flesh and skin from the varieties Fuerte, Rincon, Ryan, and Edranol indicated the presence in all extracts of the same range of acetylenic compounds, including Ib, IIIb, and IVb.

Commercial samples (1 lb each) of crude and rectified avocado flesh oil (A.G. Hersom, Kingston upon Thames, England) as used for cosmetic purposes were treated to isolate crude acetylenic fractions (crude oil, 0.3% yield, rectified oil, 0.2% yield). The tlc examination showed that they contained at least eight acetylenic substances, of identical R_f value to those from immature Zutano skin and flesh oil. By preparative tlc, melting points, and infrared spectra, it was demonstrated that the acetylenic fractions from crude and rectified commercial oil contained flavor compounds Ib, IIIb, and IVb. These commercial oils, of unknown origin, were very likely extracted from avocadoes of differing variety and maturity. The flavor compounds Ib, IIIb, and IVb therefore appear to be widespread among avocado varieties, and a relationship may exist between the content of the compounds and maturity of the avocado.

GAS CHROMATOGRAPHY OF THE COMPOUNDS IN THE PAIRS I, II, IV, AND V. Attempts were made to develop a gas chromatographic method for qualitative and quantitative estimation of the compounds. The natural substances, injected as such into various columns (DEGS, SE 30, QFI), decomposed at the oven temperatures required for short retention times. The compounds were then silylated and injected into various columns (SE 30, DEGS, Apiezon M, QFI). Although a single peak of short retention time could be obtained from all of the compounds, none of the columns used was selective enough to differentiate between the derivatives of compounds known to be structurally different. The compounds were apparently too closely related chemically to enable satisfactory separations to be obtained. It might, however, be possible to develop a total estimation of all the compounds, or of the olefinic or acetylenic groups of compounds.

This aspect is currently being investigated further, so that the relationship between content of these compounds and the maturity and processing characteristics of various avocado varieties can be studied.

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Oats and Their Dry-Milled Fractions: Protein Isolation and Properties of Four Varieties

Y. Victor Wu,* Kenneth R. Sexson, James F. Cavins, and George E. Inglett

Four varieties oats were dry-milled into break flour, reduction flour, shorts flour, shorts, bran, and hulls. The three flour fractions have protein contents not much different from those of the whole oats, but the shorts and bran fractions have about double the protein content of whole oats. The 1 *M* NaCl extract accounts for a large percentage of total nitrogen from all fractions, whereas, the 0.1 *N* acetic acid extract represents a major part of the protein from the three flour fractions. Wyndmere whole oats, as well as its break flour, reduction flour, and shorts flour, have high lysine content (4.5–4.8 g/16 g of N) and almost the same amino acid composition. The water-soluble protein has high lysine (8.1 g/16 g of N) and half cystine (5.4 g/16 g of N), and the residue has high lysine and methionine (4.1 g/16 g of N).

Although cereal grains in general have only moderate protein content and are deficient in some essential amino acids, oats have both a high protein content and a good quality protein (Cremer, 1951; Hischke *et al.*, 1968; Jones *et al.*, 1948; Murlin *et al.*, 1938; Robbins *et al.*, 1971; Smuts and Malan, 1938). Practically no work has been done on individual oat protein fractions or dry-milled fractions of oats.

Oat proteins from dry-milled fractions were isolated by solubility methods. Water-, sodium chloride-, ethanol-, acetic acid-, and sodium hydroxide-soluble proteins were obtained. The amino acid composition and the protein content of the mill fractions and the isolated proteins were

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Table I. Weight Distribution and Protein Content of Dry-Milled Oat Fractions

Fraction	Weight, % as is				Protein (N × 6.25), % dry basis[a]			
	Wyndmere	Dawn	Sioux	ND 86-3-63	Wyndmere	Dawn	Sioux	ND 86-3-63
Break flour	4.7	2.5	3.3	5.1	11.1 (10.9)[b]	13.2	9.6	11.4 (10.6)
Reduction flour	23.2	13.9	14.8	21.5	13.0 (11.9)	13.6	10.8	12.6
Shorts flour	7.8	14.1	18.1	11.8	15.9 (14.9)	14.9	12.7	16.6
Shorts, on 8XX (80 mesh)	11.9	8.9	7.8	9.1	24.5 (26.2)	24.6	21.9	26.7
Bran, on 25 mesh	18.6	25.0	21.3	22.7	24.2 (26.5)	24.1	24.0	30.8
Hulls	33.7	35.6	34.7	29.8	4.2	3.3	2.7	4.1
Groats	66.3	64.4	65.3	70.2	*c*	18.7	16.4	20.5
Whole oats[d]					14.3	14.9	12.4	15.8

