ALBUMIN α-AMYLASE INHIBITOR
FAMILIES FROM WHEAT FLOUR

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ABSTRACT

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When submitted to gel filtration on a Sephadex G-100 column, albumins from 70% extraction wheat flour gave three peaks whose molecular weights were 60,000, 24,000, and 12,000. These three peaks were coded as Peaks II, III, and IV, respectively, to stress the absence in this albumin fraction of Peak I (excluded in the column void volume) which was the main peak observed when albumins from whole wheat flour were analyzed under identical experimental conditions. Peaks II and III were active in inhibiting human saliva and *Tenebrio molitor* L. α-amylases, whereas Peak IV only inhibited the insect amylase. After treatment with sodium dodecyl sulfate in the presence or absence of β-mercaptoethanol, the molecular weight of Peak IV was unaffected, whereas about 80% of Peak II and 95% of Peak III dissociated into subunits with a molecular weight identical to that of Peak IV. The extensive dissociation of Peaks II and III was reversed upon removal of the detergent by ion-exchange chromatography, but the inhibitory activity toward α-amylase was restored only when treatment with the detergent had been carried out in the absence of β-mercaptoethanol. When submitted to polyacrylamide gel electrophoresis, Peaks II and III were found to be heterogeneous. Ten anodic components at pH 8.5 and seven cathodic components at pH 4.3 were observed in Peak III; Peak II showed 5 components at both acidic and alkaline pH values. Peak III was fractionated into four peaks by means of preparative gel electrophoresis at pH 8.5. Although no isolation of individual components could be achieved, it was possible to show that all the components of Peak III which moved toward the anode at alkaline pH (about 95% of the total) were active in inhibiting human saliva and *T. molitor* L. α-amylases. Moreover, they showed very similar amino acid compositions and circular dichroism spectra in the far and near ultraviolet. These results indicate that the components of Peak III constitute a family of closely related albumins (coded as 0.19 family from the electrophoretic mobility of the main component).

Wheat-flour albumins are a very heterogeneous group of proteins with similar solubility properties (1). As shown by typical blue-black bands obtained by submitting wheat albumins to polyacrylamide gel electrophoresis and staining them with aniline blue-black, the components of the albumin group are also related from a structural standpoint and, in this respect, clearly differ from globulin and gliadin components that gave blue-green and reddish-brown bands, respectively (2). Although for a long time it has been believed that wheat albumins were mainly enzymatic proteins (3), it is now becoming clear that the biological activity associated with the bulk of wheat albumins is the ability to inhibit a number of α-amylases from different sources (4—7). When submitted to gel filtration on Sephadex G-100, a typical albumin preparation from hexaploid wheat flour can be resolved into three heterogeneous fractions with apparent molecular weights of 60,000, 24,000, and 12,000. The 12,000 albumin fraction consists of five main components which have been individually isolated by

preparative polyacrylamide gel electrophoresis and thoroughly investigated with respect to their physicochemical properties and their ability to inhibit α-amylases from different sources (4,8). After these investigations, Silano *et al.* (4) suggested that these albumins constitute a family of closely related proteins (coded 0.28 family from the electrophoretic mobility of the main component). This hypothesis has been recently confirmed by Redman (9) who has shown the close homology existing among primary structures of some components of the 0.28 family. The 24,000 albumin fraction also contains several protein components capable of inhibiting α-amylase (5). As compared with the 0.28 family, the components of the 24,000 fraction inhibit a larger number of α-amylases from different sources and, therefore, are less specific amylase inhibitors (7). The main component of this albumin pool (coded 0.19 from its electrophoretic mobility) has been purified (10) and extensively characterized (4,10,11). Amylase inhibitors which might belong to this albumin group have been studied by Shainkin and Birk (12), Feillet and Nimmo (13), Saunders and Lang (14), and Fish and Abbott (15). Preliminary experiments concerning the 60,000 albumin fraction indicated (5,7) that α-amylase inhibitory activity is also common to some components of this albumin group. These findings disclose an unexpected relationship among a very large number of wheat-flour albumin components and give further support to the close structural similarity of the components of this protein class.

This paper deals with some investigations undertaken to further characterize the components of the 24,000 and 60,000 albumin fractions with respect to some physical properties and to their ability to inhibit α-amylase. This study also was intended to clarify the relationship existing among the albumin amylase inhibitors with different molecular weights.

MATERIALS AND METHODS

Extraction and Purification of Wheat-Flour Albumins

All the experiments described in this paper were carried out with the 70% extraction white flour (ash less than 0.5%) obtained from the wheat sample *(Triticum aestivum,* pure variety Mentana) used for previous investigations by Petrucci *et al.* (5). Wheat flour was extracted at room temperature for 3 hr in a shaker with $0.15 M$ sodium chloride (2/3 w/v). After extraction, the suspension was centrifuged for 30 min at $45,000 \times g$ and the albumin fraction was precipitated by salting out the clear extract between 0.4 and $1.8 M$ ammonium sulfate. The albumin precipitate was redissolved in water, dialyzed for 48 hr against distilled water at $4°C$, and freeze-dried.

Gel Filtration on Sephadex G-100

About 1 g of albumin was dissolved in 100 ml of $0.1 M$ ammonium acetate buffer (pH 6.8), applied on a column (110×4.5 cm) of Sephadex G-100, and eluted with the same acetate buffer at a flow rate of 60 ml/hr. The absorbance of the eluate at 280 nm was monitored (Photochrom flow analyzer, Rastelli, Italy) and recorded (Kompensograph, Siemens, Germany). Fractions corresponding to central cuts of each peak were pooled and freeze-dried. Apparent molecular weights of the protein peaks were determined according to Andrews (16) by comparing their elution volumes from the Sephadex G-100 column with those of

the following proteins of known molecular weight: cytochrome C (mol wt 12,500) and chymotrypsinogen (mol wt 25,700) from Sigma Chemical Company (St. Louis, Mo.), bovine serum albumin (mol wt 68,000) from Armour Pharmaceutical Company (Chicago, Ill.), and blue dextran 2000 from Pharmacia (Uppsala, Sweden).

