Diamine Oxidase and Transglutaminase Activities in White Lupine Seedlings with Respect to Cross-Linking of Proteins

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Enzymes extracted from 10-d-old *Lupinus albus* seedlings were able to (1) polymerize casein and (2) incorporate [^{14}C]putrescine into dimethylcasein (modified casein). High molecular weight polymers formed were visualized by SDS–polyacrylamide gel electrophoresis. [^{14}C]Putrescine incorporation was not only due to transglutaminase activity but also due to diamine oxidase. The use of diamine oxidase inhibitor allowed us to localize a transglutaminase activity in the pellet after centrifugation at 41400*g* of the filtered homogenate of seedlings and a diamine oxidase activity mainly in the supernatant. Covalent conjugation of monodansylcadaverine and [^{14}C]putrescine to dimethylcasein by enzymes contained in the 41400*g* pellet was vizualized by fluorescent detection or by autoradiography in SDS–polyacrylamide gel electrophoresis. The enzyme contained in the pellet fraction was able to polymerize not only casein but also spinach ribulose-1,5-bisphosphate carboxylase/oxygenase and 7S soybean globulins. As a control we used purified diamine oxidase from porcine kidney to check the inability of this enzyme to catalyze casein polymerization. Moreover, 54% of transglutaminase activity contained in the pellet has been solubilized by 5% (v/v) detergent (Triton X-100) contrary to high concentrated salts. Transglutaminase was recovered in the 108000*g* supernatant. This suggests that this enzyme was an integral membrane protein.

Keywords: *Diamine oxidase; transglutaminase; cross-linking; soybean proteins; RuBisCO; Lupinus albus*

INTRODUCTION

Transglutaminase (TGase) catalyzes the acyl-transfer reaction in which γ-carboxamide groups of peptide-bound glutamine residues serve as acyl donors and primary amino groups serve as acyl acceptors. Thus, this enzyme allows the post-translationnal modification of glutamine residues of proteins, which either causes protein cross-linking by γ-glutamyl ϵ-lysine peptide bridges or covalent incorporation of small molecular weight amines (Pisano et al., 1968; Folk, 1980; Lorand et al., 1981). Until the publication of reports on TGase in meristemic tissues of etioled plants of *Pisum sativum* and sprouts of *Helianthus tuberosus* tubers (soluble fraction) (Icekson and Apelbaum, 1987; Serafini-Fracassini et al., 1989), it was generally agreed that the distribution of the enzyme was restricted to animals. Recently TGase (particulate fraction) was also found in the chloroplast of alfalfa leaves (Margosiak et al., 1990) and in the leaves of silver beet and broccoli floral buds (Signorini et al., 1991). In order to diversify the supply of TGase, we have looked for a TGase in white lupine seedlings.

The most sensitive assay of TGase activity is [^{14}C]-putrescine (Pu) incorporation into dimethylcasein (DMC). The lysine residues of casein were blocked in DMC. The cross-links between glutamine and lysine residues cannot be catalyzed by TGase. Only amine incorporation into DMC occurs under these conditions. This assay is used when the available amount of enzyme is low, as is the case for plant TGases. We cannot however exclude the possibility that other enzymatic activities are involved in and interfere with TGase when this common and convenient radiolabeled incorporation assay is used.

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Indeed, it is known that plants are rich in polyamines, such as Pu, spermine, and spermidine (Smith, 1985), and that a diamine oxidase (DAO) activity is responsible for the oxidation of these compounds into aldehydes (Frederico and Angelini, 1988). The latter are able to react spontaneously with an amino group in proteins to give rise to an incorporation of the amine into protein, for instance, as a Schiff base (Lorand and Conrad, 1984). In our experiments, both DAO and TGase activities resulted in ^{14}C-labeled Pu incorporation into dimethylcasein. This is the reason why we have looked for the DAO enzyme, which could be responsible for the incorporation activity in the lupine seedlings, and we have studied its relative contribution with respect to TGase activity.

