., 1976). The total counts at each velocity for all spectra were at least 10^6.

Table I. Mössbauer Spectral Parameters of Wheat Seed, Wheat Bran, and Ferric Phytates[a]

sample	quadrupole splitting	isomer shift
wheat seeds	0.55	0.76
wheat bran	0.56	0.77
monoferric phytate		
solid	0.55	0.77
saturated soln	0.35	0.77
diferric phytate	0.60	0.76

[a] These values are in millimeters per second relative to sodium nitroprusside and were measured at 80 K. They represent for most samples an average of two or more spectra, and the reproducibility is ±0.03 mm/s.

RESULTS AND DISCUSSION

The chemical information obtained from the Mössbauer spectrum is mainly contained in the values for the isomer shift and quadrupole splitting. The isomer shift or the velocity of the centroid of the doublet is related to the s-electron density at the iron nucleus, which varies with the oxidation state and the bonding of the iron. The quadrupole splitting, the difference in velocities between the two lines in the spectrum, is influenced by the configuration of the electronic environment around the iron nucleus, and its magnitude yields information about the bonding of the iron atom.

Possibly monoferric phytate exists in a hydrated form akin to the structure in aqueous solution rather than to that in the solid. The Mössbauer spectra of the solid and its saturated solution in NH_4OAc are shown in Figure 1. These spectra show that the monoferric phytate differed in solution (quadrupole splitting = 0.35 mm/s) and in the solid phase (quadrupole splitting = 0.53 mm/s). In both spectra the isomer shift was the same (0.77 mm/s) (Table I)

The spectrum of the seeds that had been grown in a culture containing ^{57}Fe is shown in Figure 2. It is very similar to the spectrum of the solid monoferric phytate, and its parameters, quadrupole splitting and isomer shift (Table I), are identical with those in the spectrum of the solid monoferric phytate. The Mössbauer spectrum of the bran isolated from these seeds is also shown in Figure 2, and the parameters are given in Table I. The spectra of the bran, seeds, and solid monoferric phytate are very

Department of Chemistry, The Catholic University of America, Washington, D.C. 20064 (L.M.), and the Vitamin and Mineral Nutrition Laboratory, Nutrition Institute, Human Nutrition Center, Science and Education Administration, U.S. Department of Agriculture, Beltsville, Maryland 20705 (E.R.M. and R. E.).

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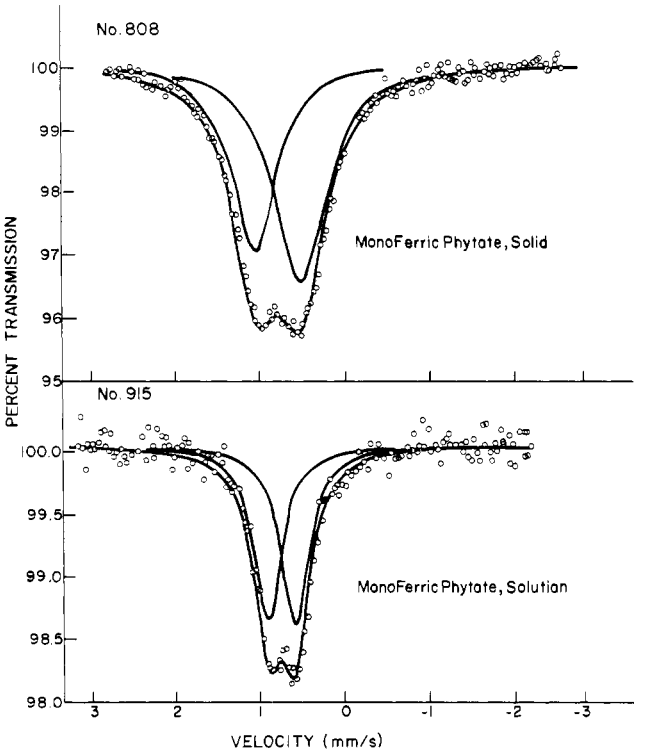

Figure 1. Mössbauer spectra of monoferric phytate at 80 K. Top (no. 808): solid, 212 mg. Bottom (no. 915): saturated solution in 1 M ammonium acetate.

similar and do change with temperature (room temperature and 80 K).

Another form of ferric phytate was found when monoferric phytate was dissolved in 2.0 M NaCl solution. The precipitate is diferric phytate, and its Mössbauer spectrum is shown in Figure 3 with the spectrum of bran. A comparison revealed that the parameter (Table I), quadrupole splitting, for diferric phytate (0.60 mm/s) differed slightly from the value for bran, seeds, and monoferric phytate (0.55 mm/s). In shape, the spectra of the bran and seeds differed from the spectrum of the diferric phytate.