[a] The standard deviation of the 37 duplicate analyses below is 0.26%. [b] Values in parentheses are for defatted fractions. [c] Not determined. [d] The nitrogen, lipid, and water for Wyndmere oats are 2.29, 5.3, 8.64%; for Dawn oats are 2.39, 5.3, 8.90%; for Sioux oats are 1.98, 4.2, 9.72%; for ND 86-3-63 oats are 2.53, 4.5, 10.30%, respectively. The nitrogen value is on a moisture-free basis while lipid value is "as-is" basis. Each lipid value is the average of four pentane–hexane extractions.

Table II. Solvent Extraction of Proteins from Mill Fractions

Fraction	Total nitrogen extracted, %					Total N in residue, %
	H_2O[a]	1 *M* NaCl	70% ethanol[b]	0.1 *N* acetic acid	0.05 *N* NaOH[b]	
ND 86-3-63 break flour	4	20		37		8
Wyndmere break flour	4	30		35		9
Wyndmere reduction flour	13	37		18		18
Wyndmere shorts flour	6	36		19		10
Wyndmere shorts	11	29	7	4	11	9
Wyndmere bran	10	33	7	1	11	10

[a] The total nitrogen extracted by water was obtained by dialyzing the 1 *M* NaCl extracts against distilled water and by freeze-drying the water-soluble fraction. After the water-soluble fraction was removed the insoluble fraction, after freeze-drying, was listed under the 1 *M* NaCl column. [b] The blank space indicated that the extraction step with the particular solvent was omitted.

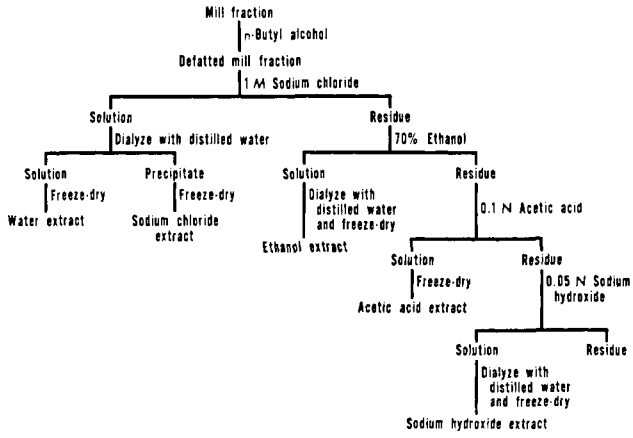
determined. Gel electrophoretic patterns were run on some isolated proteins.

MATERIALS AND METHODS

Dry Milling. The four varieties of oats were Wyndmere, Dawn, Sioux, and ND 86-3-63. They were gifts from D. C. Eboltoft, Department of Agronomy, North Dakota State University of Agriculture and Applied Science, Fargo, N.D., and grown in Fargo, N.D., in 1968. After the oats were dehulled by an Alpine pin mill, the groats were milled in a Buhler mill. Break, reduction, and shorts flours, as well as shorts, bran, and hulls, were obtained for each variety.

Isolation of Protein. Lipid was removed from the mill fractions with *n*-butyl alcohol (Jones and Dimler, 1962) before isolating protein. After lipid removal, a 100-g portion was extracted with 300 ml of 1 *M* sodium chloride in a Waring Blendor for 5 min at low speed at room temperature. The content from the blender was centrifuged at 59,100 × *g* in a Spinco Model L ultracentrifuge for 30 min. (The solution was very turbid after centrifugation at 10,400 × *g*.) The residue was similarly extracted successively three times with 1 *M* sodium chloride, distilled water (to remove sodium chloride), three times with 70% ethanol, distilled water (to remove ethanol), three times with 0.1 *N* acetic acid, distilled water (to remove acetic acid), and three times with 0.05 *N* sodium hydroxide.