Gel Filtration on Sepharose 4B

Apparent molecular weights of the protein peaks after treatment with sodium dodecyl sulfate (SDS) in the presence or absence of β-mercaptoethanol were determined by gel filtration on a Sepharose 4B column according to Janado *et al.* (17). Wheat-flour albumins were dissolved at a 0.14% concentration in $0.05M$ phosphate buffer (pH 7.0) containing 0.5% SDS. When present, β-mercaptoethanol was $0.6M$. After incubation for 72 hr at 37°C, the mixture was applied on a column (91 × 5 cm) of Sepharose 4B and eluted with the same phosphate buffer containing the detergent at a flow rate of 18 ml/hr. Chromatography was carried out at a constant temperature of 28°C, and the absorbance of the eluate at 280 nm was monitored. Reference proteins were treated under identical experimental conditions.

Removal of SDS from the effluent was achieved according to Lenard (18) by filtering the samples through a Dowex 2-X10 (Biorad, Richmond, Calif.) column (4.3 × 1.5 cm) equilibrated in the $0.05M$ phosphate buffer (pH 7.0). Then, the eluted protein was dialyzed for 48 hr against distilled water at 4°C, and freeze-dried.

Polyacrylamide Gel Electrophoresis

Anodic analytical disc electrophoresis was carried out in $0.05M$ tris-$0.383M$ glycine buffer (pH 8.5). All the conditions used for electrophoresis and for detection of electrophoretic patterns were similar to those described by Minetti *et al.* (2); bromophenol blue mobility in the system was taken as 1. Amylase inhibitor pattern was determined by slicing the gel at the end of electrophoresis. Each slice, 0.3-cm thick, was eluted for 18 hr at 4°C in 1 ml of buffer and the eluate was assayed for amylase inhibition as described below.

Cathodic analytical electrophoresis was performed in $0.35M$ tris-$0.013M$ acetic acid buffer (pH 4.3) as described in the information sheets of Shandon Scientific Company (London, England) for a gel system at pH 4.3 applying 5 mA per gel for 150 min.

Protein electrophoretic patterns were recorded by a Gilford spectrophotometer Model 2400 (Gilford Instrument Laboratories Inc., Oberlin, Ohio).

Preparative electrophoresis on polyacrylamide gels of different porosities was performed on a PD 320 column in a vertical apparatus (Canal Industrial Corporation, Rockville, Md.) according to the method of Sodini *et al.* (10). The tris-glycine buffer, pH 8.5, was used and the elution rate was 30 ml/hr. The eluate was monitored for absorbance at 280 nm and for α-amylase inhibitory activity.

Polyacrylamide gel electrophoresis in SDS was carried out according to Laemmli (19).

Ultracentrifugal Studies

Sedimentation equilibrium was run in a Beckman Model E ultracentrifuge for 72 hr at 4°C and 20,000 rpm in 0.05 M cacodylate-HCl buffer (pH 5.8) containing 0.01 M NaCl; protein concentration was 0.1%.

Optical Methods

Circular dichroism (CD) measurements were made with a Cary Model 60 spectropolarimeter equipped with a 6002 CD accessory. The protein concentration was determined spectrophotometrically according to Waddel (20), using a Gilford spectrophotometer Model 2400 for uv measurements.

Deamidation Studies

Protein solutions (at a concentration of 0.1%) in 0.2 M sodium phosphate (pH 9.9) were incubated for 72 hr at 37°C to facilitate the study of hydrolysis of amide side chains; the conditions were essentially those of McKerrow and Robinson (21). The resulting product was examined by gel electrophoresis at both acidic and alkaline pH as described above.

Amino Acid Analysis

Determination of the amino acid composition of the albumins studied was accomplished using the chromatographic technique of Spackman *et al.* (22), as previously described by Petrucci *et al.* (5).

Inhibition of α-Amylase

The inhibitory activity of wheat albumins was determined on the α-amylase from *T. molitor* L. larvae and from human saliva. *T. molitor* amylase was prepared according to Buonocore *et al.* (23); human salivary amylase was freeze-dried saliva. Amylase inhibition tests based upon starch hydrolysis were performed according to the procedure described by Silano *et al.* (4), using a barbiturate buffer (10 m M sodium barbiturate, 10 m M sodium acetate, 150 m M sodium chloride, and 0.3 m M calcium chloride). The buffer pH was 5.4 for the insect amylase and 7.0 for the human amylase. Incubation with starch was for 5 min at 37°C. One unit of inhibitor (I.U.) is the amount that causes 30% inhibition of α-amylase under the described experimental conditions. The amount of α-amylase used was that which would produce 1 mg of maltose in 5 min under the experimental conditions chosen.

RESULTS

Gel Filtration on Sephadex G-100

When the albumin fraction, purified by salting out between 0.4 and 1.8 M ammonium sulfate of salt extract from 70% extraction wheat flour, was submitted to gel filtration on a Sephadex G-100 column, three peaks of absorbance were obtained (Fig. 1a). As calculated from their retention volumes, molecular weights of the three peaks were approximately equal to 60,000, 24,000, and 12,000 (Fig. 1a, insert). As these molecular weights are very close to those exhibited by Peaks II, III, and IV, respectively, obtained from whole wheat flour albumins under identical experimental conditions, we have coded the three peaks from 70% extraction flour as Peaks II, III, and IV. This nomenclature is also intended to stress the absence, in the albumin preparation from extraction flour,

of Peak I (excluded in the column void volume) which was the main peak observed with the albumin preparation from whole wheat flour (5). As the extraction flour we have used derives from the wheat sample used by Petrucci *et al.* (5) for the investigations of whole wheat flour, these findings indicate that the albumin fraction eluted with Peak I is associated to the outer layers of the kernel and/or to wheat germ.