The Mössbauer parameters (Table I) are all in the range of those found with high-spin ferric compounds. This indicates that the monoferric phytate both in the solid form and in solution has the same iron configuration. In the solution spectrum, a single quadrupole splitting is evident (0.35 mm/s, Table I, Figure 1). There is some preliminary evidence that the spectrum of the solid form of this compound can be decomposed into four lines with two different quadrupole splittings, one of which has a value similar to that found in the solution spectrum. This suggests that there are two iron sites in the solid with different bonding to the phosphates. One of the iron sites in the solid would be identical with the site in the solution.

There are differences in the relative intensities of the lines in the spectra of seeds and bran. The differences may be related to different amounts of the two binding sites in the different preparations.

In the spectrum of the solid diferric phytate (Figure 3), both lines are equal in intensity. The parameters are similar to the parameters, but not in intensity ratio, of the doublet in the spectrum of the solid monoferric phytate fitted to two lines.

The quadrupole splitting of the solid diferric phytate differs from that of the monoferric phytate in solution, suggesting different bonding between the iron and the phosphates in the diferric than in the soluble monoferric salt.

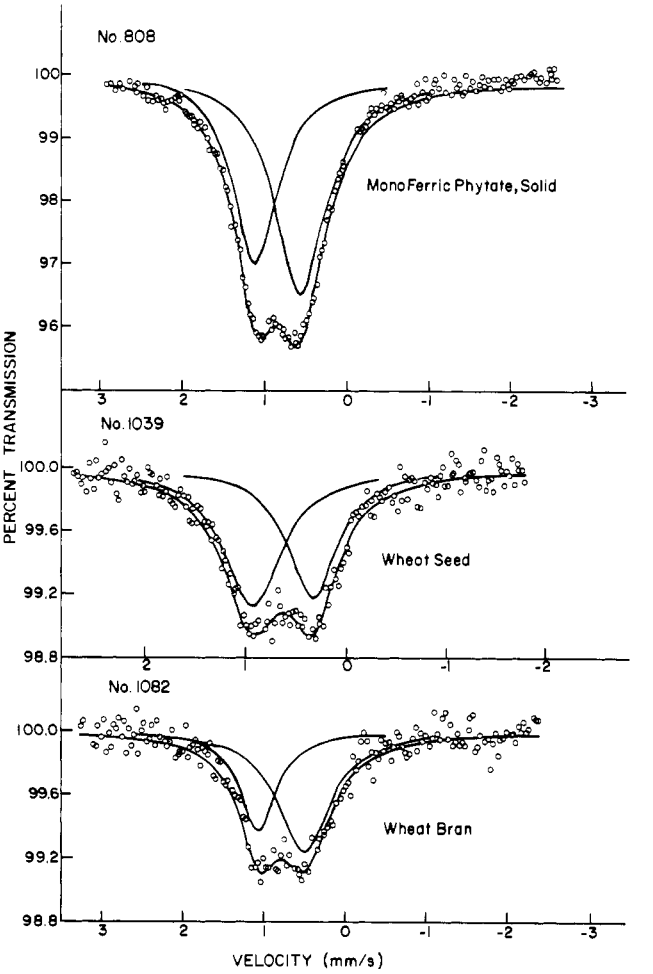

Figure 2. Mössbauer spectra of wheat seeds, wheat bran, and monoferric phytate. Top (no. 808): solid monoferric phytate, 212 mg (80 K). Middle (no. 1039): wheat seed, 1.54 g (room temperature). Bottom (no. 1082): wheat bran, 815 mg (80 K).

