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Reversible, calcium-dependent membrane association of human leukocyte 5-lipoxygenase

(arachidonic acid/leukotriene/inflammation/allergy/enzyme regulation)

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ABSTRACT **Maximal activity of human leukocyte 5-lipoxygenase requires Ca^{2+}, ATP, a microsomal membrane preparation, and two cytosolic stimulatory factors. We report here some effects of Ca^{2+} on the physical properties of the 5-lipoxygenase. When leukocytes were homogenized in the presence of 2 mM EDTA, 5-lipoxygenase was found to be a soluble enzyme. However, when Ca^{2+} was added to homogenization buffers at 0–1 mM in excess of EDTA, increasing quantities of the enzyme were recovered in the microsomal membrane fraction (100,000 × *g* pellet). The membrane-associated enzyme was resolubilized by washing pellet preparations in buffers containing 2 mM EDTA and was partially purified by anion-exchange chromatography. Studies of the stimulatory-factor requirements of the membrane-associated, resolubilized, and partially purified enzyme indicated that one of the cytosolic 5-lipoxygenase stimulatory factors exhibited a reversible, Ca^{2+}-dependent membrane association, similar to that of the enzyme itself. Ca^{2+} also caused a destabilization of the 5-lipoxygenase. Homogenates prepared in the presence of Ca^{2+} contained lower total enzyme activity, and retention of activity in these samples over time was also diminished.**

The enzyme 5-lipoxygenase catalyzes the synthesis of 5-hydroperoxy-6,8,11,14-icosatetraenoic acid (5-HPETE) from arachidonic acid, as well as the subsequent conversion of 5-HPETE to 5,6-oxido-7,9,11,14-icosatetraenoic acid (leukotriene A_4) (1–4). Thus, this enzyme is responsible for the first two steps in the pathway for leukotriene biosynthesis. Because of their potent biological activities (chemotaxis, smooth muscle contraction, increases in vascular permeability), leukotrienes have been suggested to play a significant role in the pathophysiology of immediate hypersensitivity and inflammation (5). Consequently, the regulation of 5-lipoxygenase activity has become a subject of increasing interest.

5-Lipoxygenase has now been purified from human and porcine peripheral blood leukocytes as well as from rat basophilic leukemia cells (4, 6, 7). In each case, the activity of the enzyme was shown to be dependent on Ca^{2+} and ATP, an unusual feature for lipoxygenases in general. Even greater complexity was noted in the case of the human leukocyte enzyme, which also required three other intracellular components for maximal activity. The first of these was present in the 100,000 × *g* pellet from leukocyte homogenates. The second was present in the protein precipitating from leukocyte homogenates at 60–90% saturation of ammonium sulfate, and the third was found in the nonadherent protein (pass-through fraction) obtained from chromatography of a partially purified sample on the anion-exchange resin Mono Q (Pharmacia) (6, 8). These factors are here designated 100,000 × *g* pellet, 60–90% ppt, and MQ-PTF, respectively.

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At present, little is known about the mechanism of action of the 5-lipoxygenase stimulatory factors, or what their physiological significance is for the control of 5-lipoxygenase activity in the intact human leukocyte. There can be little doubt, however, that Ca^{2+} plays a central role in the regulation of this enzyme. Accordingly, we have examined in greater detail the effects of Ca^{2+} on the properties of human leukocyte 5-lipoxygenase.

MATERIALS AND METHODS

Leukocyte Homogenates. Human leukocyte concentrates were obtained from local blood-collection centers, and the leukocytes were separated from contaminating erythrocytes by dextran sedimentation and hypotonic lysis as described (6, 9). The cells were washed once in Dulbecco's phosphate-buffered saline (GIBCO) and resuspended at a concentration of 100–250 × 10^6 per ml in KPB-1 (50 mM potassium phosphate buffer/0.1 M NaCl/2 mM EDTA/1 mM dithiothreitol/0.5 mM phenylmethylsulfonyl fluoride/0.006% soybean trypsin inhibitor, pH 7.1). In some experiments $CaCl_2$ was added just prior to cell homogenization.