MATERIALS AND METHODS

Chemicals. Proteins from bovine milk (DMC, casein, β-lactoglobulin), Pu, DAO from porcine kidney, monodansylcadaverine (MDCd), and sodium diethyldithiocarbamate (DIECA) were obtained from Sigma (St. Louis, MO); 7S and 11S globulins were purified according to the procedure of Thanh et al. (1976). Spinach ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) was supplied by Dr. Gontero, Institut J. Monod, Paris. All other reagents were of analytical grade. [1,4-^{14}C]Putrescine dihydrochloride (specific radioactivity: 108 mCi mmol^{-1}) was purchased from Amersham (Buckinghamshire, England).

Plant Material. Lupine seeds were a gift of Dr. Huygues, INRA, Lusignan, France. Seeds of white lupine (*Lupinus albus* L.) were soaked overnight in tap water and allowed to germinate on moist vermiculite at 20 °C in the darkness for 6 or 10 d. The etiolated lupine seedlings with roots (without cotyledons) were homogenized in a Waring blendor for 30 s at intermediate speed, three times, with 2 volumes of either 5 or 20 mM cold Tris-HCl buffer (pH 7.6) containing 2 mM EDTA, 0.1 mM DTT, and 1 mM benzamidine. The homogenate was then filtered through four layers of cheese cloth. The filtrate

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obtained was centrifuged at 41400*g* for 30 min. All steps were carried out at 4 °C.

When the localization of activity in different organs of the seedling was studied, every part of the seedling (roots, stems, and leaves) was separated and used as the whole seedling. For light experiments, lupine seeds germinated in the darkness and were exposed to natural light during the last 2 d. Homogenization was carried out with 20 mM Tris buffer. For sucrose extraction, under conditions of intactness of organites, 0.25 M sucrose was used for homogenization instead of Tris buffer.

TGase Extraction from the 41400*g* Pellet. The 41400*g* pellet was resuspended in homogenization of 20 mM Tris buffer containing either increasing amounts of salts (0, 150, 250, and 500 mM NaCl) or of nonionic detergent (0.1, 1, and 5% (v/v) Triton X-100). After ultracentrifugation at 108000*g* for 1 h, TGase activity (Pu incorporation in the presence of DIECA) was measured in the supernatant.

Radiolabeled Pu Incorporation Assay. [^{14}C] Pu incorporation into DMC was routinely determined by a filter paper assay (Lorand et al., 1972). The standard reaction mixture contained, in a total volume of 0.4 mL, 0.1 M Tris-HCl buffer (pH 7.5), 10 mg mL^{-1} DMC, 4.025 mM [^{14}C] Pu (1400 cpm nmol^{-1}), 3 mM $CaCl_2$, and 200 μL of lupine extract. Incubation was performed at 30 °C. Aliquots were removed at timed intervals and precipitated with 10% (w/v) TCA on filter paper (Whatman no. 3MM). The filters were washed repetitively with 5% TCA and counted for radioactivity in 10 mL of Hionic Fluor scintillation cocktail from Packard (Groningen, The Netherlands). Incorporation activity was calculated as the slope of the progress curve. A blank consisted of the homogenization buffer instead of the extract, and the values were substracted from assays to which the enzyme was added. In all cases, the assays were triplicated.

One unit (U) of incorporation activity was defined as the amount of enzyme(s) that catalyzed the formation of 1 pmol of product min^{-1} at 30 °C.

The same reaction mixture was used for visualization of [^{14}C] Pu incorporation into DMC on gel electrophoresis. Aliquots were analyzed by SDS–PAGE experiments in order to detect high molecular weight polymers either by staining with Coomassie blue or by autoradiography.

DAO Activity Assay. The DAO activity was assayed according to Okuyama and Kobayashi (1961) with the modifications of Torrigiani et al. (1989). The reaction medium contained 3 mM $CaCl_2$ and 4.025 mM Pu (1400 cpm nmol^{-1}) in 0.1 M Tris-HCl buffer, pH 7.5, and 200 μL of extract. Incubation was performed at 30 °C for 90 min. [^{14}C]Pyrroline resulting from the oxidation of [^{14}C] Pu was measured. In all cases, the assays were triplicated.

One unit of DAO was defined as the amount of enzyme that catalyzed the oxidation of 1 pmol of Pu min^{-1} at 30 °C.