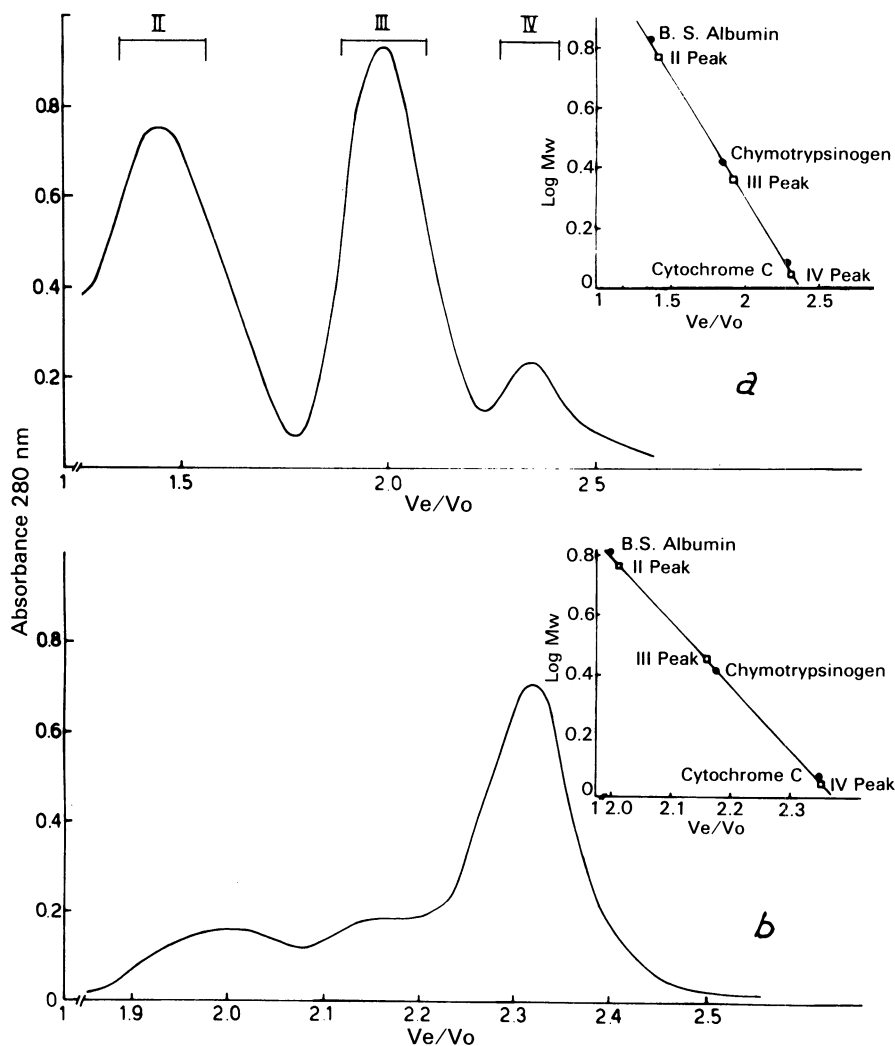Peaks II, III, and IV accounted for about 51, 40, and 9% of total absorbance,

Fig. 1. Gel filtration patterns of native and reduced wheat flour albumins (*inserts*: molecular-weight plots of reference proteins). Ve = Effluent volume; Vo = void volume; a = native albumins filtered through Sephadex G-100; and b = reduced albumins filtered through Sepharose 4B in SDS.

respectively (Fig. 1a). Fractions corresponding to central cuts of each peak of Fig. 1a (indicated by bars) were pooled, dialyzed, and freeze-dried.

When tested under identical experimental conditions, Peak II and bovine serum albumin used as a reference protein showed similar sedimentation patterns, thus confirming the molecular weight of Peak II calculated by gel filtration. Similar studies on Peaks III and IV have already been reported (4,5).

α-Amylase inhibition measurements indicated that Peaks II and III were both active in inhibiting the α-amylases from *T. molitor* L. and from human saliva, whereas Peak IV only inhibited the insect amylase. Specific inhibitory activities of the three peaks toward the two amylases are shown in Table I.

The 24,000 Albumin Fraction

The heterogeneity of Peak III has been clearly shown by polyacrylamide gel electrophoresis in both alkaline and acidic buffer systems. Ten anodic components at pH 8.5 (Fig. 2, III) and 7 cathodic components at pH 4.3 (Fig. 3, III) were distinctly separated. As has been reported previously for Peak III obtained from whole wheat flour (5), Peak III from the extraction flour also contained a few components which at pH 8.5 moved toward the cathode. Following the procedure described by Petrucci *et al.* (5), we showed that these fractions account for less than 5% of the total and are devoid of any inhibitory activity toward α-amylase.

When Peak III was submitted to preparative differential disc electrophoresis on polyacrylamide gel in the alkaline buffer system, four peaks of absorbance and of α-amylase inhibitory activity (III$_a$, III$_b$, III$_c$, and III$_d$) were obtained (Fig. 4b). The correspondence of absorbance and amylase inhibition profiles was excellent. The four peaks were all active toward both human saliva and *T. molitor* L. amylases (Table I). As shown by polyacrylamide gel electrophoresis in both alkaline (Fig. 2, III$_a$−III$_d$) and acidic (Fig. 3, III$_a$−III$_d$) buffer systems, none

TABLE I
Effect of Sodium Dodecyl Sulfate upon Specific Inhibitory Activities
toward Human Saliva and *Tenebrio molitor* L.
α-Amylases of Albumin Fractions from Wheat Flour

