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PARTITION OF CATALASE AND ITS PEROXIDASE ACTIVITIES IN HUMAN RED CELL MEMBRANE

EFFECT OF ATP DEPLETION

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Summary

Partititon of catalase (hydrogen-peroxide:hydrogen-peroxide oxidoreductase EC 1.11.1.6) and peroxidase (donor:hydrogen-peroxide oxidoreductase EC 1.11.1.7) activities between the red cell membrane and the cytosol were studied under various experimental conditions. A small but significant amount of catalase (1.6%) was retained on human red cell membranes prepared by hemolysing washed red cells with 30 volumes of 10 mM Tris buffer, pH 7.4. Membrane-bound catalase had a relatively higher peroxidase activity than the soluble enzyme fraction. Polyacrylamide gel electrophoresis in sodium dodecyl sulfate of the solubilized membranes demonstrated catalase to be a single band with a molecular weight of 60 000.

Membranes prepared from adenosine triphosphate-depleted red cells depicted a two to three-fold increase in catalase activity, as well as an increase in 60 000 molecular weight band on polyacrylamide gel electrophoresis. The extra amount of retained catalase was a less efficient peroxidase than found in fresh membranes. The binding of catalase to ATP-depleted red cell membranes was dependent upon both pH and hemolysing ratio. Red cells incubated at pH 7.1 demonstrated a decrease in bound catalase, as did membranes prepared from red cells hemolysed at 1:100 dilution. β-Mercaptoethanol decreased the catalase activity in the membranes and increased the *o*-dianisidine peroxidase activity without any significant effect on the 60 000-dalton band.

Introduction

We have demonstrated that 3-(3,4-dihydroxyphenyl)-L-alanine (L-DOPA) reduced the formation of acetylphenylhydrazine-induced Heinz bodies in human glucose-6-phosphate dehydrogenase (EC 1.11.49) deficient red blood cells [1]. The mechanism of action was though to be mediated through the peroxidase activity of catalase with L-DOPA acting as an enzyme substrate. Subsequently, L-DOPA peroxidase activity was isolated from a human hemoglobin-free red cell lysate and was found to co-purify with catalase [2].

Heinz bodies may result from oxidative denaturation of hemoglobin and attach themselves to the red cell membrane [3]. Therefore, biochemical reactions protecting both the membrane and cytosol against oxidation are worth investigating. The peroxidase activity of catalase is likely to be more responsible for removal of low concentrations of peroxidase in the cell [4,5]. Thus, the peroxidase activity of catalase is probably the true function as was initially proposed by Keilin and Hartree [4]. Therefore, the present investigation was undertaken to study the partition of catalase between the soluble and membrane fractions and to determine the ratio of catalase and the peroxidase activity of catalase existing in the various compartments from fresh red cell membranes.

Since in vitro incubations of erythrocytes in a glucose-free medium may mimic the oxidative stress to which the cells are subjected to in vivo [6], adenosine triphosphate (ATP) depleted human erythrocyte membranes were also investigated for the partioning and altering of catalase.

Materials and Methods

Red cell isolation

Fresh whole blood was collected in heparinized containers from normal donors. The red blood cells were separated by centrifgutaion and both plasma and buffy coat were removed by aspiration. The cells were washed 3 times with tris · HCl buffer, pH 7.4, 10 mM containing NaCl, 150 mM.

Membrane preparation

Red cell membranes were prepared by hemolysing washed red cells with 10, 30 or 100 volumes of 10 mM Tris, pH 7.4, at 4°C. Subsequently, the membranes were washed 3 times with the same buffer at 4°C. Washed membranes were then counted as ghosts in a Coulter counter, Model S [7], and 0.1 ml was dissolved in 0.05 ml of 0.2% Triton X-100, diluted 1 : 10 with 10 mM Tris, pH 7.4, and immediately assayed for enzyme activity as described below. Hemoglobin content of ghosts was determined by the modified benzidine method [8], and expressed as mg of hemoglobin/10^{10} ghosts.

Enzyme assays

Catalase was measured by the spectrophotometric method of Lück [9] The incubation mixture contained a final volume of 3.0 ml, 12.5 mM hydrogen peroxide, 60 mM potassium phosphate buffer, pH 7.0. One unit of enzyme

cleaves 1 μmol of substrate per min at 26°C at 240 nm. DOPA peroxidase activity was determined employing the rate of change of absorbance extinction at 330 nm for DOPAchrome [10]. The incubation mixture, total volume of 1.0 ml, contained 1 mM of sodium salt of L-DOPA, 100 mM potassium phosphate buffer, pH 7.4, 8 mM H_2O_2 incubated at 25°C, and the solubilized membrane solution. The increase of absorbance at 330 nm was recorded in a Beckman Kintrac spectrophotometer with an automatic recording device for a period of 5 min at 25°C. One unit of DOPA peroxidase was equivalent to 1 μmol of substrate used per min at 25°C, calculated and compared from a standard curve of L-DOPA incubated with horseradish peroxidase containing an equivalent amount of Triton X-100 and H_2O_2.