Cell suspensions were homogenized at 0–4°C by using a Kontes Microsonic cell disruptor at power level 3.75. The total 60-sec sonication time was delivered in three intervals of 20 sec, separated by 15–30 sec. The resulting sonicates were then subjected to centrifugations as indicated for individual experiments.

Preparation and Release of Membrane-Associated 5-Lipoxygenase, Followed by Anion-Exchange Chromatography. A leukocyte homogenate was prepared from 80 ml of a leukocyte suspension (250 × 10^6 cells per ml) in KPB-1 containing 4 mM $CaCl_2$. Fifty-five milliliters of this homogenate was subjected to centrifugation at 100,000 × *g* for 75 min. The resulting pellet was resuspended in 12 ml of TEA-1 (35 mM triethanolamine acetate/2.8 mM EDTA/1 mM dithiothreitol, pH 7.3), and subjected to a second centrifugation (100,000 × *g*, 75 min). The resulting 10 ml of pellet-wash supernatant (pws) was diluted with 5 ml of TEA-2 (25 mM triethanolamine acetate/2 mM EDTA/1 mM dithiothreitol/20 μM ferrous ammonium sulfate/30% (vol/vol) glycerol, pH 7.8).

Anion-exchange high-performance liquid chromatography (HPLC) was performed using a "fast protein liquid chromatography" system (Pharmacia) enclosed in a refrigerated cabinet maintained at 10°C. HPLC buffers were saturated with argon and thoroughly degassed before use. The diluted pws was applied to a column (5 × 50 mm) of the anion-exchange resin Mono Q (HR 5/5, Pharmacia) equilibrated in

Abbreviations: 5-HPETE, 5-hydroperoxy-6,8,11,14-icosatetraenoic acid; 60–90% ppt, protein precipitated from human leukocyte 10,000 × *g* supernatant at 60–90% saturation with ammonium sulfate; MQ-PTF, Mono Q pass-through fraction; pws, pellet-wash supernatant.

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TEA-3 (TEA-2 at pH 7.3). After removal of nonadsorbed protein (pass-through fraction), the column was developed at a flow rate of 0.5 ml/min, using a 40-ml gradient of TEA-3 to TEA-4 (TEA-3/0.4 M sodium acetate). Fractions (1 ml) were collected beginning with the gradient elution. 5-Lipoxygenase activity was eluted in fractions 18–20.

Purification of 5-Lipoxygenase. 5-Lipoxygenase was purified from leukocyte homogenates by a combination of ammonium sulfate fractionation, gel-filtration chromatography (AcA 44, LKB), and HPLC on hydroxyapatite (HPHT, Bio-Rad) and Mono Q (Pharmacia) columns. These methods are presented in detail elsewhere (6).

Preparation of Stimulatory Factors. The 60–90% ppt, MQ-PTF, and 100,000 × g pellet factors were prepared and utilized exactly as described (6, 8) except that assay samples contained 50 μl of 60–90% ppt and 100 μl of 100,000 × g pellet.

Enzyme Assay. 5-Lipoxygenase activity was assayed at 37°C and pH 7.5 for an incubation period of 10 min with 100 nmol of [^{14}C]arachidonic acid (1.5 mCi/mmol; 1 Ci = 37 GBq) in a total volume of 1 ml. Unless otherwise stated, assay mixtures contained 2 mM ATP, 3 mM $CaCl_2$, and 20 μM mixed, nonenzymatically generated hydroperoxyicosatetraenoic acids as stimulatory factors. Details of the assay conditions and the analysis of samples by extraction, silicic acid column chromatography, and HPLC have been published (6). One unit of enzyme activity is defined as the quantity that produces 1 nmol of 5-HPETE under standard assay conditions. In experiments in which samples contained stimulatory factors, corresponding blanks containing only those factors were also assayed, and the resulting blank values were subtracted from the sample values. The data reported are the representative results of three or more reproducible experiments in which single samples were assayed.

