The Two Calcium-binding Proteins, S100A8 and S100A9, Are Involved in the Metabolism of Arachidonic acid in Human Neutrophils*

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Recently, we identified the two myeloid related protein-8 (MRP8) (S100A8) and MRP14 (S100A9) as fatty acid-binding proteins (Klemp, M., Melkonyan, H., Nacken, W., Wiesmann, D., Holtkemper, U., and Sorg, C. (1997) FEBS Lett. 408, 81–84). Here we present data that the S100A8/A9 protein complex represents the exclusive arachidonic acid-binding proteins in human neutrophils. Binding and competition studies revealed evidence that (i) fatty acid binding was dependent on the calcium concentration; (ii) fatty acid binding was specific for the protein complex formed by S100A8 and S100A9, whereas the individual components were unable to bind fatty acids; (iii) exclusively polyunsaturated fatty acids were bound by S100A8/A9, whereas saturated (palmitic acid, stearic acid) and monounsaturated fatty acids (oleic acid) as well as arachidonic acid-derived eicosanoids (15-hydroxyeicosatetraenoic acid, prostaglandin E2, thromboxane B2, leukotriene B4) were poor competitors. Stimulation of neutrophil-like HL-60 cells with phorbol 12-myristate 13-acetate led to the secretion of S100A8/A9 protein complex, which carried the released arachidonic acid. When elevation of intracellular calcium level was induced by A23187, release of arachidonic acid occurred without secretion of S100A8/A9. In view of the unusual abundance in neutrophil cytosol (approximately 40% of cytosolic protein) our findings view of the unusual abundance in neutrophil cytosol. In areas of acute inflammation, polymorphonuclear leukocytes, expressing the membrane-associated heterodimer S100A8/A9, are the predominant cell type. These cells have been shown to release high amounts of tumor necrosis factor-α and interleukin-1β, indicating that S100A8/A9 surface expression is restricted to activated or recruited phagocytes (7). These phagocytes perform several host defense functions, such as phagocytosis of invading microorganisms and cell debris, release of proteolytic enzymes, and generation of reactive oxygen metabolites. In addition, they release a number of arachidonic acid-derived eicosanoids, which amplify or perpetuate the acute inflammatory response. This subset of phagocytes is present in acute but absent in chronic inflammatory disorders (14). These findings have led to the assumption that S100A8 and S100A9 affect leukocyte trafficking and display a propagating role in inflammatory responses. Although there are a number of hypotheses, the exact functions of both proteins remain unknown.

Recently, we and others showed that S100A8/A9 heterodimers specifically bind (poly)unsaturated fatty acids in a calcium-dependent manner (21, 22), making them a good can-
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For immunoprecipitation cytosolic proteins (10 μg) were preincubated with 1 μM [3H]AA in the presence of calcium for 1 h at 0 °C, and increasing concentrations of 27E10 were added. After incubation for 1 h at 0 °C, antigen-antibody complex was removed by protein G-Sepharose, washed three times with Lipidex binding assay buffer, and radioactivity was determined. Lipidex suspensions were added. After mixing, the samples were further incubated on ice for 60 min. Equilibrium binding of the fatty acid to both the proteins and to Lipidex was completed within the period of the assay (data not shown).

RESULTS

S100A8/A9 Exclusively Binds Arachidonic Acid in a Ca<sup>2+</sup>-Dependent Manner—Using proteins either from human or murine origin, two groups have shown independently that S100A8/9 specifically binds fatty acids in a Ca<sup>2+</sup>-dependent manner. Both studies differ in so far as the protein complex formed by the purified proteins from human keratinocytes binds unsaturated as well as polyunsaturated fatty acids with comparable affinities (22). In contrast, the heterodimer of murine recombinant S100A8 and S100A9 specifically binds arachidonic acid and shows only little affinity toward oleic acid (21). Therefore, we purified S100A8 and S100A9 from human neutrophils and investigated their specificity toward various fatty acids. The S100A8 and S100A9 proteins from human neutrophils were prepared from leukocyte-rich blood fractions ("buffy coat") according to Müller et al. (24). S100A8 and S100A9 were purified as described by van den Bos et al. (25) with some modifications. Prior to use, the proteins were rechromatographed by anion-exchange chromatography using a UnoQ column (Bio-Rad).