O-Dianisidine peroxidase activity in the red cell ghosts was measured according to a modification of the method of Dvorak et al. [11] by following the oxidation of *o*-dianisidine in the presence of H_2O_2. The incubation mixture, total volume of 1.0 ml, contained 1 mM of *o*-dianisidine, 10 mM sodium phosphate buffer, pH 7.3, and hydrogen peroxide, 8.0 mM. The rate of change of absorbance coefficient at 460 nm was determined with various concentrations of H_2O_2 and Triton, and compared with values obtained using a standard horseradish peroxidase. Because of the difficulty in maintaining H_2O_2 levels during the peroxidase assay, the hemoglobin-free enzyme lysate and ghost preparations required dilutions to keep the catalase background level the same. Additionally, a concentration of 8 mM H_2O_2 was necessary in order to obtain maximum peroxidase activity.

Membrane enzyme elution

Catalase was eluted from fresh and adenosine triphosphate-depleted erythrocyte membranes according to a modification of the method of Kant and Steck [12]. 0.5 ml of membranes were added to an equal volume of a 10 mM Na phosphate buffer, pH 8.0, with varying concentrations of sodium chloride, and kept on ice for 60 min. Following centrifugation at 20 000 rev./min for 10 min in a Sorvall SS34 rotor, the supernatant solution was collected and assayed for catalase and peroxidase activity. The remaining ghost pellet was solubilized with Triton X-100 and assayed for residual enzyme activity.

Enzyme inhibition

The solubilized ghosts were incubated with two known catalase inhibitors, 3 amino-1,2,4-triazole [13], and 1 mM sodium azide [14], at 37°C for 10 min and then re-assayed for catalase, peroxidase and DOPA peroxidase activities in the continued presence of the inhibitor.

Hemoglobin-free red cell lysate

Hemoglobin-free red cell lysates were prepared according to the method of Hennessey et al. [15] and were assayed for catalase, *o*-dianisidine peroxidase and DOPA peroxidase activities and hemoglobin content.

In vitro aging-ATP depleted erythrocytes

Fresh washed red cells were suspended in a siliconized glass flask in a buffer containing glycylglycine, 50 mM, KCl 5 mM, and $CaCl_2$ 2 mM, and 117 mM

NaCl. The pH of the buffer was adjusted to 7.4 and the final osmolarity was 290 mosM. The hematocrit was 10% and the flasks were incubated at 37°C for 24 h in a shaking water bath at 100 oxcillations per min. Sterile glassware was used and penicillin, 100 units/ml, and streptomycin, 0.1 mg/ml, were added to the incubation flasks. The results, however, were not significantly different with or without antibiotics. The pH was adjusted with either 0.1 M isotonic HCl or NaOH at 6 and 10 h of incubation. After the incubation was completed, an aliquot of the cell suspension was removed, deproteinized with perchloric acid, and ATP was measured [16] and found to be less than 0.1% of the original cell ATP level.

The amount of hemolysis was quantitated by determining heomglobin concentration [8] in the supernatant, which was always less than 1% of the original hemoglobin concentration. The final pH was determined and the cells were washed 3 times in a 10 mM Tris-buffered saline, pH 7.4 or 7.1. In certain incubations β-mercaptoethanol, 0.5 mM or 1.0 mM, was added at the start of the experiment.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis

The discontinuous gel system described by Laemmli [17] was employed in cylindrical gel electrophoresis. The "stacking" (0.5 × 1.0 cm) and the "separating" (0.6 × 8.0 cm) gels contained 2.67% and 8% acrylamide respectively, in 0.1% sodium dodecyl sulfate.

The electrophoresis buffer was composed of 0.05 mM Tris, 0.38 M glycine, 0.002 M EDTA, and the pH was asjusted to 8.3. The membrane sample was dissolved in 1% sodium dodecyl sulfate with 0.025 M dithiothreitol, 0.05 M Tris, 0.25 M sucrose, pH 7.5, and was stored frozen at −40°C. This was subsequently heated to 100°C for 1 or 1.5 min just prior to gel electrophoresis. A typical electrophoresis was carried out at 1 mA per gel for about 7 h. After electrophoresis, gels were stained and destained according to Fairbanks et al. [18] except that Coomassie Blue was not introduced into the destaining solution. The gels were scanned using a densitometer (Quick Scan Jr., Helena Co.) at 545 mμ. The numbering of major protein bands followed the method of Fairbanks et al. [18] except the region between band 3 and 5 including band 4.5 [19]. In addition, the identification of major protein banks was checked by specific elutions of ghost proteins with low ionic strength medium and isotonic salt solutions [18].