Protein Assay. Protein concentrations were estimated by the method of Bradford (10), with bovine serum albumin as a standard.

RESULTS

Effect of Ca^{2+} and Ca^{2+} Ionophore A23187 on 5-Lipoxygenase Activity in Human Leukocyte Homogenates. Previous studies had shown that 5-lipoxygenase activity in human leukocyte homogenates was highly dependent upon the presence of free Ca^{2+} in the assay mixtures. Enzyme activity was nearly undetectable until the concentration of added Ca^{2+} exceeded that of EDTA (1.6 mM final concentration). Activity was maximized at approximately 3 mM added Ca^{2+}. Similar results were noted for the leukotriene A_4 synthase activity of the enzyme (1). Because of this requirement for Ca^{2+} in the cell-free system, and because Ca^{2+} ionophore A23187 stimulates 5-lipoxygenase activity in intact neutrophils (11), we questioned whether exposure of leukocytes to Ca^{2+} in the presence or absence of ionophore prior to cell homogenization would result in an increased yield of enzyme in the resulting homogenates. This hypothesis was tested in experiments in which leukocytes were suspended in buffers containing 2 mM EDTA plus 0–3 mM $CaCl_2$ and incubated for 5 min at 37°C in the presence or absence of the ionophore. 5-Lipoxygenase activity was assayed in 10,000 × g and 100,000 × g supernatants, after cell homogenization. As shown in Table 1, we observed that the presence of Ca^{2+} (2–3 mM) resulted not in improved, but rather in reduced, enzyme recovery. Furthermore, exposure of the cells to ionophore caused a still greater loss of activity, which was observed even in the absence of exogenous Ca^{2+}. These findings were more marked in 100,000 × g than 10,000 × g supernatants.

Ca^{2+}-Dependent Membrane Association of 5-Lipoxygenase. There are many possible explanations for the data in Table 1. Ca^{2+} might have had a direct, destabilizing effect on the 5-lipoxygenase, or it might have caused enzyme degradation through the activation of Ca^{2+}-dependent proteases. Alternatively, Ca^{2+} may have activated a specific inhibitor of the enzyme, though this seems less likely in view of the stimulatory effect of Ca^{2+} on 5-lipoxygenase activity. Finally, Ca^{2+} may also have induced an association of the enzyme with a cellular membrane fraction.

Table 1. Exposure of human leukocytes to Ca^{2+} and ionophore A23187 decreases recovery of 5-lipoxygenase activity in 10,000 × g and 100,000 × g supernatants

	5-Lipoxygenase activity, units/ml			
	10,000 × g		100,000 × g	
Ca^{2+}, mM	− A23187	+ A23187	− A23187	+ A23187
0	29.7	21.2	21.5	12.0
2	19.1	12.6	6.6	4.2
2.5	18.2	11.8	6.9	4.8
3.0	14.8	9.2	6.1	3.6

Leukocytes were suspended in KPB-1 containing the indicated concentrations of added $CaCl_2$. The cells were then incubated for 5 min at 37°C in the presence (2 μM) or absence of Ca^{2+} ionophore A23187. After cell homogenization, 5-lipoxygenase activity was assayed in 10,000 × g and 100,000 × g supernatants.

To investigate this latter possibility more thoroughly, we performed experiments in which leukocytes were suspended in homogenization buffer containing 2 mM EDTA, and Ca^{2+} was added to give concentrations of 2–3 mM (0–1 mM in excess of EDTA). The cells were homogenized, and 5-lipoxygenase activity was assayed in various subcellular fractions. The results (Table 2) showed that, in the absence of Ca^{2+}, the 5-lipoxygenase was essentially a soluble enzyme. The 3–8% activity in the pellet fractions could readily be explained by the fact that the pellets had not been washed, and to the presence of unbroken cells (1000 × g pellet). As Ca^{2+} concentration was increased, however, we noted a marked decrease in supernatant activity and an appearance of increased activity in the 100,000 × g pellet. Thus, at 3 mM added Ca^{2+}, more enzyme was recovered in the 100,000 × g pellet than in the corresponding supernatant. These data indicated that the 5-lipoxygenase displayed a Ca^{2+}-dependent association with the microsomal membrane fraction.