MDCd Incorporation into DMC. The standard reaction mixture was identical to the Pu incorporation assay. MDCd (1 mM) replaced Pu. Incubation was performed at 30 °C for suitable times. Aliquots were taken out and submitted to SDS–PAGE in order to detect high molecular weight polymers either by staining with Coomassie blue or under UV light (365 nm) in order to reveal the dansyl fluorophore.

Cross-Linking Experiments. The standard reaction mixture contained, in a total volume of 0.4 mL, 0.1 M Tris-HCl buffer (pH 7.5), 10 mg/mL substrate protein except for RuBisCO (2.6 mg/mL), 3 mM $CaCl_2$, and 200 μL of the lupine extract. Incubation was performed at 30 °C. At suitable times, aliquots were taken out and submitted to SDS–PAGE in order to detect the product polymerized through the intermolecular cross-linking catalyzed by TGase.

Polyacrylamide Gel Electrophoresis Analysis. MDCd or [^{14}C] Pu incorporations into DMC and the cross-linkings were analyzed by using vertical slab gels (NOVEX precast gels) in the presence of 1% SDS. The samples were diluted in a 50 mM Tris-HCl buffer (pH 6.8) containing 10% (v/v) glycerol, 2% (w/v) SDS, and 5% (v/v) 2-mercaptoethanol and were heated at boiling water temperature for 3–5 min. SDS–PAGE was performed using the Tris–glycine buffer system described by Laemmli (1970) with 4–20% continuous gradient acrylamide gel. Ten micrograms of the denatured protein was loaded, except for the MDCd incorporation experiment (20 μg).

Protein Estimation. Protein concentration was determined according to the Bradford (1976) method (Bio-Rad protein assay) using bovine serum albumin (Sigma) as the standard.

Table 1. Transglutaminase ([^{14}C]Pu Incorporation into DMC) and DAO Activities of Various Fractions of Lupine Extract Prepared with 20 mM Tris-HCl as Extraction Buffer[a]

fraction	[^{14}C]Pu incorporation (U/g of wet tissue)	DAO activity (U/g of wet tissue)	protein (mg/g of wet tissue)
filtrate	185 ± 12	731 ± 25	2.7
with DIECA	36 ± 10	0	
41400*g* supernatant	82 ± 8	402 ± 49	1.8
with DIECA	7 ± 7	0	
41400*g* pellet	85 ± 12	154 ± 7	0.86
with DIECA	32 ± 11	0	

[a] The filtrate, the 41400*g* supernatant, and the 41400*g* pellet were assayed as described under Materials and Methods.

RESULTS AND DISCUSSION

It is well known that transglutaminases, like guinea pig liver transglutaminase and human factor XIIIa (Ikura et al., 1980; Traoré and Meunier, 1991), are responsible for putrescine incorporation into DMC and protein polymerization. Also, if it is found that an enzymatic activity in purification fractions of lupine seedlings gives rise to these incorporation–polymerization reactions, TGase has to be involved but, as already told, without ruling out a possible secondary activity.

Six-day-old seedlings were grown in the dark, crushed in a 20 mM Tris buffer, and filtered. Incorporation activity was detected in the filtrate (Table 1). But this incorporation of [^{14}C] Pu was not absolutely specific of TGase. Indeed it is known that an enzyme, DAO, is able to catalyze the transformation of Pu into an aldehyde (γ-aminobutyraldehyde). That can be incorporated into dimethylcasein as Schiff base. To check whether the incorporation was due to this secondary reaction, we performed a DAO assay on the filtrate. Indeed DAO activity was found in the filtrate (Table 1).