Standard polyacrylamide gel electrophoresis without sodium dodecyl sulfate

Ghost preparations were dissolved in 1% Triton X-100 and 20 μg protein was used for analytical polyacrylamide gel electrophoresis as described by Davis [20]. The cathode chamber contained Tris/glycine, 52 mM, pH 8.9, and the anode chamber contained Tris · HCl, 100 mM, pH 8.2. Acrylamide concentration of the spacer gels was 2.5 % and that of the resolving gel was 7.5%. Where specified, the electrophoresis was carried out in a single buffer system using Tris · glycine, 52 mM, pH 8.3, without a spacer gel. In all experiments, a 2 mA current per gel was applied for 2.5 h. Gels were stained in the dark for DOPA peroxidase by incubating in L-DOPA solution 1 mg/ml in 0.1 M potassium phosphate buffer [2]. DOPA peroxidase appeared as a solid dark staining band.

Catalase was stained on gels according to the method of Gregory and Fridovich [21] and appeared as an achromatic band flanked by dark brown peroxidase bands. Unstained gels were subjected to second dimensional gel electrophoresis in the presence of sodium dodecyl sulfate according to the method of Liu, S.C., Fairbanks, G. and Palek, J. (unpublished) in order to define whether the catalase and DOPA peroxidase bands were a single protein and to determine more accurately the molecular weight.

Two-dimensional gel electrophoresis

The first dimensional gel was transferred to the top of a 6 mm thick slab of a discontinuous gel system of Laemmli [17] and sealed with Agarose solution containing 1% sodium dodecyl sulfate and 0.04 M dithiothreitol. Electrophoresis was carried out at about 20 mA per slab for 18 h. Procedures for the staining and destaining were similar to those used in the one-dimensional electrophoresis [18].

Results

Membrane partition

Catalase activity in the original hemolysate was 45 125 units (Table I). The recovery of catalase activity through DEAE cellulose was over 95% with removal of over 99% of the original hemoglobin. A small amount of catalase activity was retained in the original membrane despite repeated washing as others have observed [22].

The high concentration of hemoglobin present in the original hemolysate made it difficult to measure accurately any peroxidase activity. The peroxidase activity in the DEAE-treated hemolysate was 224 and 145 units for *o*-dianisidine and DOPA peroxidase, respectively (Table I). The concentration of H_2O_2 in the assay was increased until maximum rates were observed because of the high catalase activity.

The membrane also contained peroxidase activity that remained in the membrane after exhaustive washing. The peroxidase activity of the membrane

TABLE I

CATALASE AND PEROXIDASE ACTIVITY IN ERYTHROCYTES AND MEMBRANES

Ghosts were prepared from fresh erythrocytes and dissolved in Triton (as described in Materials and Methods). Units are expressed as μmol/min per $1 \cdot 10^{10}$ erythrocytes from hemoglobin-free lysate or from $1 \cdot 10^{10}$ ghosts ± 1 S.D. Samples were obtained from 4 different experiments.

	Hemoglobin (mg/10^{10} cells or ghosts)	Enzyme activity (units)		
		Catalase	*o*-Dianisidine peroxidase	DOPA peroxidase
Packed erythrocytes $1 \cdot 10^{10}$	330 ± 35	45.125 ± 1500	— *	— *
Hemoglobin-free hemolysate	0.67 ± 0.09	43.300 ± 2000	224 ± 12	145 ± 19
Fresh ghosts $1 \cdot 10^{10}$	0.08 ± 0.01	725 ± 150	51.4 ± 12	38 ± 4
+ 50 mM amino 1,2,4-triazole	—	0	12 ± 6	8 ± 3
+ 1 mM sodium azide	—	0	10 ± 6	12 ± 5

* Only hemoglobin-free lysates were assayed for peroxidase because hemoglobin interfered.

relative to the catalase activity was much higher than in the DEAE-treated hemolysate.

The increased peroxidase to catalase ratio in the membrane suggests an altered membrane enzyme or additional peroxidase present in the membrane. The ratio of *o*-dianisidine peroxidase to DOPA peroxidase activity in the hemolysate and membrane was similar suggesting a common enzyme specificity.

When fresh ghosts were exposed to various salt concentrations, 0.02 M to 0.08 M sodium chloride, in an attempt to determine a binding constant, two-thirds of the catalase, peroxidase and DOPA peroxidase were solubilized. About 310 units of catalase, 17 units of *o*-dianisidine peroxidase, and 13 units of DOPA peroxidase remained firmly bound to the membranes, thus failing to demonstrate a selective salt elution profile.