Effect of Ca^{2+} on Recovery and Stability of 5-Lipoxygenase. Close inspection of Table 2 indicates that the recovery of total 5-lipoxygenase activity from the 100,000 × g centrifugation was considerably less than 100%, and that this effect was most notable as Ca^{2+} concentrations were increased. Previous experiments performed in the absence of Ca^{2+} had

Table 2. Effect of Ca^{2+} on subcellular localization of 5-lipoxygenase

	Total 5-lipoxygenase activity, units			
Fraction	0 mM Ca^{2+}	2 mM Ca^{2+}	2.5 mM Ca^{2+}	3 mM Ca^{2+}
Homogenate	1030	1040	1040	846
Supernatants				
1,000 × g	1170	1090	1130	808
10,000 × g	1080	937	1000	655
100,000 × g	827	673	460	98.8
Pellets				
1,000 × g	81.1	65.4	56.4	87.3
10,000 × g	32.1	34.7	41.3	62.1
100,000 × g	61.3	53.4	104	289

Leukocytes were suspended in KPB-1 containing the indicated concentrations of added Ca^{2+}. After homogenization, samples were subjected to centrifugation at 1000 × g (15 min), 10,000 × g (15 min), and 100,000 × g (75 min). 5-Lipoxygenase activity was determined in the resulting supernatants and pellets. Pellet samples were assayed in the presence of 50 μl of the 60–90% ppt (see text).

indicated that the 100,000 × *g* pellet contains a 5-lipoxygenase stimulatory activity (6, 8). In the experiments described in Table 2, the 100,000 × *g* supernatants were assayed in the absence of this 100,000 × *g* pellet stimulatory factor, resulting in an underestimation of their enzyme activity. Reconstitution experiments indicated that this mechanism fully accounted for the apparent loss of activity (20–25%) observed upon centrifugation (100,000 × *g*) of samples prepared in the absence of Ca^{2+}. Thus recombination of 100,000 × *g* supernatants and pellets fully restored the activity observed in the original 10,000 × *g* supernatants. However, when samples were prepared in the presence of Ca^{2+}, 40–45% of the enzymatic activity was lost upon centrifugation at 100,000 × *g*, and only about half of this activity could be recovered in reconstitution experiments. Thus, in the presence of Ca^{2+}, centrifugation and resuspension resulted in a true loss of enzymatic activity, about 20–25%.

In addition to the deleterious effects of Ca^{2+} during centrifugation, we noted that the activities of homogenates prepared in the presence of Ca^{2+} (1–2 mM in excess of EDTA) were also reduced by 15–20%. Further, the stability of the enzyme with time was diminished in the presence of Ca^{2+}. For example, after 10 hr at 4°C, 10,000 × *g* supernatants incubated in the absence of Ca^{2+} retained 80% of their activity as compared to samples containing 3 mM Ca^{2+} (2 mM EDTA), in which only 48% of the activity remained. Thus it appears that the stability of 5-lipoxygenase is reduced at high Ca^{2+} concentrations.

Effect of Stimulatory Factors on Membrane-Associated 5-Lipoxygenase. It is important to note that the 5-lipoxygenase activities in the 100,000 × *g* pellets shown in Table 2 were detected only upon the inclusion of the 60–90% ppt stimulatory factor in the assay mixtures. Fig. 1*A* summarizes the results obtained when we investigated the effects of the various stimulatory factors on the 5-lipoxygenase activity of a 100,000 × *g* pellet prepared in the presence of Ca^{2+} (4 mM). Clearly, this sample would not require the addition of the 100,000 × *g* pellet factor, since it already contained that factor. However, the 60–90% ppt factor effected a marked (4- to 8-fold) 5-lipoxygenase stimulation. In contrast, addition of the MQ-PTF caused no further augmentation of activity and was even slightly inhibitory.