Legume DAO contains atoms of copper essential for its activity (Smith and Barker, 1988). Diethyldithiocarbamate (DIECA), a copper chelating agent, is a known inhibitor of DAO (Suzuki, 1973). The use of this inhibitor allowed the determination of the cellular localization of DAO and TGase. Results shown in Table 1 indicated that DAO was present in the supernatant (obtained from the filtrate centrifugated at 41400*g*) and that TGase was localized in the pellet. DAO and TGase were contained in the organites. TGase seemed to be bound to membranes while DAO was free. When the seedlings were extracted with 0.25 M sucrose, a condition of intactness of organites, incorporation activity was highest in the pellet (86%). After disruption of the organites with Tris-HCl medium, DAO activity was mainly found in the supernatant. TGase activity in the pellet has already been described for alfalfa leaves (Margosiak et al., 1990). In order to solubilize the TGase, the 41400*g* pellet was extracted by increasing salt concentrations and by the use of detergent. Only the detergent Triton X-100 (from 1% (v/v)) allowed the activity recovery in the supernatant after a centrifugation at 108000*g* for 1 h (Table 2). This experiment

Table 2. Extraction of TGase Activity from the 41400*g* Pellet by Use of NaCl and Triton X-100[a]

	NaCl (mM)[b]				Triton X-100 (% (v/v))[b]		
	0	150	250	500	0.1	1	5
activity recovery (%)	10	9	6	7	5	44	54

[a] Results represent activity recovery (%) from the 41400*g* pellet. [^{14}C] Pu incorporation into DMC in the presence of 1 mM DIECA by the 108000*g* supernatant resulting from extraction by different buffers. [b] Tris extraction buffer (20 mM) different amounts of these compounds as indicated.

Table 3. [^{14}C]Pu Incorporation into Different Proteins by the 41400*g* Pellet

	relative rate incorporation (%)[a]		incorporation recovered (%) + DIECA/−DIECA
substrates	−DIECA	+DIECA (1 mM)	+DIECA (1 mM)
dimethylcasein	100*	100$^+$	59
casein	65	59	53
β-lactoglobulin	106	34	18.5
7S globulins	127	100	50
11S globulins	285	170	35
RuBisCO	78	56	42

[a] 100* = 100$^+$ = 6.9 U mL^{-1} (mg of substrate)$^{-1}$.

confirmed that the TGase contained in the 41400*g* pellet either was an integral membrane protein or was bound to the integral membrane protein. The nature of the membrane has yet to be determined, but it is not one of a microsome (41400*g* for 30 min is not sufficient to sediment microsomes).

The amount of activity in the filtrate was higher in the presence of 1 mM benzamidine (a serine protease inhibitor) (170 U (g of wet tissue of seedling)$^{-1}$) than in its absence (140 U g^{-1}). Specific activity was unchanged (38 U (mg of protein)$^{-1}$). The addition of a competitive thiol protease inhibitor, antipain (1 μg mL^{-1}) with benzamidine during the extraction gave a small decrease of specific activity (30 U mg^{-1}) and of extracted total activity. This suggests that a cysteine residue was involved in the active site of enzyme as it is the case for other TGases. Therefore, experiments were carried out in the presence of benzamidine.

The variety Lublanc incorporated more Pu than Lucky (170 U (g of wet tissue^{-1}) against 53 U g^{-1}). The addition of a phenol chelator, 10% (w/w) polyvinylpyrrolidone (PVP), in the homogenization buffer did not improve either the specific activity or the extracted total activity. The latter result did not depend on the age of lupine (6 or 10 d). The presence of light during the last 2 d before the end of the germination had no pronounced effect on the specific activity (data not shown). It was higher in roots (36 U mg^{-1}) than in leaves or stems (10 U mg^{-1}). But 55% of the extracted total units was found in stems and 41% in roots. It is the main reason why the whole seedlings were crushed in our experiments.

The incorporation was also dependent on the buffer nature and the ionic strength. Tris-HCl buffer was better than sucrose, and 20 mM Tris buffer concentration was the optimum.

Different proteins were used as substrates and amine acceptors for Pu incorporation by enzymes contained in the 41400*g* pellet. Results in Table 3 show that 7S and 11S globulins were the best acceptors. β-Lactoglobulin and DMC were good substrates, with RuBisCO being the poorest substrate. In order to explain the discrepancy between DMC and the whole casein, one can infer that the treatment of casein to obtain DMC unfolded the structure with the exposure of reactive groups (carboxamide) while the more compact structure of native caseins prevented access of these groups to the enzyme. Moreover, this can be explained by a competition between polymerization and incorporation for glutamine residues. Glutamine residues involved in isopeptidic bonds cannot be substrates for Pu incorporation. When Pu incorporation was measured in the presence of DIECA, incorporation corresponded to TGase activity. The TGase specificity for proteins substrates can be estimated. The best substrates for this lupine TGase were soybean proteins (11S and 7S globulins) and DMC, next are the unmodified casein and spinach RuBisCO. In the presence of DIECA, incorporation inhibition was important in the case of β-lactoglobulin. This can be explained by the presence of numerous amino groups on side chains which were substrates for a DAO-mediated incorporation.