	Specific Inhibitory Activity (I.U./μg protein)			
	Human salivary amylase		*Tenebrio molitor* amylase	
Albumin Fraction[a]	No treatment	After treatment with 0.5% SDS	No treatment	After treatment with 0.5% SDS
Peak II	0.8 ± 0.3	0.2 ± 0.04	0.4 ± 0.1	0.2 ± 0.03
Peak III	3.0 ± 0.6	1.6 ± 0.40	4.0 ± 1.0	2.0 ± 0.40
Peak III$_a$	2.8 ± 0.6	Not tested	3.0 ± 0.6	Not tested
Peak III$_b$	4.5 ± 1.0	Not tested	3.5 ± 0.9	Not tested
Peak III$_c$	4.6 ± 0.9	Not tested	3.4 ± 0.8	Not tested
Peak III$_d$	3.6 ± 0.7	Not tested	4.5 ± 1.0	Not tested
Peak IV	Not active	Not active	5.1 ± 1.1	2.3 ± 0.50

[a]Wheat albumin fractions were incubated for 72 hr at 37°C in phosphate buffer (pH 7.0) in the presence or absence of 0.5% SDS. At the end of the incubation, SDS was removed by ion-exchange chromatography. The inhibition unit (I.U.) is defined in the text under **Inhibition of α-Amylase**. All the albumin samples were tested at a maximum concentration of 2 μg/ml in the incubation mixture. Mean values of three determinations ± standard deviation are given.
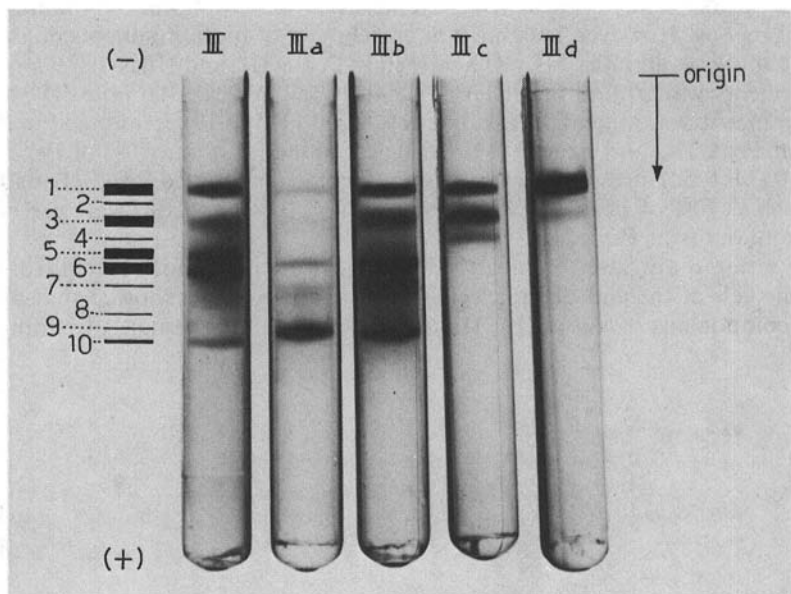
Fig. 2. Polyacrylamide-gel electrophoretic patterns at pH 8.5 of Peak III and of the fractions obtained from Peak III by preparative electrophoresis.
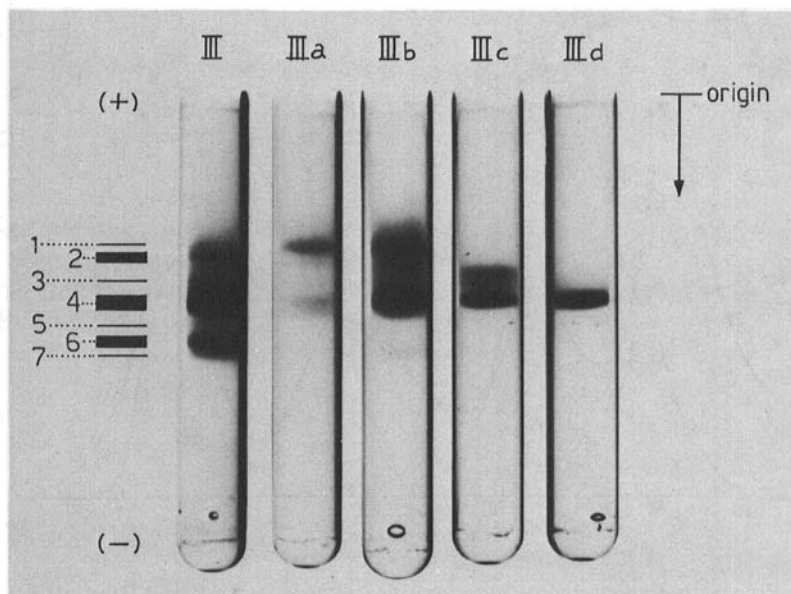

Fig. 3. Polyacrylamide-gel electrophoretic patterns at pH 4.3 of Peak III and of the fractions obtained from Peak III by preparative electrophoresis.

of the peaks eluted from the preparative electrophoretic column was homogeneous. However, a significant enrichment of the components migrating more rapidly at alkaline pH was achieved in Peaks III_a and III_b, whereas slower components were found in higher concentrations in Peaks III_c and III_d. From densitometric scanning of the alkaline gels, band 1 ($M_b 0.19$) accounted for about 90% of Peak III_d and band 3 ($M_b 0.27$) accounted for about 80% of Peak III_c. Band 9 ($M_b 0.52$), present in a small amount in unfractionated Peak III, made up as much as 70% of Peak III_a; bands 5 and 6 ($M_b 0.36$ and 0.38) were present in larger amounts in Peak III_b.