Immunoprecipitation of S100A8/A9 from Human Neutrophils—Human neutrophils were prepared from leukocyte-rich blood fractions ("buffy coat") according to Müller et al. (24). S100A8 and S100A9 were purified as described by van den Bos et al. (25) with some modifications. Prior to use, the proteins were rechromatographed by anion-exchange chromatography using a UnoQ column (Bio-Rad).

**EXPERIMENTAL PROCEDURES**

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**Incorporation of [14C]AA into HL-60 Cells**—Incorporation of [14C]AA into HL-60 cells was performed analogously to Kerkhoff et al. (26) with some modifications. For differentiation into a neutrophil-like phenotype, HL-60 cells were cultured at a density of 1×10⁶ cells/ml in the presence of 1,250 M [3H]AA in the absence of calcium for 1 h at 0 °C. The HL-60 cells were washed and viability was determined using trypan blue exclusion. Incubation of HL-60 cells with 10 μM [3H]AA in the absence of calcium resulted in a 95% incorporation of the fatty acid into the cells, as determined by liquid scintillation analysis. The HL-60 cells were harvested by centrifugation at 400 × g for 10 min at room temperature and washed three times with RPMI 1640 supplemented with 0.1% (w/v) fatty acid-free bovine serum albumin (BSA).

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**AA/BSA complexes were formed by the addition of 25 μl of [14C]AA (specific activity 56 mCi/ml) to 228 μl of AA (1 mM in ethanol). The solution was dried under N₂ and the fatty acids were resuspended in 2.5 ml of Hepes-buffered RPMI 1640 supplemented with 1% (w/v) fatty acid-free BSA and sonicated at 4 °C for 5 min at 20 watts. The Me₂SO-treated cells were adjusted to a cell density of 2×10⁶ cells/ml in Hepes-buffered RPMI 1640/0.1% (w/v) fatty acid-free BSA and labeled at 37 °C for 2 h by the addition of 1,250 M [14C]AA/BSA complex (final arachidonic acid concentration of 10 μM). At the end of labeling cells were washed three times with RPMI 1640/0.1% (w/v) fatty acid-free BSA. For lipid analysis the lipids were extracted according to the method of Bligh and Dyer (27) and analyzed by high performance thin layer chromatography as described by Kerkhoff et al. (26). Typical value range for incorporation of arachidonic acid was 100,000 dpm/×10⁶ cells. Under these conditions, more than 90% of the total radioactivity was incorporated into cellular lipids.

For stimulation experiments, 5×10⁶ HL-60 cells/900 μl were added to 100 μl of 10 μM A23187, 100 nM PMA, or a combination of A23187/PMA in polypropylene tubes and incubated at 37 °C for different time intervals as indicated. The stimulation was terminated by placing samples on ice followed by centrifugation in an Eppendorf centrifuge at 14,500 rpm for 2 min at 4 °C. Aliquots of the supernatants were used for determination of AA release and immunoprecipitation as described above.

**Immunoprecipitation of S100A8/A9**—The S100A8/A9 heterodimer-specific monoclonal antibody 27E10 was purified from hybridoma supernatants using protein G-coupled Sepharose as described by the manufacturer (Amersham Pharmacia Biotech). The immunoprecipitation experiments were performed according to Roth et al. (28) with some modifications. Briefly, aliquots of the cell supernatants were subjected to preadsorption by incubation for 1 h after the addition of 100 μl rabbit IgG (Calbiochem), followed by incubation for 1 h after the addition of protein G-Sepharose (Amersham Pharmacia Biotech). After centrifugation for 10 min at 14,000 × g, 1 μg/ml nonspecific mouse IgG1 or 1 μg/ml monoclonal antibody 27E10 were added to the supernatants and incubated for 1 h. Protein G-Sepharose (30 μl/ml) was added, and samples were further incubated for 1 h. Sepharose was collected by centrifugation, and the supernatants were discarded. The pellets were resuspended with 0.1% SDS and transferred to scintillation vials, and radioactivity was determined after the addition of 5 ml of scintillation liquid in an LKB 1211 Rackbeta counter.