Amino 1,2,4-triazole and sodium azide completely inhibited ghost catalase whereas *o*-diansidine and DOPA peroxidase activities were inhibited 70—80%.

ATP depletion studies

Membrane-bound catalase and peroxidase were investigated in cells undergoing the stress of ATP depletion. Incubation of ATP-depleted red cells for 24 h at pH 7.45, resulted in about 2.5-fold increase in the retention of catalase in the ghosts (Table II). However, a commensurate increase in *o*-dianisidine peroxidase or DOPA peroxidase activity was not observed.

The addition of 2 mM $CaCl_2$ to the incubation media, pH 7.45, during ATP depletion was associated with an increase in the hemoglobin retention in the ghost preparation without an additional increase in catalase or peroxidase enzymes. Lowering of the pH to 7.1 during the incubation of red cells decreased membrane-bound catalase to 964 units (Table II). An even greater effect on catalase binding to ghosts was noted with 1.0 M β-mercaptoethanol and 2 mM $CaCl_2$ which resulted in a significant increase in the retention of hemoglobin and *o*-dianisidine activities in the ghosts. When ATP-depleted red cells were hemolysed with varying ratios of the hemolysing solution to erythrocytes, the

TABLE II

CATALASE AND PEROXIDASE ACTIVITY IN ATP-DEPLETED HUMAN RED CELL GHOSTS $1 \cdot 10^{10}$

Ghosts were prepared from ATP-depleted erythrocytes (as described in Materials and Methods) and dissolved in Triton X-100 and assayed for enzyme activity. ATP levels were 0.1% of the original ATP. Units are expressed as μmol substrate per min per $1 \cdot 10^{10}$ ghosts ± 1 S.D. based on separate assays from 4 experiments.

Ca (2 mM)	pH	Mercapto-ethanol (mM)	Hemoglobin (mg/$1 \cdot 10^{10}$ ghosts)	Enzyme activity (units)		
				Catalase	*o*-Dianisidine peroxidase	DOPA peroxidase
+	7.45	—	0.32 ± 0.06	1935 ± 100	62 ± 6	38 ± 4
—	7.45	—	0.13 ± 0.08	1925 ± 115	59 ± 7	—
+	7.10	—	0.24 ± 0.15	964 ± 175	56 †	—
+	7.45	0.5	0.32 ± 0.18	1100 ± 150	158 †	—
+	7.45	1.0	1.42 ± 0.14	220 ± 50	430 ± 140	—

† Based on two separate assays.

TABLE III

EFFECT OF THE CHANGE IN THE RATIO OF THE ERYTHROCYTES TO HEMOLYSING SOLUTION ON THE BINDING OF CATALASE TO GHOSTS PREPARED FROM ATP-DEPLETED ERYTHROCYTES

The ghosts were prepared from 24-h ATP-depleted red cells incubated at 7.45 pH, 2 mM Ca^{2+}. Units are expressed as μmol (substrate) per min per $1 \cdot 10^{10}$ ghosts.

Hemolysis ratio	Hemoglobin mg/ghosts M 10^{10}	Catalase units	PAGE * SDS band 4.5/3 ratio
1 : 10	0.99	3230	0.231
1 : 30	0.33	1960	0.137
1 : 100	0.08	1120	0.106

* Polyacrylamide gel electrophoresis with SDS internal standardization ratio between band 4.5 to band 3.

retention of catalase in the ghosts was dependent upon the initial volume of the hemolysing solution (Table III). A 1 : 10 ratio of erythrocytes to hemolysing solution resulted in the maximum catalase retention (3230 units) by the ghosts, while a 1 : 100 ratio resulted in the lowest retention of catalase (1120 units).

Polyacrylamide gel electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis electrophore-