Resolubilization of Membrane-Associated 5-Lipoxygenase. In order to better understand the behavior of the membrane-associated enzyme, efforts were next initiated to resolubilize the 5-lipoxygenase from the membrane. Duplicate 100,000 × *g* pellets were prepared in the presence of 4 mM Ca^{2+} (2 mM EDTA) to maximize membrane association. One sample was resuspended in buffer containing 2 mM EDTA, and the second in the same buffer supplemented with 4 mM $CaCl_2$. After a second centrifugation at 100,000 × *g*, 5-lipoxygenase activity was assayed in the initial resuspended pellets as well as in the final supernatants and pellets. Fig. 2 shows that the presence of Ca^{2+} during the initial resuspension had little effect on 5-lipoxygenase activity. However, after the second centrifugation, a marked difference was noted in the two samples. In the absence of the cation, 64% of the enzyme that was originally membrane-associated was now recovered in the pws sample, whereas 7% remained in the pellet. In contrast, when Ca^{2+} was included in the wash buffer, only

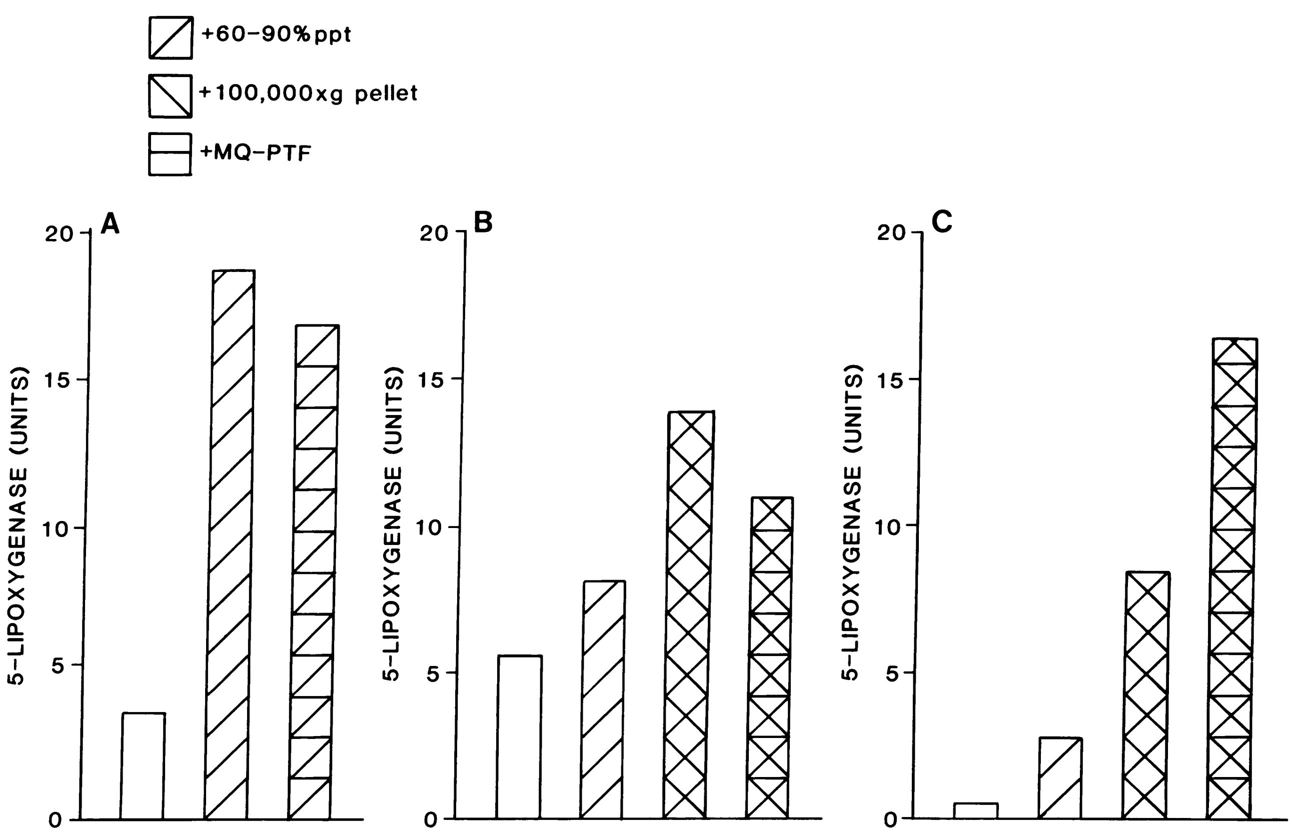

FIG. 1. Stimulatory-factor requirements of various lipoxygenase preparations. (*A*) Leukocytes were suspended in KPB-1 with 4 mM $CaCl_2$ and homogenized. Supernatants (10,000 × *g*) were prepared and subjected to centrifugation at 100,000 × *g* (60 min). The pellets were resuspended in KPB-1 and assayed for 5-lipoxygenase activity alone or in the presence of 60–90% ppt and MQ-PTF as indicated. (*B*) A pws was prepared, as described in the legend to Fig. 2, and assayed for 5-lipoxygenase activity alone or in the presence of the indicated stimulatory factors. (*C*) A pws was subjected to chromatography on Mono Q (see *Materials and Methods* and Fig. 3). Enzyme-containing fractions were combined and assayed for 5-lipoxygenase activity with the addition of the various stimulatory factors.