The abbreviations used are: AA, arachidonic acid; BSA, bovine serum albumin; PMA, phorbol 12-myristate 13-acetate; PAGE, polyacrylamide gel electrophoresis.
The binding of [3H]AA to the S100A8/A9 protein complex formed (33). The native proteins were used in different combinations to analyze whether the individual components of the heterodimer and/or the S100A8/A9 protein complex were able to specifically bind AA. Protein-bound AA was discriminated from unbound fatty acid by the Lipidex assay. We found that both S100A8 and S100A9 were not able to bind the fatty acid either in the absence or presence of calcium. Exclusively, the native S100A8/A9 heterodimer showed fatty acid binding capacity in the presence of calcium (Fig. 1, A and B). The binding of [3H]AA to the S100A8/A9 protein complex was prevented by the addition of EDTA, although the presence of EDTA does not destabilize the protein complex once being formed (33).

Next, we investigated the molar concentrations of free Ca$^{2+}$ required for the [3H]AA binding. As shown in Fig. 1C, the S100A8/A9 showed significant AA binding at 0.2 μM calcium, and the AA binding capacity reached a plateau for values greater than 1 μM calcium. An IC$_{50}$ value of about 0.5 μM calcium was calculated, indicating that the calcium concentration required for AA binding to the S100A8/A9 complex is within the physiological range. Then a binding isotherm was obtained for the S100A8/A9 heterodimers using increasing amounts of [3H]AA. The binding of AA to S100A8/A9 in the absence of calcium was defined as nonspecific binding. Arachidonic acid was bound by the S100A8/A9 protein complex in a saturable manner (Fig. 2), and the corresponding Scatchard plot revealed a single class of binding sites for AA with a $K_D$ of 0.2 μM (Fig. 2 inset) and a stoichiometry of 0.4 mol of fatty acid/mol of S100A8/A9 heterodimer. This low value might be due either to the existence of S100A8/A9 isoforms, which did not display fatty acid binding capacity, or to the calcium-induced formation of higher order species of S100A8/A9 heteromers. Therefore, we performed size-exclusion chromatography followed by SDS-PAGE analysis to investigate the association state of the S100A8/A9 protein complex. As shown in Fig. 3, in the absence of calcium the elution profile showed only one peak corresponding to 34-kDa proteins. The monomeric proteins could not be detected. Upon the addition of calcium, the elution profiles showed two peaks corresponding to 34- and 48-kDa proteins, respectively. Then the proteins were incubated with radiolabeled AA in the presence of calcium and analyzed by size-exclusion chromatography. The different peaks were collected, and the radioactivity was determined in these fractions. It is obvious from Table I that the radiolabeled AA comigrates with both protein complexes, indicating that both protein complexes display AA binding capacity.

To answer the question whether S100A8/A9 exclusively binds AA, we performed binding and competition studies with various fatty acids as well as AA metabolites. Purified S100A8/A9 was incubated with 1 μM [3H]AA and a final concentration of 5 mM calcium in the presence of increasing concentrations of the various competitors as indicated. As shown in Fig. 4B, binding of [3H]AA to the S100A8/A9 protein complex was competed in a concentration-dependent manner by increasing concentrations of AA and α-linolenic and γ-linolenic acids, whereas saturated fatty acids, such as palmitic and stearic acids, and the monounsaturated fatty acid oleic acid were poor competitors (Fig. 4A). In addition, none of the tested AA metabolites were able to displace AA from the S100A8/A9 protein complex (Fig. 4A). It is of interest to note that the arachidonic trifluoromethyl ketone (AA-COClF3) as well as 15-...
hydroxyeicosatetraenoic acid, which did not compete for AA binding to S100A8/A9, exhibit structural similarity to AA. Analogous experiments with [3H]oleic acid as ligand revealed that the ligand was bound by S100A8/A9. However, there was no displacement by increasing concentrations of unlabeled oleic acid (in the concentration range 0–20 μM). Therefore, this binding was regarded as nonspecific binding to S100A8/A9 (data not shown).

S100A8/A9 Represents the Major AA-binding Protein in Human Neutrophils—Because of their unusual abundance in the human neutrophilic cytosol (approximately 40–50% of the cytosolic protein) (34), we assessed the AA binding capacity in the cytosolic fraction. Cytosolic proteins from human neutrophils were prepared as described under “Experimental Procedures,” and increasing protein concentrations were incubated with 1 μM [3H]AA in the absence and presence of calcium. The amount of protein-bound AA was determined using the Lipidex assay, which is widely used for the measurement of affinity constants for the binding of fatty acids to fatty acid-binding proteins. Apparently, no AA binding capacity was present in the cytosolic fraction from human neutrophils in the absence of calcium. By the addition of calcium, the formation of S100A8/A9 heterodimers was induced, and consequently, there was a protein concentration-dependent increase of the AA binding capacity in the cytosol (Fig. 5A). In analogous experiments, lymphocytic cytosol was used as the control because S100A8 and S100A9 are absent in lymphocytes. Thus, an AA binding capacity was not present either in the absence or in the presence of calcium (Fig. 5B).