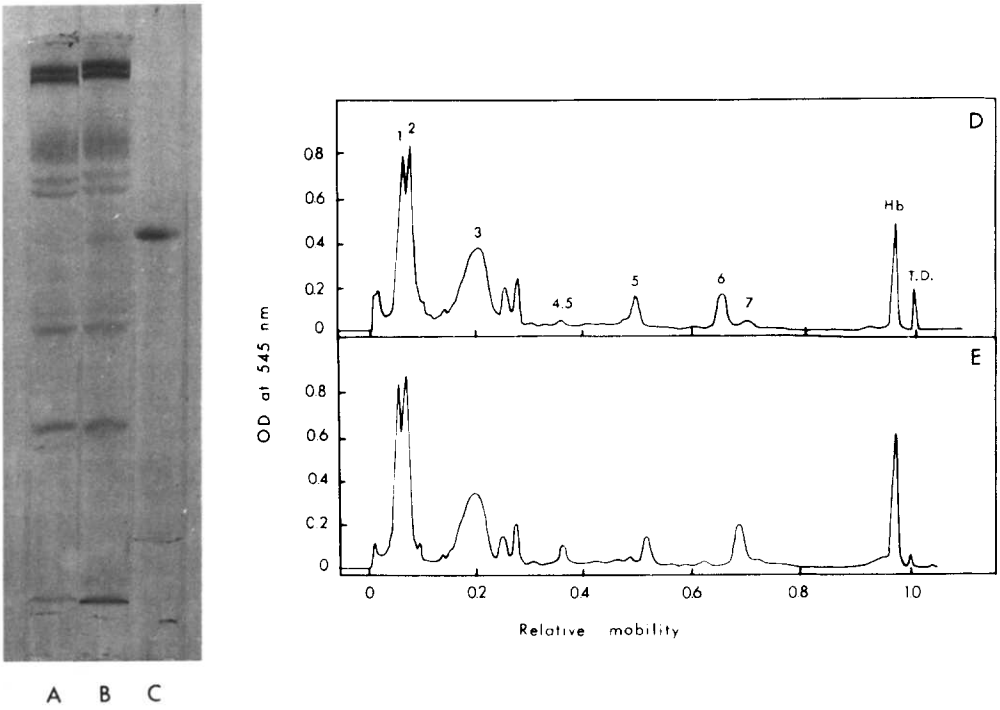

Fig. 1. SDS gel electrophoretograms and densitometric scans of ghosts isolated from fresh and ATP-depleted erythrocytes. Discontinuous gel system of Laemmli [19] was employed with "stacking" and "separation" gels containing acrylamide 2.67% and 8% respectively. (A), Fresh ghosts (25 μg); (B), ATP-depleted ghosts (25 μg); (C), beef liver catalase (2 μg); (D), scan of gel (A); (E) scan of gel (B).

togram and densitometric scans of ghosts isolated from the fresh and ATP-depleted red cells (Fig. 1) indicated an increased retention of (a) band 4.5 [19] (M_1 60 000) and (b) hemglobin, in ATP-depleted ghosts; beef liver catalase (Fig. 1c) appeared to have the same mobility as band 4.5.

When ATP-depleted, Triton-dissolved membranes were subjected to polyacrylamide gel electrophoresis without sodium dodecyl sulfate, and stained for DOPA peroxidase and catalase, identical migrating achromatic bands of catalase and dark staining bands of DOPA peroxidase were demonstrated (Fig. 2, a and b). Furthermore, when unstained Triton acrylamide gels were further subjected to second dimensional slab gel electrophoresis in the presence of sodium dodecyl sulfate, which separated protein according to size (apparent molecular weight), a single protein of 60 000 daltons (band 4.5) appeared on the slab migrating correspondingly under the band stained in the first dimension for catalase and DOPA peroxidase activity (Fig. 2c). Additionally, Fig. 3 demonstrated