DAO activity in lupine seeds interfered with TGase activity when assayed with the incorporation assay. One can wonder whether DAO activity was also responsible for the usual reaction catalyzed by TGase, e.g., polymerization of proteins.

The incorporation of Pu into DMC catalyzed by DAO occurs as the following:

$$NH_2\text{-}(CH_2)_3CH_2\text{-}NH_2 + O_2 + H_2O \xrightarrow{DAO} \underbrace{NH_2\text{-}(CH_2)_3\text{-}CHO}_{\mathbf{R}} + NH_3 + H_2O_2$$

$$R\text{-}CHO + DMC\ (NH_2CO) \rightarrow R\text{-}CH{=}N\text{-}CO\text{-}DMC + H_2O$$

$$R\text{-}CH{=}NCO\text{-}DMC + n(R\text{-}CHO) \rightarrow \text{poly Pu proteins}$$

But since Pu is a low MW polyamine, the MW of the polymerization products (poly Pu proteins) will hardly exceed the MW of DMC, e.g., no HMW polymers will be observed on SDS−PAGE.

The only way to obtain HMW polymers with DAO (which then interfers with TGase) is to suppose that DAO oxidizes both NH_2 groups leading to HMWP by imine bridges:

...P-CO-N=CH-Pu-CH=N-CO-P...

This situation is not likely expected to occur since DAO oxidizes only one NH_2 group of a diamine molecule. However one cannot exclude the presence of the amine oxidase (AO) with the DAO leading to imine bridges. In order to check that TGase is only responsible for polymerization of proteins, we have performed the following experiments.

A mixture of [^{14}C] Pu and DMC submitted to the action of lupine extracts was studied by SDS−PAGE (Figure 1A). The filtrate and especially the 41400*g* pellet gave rise to the formation of HMW polymers that did not enter into the gel. HMW polymer formation was not prevented by the presence of DIECA. The 41400*g* supernatant exhibited no polymerization activity (data not shown). Moreover, the absence of polymerization was confirmed by the use of commercial DAO (Figure 1B). Therefore, as already supposed, no imine bridges were made by DAO or AO. The same results were observed for the incorporation of [^{14}C] Pu into casein and RuBisCO (Figure 1A). In both cases, HMW polymers were formed. These polymers were built via Pu