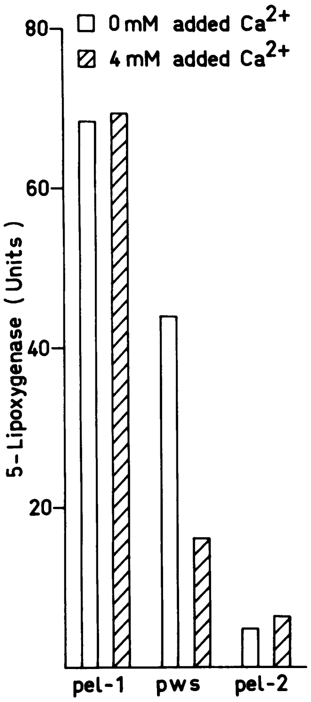

FIG. 2. Release of 5-lipoxygenase from membranes by removal of Ca^{2+}. Leukocytes were suspended (200×10^6 cells per ml) in KPB-1, 4 mM $CaCl_2$ was added, and the cells were homogenized by sonication. Two 10-ml aliquots of the sonicates were then sequentially subjected to centrifugation at $10,000 \times g$ (15 min) and $100,000 \times g$ (75 min). The $100,000 \times g$ pellets were resuspended in 4 ml of KPB-2 in the presence or absence of 4 mM $CaCl_2$ and subjected to a second centrifugation. The pellets were then resuspended in 1 ml of KPB-1 (no $CaCl_2$). 5-Lipoxygenase activity was determined on the resuspended $100,000 \times g$ pellets after the first (pel-1) and second (pel-2) centrifugations and on the pws. All assays were performed under standard conditions at a final common Ca^{2+} concentration of 3 mM. The data presented indicate the total enzyme activity in each sample. Initially, the $10,000 \times g$ and $100,000 \times g$ supernatants contained 36 units and 12 units of 5-lipoxygenase activity per ml, respectively.

23% of the original activity was recovered in the supernatant, while the second pellet retained 9%. These data indicated that the enzyme could be effectively released from the membrane by the EDTA wash procedure. They also demonstrated that it was difficult to maintain the enzyme in the membrane-associated form, even in the presence of Ca^{2+}. The fact that recovery of activity was lower in the presence of Ca^{2+} (32%) than in its absence (73%) was consistent with our prior observations of the destabilization of the enzyme by Ca^{2+}, although the effect was more striking here. This result suggests that the presence of Ca^{2+} during centrifugation may be more damaging for the enzyme if it is membrane-associated or that prolonged membrane-association itself may lead ultimately to loss of enzyme activity.