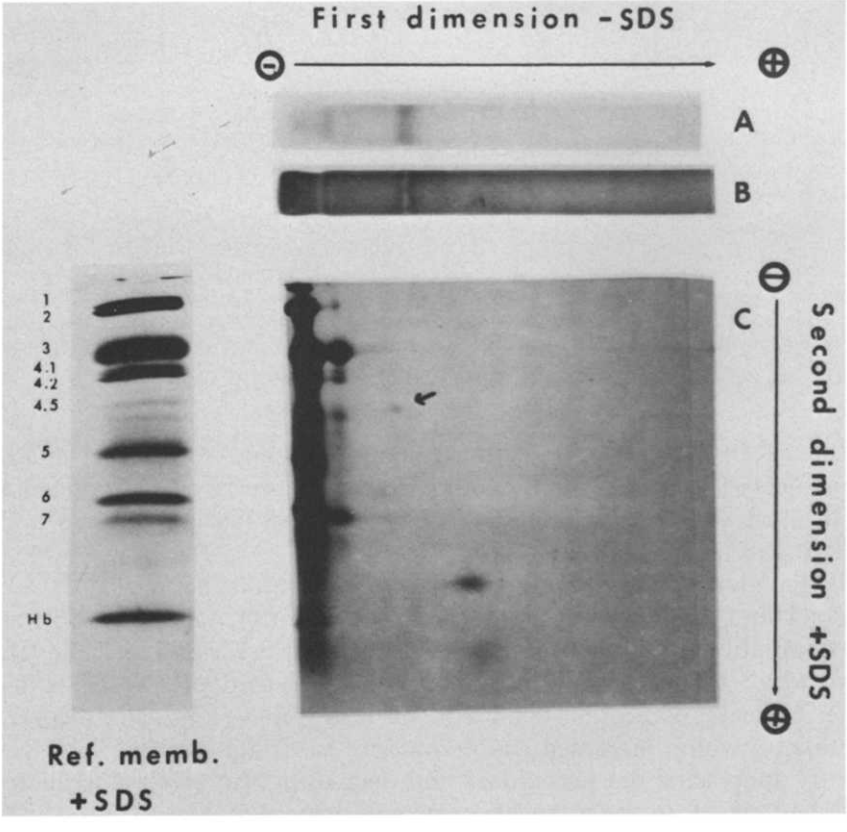

Fig. 2. Two-dimensional gel electrophoretogram of ghosts isolated from ATP-depleted erythrocytes. Membrane sample (20 μg) treated with 1% Triton X-100, subjected to first dimensional polyacrylamide gel electrophoresis without SDS, and then followed a second dimensional slab gel electrophoresis with 0.1% SDS. Parallel gels of first dimensional electrophoresis were stained for (A), DOPA peroxidase, dark band; (B), catalase and peroxidase manifested by achromatic area flanked by dark areas; (C), slab gel stained with Coomassie Blue band 4.5 lines up with achromatic area of catalase (arrow depicting band 4.5).

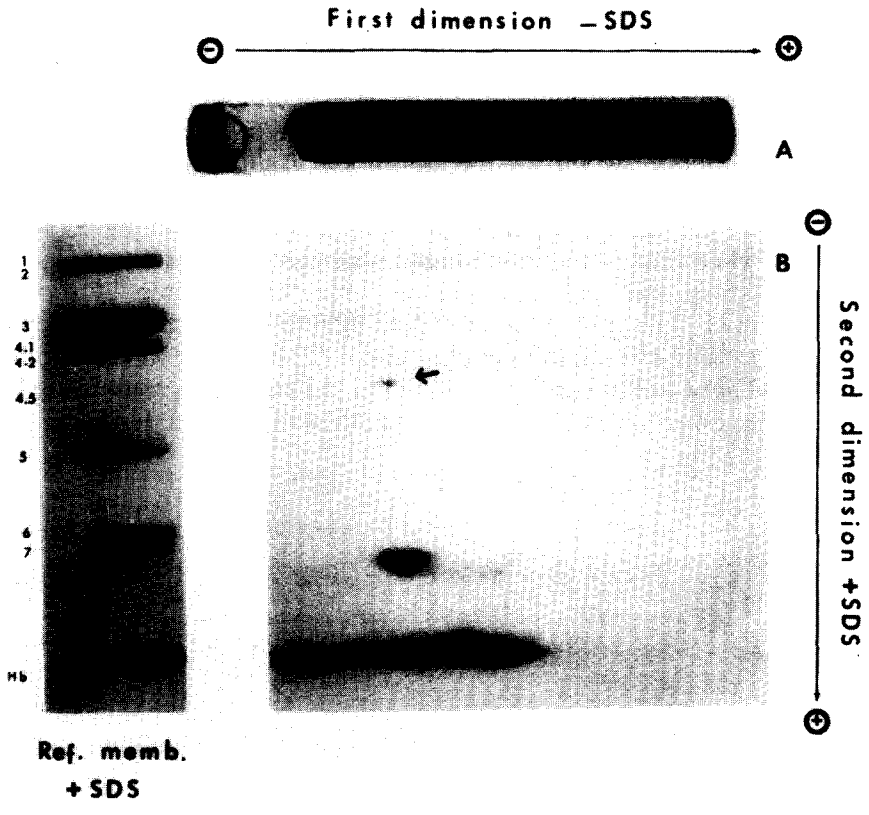

Fig. 3. Two-dimensional gel electrophoretogram of fresh erythrocyte. Packed erythrocyte, 5 λ, treated with 1% Triton X-100 were subjected to electrophoresis similar to that in Fig. 3. (A), catalase stain; (B), slab gel stained with Coomassie Blue. (Arrow depicting band 4.5).

that when 0.5 ml of washed, fresh, intact packed red cells, solubilized in 1% Triton and subjected to two-dimensional gel electrophoresis also produced a single protein spot of 60 000 daltons on the slab, representing catalase and peroxidase activities in the red cells.

When ATP-depleted red cells were lysed at various hemolsying ratios, 1 : 10, 1 : 30 and 1 : 100, (Fig. 4), a decrease in the intensity of protein band 4.5 was noted to be compatible to enzyme activity measurement described in Table III. Furthermore, when red cells were incubated at pH 7.1 to deplete ATP, a decrease in the intensity of protein band 4.5 was also noted (Fig. 4). However, β-mercaptoethanol, which increased the peroxidase to catalase ratio (Table II) by significantly increasing the peroxidase and decreasing the catalase activity, had no significant effect on the intensity of protein band 4.5 (Fig. 4).

