A Heterocomplex Formed by the Calcium-binding Proteins MRP8 (S100A8) and MRP14 (S100A9) Binds Unsaturated Fatty Acids with High Affinity*

We show that unsaturated fatty acids (FAs) bind reversibly and with high affinity to a heterocomplex of 34 kDa (FA-p34) formed by the non-covalent association of two calcium-binding proteins of the S100 family: MRP8 (S100A8) and MRP14 (S100A9). Fatty acid-competition studies on the [3H]oleic acid-FA-p34-complex show that oleic, α-linoleic, γ-linolenic, and arachidonic acids have IC50 values of about 1 μM, whereas palmitic and stearic acids are poor competitors. The binding of arachidonic acid is saturable with a single class of binding site per FA-p34, and a dissociation constant (Kd) of 0.13 μM is calculated. The individual subunits MRP8 and MRP14 show no binding properties for fatty acids, whereas a p34 complex reconstituted in vitro by the recombinant molecules exhibits binding properties, suggesting that the fatty acid-binding site of FA-p34 is created through heterocomplex formation. Furthermore, we demonstrate that lowering free Ca2+ levels to 16 nM results in a loss of the fatty acid-binding capacity of purified FA-p34. In calcium-induced differentiating keratinocytes, the amounts of FA-p34 are increased in the particulate (2.0 ± 0.5 pmol of [3H]oleic acid/mg protein) and in the cytosolic (4.5 ± 0.6 pmol of [3H]oleic acid/mg protein) fractions, whereas no FA-p34 can be detected in non-differentiated cultured keratinocytes.

In abnormally differentiated keratinocytes (psoriasis) and in human polymorphonuclear leukocytes, FA-p34 is highly expressed (31.35 ± 1.6 and 34.95 ± 17.9 pmol of [3H]oleic acid/mg protein, respectively), pointing toward a role for this heteromer in mediating effects of unsaturated fatty acids in a calcium-dependent way during cell differentiation and/or inflammation.

Fatty acids (FAs)1 are implicated in energy delivery, membrane synthesis, in the lipid barrier function of epidermis, in inflammation, and they modulate gene expression (1–3). FAs are hydrophobic, labile molecules forming poor-soluble complexes with intracellular calcium ions, which are required for keratinocyte differentiation. Therefore, FAs need to be solubilized, stabilized, and translocated by specific carrier proteins (4). Three distinct families of lipid-binding proteins, i.e. extracellular albumin, the cytoplasmatic fatty acid-binding proteins (FABPs), and the peroxisome proliferator-activated nuclear receptors (2, 3) are thought to mediate the biological activities of FAs. Skin represents a very active lipid-synthesizing tissue in mammals. Two recent reports describe that human keratinocytes express the epidermal FABP, which is highly up-regulated in the hyperproliferative and inflammatory skin disease psoriasis (5–7). The epidermis lacks the Δ5- and Δ6-desaturases (8), and, therefore, essential FAs like linoleic and arachidonic acid must be acquired from circulation. E-FABP binds stearic, oleic, and linoleic acid but has no affinity for arachidonic acid and a very low affinity for the nearby precursor, linolenic acid (6). Since no other FABPs have been detected in keratinocytes so far, we investigated whether a carrier protein, capable to bind unsaturated fatty acids like linoleic and arachidonic acid, might exist in psoriatic skin and cultured human keratinocytes.

We characterized a fatty acid-binding heteromer of 34 kDa (FA-p34) isolated from human keratinocytes, which is composed of MRP8 (also referred to as cystic fibrosis antigen, the L1 light chain, p8, calgranulin A, S100A8) and MRP14 (L1 heavy chain, p14, calgranulin B, S100A9) (for a review, see Ref. 9). These proteins belong to the S100 family of Ca2+-binding proteins (reviewed in Ref. 10), and both molecules are highly up-regulated in psoriatic skin (5, 11–14). Furthermore, for both molecules several post-translational modifications and the formation of high molecular weight complexes in vivo have been reported (15–18). In this report we show that FA-p34 represents a novel class of FA-binding proteins and discuss its possible role in mediating the biological activities of unsaturated fatty acids.