By testing α-amylase inhibitory activity of protein fractions eluted from the alkaline gels at the end of the electrophoretic analysis, we showed that all the main components of Peaks III_a, III_b, III_c, and III_d inhibit human saliva amylase
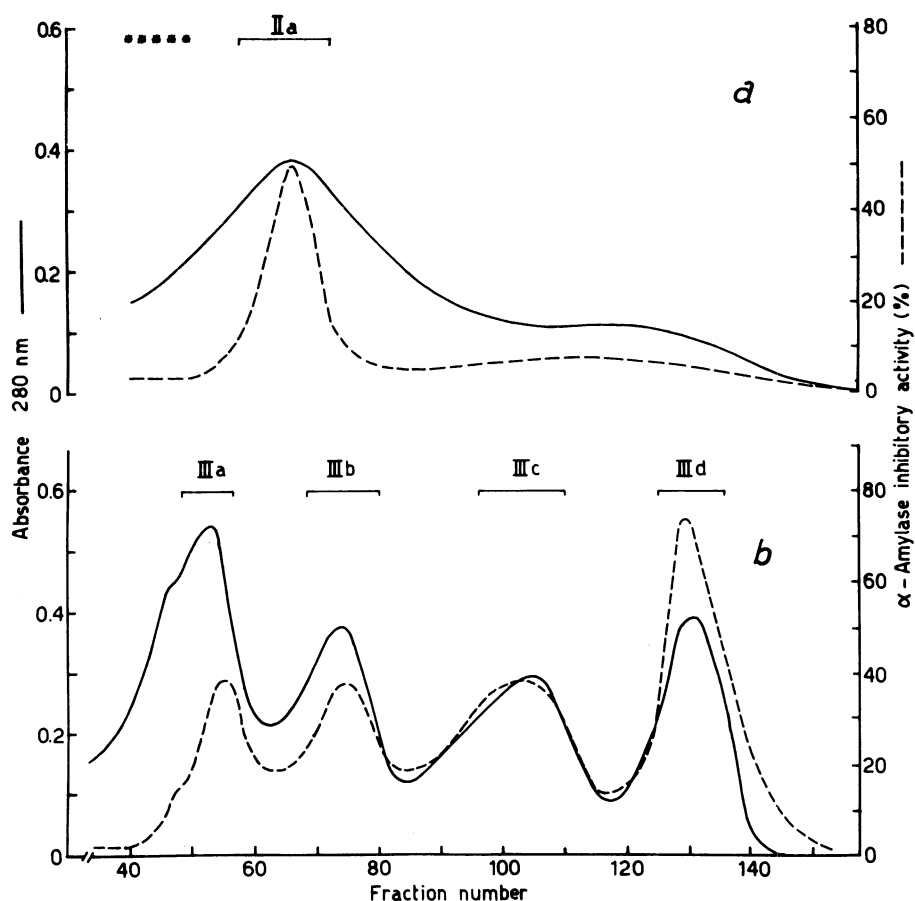

Fig. 4. Preparative gel electrophoretic fractionations of Peaks II and III at pH 8.5. a = Peak II, and b = Peak III (in Fig. 4a, fractions indicated by asterisks contained amylase activity).

(Fig. 5) and *T. molitor* L. amylase (not shown in the figure).

Circular dichroism spectra of Peaks III_a, III_b, III_c, and III_d in the far and near ultraviolet were not significantly different from those of unfractionated Peak III. They were all similar to those reported by Silano *et al.* (4) for the 0.19 albumin inhibitor that, as mentioned above, makes up about 90% of Peak III_d.

No obvious differences were noted among the amino acid compositions of these four peaks and that of Peak III reported by Petrucci *et al.* (5), although the accuracy of the determination was not sufficient to rule out differences of one or
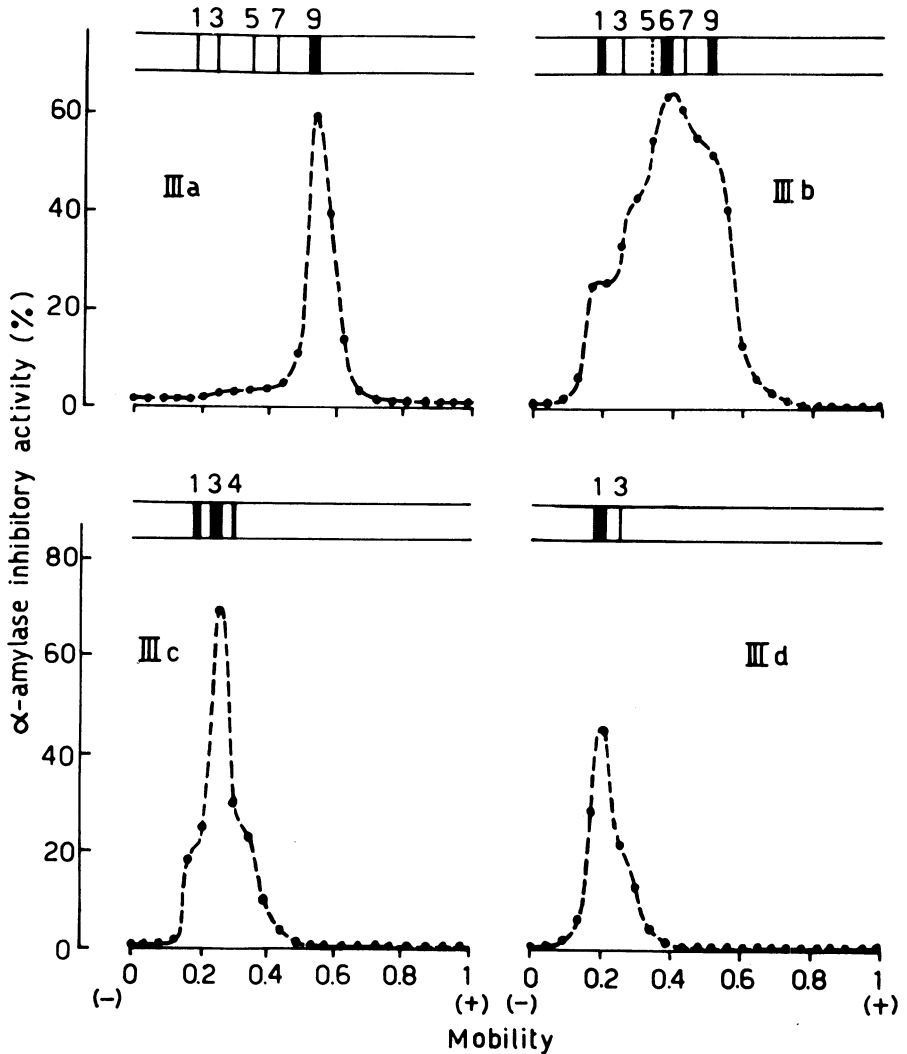

Fig. 5. Protein and α-amylase inhibitor gel-electrophoretic patterns at pH 8.5 of the fractions obtained from Peak III by preparative electrophoresis.

even two residues for some of the amino acids on a 24,000 mol-wt basis.