Discussion

Decomposition of H_2O_2 in red cells is carried out mainly through the enzymes, catalase and glutathione peroxidase, which are considered to be the

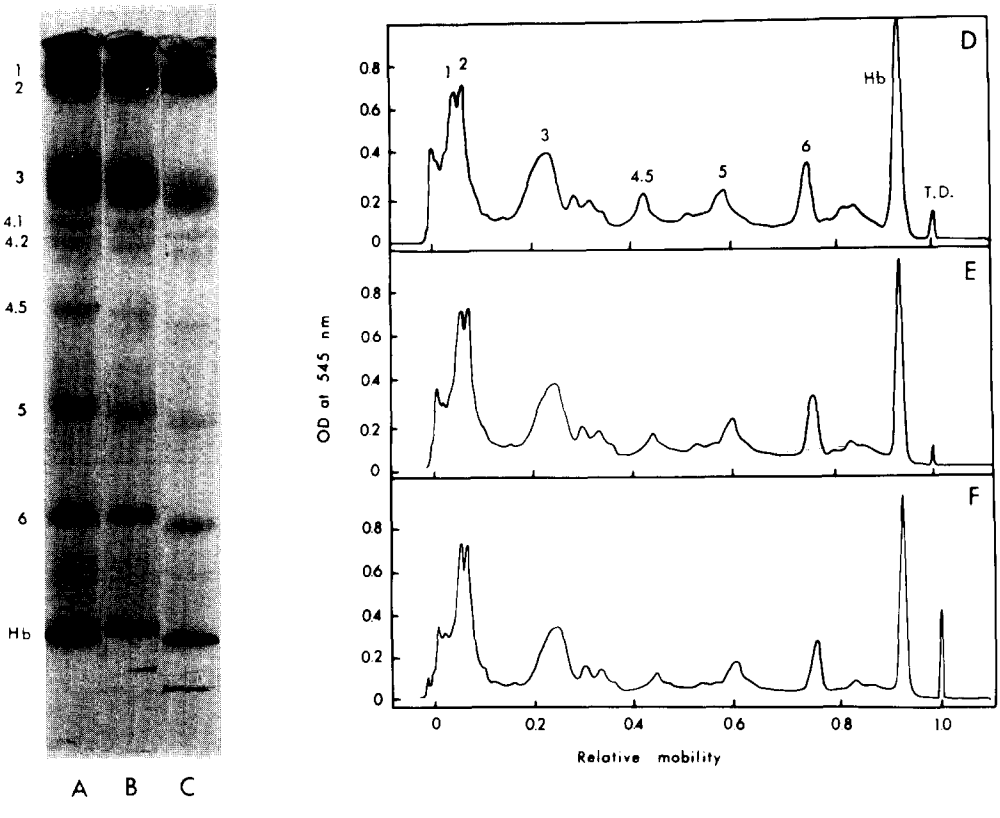

Fig. 4. SDS gel electrophoretograms and densitometric scans of ATP-depleted erythrocyte ghosts prepared with different lysis conditions. Washed cells were hemolysed at 0—4°C with volume ratio of packed cells to buffer: (A), 1 : 10; (B), 1 : 30; (C), 1 : 100; (D), scan of A; (E), scan of B; (F), scan of C.

main defense mechanism against oxidative damage [23,24,5]. Compartmentalization of these enzymes between the membrane and the soluble fraction of the red cell, under varying experimental conditions, has however, never been performed.

Catalase exhibits DOPA peroxidase activity [2] as both enzyme activities copurify and have identical chromatographic and electrophoretic properties.

In the present report, we have confirmed that only a very small percentage (1.6%) of the total catalase is bound to erythrocyte ghosts. Furthermore, the peroxidase to catalase activity ratio of the membrane bound enzyme is about 16 times higher than in the major soluble fraction. Triton X-100-solubilized membranes, subjected to polyacrylamide gel electrophoresis, demonstrated a single band with catalase activity which stained for both peroxidase and DOPA peroxidase activities (Fig. 2).

When unstained gels from Triton-solubilized membranes were subjected to two-dimensional sodium dodecyl sulfate slab gel electrophoresis, a single protein band with a molecular weight of 60 000 (band 4.5) was observed similar to that witnessed with commercial beef liver catalase, indicating a common molecular weight monomer, making the possibility of additional non-specific perox-