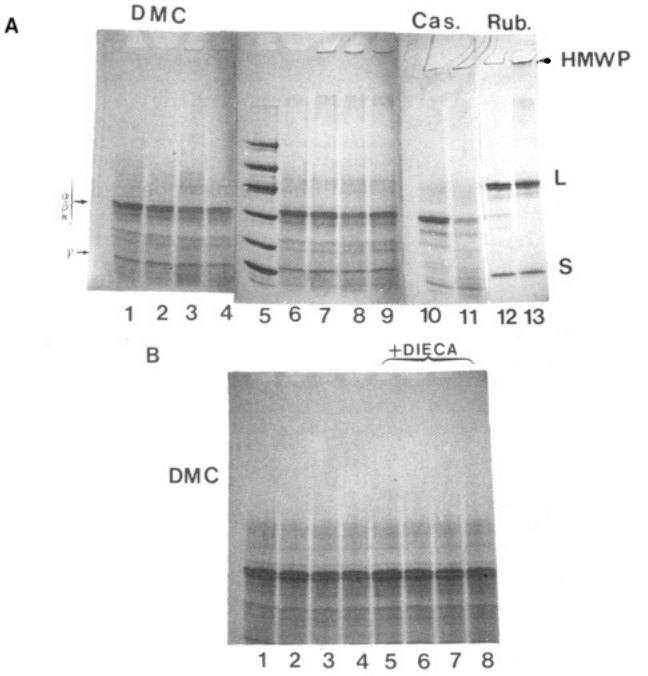

Figure 1. [^{14}C] Pu incorporation into dimethylcasein, casein, and RuBisCO by lupine extracts and by DAO. The mixture [^{14}C] Pu and proteins submitted to the action of lupine extracts was studied by SDS–PAGE. (A) Effect of time and DIECA on the action of the filtrate with respect to DMC. Lanes 1–3: 0, 9, and 20 h; lane 4: 20 h in the presence of 1 mM DIECA. Lane 5: protein markers; from the top: 94, 67, 43, 30, 20.1, and 14.4 kDa. Effect of time and DIECA on the action of the pellet with respect to DMC. Lanes 6–8: 0, 9, and 20 h; lane 9: 20 h in the presence of 1 mM DIECA. Effect of time on the action of the pellet with respect to casein (lanes 10 and 11: 0 and 20 h) and to RuBisCO (lanes 12 and 13: 0 and 20 h). (B) Effect of time and DIECA on the action of commercial DAO (0.5 mg/mL) with respect to DMC. Lanes 1–4: 0, 8, 15, and 22 h; lanes 5–8: in the presence of 1 mM DIECA (for the same time intervals as lanes 1–4, respectively).

bridges between proteins molecules. On autoradiograms, the label appeared at the top of the wells corresponding to the Coomassie stained bands, confirming the formation of HMW polymers (data not shown).

These results were confirmed by the polymerization of casein by the 41400*g* pellet (Figure 2). Under these conditions, (D)AO cannot be involved because these experiments were carried out without polyamine. The DTT slowed down the rate of the polymerization of casein, and the Ca^{2+} increased it.

The 41400*g* pellet also polymerized DMC (Figure 3). The latter result proves that all lysyl NH_2 groups are not methylated in the DMC. RuBisCO was not a good substrate for the TGase contained in the 41400*g* pellet. Some polymers were observed at 65 and 94 kDa, but no additional HMW polymers were seen with respect to the control (Figure 3). Indeed, the HMW polymers observed were due to the insoluble fraction of RuBisCO. β-Lactoglobulin and 7S soybean globulins were also substrates for polymerization (Figure 4). It was noted that α and α' subunits of 7S globulin were preferentially used. The efficiency with which a protein can be used as a substrate by transglutaminase is known to be influenced by the amino acid sequence around the reactive glutamyl residues (Gorman and Folk, 1980). Thus, the primary structure of a protein is of greater importance in assessing its ability to act as a substrate than its absolute lysine and glutamine content. That may be the reason why lupine TGase acts differently according to the substrate.