The possibility that the components of Peak III are simply deamidated forms of the 0.19 albumin has been tested by submitting Peak III$_d$ to deamidation in phosphate buffer, pH 9.9, at 37°C for 72 hr. Several extra bands with greater mobilities were observed at pH 8.5 (Fig. 6). Electrophoresis at pH 4.3 produced no new bands, however. Since the components of Peak III had different mobilities at pH 4.3 as well as at pH 8.5, we concluded that the proteins must differ in ways other than substitution of glutamic or aspartic acid for glutamine or asparagine in the primary structure.

The 60,000 Albumin Fraction

When submitted to gel electrophoresis under the experimental conditions used for Peak III, Peak II showed five anodic components at pH 8.5 and five cathodic components at pH 4.3.

No fractionation of the components of Peak II could be achieved by means of preparative disc electrophoresis on polyacrylamide gel in the alkaline buffer system, and only one peak (Peak II$_a$) of absorbance and amylase inhibitory activity was obtained (Fig. 4a). The electrophoretic patterns of Peak II$_a$ at both acidic and alkaline pH values were not significantly different from those of Peak II.

Dissociation by SDS

When the unfractionated wheat-flour albumins were incubated for 72 hr with 0.5% SDS and 0.6M β-mercaptoethanol and the incubation mixture was submitted to gel filtration on a Sepharose 4B column equilibrated in a SDS-
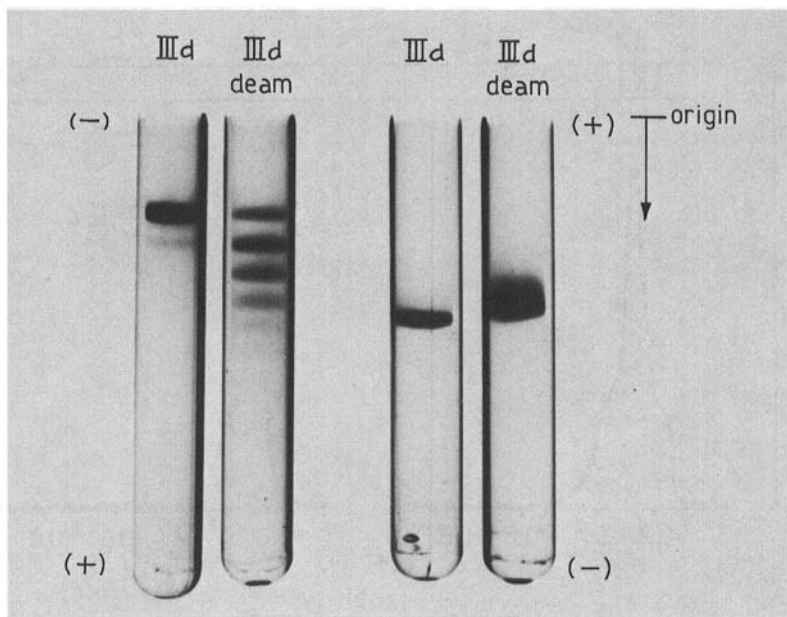

Fig. 6. Polyacrylamide-gel electrophoretic patterns at pH 8.5 (left) and at pH 4.3 (right) of native and deamidated Peak III$_d$.

containing buffer, three peaks of absorbance were obtained (Fig. 1b). β-Mercaptoethanol (not shown in the figure) was eluted long after the main peak of Fig. 1b. In contrast with the results obtained in the absence of the detergent (Fig. 1a), the main peak of absorbance (about 80% of total absorbance) corresponded to cytochrome C in Ve/Vo (Fig. 1b, insert). It appears that the components of the 60,000 and 24,000 albumin fractions undergo dissociation upon treatment with the detergent and the reducing agent. These findings were confirmed by separately submitting Peaks II, III, and IV from Fig. 1a to reduction with β-mercaptoethanol and to gel filtration on the Sepharose 4B column (Fig. 7) or to polyacrylamide gel electrophoresis in SDS. As shown in Fig. 7, the molecular weight of Peak IV was unaffected, whereas Peak III split into two peaks (Peaks III_A and III_B), and Peak II split into three peaks (Peaks II_A, II_B, and II_C). The molecular weight of Peak III_A (about 95% of Peak III) was very close to that of cytochrome C (Fig. 7, insert) and identical with that determined for Peak IV under the same experimental conditions as well as in the absence of the dissociating agent. Peak III_B exhibited a molecular weight identical with that of the undissociated Peak III (Fig. 1a). These results are consistent with those previously obtained with Peak III from whole wheat flour (5), as well as with the purified 0.19 inhibitor (4) which in the presence of $5M$ guanidine hydrochloride exhibited a molecular weight of about 12,000, equal to about one-half of the molecular weight determined in the absence of the dissociating agent. Peak II_A (about 80% of Peak II) exhibited a retention volume identical with that of Peak IV, whereas the molecular weights of Peak II_B and II_C (about 10% each of Peak II) were identical to those of the undissociated Peaks III and II, respectively (Fig. 1a).