The water-soluble fraction and the salt-soluble fraction were separated from 1 *M* sodium chloride extract by dialysis against distilled water at 4°C and freeze-dried. The sodium hydroxide and ethanol extracts were dialyzed against distilled water at 4°C and freeze-dried. The acetic acid extract was freeze-dried directly without dialysis. For some mill fractions the ethanol extraction was deleted because of the small amount of prolamin present (2% of N of Wyndmere reduction flour). The sodium hydroxide extraction was used only on the shorts and bran. The percentage of nitrogen in each freeze-dried fraction was determined by duplicate micro-Kjeldahl analysis, and the total nitrogen in each fraction was calculated. The protein isolation procedure is summarized by a diagrammatic scheme.



Amino Acid Analysis. In preparing samples for a Beckman Spinco Model 120 amino acid analyzer, they were hy-

drolyzed in reflux constant boiling HCl for 24 hr. The hydrolyzed products were evaporated to dryness, and the residue was dissolved in pH 2.2 citrate buffer. After the peaks were integrated electronically, the amino acid data were computed automatically (Cavins and Friedman, 1968).

Electrophoresis. Starch gel electrophoresis was done in aluminum lactate buffer containing 3 *M* urea at pH 3.1 in 17% starch gel, and nigrosine dye was used for the staining (Cluskey, 1964). Polyacrylamide gel electrophoresis was carried out in 5% polyacrylamide gel with aluminum lactate buffer containing 3 *M* urea at pH 3.1 (Jones and Beckwith, 1970).

RESULTS AND DISCUSSION

Weight Distribution and Protein Content. Weight distribution and protein content of dry-milled fractions from four varieties of oats are given in Table I. Whole oats had a protein range from 12.4 to 15.8%. When the hulls were removed from the oats the resulting groats had from 4 to 5% more protein. This substantial increase in protein content of groats results from the large hull fraction (29.8–35.6%) and the low protein content of the hulls (2.7–4.2%). The three flour fractions have protein contents not greatly different from those of the whole oats. Break flour has the lowest protein and the shorts flour has the highest protein among the three flour fractions, which represent from 30.5 to 38.4% of the whole oats. The shorts and bran fractions have by far the highest protein content (24.2–30.8%), about double those of the whole oats. Shorts and bran together account for from 29.1 to 33.9% of the whole oats. Protein contents of defatted dry-milled fractions, shown in parentheses in Table I, do not differ greatly from the corresponding fractions not defatted. The protein (or nitrogen) removed by lipid extraction was not studied.

Protein Isolates. The successive solvents used to extract protein from mill fractions are listed from left to right in Table II. Table II shows that the 1 *M* NaCl extract after the water-soluble fraction was removed (globulin) accounts for a large percentage of total nitrogen extracted from all dry-milled fractions. The 0.1 *N* acetic acid extract is also an important protein fraction in the three flour fractions, with break flour having the highest percentage. The acetic acid extract, however, is only a minor protein fraction for the shorts and bran fractions. The 70% ethanol extract (prolamin) is also a minor protein fraction, while the water extract (albumin) accounts for from 4 to 13% total nitrogen. The protein distribution of shorts and bran fraction are almost the same, and the 0.05 *N* NaOH extracts 11% of total nitrogen. The two break flours in Table II have similar protein distribution except the ND 86-3-63 variety has lower 1 *M* NaCl soluble fraction. The final residue after solvent extraction has from 8 to 18% of total nitrogen.

The total nitrogen accounted for varied from 69 to 86%. Part of the nitrogen loss was due to the numerous steps involved in the isolation procedures, and another possibility was the presence of small molecular weight nitrogen compounds in the dry-milled fractions that were not recovered as protein after dialysis.

The nitrogen analysis of solid extracts from dry-milled fractions is shown in Table III. The high percentage of nitrogen contained in the 1 *M* NaCl, 70% ethanol, and 0.1 *N* acetic acid extracts from the three flours indicated that those extracts are largely all protein. The considerably lower nitrogen content of the water, 0.05 *N* NaOH, and 0.1 *N* acetic acid extracts from shorts and bran indicated the presence of considerable nonprotein material in addition to protein. The percentage protein was not calculated by N × 6.25 for Table III because some proteins have higher than 16% nitrogen. When the percentage nitrogen of protein was calculated from the amino acid analysis, it was from 92 to 94 in NaCl extract, from 91 to 95 in acetic acid extract, from 83 to 91 in ethanol extract, and from 56 to 65 in water extract.