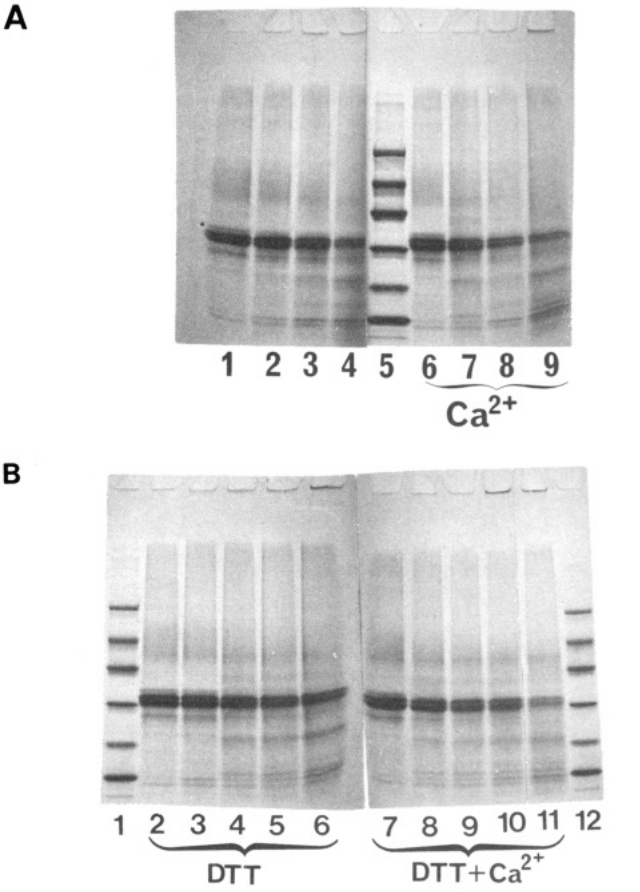

Figure 2. SDS–PAGE of casein subjected to the lupine pellet (10 mg/mL of casein). Effect of $CaCl_2$ and of DTT. (A) Effect of $CaCl_2$. Lanes 1–4: 0, 3, 9, and 21 h, pellet alone; lanes 6–9: 0, 3, 9, and 21 h, pellet with 3 mM $CaCl_2$. Lane 5: protein markers; from the top: 94, 67, 43, 30, 20.1, and 14.4 kDa. (B) Effect of DTT. Lanes 2–6: 0, 3, 9, 21, and 48 h, pellet with 10 mM DTT; lanes 7–11: 0, 3, 9, 21, and 48 h, pellet with 3 mM $CaCl_2$ and 10 mM DTT. Lanes 1 and 12: protein markers; from the top: 94, 67, 43, 30, 20.1, and 14.4 kDa.

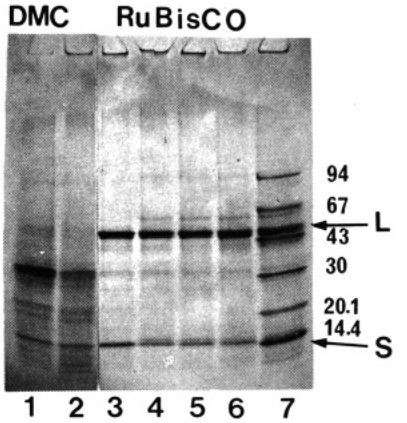

Figure 3. SDS–PAGE of DMC and spinach RuBisCO subjected to the pellet fraction of lupine. Lanes 1 and 2: 10 mg/mL DMC; 0 and 21 h. Lanes 3–6: 2.6 mg/mL of spinach RuBisCO; 0, 9, 21, and 48 h. Lane 7: protein markers, from the top: 94, 67, 43, 30, 20.1, and 14.4 kDa and RuBisCO (L subunit, 54 kDa, and S subunit, 15 kDa).

The spectrum of substrate specifity of lupine TGase is different from one of the human placental factor XIIIa, an animal TGase. Globulins were better substrates than caseins with respect to lupine TGase (Table 3), when any significant difference of specificity between both substrates was observed for factor XIIIa (Traoré and Meunier, 1991; Siepaïo and Meunier, 1995). The

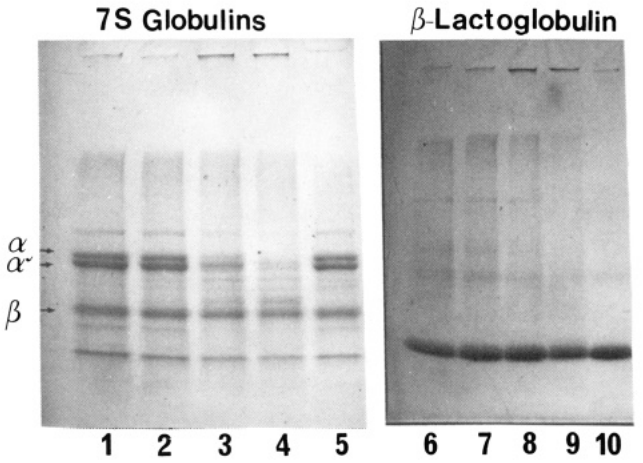

Figure 4. SDS–PAGE of 7S soybean globulins and β-lactoglobulin subjected to the pellet fraction of lupine. Lanes 1–4: 10 mg/mL 7S globulins; 0, 1, 10, and 20 h; lane 5: 10 mg/mL 7S globulins in the absence of pellet; 20 h. Lanes 6–9: 10 mg/mL β-lactoglobulin: 0, 1, 10, and 20 h; lane 10: 10 mg/mL β-lactoglobulin in the absence of pellet; 20 h.