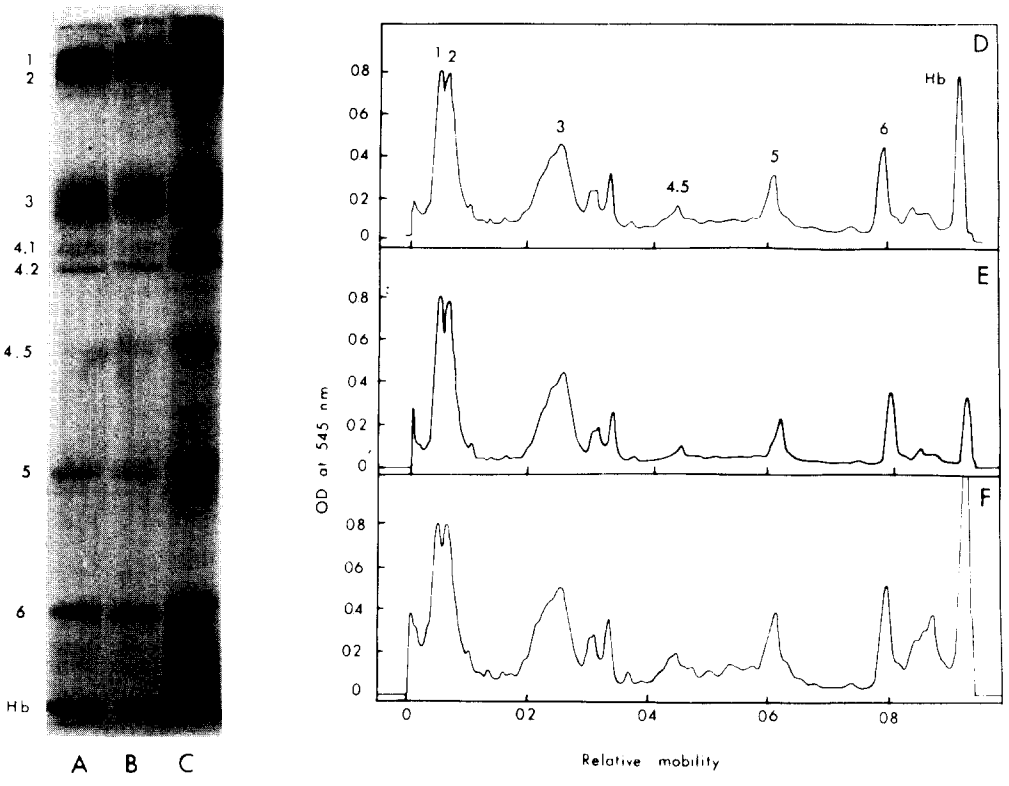

Fig. 5. SDS gel electrophoretograms and densitometric scan of ghosts isolated from ATP-depleted erythtocytes incubated in isotonic buffer at: (A), pH 7.4; (B), pH 7.1; (C), pH 7.4 with 0.5 mM mercaptoethanol.

idase activity less likely (Fig. 3c 1). In other words, catalase and DOPA peroxidase activity are derived from the same protein as judged from identical mobilities in two different electrophoresis systems; Triton polyacrylamide gel electrophoresis which fractionated proteins according to their size and charge, and sodium dodecyl sulfate polyacrylamide gel electrophoresis which separated according to the molecular weight. Finally, the inhibition of catalase, DOPA peroxidase, and *o*-dianisidine peroxidase by amino 1,2,4-triazole (Table II) indicated that catalase was probably responsible for both L-DOPA and *o*-dinisidine peroxidase activity of catalase.

Several membrane-bound enzymes have been shown to have different functional properties from the isoenzymes identified in the soluble fractions [25]. Since we have already demonstrated that catalase and DOPA peroxidase activities are exhibited by the same protein [2], the higher peroxidase to catalase ratio in the membrane-bound species of catalase would indicate that the soluble and the bound form of the protein have different catalactic and peroxidase activities.

It is well documented that catalase has a molecular weight of approximately 240 000 and is a tetramer of identical subunits. During purification, it is very sensitive to oxidative dissociation to dimer and monomer forms having the molecular weights of 120 000 and 60 000 respectively [26]. Aebi [27] has demon-

strated that the monomeric form loses its catalase activity. Thus, the possibility exists that the membrane bound from of catalase is more likely the monomeric form. However, this study could not determine whether the membrane-bound enzyme has altered kinetic properties or is a dissociated monomer form.

There are several plausible explanations for the increased retention of the high molecular weight (240 000) catalase to the membrane of the ATP-depleted red cells. There is evidence that ghosts from ATP-depleted red cells undergo membrane rearrangement [28] with an increased binding of cytoplasmic components to the membrane's inner surface [7,29]. In addition, ATP-depleted ghosts have decreased sodium permeability and increased membrane rigidity, resulting in resistance to changes to volume [30]. This alteration in permeability and decrease in membrane elasticity may explain the increased retention of catalase in ATP-depleted red cells which, as demonstrated in Table II and Fig. 4, may be overcome by increasing the hemolytic ratio during the initial ghost preparation.

An alternative possibility is that catalase is retained in a small percentage of the ghost population. Bodemann and Passow [31] have demonstrated a heterogeneous ghost population, thus, ATP depletion may increase the fraction retaining catalase.

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