Table III. Nitrogen Analysis of Solid Protein Extract from Dry-Milled Oat Fractions

	N in extract, % (dry basis)				
Fraction	H_2O	1 *M* NaCl	70% ethanol[a]	0.1 *N* acetic acid	0.05 *N* NaOH[a]
ND 86-3-63 break flour	10.1	16.9		16.7	
Wyndmere break flour	9.3	17.6		16.8	
Wyndmere reduction flour	11.6	16.6	15.7	16.5	
Wyndmere shorts flour	11.7	17.2		17.0	
Wyndmere shorts	7.7	17.3	15.0	9.7	7.4
Wyndmere bran	10.8	16.5	16.0	10.1	10.6

[a] The blank space indicated that the extraction step with the particular solvent was omitted.

Amino Acid Composition. The amino acid composition of mill fractions from Wyndmere oats is given in Table IV. Kwolek and Cavins (1971) concluded that amino acid results from independent samples of the same protein should agree to within 25% (17.7% for glutamic acid to 42.3% for half cystine) about 19 times out of 20. Differences greater than these indicate a significant difference between two observations. Break, reduction, and shorts flours have the same amino acid composition except the shorts flour has lower methionine. Lysine levels of these flours are from 4.5 to 4.8 g/16 g of N and methionine, 0.1–0.4 g/16 g of N. Shorts and bran have the same amino acid composition, but they have higher half cystine, valine, and methionine and lower lysine and proline values compared with flours. The percentage of nitrogen recovered for whole oats and their fractions from amino acid analysis varied from 92.4 to 97.4, except hulls, where the average of two runs had a value of 45.7. The low nitrogen recovered for oat hulls is in general agreement with 28 samples of seed coat and pericarp from different species of plants where about half the nitrogen represents amino acids (VanEtten *et al.*, 1961). The level in hulls of each amino acid is considerably lower than in other fractions except for proline, half cystine, and methionine. If the amino acid composition of hulls, shorts, and bran is adjusted to 100% nitrogen recovery, then shorts, bran, and hulls have similar amino acid composition except that the hulls have considerably more half cystine, methionine, and proline but lower isoleucine and leucine values.

Sioux, Dawn, and ND 86-3-63 oats have the same amino acid composition as Wyndmere oats, and the lysine and methionine levels are from 4.0 to 4.5 and from 1.3 to 1.4 g/16 g of N, respectively. Dawn, Sioux, and ND 86-3-63 groats have about the same amino acid composition as the oats from which they are milled, except that Sioux groats showed lower levels of lysine and histidine, ND 86-3-63 groats showed lower level of proline, and Sioux and ND 86-3-63 groats had higher levels of valine. The amino acid composition of ND 86-3-63 break flour is similar to that of

Table IV. Amino Acid Composition in Mill Fractions from Wyndmere Oats (g/16 g of N)

Amino acids[a]	Oats	Break flour	Reduction flour	Shorts flour	Shorts	Bran	Hulls
Lysine	4.5	4.5	4.8	4.7	3.6	3.5	2.2
Histidine	2.4	2.3	2.3	2.3	2.0	2.1	1.0
Ammonia	2.8	2.9	2.8	2.6	2.5	2.5	0.7
Arginine	8.1	8.1	8.0	7.9	7.8	7.6	3.7
Aspartic	8.5	8.6	8.2	8.3	7.8	7.2	4.2
Threonine	3.4	3.2	3.3	3.2	3.3	3.2	1.9
Serine	4.8	4.8	4.7	4.8	4.3	4.5	2.2
Glutamic	21.6	23.7	22.0	23.2	19.5	20.5	8.7
Proline	5.1	5.3	5.0	5.6	3.6	3.8	3.1
Glycine	4.9	4.5	4.6	4.7	4.8	4.7	2.4
Alanine	4.5	4.2	4.5	4.5	4.6	4.5	2.7
Half cystine	2.2	1.3	1.4	1.6	2.5	2.5	2.1
Valine	5.0	5.0	5.0	5.0	8.4	8.0	5.5
Methionine	1.3	0.4	0.3	0.1	2.0	1.6	2.2
Isoleucine	3.8	3.9	3.8	3.7	3.6	3.6	1.3
Leucine	7.6	7.7	7.6	7.6	7.2	7.2	2.6
Tyrosine	3.8	4.0	3.8	3.8	3.9	3.8	2.0
Phenylalanine	5.3	5.3	5.2	5.1	5.0	5.0	2.2

[a] Tryptophan not determined.