EXPERIMENTAL PROCEDURES

Ligands—[9,10-3H(O)]Oleic acid (specific activity, 9.2 Ci/mmol), [5,6,8,9,11,12,14,15-3H(N)]arachidonic acid (100 Ci/mmol), and 45CaCl2 (21.9 mCi/mg) were purchased from DuPont NEN (Regensdorf, Switzerland). Retinoic acid, α-linoleic acid, α- and γ-linolenic acid, palmitic acid, and stearic acid were all obtained from Sigma.

Cell Cultures and Tissue Isolation—Normal human keratinocytes from foreskin were cultured in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc., Switzerland) containing 1.3 μM Ca2+ and 10% fetal calf serum (19); after stratification, differentiating keratinocytes were separated from the non-differentiated cells by the low Ca2+ switch method as described (20). Human polymorphonuclear leukocytes (PMNL) from healthy volunteers were isolated as described (21). Psoriatic scales were obtained by gentle scraping of lesional skin from volunteer psoriatic patients. Normal human skin was obtained using a keratome set at 180 μm on skin biopsies from patients that have undergone plastic surgery. All cells and tissue samples were kept frozen at −20 °C until use.

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Preparation of Proteins Fracions—Keratinocytes (about 300 mg of lyophilized cells) were homogenized using a Polytron tissue homogenizer in 1.5 ml of Tris buffer (50 mM Tris/HCl, 25 mM NaCl, 2.5 mM EDTA, 1 mM dithiothreitol, pH 7.5) and centrifuged at 30 min at 100,000 × g. This supernatant is referred to as the cytosolic fraction. The cell debris were centrifuged at 10,000 × g for 90 min and centrifuged at 100,000 × g for 10 min, and the supernatant was discarded. This washing procedure was repeated twice. The pellet constituted of cellular debris is called particulate fraction. The corresponding pellet were aliquoted and frozen at −20 °C for storage.

About 5 g of psoriatic scales or normal skin were homogenized as above in 15 ml of Tris buffer and subsequently centrifuged at 100,000 × g for 1 h at 4 °C to obtain the cytosolic fraction. The corresponding pellet was washed in 5 ml Tris buffer to remove residual cytosolic proteins; the washed pellet was centrifuged at 10,000 × g for 15 min, and the supernatant was then discarded. This washing procedure was repeated twice. The pellet constituted of cellular debris is called particulate fraction. The pellet was then treated with 15 ml of high salt KCl buffer for 90 min and centrifuged at 10,000 × g for 15 min. This procedure was repeated once. The supernatants were collected and concentrated using centrifugal microconcentrators (Amicon) with a molecular mass cut-off of 3000 Da. This preparation contains soluble proteins dissociated from the particulate fraction by the high salt KCl buffer and is named high salt extractable protein fraction, HSEPF. Cytosolic proteins and HSEPF were aliquoted and frozen at −20 °C for storage.

For 90 min and centrifuged at 10,000 × g for 1 h at 4 °C to obtain the cytosolic fraction. The corresponding pellet was centrifuged at 10,000 × g for 15 min, and the supernatant was discarded. This washing procedure was repeated twice. The washed pellet was centrifuged at 10,000 × g for 10 min, and the supernatant was discarded. This washing procedure was repeated twice. The pellet was then resuspended in 5 ml high salt KCl buffer and incubated at 4 °C overnight. The supernatant referred to as HSEPF was separated from insoluble material by centrifugation at 12,000 × g for 10 min. The procedure was repeated once, and the HSEPFs were pooled, aliquoted, and stored at −20 °C. Protein concentrations were estimated by the colorimetric method described in Ref. 22 using human serum albumin as standard.

For keratinocytes and PMNL were separated by SDS-PAGE (15%), and differentiating and non-differentiated cultured human keratinocytes, and PMNL were separated by SDS-PAGE (15%) and subsequently blotted onto a nitrocellulose membrane (Electrnan, BDH Laboratory Supplies, United Kingdom). Membranes were incubated in PBS containing 0.5% skimmed dry milk, 0.2% Tween 20. mAbs directed against recombinant MRP8 and MRP14 were separated by SDS-PAGE (15%), and subsequently blotted onto a polyvinylidene difluoride membrane (Millipore) before probing with 2 μCi/ml of 45CaCl for 20 min in 10 mM imidazole, 5 mM MgCl2, 50 mM KCl at pH 7.8 as described previously (26).