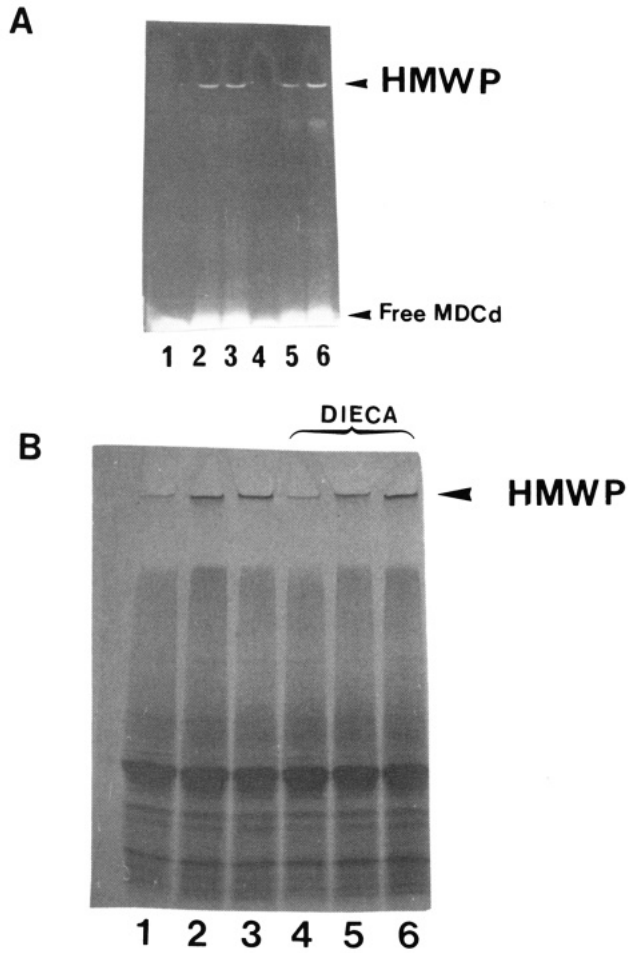

Figure 5. MDCd incorporation into DMC by lupine pellet as visualized by the fluorescence emission of MDCd and the polymerization by gel staining. Effect of time and DIECA on the action of the pellet. Lanes 1–3: 0, 10, and 20 h, respectively; lanes 4–6: 0, 10, and 20 h, respectively, in the presence of 1 mM DIECA. Gel under UV on A and gel staining by Coomassie blue on B.

specificity of guinea pig liver TGase was still different (Ikura et al., 1980). So it is possible to perform polymerization of a great lot of proteins by choosing the right TGase.

The incorporation of MDCd into DMC by filtrate and especially by pellet (Figure 5) to give HMWP was supported by TGase implication. The appearance of HMW polymers could be observed either by Coomassie blue staining or under UV (Figure 5B,A, respectively). In this latter case, a fluorescent spot could be seen on top of the wells where the blue stains appeared. Even if DAO was active against derivatives of diamines, polymerization was observed in the presence of DIECA, e.g., the TGase which was involved. This polymerization occurred because all the NH_2-Lys of DMC were not methylated or because all the cadaverine molecules were not dansylated. If all casein Lys were methylated or all the cadaverine molecules were dansylated, polydansyl casein molecules of low MW could be obtained.

In conclusion, there is a DAO activity in the filtrate and in the supernatant (a contamination in the pellet) and a TGase activity in the filtrate and in the pellet. It is clear that the polymerization activity of the pellet was due to TGase and not to DAO contaminating the pellet. But in order to follow the TGase activity during the purification, the [^{14}C] Pu assay can be used only in the absence of DAO activity or with a blank in the presence of DIECA. Moreover, the substrate specificity of this new TGase is different from one of the other sources.

ABBREVIATIONS USED

DIECA, diethyldithiocarbamic acid sodium salt; DMC, dimethylcasein; MDCd, monodansylcadaverine; DTT, dithiothreitol; HMWP, high molecular weight polymers; EDTA, ethylenediaminetetraacetic acid tetrasodium salt; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; TGase, transglutaminase; DAO, diamine oxidase; AO, amine oxidase; RuBisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; TCA, trichloroacetic acid; Pu, putrescine; Ci, Curie.

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