Table V. Amino Acids of Oat Extracts in g/16 g of Nitrogen

Amino acids	H_2O-soluble[a] (5)	NaCl-soluble (4)	Alcohol-soluble (3)	Acetic acid-soluble (4)	Residue
Lysine	8.1 ± 1.0	4.2[b] ± 0.6	0.7 ± 0.1	2.9 ± 0.3	5.9
Histidine	2.4[b] ± 0.6	2.6[b] ± 0.3	0.9 ± 0.1	2.6[b] ± 0.3	3.3
Ammonia	1.7 ± 0.4	2.3 ± 0.2	4.2 ± 0.8	3.4 ± 0.3	1.0
Arginine	7.9[b] ± 2.0	9.3 ± 0.3	3.5 ± 0.3	7.9 ± 0.2	11.2
Aspartic	8.1 ± 0.6	9.4 ± 0.6	1.7[b] ± 0.3	6.5[b] ± 1.1	9.6
Threonine	4.7 ± 0.4	3.5 ± 0.2	1.4 ± 0.2	2.8 ± 0.2	4.7
Serine	7.0[b] ± 1.0	4.9 ± 0.3	1.9 ± 0.2	3.8 ± 0.4	5.1
Glutamic	16.1 ± 1.1	21.1 ± 1.5	42.1 ± 3.8	33.4[b] ± 3.8	20.3
Proline	6.7[b] ± 1.4	4.2[b] ± 0.4	8.4[b] ± 2.4	7.3[b] ± 1.3	5.2
Glycine	7.6 ± 0.6	4.5 ± 0.2	0.8[b] ± 0.1	3.0[b] ± 0.5	5.6
Alanine	6.3 ± 0.3	4.4 ± 0.3	3.5[b] ± 0.5	4.1 ± 0.1	6.2
Half cystine	5.4[b] ± 1.8	1.2[b] ± 0.3	2.8[b] ± 1.1	1.9 ± 0.1	2.6
Valine	4.7[b] ± 0.5	5.2 ± 0.4	6.7[b] ± 3.7	6.0 ± 0.2	11.6
Methionine	0.5[b] ± 0.3	0.7[b] ± 0.5	2.2[b] ± 0.9	1.2[b] ± 0.4	4.1
Isoleucine	3.1 ± 0.0	4.5 ± 0.3	2.8 ± 0.3	4.1 ± 0.2	5.0
Leucine	6.4 ± 0.2	7.5 ± 0.4	11.2 ± 0.9	9.4 ± 0.7	9.4
Tyrosine	4.0[b] ± 0.6	4.2 ± 0.2	2.1 ± 0.3	3.9 ± 0.3	5.3
Phenylalanine	3.6[b] ± 0.7	5.8 ± 0.4	6.7[b] ± 0.9	6.9 ± 0.2	7.0

[a] The first figure is the average value and the second value standard deviation. The number in parentheses following each extract indicates the number of amino acid analyses used for the calculation. [b] There is significant difference between the highest and the lowest values in this average.

Wyndmere break flour, except the former has higher valine and methionine values. The high lysine content of Wyndmere oats and its mill fractions (Table IV) is exceptional for cereals, which have low lysine in general.

The amino acid distribution of oat extracts is shown in Table V. Values given under the water-soluble fraction represent the average of analyses of Wyndmere break flour, reduction flour, shorts flour, and ND 86-3-63 break flour. The highest levels of lysine (8.1%) and half cystine (5.4%) are observed in this water-soluble fraction (Table V). The value for NaCl extract is the average of analyses of Wyndmere break flour, reduction flour, shorts flour, and ND 86-3-63 break flour.

The value for alcohol-soluble extract in Table V is the average of analyses of Wyndmere reduction flour and Wyndmere shorts. Lysine content of the alcohol-soluble fraction is quite low, but the methionine value is rather high. The acetic acid-soluble value is the average of analyses of Wyndmere break flour, reduction flour, shorts flour, and ND 86-3-63 break flour. These acetic acid-soluble fractions were obtained after the water- and NaCl-soluble fractions had been removed. The residue obtained after water, NaCl, acetic acid, and ethanol-soluble fractions were removed from Wyndmere reduction flour has high valine and methionine compared with other extracts in Table V.