RESULTS

Analysis of [3H]Oleic Acid-binding Proteins—Protein extracts from various samples were analyzed for proteins with fatty acid-binding capacity using gel filtration-high pressure liquid chromatography and [3H]Oleic acid as a ligand. The radioactive elution profile of the cytosolic fraction from differentiating keratinocytes showed one large radioactive peak at 94 kDa (referred to as FA-p34), which virtually abolished with the addition of an excess of unlabeled ligand (Fig. 1A), demonstrating high binding specificity. The excess of unlabeled oleic acid revealed another radioactive peak at 15 kDa (Fig. 1A). This peak corresponded to [3H]Oleic acid bound to E-FABP, since it co-eluted with purified human E-FABP. The appearance of the E-FABP peak upon gel filtration can be explained by the finding that E-FABP has a higher Kd value of 2.5 μM (measured with dextran-coated charcoal) than the value described earlier using another technique (20). Thus, a higher concentration of oleic acid was needed to saturate E-FABP binding sites. The other eluted radioactive peaks were either excluded (Vθ) or included (free excess [3H]Oleic acid) from the gel matrix.

Analysis of HSEPF of differentiating keratinocytes showed, besides minor radioactive peaks, a large radioactive peak co-eluting with cytosolic FA-p34 (Fig. 1B). No E-FABP could be detected in the HSEPF, confirming that FABPs are essentially cytosolic. The binding was specific since a molar excess of

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unlabeled oleic acid almost abolished the radioactive peak, whereas a molar excess of retinoic acid had no effect on \[^3H\]oleic acid binding to FA-p34. A UV trace experiment at 280 nm from the eluted material of cytosolic proteins and HSEPF from differentiating keratinocytes is shown Fig. 1E. As lesional psoriatic skin contains high levels of E-FABP, revealing high FA-traffic (6), the presence of FA-p34 was investigated in this tissue. HSEPF of scales showed a radioactive peak at 34 kDa, which was specific since it was almost abolished by a molar excess of unlabeled oleic acid (Fig. 1C). The radioactive elution profile of normal skin (cytosol and HSEPF) and psoriatic skin (cytosolic fraction) showed no and weak levels, respectively, of FA-p34 (data not shown). All elution times and ligand specificities observed for the radioactive peak at 34 kDa were identical for all samples investigated, suggesting that these \[^3H\]oleic acid-binding proteins were identical FA-p34 species. To quantify the binding capacity of FA-p34 from various samples, the radioactive peak of \[^3H\]OA-FA-p34 complex was integrated. The amounts of FA-p34 from various samples are summarized in Table I.

Purification of FA-p34—As HSEPF of psoriatic scales contains about 5 times more FA-p34 than the cytosolic fraction of differentiating keratinocytes, scales were used for the purification of FA-p34. Three purification steps were necessary to obtain a homogenous protein peak of FA-p34 when analyzed by gel filtration chromatography (Fig. 1D). About 389 \(\mu g\) of FA-p34 was obtained representing a yield of 0.0078% of starting material. Purified FA-p34 conserved its binding property and specificity during the different purification steps, since the FA-p34 peak co-eluted on Superose with the radioactive peak of \[^3H\]OA-FA-p34 and was abolished by the addition of a molar excess of oleic acid.

Analysis of FA-p34 by SDS-PAGE and Partial Amino Acid Sequencing—Analysis of FA-p34 by SDS-PAGE (15%) under non-reducing conditions revealed the presence of two Coomassie-stained protein bands of about 14 and 8 kDa (Fig. 2A, lane 1), suggesting that FA-p34 is a heterocomplex consisting of two non-covalently associated proteins. To unravel the identity of the two subunits, both proteins were digested with trypsin. Proteolysis yielded 7 peptides for the 8-kDa subunit and 15 for the 14-kDa subunit. One peptide of each digested subunit was randomly selected and sequenced. The sequences were GNFLHAVRYD for the peptide obtained from the 8-kDa subunit and LTWASHEK for the peptide from the 14-kDa subunit. By sequence comparison with the published sequences, the 8-kDa subunit was identified as MRP8 and the 14-kDa subunit as