Next, aliquots of the cytosolic proteins from human neutrophils (10 μg) were incubated with 1 μM [3H]AA to induce formation of S100A8/A9. Then increasing amounts of the human S100A8/A9-specific monoclonal antibody 27E10 were added, and the antigen-IgG complex was removed by protein G-coupled Sepharose. The supernatants were applied to a Lipidex assay to determine the amount of the remaining protein-bound fatty acids. It is obvious from Fig. 5C that the radiolabeled ligand was removed from the binding solution with increasing concentrations of 27E10 antibodies, indicating a strong interaction between AA and the S100A8/A9 protein complex. Correspondingly, the amount of AA bound by S100A8/A9 was decreased in the binding solution with increasing concentrations of 27E10 antibodies.

S100A8/A9 Secreted from Neutrophil-like HL-60 Cells Binds PLA2-released AA—It has been shown that S100A8/A9 is re-
were incubated with 1 ng/ml of cytosolic protein either from neutrophils or lymphocytes (A) and lymphocytes (B). Increasing concentrations of cytosolic protein from human neutrophils (10 μg) were incubated with 1 μM [3H]AA in the presence of calcium, and after addition of increasing concentrations of 27E10, the antigen-antibody complex was removed by protein G-Sepharose. The protein-bound [3H]AA was found in the antigen-antibody complex (●). In the supernatant the protein-bound fatty acids were separated from nonbound fatty acid using the Lipidex assay (●). Therefore, aliquots of the cell supernatants were applied to immunoprecipitation with the monoclonal antibody 27E10. The immunoprecipitate of the cell supernatant of A23187-stimulated neutrophil-like HL-60 cells contained significant amounts of the radiolabeled AAs, whereas the amount of radiolabeled AA in the immunoprecipitate of the cell supernatant of A23187-stimulated neutrophil-like HL-60 cells was found to be negligible. It is obvious from Fig. 6B that the S100A8/A9 secretion coincides in time with the AA release. The maximum of radiolabeled AA in the immunoprecipitate was achieved in the cell supernatant of neutrophil-like HL-60 cells stimulated with the combination of A23187 and PMA (Fig. 6B). Neutrophil-like HL-60 cells have an exceedingly high capacity to release AA, and only little of the liberated AA is converted into eicosanoids. In addition, a low level of immunodetectable cyclooxygenase-2 was expressed in unstimulated HL-60 cells. The cyclooxygenase-2 protein expression was induced by both agents within hours (data not shown). Therefore, the radiolabeled AA in the immunoprecipitate is considered to represent nonmetabolized AA bound by the secreted S100A8/A9 protein complex. Whether AA and S100A8/A9 protein complex were secreted either in a concerted or independent manner remains unclear.

DISCUSSION

The investigation of the AA binding properties by using purified S100A8 and S100A9 from human neutrophils revealed that exclusively the S100A8/A9 protein complex binds AA in a calcium-dependent manner (Fig. 1, A and B). The estimated calcium concentration required to induce fatty acid binding was within the physiological range (Fig. 1C). The fact that the individual components of the protein complex were unable to bind fatty acids either in the absence or in the presence of calcium leads to the assumption that docking of the two sub-
units creates an asymmetric fatty acid-binding site located at the interface between the subunits. In preliminary studies, different molar ratios of S100A8 and S100A9 were used in the presence of calcium to induce protein complex formation followed by the determination of AA binding capacity. They indicated that the protein complex that is able to bind AA consists of equal moles of S100A8 and S100A9.2 This result is in accordance with other studies (33).