The standard deviation of each amino acid in each of the four extracts is calculated, and the standard deviation follows the average value of each amino acid. If the difference between the highest and lowest values is significant, then the average amino acid value is marked by a superscript.

Electrophoretic Patterns. The gel electrophoretic patterns of water extract from Wyndmere reduction flour showed a single fast blurred band with trailing. Sodium chloride extract from Wyndmere break flour showed four slow bands (one heavy, one light, and two moderately heavy) and a fast blurred band. The ethanol and butanol extracts from Wyndmere reduction flour had the same electrophoretic patterns and they showed three moderately heavy bands (one relatively rapid and two slow ones). The acetic acid extract from Wyndmere break and reduction flour both showed three slow bands (one heavy and two lighter ones). These patterns indicated that most of the proteins contain more than one component, with the possible exception of water extract.

Ewart (1968) extracted proteins from oat flour with 0.04 *M*

NaCl, water, 70% ethanol, and 0.1 *N* acetic acid. The nitrogen content of his extracts varied from 0.4 to 7.7%, and two-thirds of the nitrogen remained in the residue. Although his work is on oat flour while our results are on the individual dry-milled fractions, the percentages of total nitrogen extracted by water and by 70% ethanol in his study are comparable with those in Table II. On the other hand, the percentage of total nitrogen extracted by 1 *M* NaCl and 0.1 *N* acetic acid was much higher in our studies. Our higher recovery of globulin may be a result of the higher concentration of NaCl used. In addition, there may be differences among the oats used for extraction. Our nitrogen recovery calculated from Table II was not far from the 82.7% value reported by Ewart. Starch gel electrophoresis in 8 *M* urea of his 70% ethanol extracts of oat flour showed three bands, the same number as we obtained from Wyndmere break flour. Five bands were observed in starch gel electrophoresis by Elton and Ewart (1962) from their oat flour dispersion in aluminum lactate buffer, which dissolved only 23% protein.

Waldschmidt-Leitz and Zwisler (1963) isolated albumin, globulin, and avenin from defatted oat flour and studied the amino acid composition. Because their nitrogen recovery was very low (less than 5%), their results may not be representative. For this reason, no comparison was made between their results and Table V. The amino acid composition of Wyndmere oats in Table IV agrees quite well with compositions reported by Hischke *et al.* (1968) for seven varieties of oat groats and with the mean amino acid composition of 289 oat groats as established by Robbins *et al.* (1971).

The availability of high-protein oats, the favorable solubility properties of oat proteins, and their well-known nutritive value indicate a bright future for low-cost, high-protein, and highly nutritious food products being made from oat fractions.

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Grain Sorghum Glutelin: Isolation and Characterization

Alfred C. Beckwith

Glutelin accounts for more than half of the total protein content in three grain sorghum hybrid flours. A procedure has been developed to isolate this major fraction of highly insoluble proteins. Changes in glutelin solubility, gel electrophoretic, and chromatographic properties accompanying the cleavage of protein disulfide bonds are described.

In describing the protein composition of three hybrid grain sorghums, Jones and Beckwith (1970) noted that less than half the total protein nitrogen in the flour fractions was extractable protein nitrogen. A method has now been developed for isolating the major protein fraction, hereafter called glutelin. This glutelin is not extracted from flour with common neutral or weakly acidic solvents. Basic solvents were not employed, to avoid possible chemical decomposition of macromolecules. Physical-chemical properties of the isolated glutelin were roughly determined, as well as those of the product left after disulfide bond disruption.

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MATERIALS AND METHODS

Guanidine hydrochloride (GHCl, Eastman Organic Chemicals white label grade) was recrystallized by the method of Kolthoff *et al.* (1957). When used in reaction solvents, GHCl was further purified by the procedure of Wu and Dimler (1964). β-Mercaptoethanol, also purchased from Eastman Organic Chemicals, was redistilled under nitrogen. The fraction boiling at 153–155°C was collected and used as a disulfide bond reducing agent. Acrylonitrile (Eastman's yellow label) was used as a sulfhydryl blocking agent without further purification. Sephadex G-150 crosslinked dextran was purchased from Pharmacia Fine Chemical Inc. Twice-recrystallized hog pancreas α-amylase came from the Mann Research Laboratories. The stated activity of the enzyme