Having resolubilized the enzyme, we investigated the effects of the various stimulatory factors on the activity of the pws sample. Fig. 1*B* shows that the 5-lipoxygenase activity in the pws sample, like that of the membrane-associated enzyme (Fig. 1*A*), was stimulated by the 60–90% ppt. Furthermore, since the pws sample lacked the membrane component, it was now also stimulated by a fresh $100,000 \times g$ pellet (prepared in the absence of Ca^{2+}). However, as we observed for the membrane-associated samples (Fig. 1*A*), the MQ-PTF caused no further augmentation of 5-lipoxygenase activity in the pws sample (Fig. 1*B*).

Demonstration of the MQ-PTF in the pws Samples. We had observed (Fig. 1 *A* and *B*) that the 5-lipoxygenase activity in neither the $100,000 \times g$ pellets nor the pws samples was stimulated by the MQ-PTF. One possible explanation for this observation was that the stimulatory factor in the MQ-PTF exhibited a reversible, Ca^{2+}-dependent membrane association, similar to that of the enzyme itself. In this case, both the pellet and pws samples would already contain this factor and would, therefore, not respond to exogenously added MQ-PTF.

In order to test this hypothesis, it would be necessary to separate the 5-lipoxygenase in the pws from the MQ-PTF factor. Prior purification studies indicated that this could be accomplished by Mono Q chromatography, so the pws sample was subjected to this procedure. A peak of 5-lipoxygenase activity was recovered that exhibited the same chromatographic behavior as 5-lipoxygenase purified from human leukocyte $10,000 \times g$ supernatants (6). The identity of the enzyme was further supported by $NaDodSO_4$/polyacrylamide gel electrophoresis, which showed a prominent band having the same R_f value as the purified enzyme (data not shown). Fig. 1*C* demonstrates the stimulatory factor requirements of the enzyme obtained from chromatography of the pws. The activity of this preparation was clearly increased by all three factors, including the MQ-PTF.

These results indicated that we had isolated a 5-lipoxygenase preparation from the Mono Q column that did not contain the MQ-PTF stimulatory factor. As shown in Fig. 3, chromatography of the pws on Mono Q also yielded a pass-through fraction that possessed typical MQ-PTF factor stimulatory activity. This was demonstrated by its stimulatory effect on a preparation of partially purified enzyme lacking this factor. Thus, we could conclude that the pws sample did, in fact, contain both 5-lipoxygenase and the MQ-PTF factor and that these two components had been successfully separated by Mono Q chromatography.

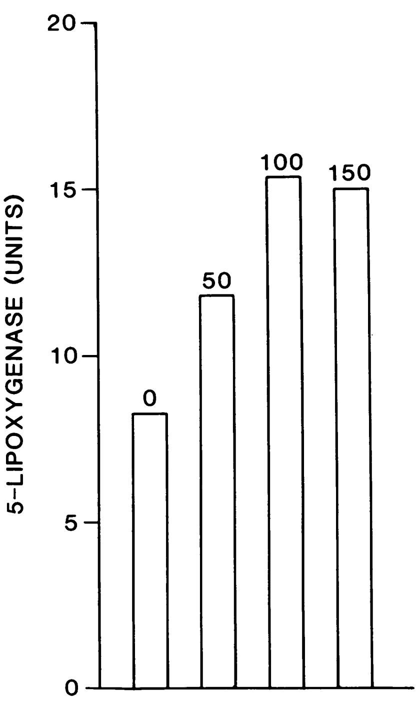

FIG. 3. MQ-PTF factor stimulatory activity from the pws. A pws was prepared as described (Fig. 2) and subjected to Mono Q chromatography. The nonadherent protein (pass-through fraction) from the column was collected and concentrated by ultrafiltration to 10 ml. Samples (150 μl) of the enzyme obtained from the Mono Q column were then assayed in the presence of the 60–90% ppt, the $100,000 \times g$ pellet, and the indicated quantities (0, 50, 100, and 150 μl) of the concentrated pass-through-fraction sample.