Polyacrylamide gel electrophoretic patterns of reduced Peaks III and IV in SDS showed a very intense band with mobility slightly higher than that of cytochrome C. Peak III, but not Peak IV, also showed a very faint band with a mobility comparable to that of chymotrypsinogen. When tested under identical experimental conditions, Peaks III_a, III_b, III_c, and III_d from the preparative gel electrophoretic column did not show the faint band with lower mobility, suggesting that the small amount of Peak III that does not dissociate in the presence of the detergent is removed by preparative gel electrophoresis and, therefore, is cathodic at pH 8.5. Finally, the electrophoretic pattern of Peak II, in addition to the two bands found in Peak III, a minor band with mobility close to that of bovine serum albumin was evident.

The extensive dissociation of Peaks II and III, as monitored by gel electrophoresis, was also observed after incubation of these two peaks for 72 hr at 37°C with SDS in the absence of β-mercaptoethanol. These results, together with those obtained using guanidine hydrochloride by Petrucci *et al.* (5), indicate that the reducing agent is not necessary for dissociating the components of Peaks II and III.

Reversibility of the Dissociation Induced by SDS

To evaluate the reversibility of the dissociation of Peaks II and III induced by reduction with β-mercaptoethanol in the presence of SDS, fractions corresponding to Peaks II_A and III_A of Fig. 7 were pooled and submitted to ion-exchange chromatography to remove the detergent. After this treatment, the molecular weights of Peaks II_A and III_A, as determined by gel filtration on
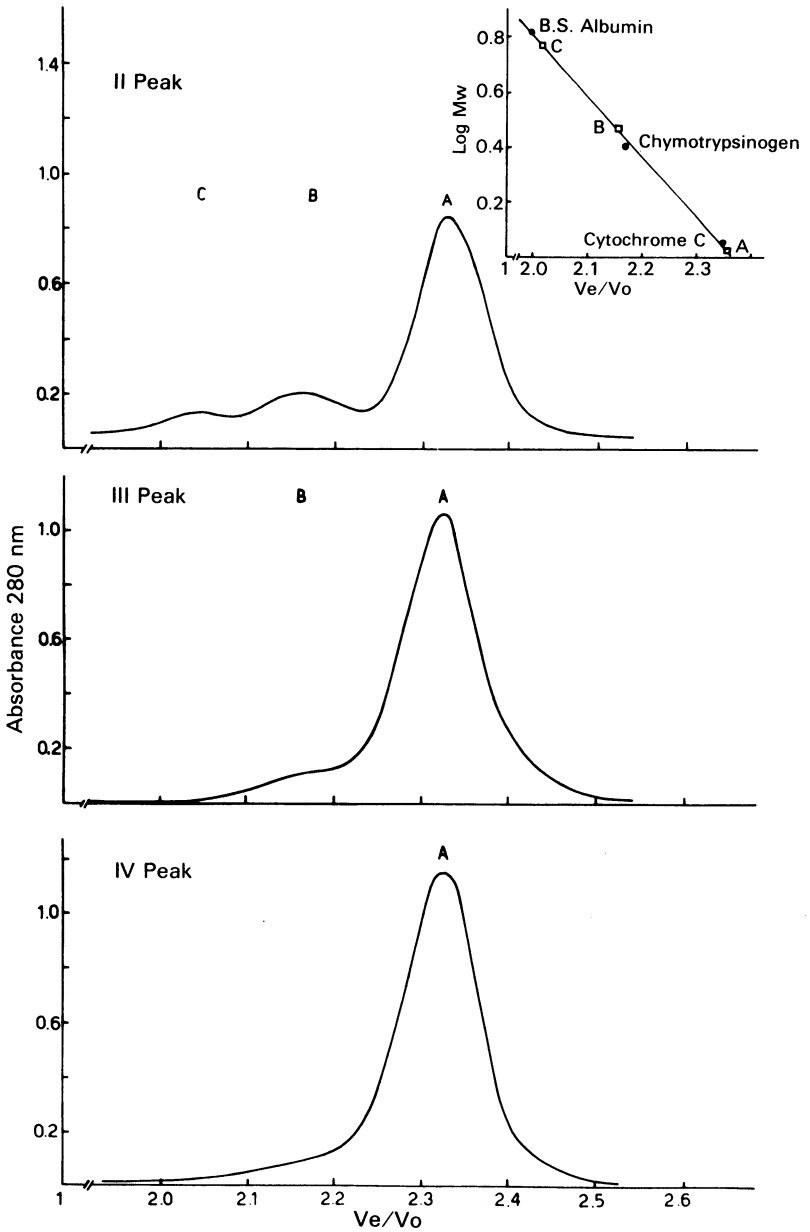
Fig. 7. SDS gel-filtration patterns on Sepharose 4B of Peaks II, III, and IV from Fig. 1a after reduction with β-mercaptoethanol. *Insert:* molecular-weight plot of reference proteins.

Sephadex G-100 under the conditions of Fig. 1a, were 60,000 and 24,000, respectively. Although molecular weights of Peaks II and III were recovered upon removal of the detergent, inhibitory activities of these two peaks toward the mammalian and insect amylases as well as that of Peak IV could not be restored by such treatment. The irreversible denaturation of the components of Peaks II, III, and IV was also indicated by the fact that no distinct electrophoretic pattern could be obtained under the conditions of Fig. 2 from the treated peaks. However, when the treatment with SDS had been carried out in the absence of the reducing agent, only a partial loss of inhibitory activity was observed upon removal of the detergent (Table I). In this case, gel electrophoretic patterns of the three peaks after removal of the dissociating agent were identical with those observed before the treatment.