The binding studies revealed that arachidonic acid binding was saturable and had specific binding characteristics. A $K_D$ of 0.2 μmol and a stoichiometry of 0.4 mol of fatty acid/mol of S100A8/A9 heterodimer was derived from Scatchard analysis (Fig. 2). Klotz (35) proposed that Scatchard analysis of ligand is only valid if an S-shaped curve for binding is obtained. This was not possible in the present investigation due to the limited binding capacity of the Lipidex material. Thus, the actual number of fatty acid molecules bound by the protein complex may be overestimated as outlined by Klotz (35). On the other hand, it is worth mentioning that upon calcium binding, conformational changes lead to the exposure of hydrophobic surfaces. Therefore, the amount of protein-bound AA may be underestimated in the Lipidex assay, although the microcentrifuge tubes were precoated with poly(propylene glycol) to minimize non-specific adsorption. Both limitations should be taken into account but do not change the interpretation that a part of the S100A8/A9 complex was able to bind AA (Fig. 2).

Alternatively, the calculated stoichiometry is based on the assumption that the multimer displaying the AA binding capacity represents a heterodimer. However, whether S100A8/A9 represents a heterodimer or an oligomer containing S100A8 and S100A9 at equimolar concentrations is still in debate. We performed gel filtration with the native proteins followed by SDS-PAGE analysis (Fig. 3). In the absence of calcium the nonmonomeric proteins could not be detected, indicating a strong interaction of S100A8 and S100A9 also in the absence of calcium. This assumption is also confirmed by the finding that the S100A8/A9 complex formation is not interfered by Ca$^{2+}$-chelation (33). The heterodimeric protein complex showed an apparent $M_r$ of 34 kDa in accordance to Siegenthaler et al. (22). However, several biophysical determinations confirm that the heterodimer has a $M_r$ of 24 kDa (33, 36). Thus, the heterodimeric protein complex is assumed to show an unusual migration in size-exclusion chromatography. In addition to the heterodimer, a protein complex with a $M_r$ of 48 kDa was analyzed after the addition of calcium, and radio labeled AA comigrated with both protein complexes, indicating that both complexes display AA binding capacity (Table I). The 48-kDa protein is assumed to represent a tetrameric protein complex consisting of two molecules of S100A8 and two molecules of S100A9. The existence of the tetramer is confirmed by a recently published study from our group by ultraviolet matrix-assisted laser desorption/ionization mass spectrometry (36).

In addition, both S100A8 and S100A9 have some post-translational modifications (33, 36), which may influence complex formation as well as fatty acid binding properties. However, phosphorylation of S100A9 within purified S100A8/A9 protein complexes did not affect the AA binding capacity (data not shown).

The competition studies clearly indicate that exclusively polyunsaturated fatty acid are bound by the protein complex in a saturable and reversible manner, whereas saturated fatty acids, such as palmitic and stearic acids, and the monounsaturated oleic acid were poor competitors. The S100A8/A9 protein complex showed the highest specificity toward arachidonic acid. In addition, the S100A8/A9 complex did not show any affinity for AA-derived eicosanoids (Fig. 4, A and B). These assays give insights into the nature of the fatty acid binding site. The affinity was significantly influenced by (i) the polar heads, (ii) the number of the double bonds, and (iii) their position within the fatty acid molecule. The identification of this binding pocket is currently under investigation in our laboratory. The specificity toward polyunsaturated fatty acids as well as the reversibility of the binding to the protein complex excludes the possibility that merely a solvation of insoluble fatty acid calcium salts occurs. These results also argue against a coordination of the fatty acid by one of the calcium ions of the S100A8/A9 complex. In contrast to our results, Siegenthaler et al. (22) found that S100A8/A9 shows specificity toward various fatty acids. This finding may be due to the fact that they used purified proteins from human keratinocytes for their binding and competition studies.

In the present study we could show for the first time that the S100A8/A9 protein complex represents the only AA binding capacity in the neutrophilic cytosol. This finding is corroborated by the facts that (i) there was only a low capacity for AA binding in the neutrophilic cytosol in the absence of calcium (Fig. 5A); (ii) the AA binding capacity in the cytosol was increased in a protein concentration-dependent manner after the addition of calcium, which induces the formation of S100A8/A9 protein complexes (Fig. 5A); and (iii) the AA binding capacity in the cytosol was depleted by immunoprecipitation with the S100A8/A9 protein complex-specific monoclonal antibody 27E10 (Fig. 5C). Furthermore, our conclusion is supported by the finding that members of the fatty acid binding protein family are not expressed in human neutrophils (37). Activated neutrophils, the predominant cell type present in areas of acute inflammation, release arachidonic acid as well as arachidonic acid-derived eicosanoids into the plasma where they may amplify or perpetuate the acute inflammatory response. Our novel finding that the S100A8/A9 protein complex represents the exclusive AA binding capacity in the neutrophilic cytosol together with their unusual abundance in human neutrophilic cytosol (34) clearly indicate that S100A8/A9 complexes play an important role in the AA metabolism of neutrophils. The calcium-induced binding of AA points to a role of S100A8/A9 in the mobilization, metabolism, or release of AA.