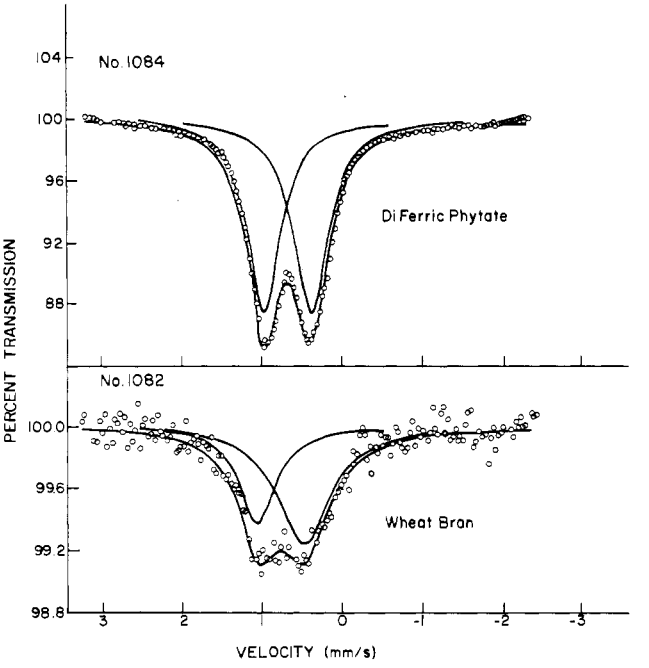

Figure 3. Mössbauer spectra of wheat bran and differic phytate. Top (no. 1084): solid diferric phytate, 212 mg (room temperature). Bottom (no. 1082): wheat bran, 815 mg (80 K).

These Mössbauer spectra indicate that the iron that is associated with the phytate in situ in the bran is in the

same combination as that in monoferric phytate. This conclusion concerning the nature of most of the endogenous iron in wheat bran explains the finding that the iron in bran and monoferric phytate was equally bioavailable (Morris and Ellis, 1976).

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Laboratory Comparisons of Polyphenols and Their Repellent Characteristics in Bird-Resistant Sorghum Grains

Roger W. Bullard,* Melvyn V. Garrison, Stephen R. Kilburn, and John O. York

Laboratory evaluations of repellency and polyphenol composition were conducted for 15 varieties of bird-resistant (BR) sorghums. Tests involved the weaver finch (*Quelea quelea*) and the red-winged blackbird (*Agelaius phoeniceus*). We compared BR varieties with a bird-susceptible (Martin X) sorghum by using a two-choice paired preference test under light and reduced lighting (near darkness) conditions. The most important observation of this study was recognition of the diversity of polyphenolic properties among BR sorghums. The 15 varieties were separated into three groups (seven least, seven intermediate, and one most preferred) based on preference response. In most instances, polyphenol values from modified vanillin–HCl and Sephadex LH-20 gel permeation chromatography analyses of these varieties could be placed in similar groups. The seven least preferred sorghums, with the exception of WGF, were uniform in polyphenol properties whereas substantial variation occurred among the remaining eight varieties. Several observations involving test conditions, bird species, and sorghum properties were also discussed.

Bird damage to sorghum crops [*Sorghum bicolor* (L.) Moench] is so severe in many parts of the world that control measures must be taken or most of the crop will be lost. The most common practice is to grow sorghums which are astringent during the immature stages when bird damage is normally the highest (Doggett, 1957; McMillian et al., 1972). In many varieties, however, the polyphenolic tannins which impart bird-repellent properties are also present in the mature grain and lower their palatability and nutritional qualities for the consumer (Harris, 1969; Mabbayad and Tipton, 1975). The result is that these high-tannin "bird-resistant" (BR) sorghums have less value on the export market (Price et al., 1979) and farmers that produce them are at an economic disadvantage. Many farmers in this country have need to include sorghum in their crop rotation program, and the economic disadvantage is especially serious for farmers in arid regions of the world. Many cannot otherwise protect their sorghum crops and cannot grow wheat, corn, or rice as an alternative.

Currently, the repellent characteristics of BR sorghums are attributed to the group of polyphenolics known as "condensed tannins". Specifically, the references are usually to proanthocyanidins which are a series of condensed flavon-3-ol and flavan-3,4-diol molecules of increasing complexity. The term "tannin" is generally reserved for those polymers having molecular weights between 500 and 3000 which form stable complexes with proteins (Ribereau-Gayon, 1972). In the human mouth, these tannins elicit an "astringent response"—a contracting or drying feeling caused by the precipitation of proteins in saliva and on mucousal surfaces (Joslyn and Goldstein, 1964; Singleton and Noble, 1976). Astringency generally increases with increased polymerization up to an intermediate molecular weight (e.g., hexamer or heptamer) and then decreases as the molecule becomes insoluble and too large to effectively bind with proteins (Goldstein and Swain, 1963; Ribereau-Gayon, 1972). Apparently, any characteristic that influences the protein binding properties of a molecule also influences its activity in other biochemical processes such as leathering of hides (Gus-

Denver Wildlife Research Center, Federal Center, Denver, Colorado 80225 (R.W.B., M.V.G., and S.R.K.), and University of Arkansas, Fayetteville, Arkansas 72701 (J.O.Y.).