DISCUSSION

Although we have not individually isolated the components of the 24,000 albumin pool from wheat flour, our data on partially purified fractions provide considerable evidence for the close relationship of the components of this albumin group and, together with the previous findings of Petrucci *et al.* (5), justify referring to these albumins as a family of proteins (coded as 0.19 family from the electrophoretic mobility of the main component). Even though differences in gel electrophoretic mobilities among the components of the 24,000 albumin pool indicate that the components differ in amino acid composition, the identity of circular dichroism spectra provides strong evidence that these components are closely related, having largely identical conformational structures and amino acid sequences. A definite confirmation of the close relationship existing among the components of the 0.19 family is given by their common activity in inhibiting human salivary and *T. molitor* L. α-amylases, as well as by their ability to reversibly dissociate into subunits of molecular weight 12,000 in the presence of dissociating agents. Moreover, all the components of the 0.19 family were irreversibly denatured by a treatment with SDS and β-mercaptoethanol, but not with SDS alone. These findings suggest that intramolecular disulfide bonds are necessary for inhibitor functionality, and demonstrate that apparently no intersubunit disulfides are present in native protein inhibitors. Actually, random intersubunit disulfide bonds, formed upon removal of SDS and β-mercaptoethanol, might be responsible for the production of inactive proteins from reduced 24,000 inhibitors.

Our preliminary investigations of the 60,000 albumin fraction have shown that as much as 80% of the total protein of this fraction is formed of subunits with a molecular weight of 12,000, which is identical with that of the subunits of the 0.19 family and with that of the components of the 0.28 family (Peak IV). Although several amylase inhibitor components have been described in the 60,000 albumin fraction (5), and the subunits of this fraction showed the same ability as the components of the 0.19 family to reassociate upon removal of SDS, definite evidence is still lacking that α-amylase inhibitory activity is associated with the protein components able to dissociate upon addition of the detergent. Further investigations, possibly using purified inhibitors from this albumin pool, are needed to clarify this point.

The scheme shown in Fig. 8 summarizes diagrammatically all the information available on albumin amylase inhibitors from wheat, as well as the known and

inferred relationships among the different inhibitor groups. Moreover, the scheme points out that the understanding of the relationship existing among the subunits of the 24,000 and 60,000 inhibitors and the 12,000 inhibitors is the key step for the further development of knowledge on protein α-amylase inhibitors from wheat. As a working hypothesis, it is suggested that albumin α-amylase inhibitors from wheat flour are coded by a small number of structurally related genes, possibly derived by mutation from a common ancestor, and that some
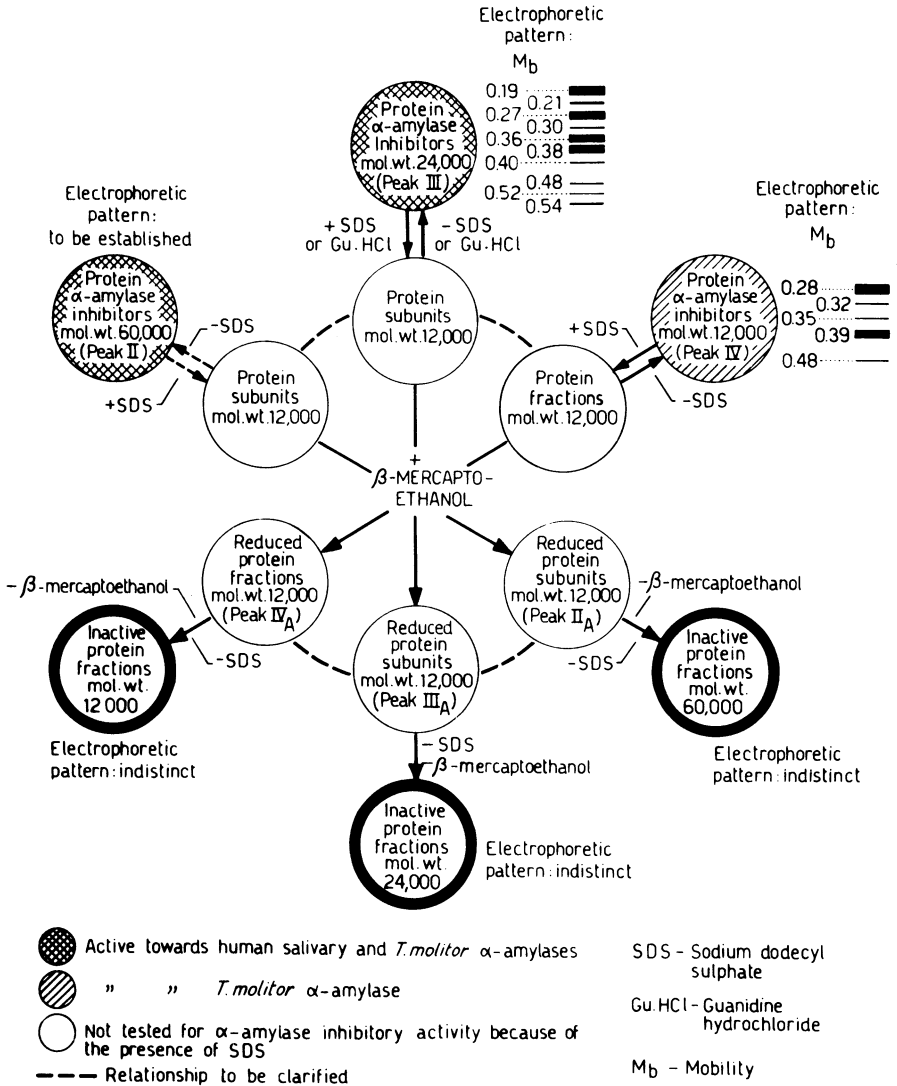
Fig. 8. Scheme summarizing the information available on albumin α-amylase inhibitors from wheat and the known and inferred relationships among different inhibitor groups.

mutant genes have produced albumins which are able to associate, thus giving rise to polymer components. Fingerprinting and sequencing studies of subunits of the 24,000 and 60,000 amylase inhibitors, to be compared to those available for the components of the 0.28 family (9), are needed to test the hypothesis of the monophyletic origin of such a large number of wheat albumins.

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