Therefore, the neutrophil-like HL-60 cells (for review, see Ref. 38) were used to study the release of AA as well as the secretion of S100A8/A9 heterodimers (Fig. 6). Arachidonic acid release was induced by either an increase of the intracellular calcium level by the calcium ionophore A23187 or an activation of protein kinases by the phorbol ester PMA (39–41). S100A8/A9 was secreted from neutrophil-like HL-60 cells by PMA, whereas the calcium ionophore A23187 was ineffective to induce the S100A8/A9 secretion. This finding was confirmed by the metabolic labeling of de novo-synthesized S100A8/A9 with [14C]leucine (data not shown), indicating that, in addition to monocytes as described by Rameses et al. (16), neutrophils also secreted the protein complex after activation of protein kinase C. The immunoprecipitation experiments of the cell supernatants revealed that (i) the secreted S100A8/A9 protein complex binds fatty acids and (ii) the majority of the simultaneously released AA was bound by the protein complex. Furthermore, the time courses of both AA release and S100A8/A9 secretion were found to be similar (Fig. 6). In contrast, the stimulation with A23187 led to a rapid release of AA but not to secretion of S100A8/A9. Consequently, no significant amounts of radio labeled fatty acids were determined in the immunoprecipitate. Still, the functional consequences of AA release either in the absence of S100A8/A9 secretion (as induced by calcium iono-

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2 C. Kerkhoff, unpublished observations.
TABLE II

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Bound (^{3}H)AA pmol/10 μg protein</th>
</tr>
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<tbody>
<tr>
<td>S100A8/A9</td>
<td></td>
</tr>
<tr>
<td>In the absence of 5 mM CaCl(_2)</td>
<td>6.6 ± 2.7</td>
</tr>
<tr>
<td>In the presence of 5 mM CaCl(_2)</td>
<td>46.7 ± 4.7</td>
</tr>
<tr>
<td>Hypochlorite-treated S100A8/A9</td>
<td></td>
</tr>
<tr>
<td>In the absence of 5 mM CaCl(_2)</td>
<td>6.3 ± 4.9</td>
</tr>
<tr>
<td>In the presence of 5 mM CaCl(_2)</td>
<td>43.6 ± 3.7</td>
</tr>
</tbody>
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phore) or in parallel to S100A8/A9 secretion (as induced by PMA) remain unclear. The S100A8/A9 secretion from human neutrophils is also induced by the chemoattractants formylmethionyleucylphenylalanine and complement 5a (42), indicating that this mechanism is functional following receptor-dependent activation of cells.

It is worth mentioning that apart from phosphorylation of S100A9, also oxidative modification of the protein complex by hypochlorite did not affect the AA binding capacity (Table II). The latter finding is of interest, since upon PMA stimulation hypochlorite did not affect the AA binding capacity (Table II).

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REFERENCES

32. Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., see Refs. 55 and 56. However, the mechanism by which arachidonate as well as eicosanoids, once biosynthesized, leave the producer cell to reach their target cells is still poorly understood. In addition, arachidonic acid is liberated from cellular phospholipids, but the activities of the AA-metabolizing enzymes are detectable within hours after stimulation (57–59). This finding was confirmed by cell stimulation experiments.
33. Both PMA and A23187 led to a time-dependent increase in the level of immunodetectable cyclooxgenase-2 (data not shown). In parallel, the enhanced calcium level also induces S100A8/A9 protein complex formation and its AA binding capacity. Thus, it could be envisioned either that the AA/S100A8/A9 complex may function as an intermediate reservoir or that the translocation of S100A8/A9 is accompanied by AA transport as suggested by Roulin et al. (37). Further investigations have to elucidate the exact nature of the protein complex which exhibits AA binding capacity as well as its role in the biological events caused by eicosanoids. They will provide further insights into the molecular mechanisms of inflammatory responses.

For example, it has been shown that endothelial cells utilize eicosanoids. They will provide further insights into the molecular mechanisms of inflammatory responses.
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