DISCUSSION

The data presented here indicate that Ca^{2+} has three major effects on human leukocyte 5-lipoxygenase. First, Ca^{2+} is absolutely required for 5-lipoxygenase activity. Second, Ca^{2+} decreases the stability of the enzyme. Third, Ca^{2+} causes a reversible association of the enzyme with cellular microsomal membranes. The divalent-cation specificity of these phenomena has not been examined in detail; however, Mg^{2+} at the same concentrations had none of these effects on the enzyme.

The destabilizing effect of Ca^{2+} on the 5-lipoxygenase was seen in three ways, including a decrease in total homogenate activity, an increased loss of activity over time, and a decreased recovery of enzyme during centrifugation and pellet-resuspension procedures. However, this effect was seen at much higher Ca^{2+} concentrations than those required for 5-lipoxygenase activation. It is therefore unlikely that such destabilization is relevant to the regulation of enzyme activity in the intact cell. Furthermore, despite the inclusion of protease inhibitors in the buffers, one cannot rule out that Ca^{2+}-dependent proteases may contribute to this phenomenon. Regardless of the mechanism, these data are of practical value for those working with this enzyme and suggest the value of EDTA or EGTA as a stabilizing agent.

In interpreting the results concerning the Ca^{2+}-dependent membrane association of the 5-lipoxygenase, the data in Fig. 2 are particularly problematic. Ideally, one would expect to have recovered considerably more activity in the pellet fraction after the second centrifugation in the presence of Ca^{2+}. Although there remains no clear explanation for the poor recovery of enzyme from this sample, it is interesting to compare these results with previous findings concerning the 5-lipoxygenase stimulatory activity of the $100,000 \times g$ pellet. In these earlier studies, we observed that the $100,000 \times g$ pellet (prepared in the absence of Ca^{2+}) could significantly stimulate 5-lipoxygenase activity. However, if the membrane was washed by subjecting it to a second centrifugation and resuspension, the stimulatory activity was totally lost and could not be recovered in the wash supernatant or in reconstituted samples (8). Thus the stimulatory activity of the membrane displays similar behavior on pellet washing as does the membrane-associated 5-lipoxygenase activity, and we speculate that both phenomena may be explained by some basic property of the membrane itself.

The most important question concerning the membrane association of 5-lipoxygenase is whether this phenomenon is physiologically relevant. A most compelling argument against this possibility comes from the fact that large quantities of enzyme were associated with the membrane only at very high Ca^{2+} concentrations (>1 mM in excess of EDTA). On the other hand, it is also possible that the techniques used for these studies underestimate the degree of membrane-association at more physiological concentrations of Ca^{2+}. In support of this possibility are the decreased stability of the enzyme in the presence of Ca^{2+}, the ease of reversibility of the membrane association, and the difficulty in maintaining the membrane-associated 5-lipoxygenase activity even in the presence of Ca^{2+}. Further, we have noted that the harshness of the sonication procedure can markedly affect the amount of enzyme that is recovered in the membrane fraction at any given Ca^{2+} concentration (unpublished observations).

In favor of a physiological role for the Ca^{2+}-dependent membrane association of the 5-lipoxygenase is the fact that the membrane fraction involved is the same fraction that has been shown to have a stimulatory effect on 5-lipoxygenase activity. Furthermore, another 5-lipoxygenase stimulatory factor, the MQ-PTF factor, appears to exhibit a similar membrane-association. These observations lead to the attractive hypothesis that neutrophil activation, leading to increased intracellular Ca^{2+} concentrations, might result in a translocation of the enzyme and MQ-PTF factor to the membrane in some form of activated complex. However, the 5-lipoxygenase can be activated by Ca^{2+} in the absence of the microsomal membrane and the MQ-PTF, so this mechanism is clearly not required for this most important effect of Ca^{2+} on the enzyme. Therefore, the exact relevance of the Ca^{2+}-dependent membrane association of 5-lipoxygenase to the regulation of the enzyme remains a matter of speculation.

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