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**Fig. 1.** Gel filtration analysis of \[^3H\]oleic acid-binding proteins from various samples. The panels represent radioactive elution profiles obtained from Superose column of cytosolic proteins (A), HSEPF (B) of cultured differentiating human keratinocytes, from HSEPF from psoriatic scales (C), and of FA-p34 (35 \(\mu g\)) after the final purification step (D). Proteins were incubated with 1.2 \(\mu M\) \[^3H\]OA in the presence (\(\Box\)) or absence (\(\Box\)) of a 200-fold molar excess of unlabeled oleic acid or in the presence of a 200 molar excess of retinoic acid (\(\odot\)), as described under “Experimental Procedures.” The protein elution profile was measured by absorption at 280 nm (solid line). The specific radioactive peak, eluted at 34 kDa, corresponds to FA-p34. Human albumin (referred to as alb) and E-FABP were used as standards. A UV trace experiment at 280 nm of eluted material from cytosolic proteins (solid line) and HSEPF (dotted line) of differentiating keratinocytes is shown in E. The hatched box shows the MRP14 immunoreactive fractions, and the closed box represents the MRP8 immunoreactive fractions measured by SDS-PAGE immunoblotting as described in Fig. 3.
TABLE I
Levels of FA-p34 in human keratinocytes and in polymorphonuclear leukocytes

| Samples                          | FA-p34 |
|----------------------------------|--------|
| Cultured human keratinocytes     |        |
| Ca\(^{2+}\)-induced differentiation |        |
| Particulate                     | 2.0 ± 0.5 |
| Cytosol                         | 4.5 ± 0.6 |
| Non-differentiated               |        |
| Particulate                     | NM     |
| Cytosol                         | NM     |
| Psoriatic scales                 |        |
| Particulate                     | 31.35 ± 1.6 |
| Cytosol                         | 3.64 ± 0.18 |
| Normal skin                     |        |
| Particulate                     | NM     |
| Cytosol                         | NM     |
| Polymorphonuclear leukocytes     |        |
| Particulate                     | NM     |
| Cytosol                         | 349.8 ± 17.9 |

MRP14 (27). In addition, SDS-PAGE analysis of rMRP8 and rMRP14 revealed an identical mobility with the subunits of FA-p34 (Fig. 2A, lanes 2 and 3), suggesting that the rMRPs are very similar to the MRPs composing FA-p34.

**Ca\(^{2+}\) Binding Studies of the FA-p34 Subunits**—Since MRP8 and MRP14 are two calcium-binding proteins, we investigated whether the individual components of FA-p34 are able to bind Ca\(^{2+}\). Using the overlay technique (26), the direct autoradiography showed that \(^{45}\)Ca\(^{2+}\) bound to MRP8 and MRP14 separated from the purified FA-p34 complex by SDS-PAGE (Fig. 2B, lane 1) as did the recombinant proteins (lanes 2 and 3). The strong radioactive bands of native and recombinant MRP14 suggest higher calcium-binding capacity of MRP14 compared with MRP8.

**Expression Studies of MRP8 and MRP14**—By protein-blot analysis using mAbs directed against MRP8 (Fig. 3A) and MRP14 (Fig. 3B), we studied the expression of these proteins in cytosolic fractions and HSEPF from the various samples. In normal human skin (lanes 1 and 2) MRPs were not detectable. In non-differentiated keratinocytes (lanes 3 and 4), only MRP8 was detectable. In contrast, high amounts of MRPs were found in the cytosol and in HSEPF of differentiating keratinocytes (lanes 5 and 6), psoriatic scales (lanes 7 and 8), partially purified FA-p34 from PMNL (fraction 16 from Fig. 8) (lane 9), and purified FA-p34 from psoriatic scales (lane 10). The immunoreactive bands of the rMRP8 and rMRP14 used as standards (lanes 11 and 12) showed identical electrophoretic mobilities as the MRPs from the samples.

**Ligand Binding Studies of Purified FA-p34**—A saturation curve at the equilibrium was obtained for purified FA-p34 using increasing amounts of \([^{3}H]\)oleic acid (Fig. 4A). The straight line of the Scatchard plot indicates a single class of binding site for arachidonic acid with a \(K_d\) of 0.13 \(\mu\)M (representative value of two independent experiments) (Fig. 4B). The calculated number of binding sites per FA-p34 was about 0.3. This low value might be explained by (i) an overestimation of the protein concentration measured by the colorimetric method used, compared with the intrinsic concentration of FA-p34, and (ii) the presence of FA-p34 isoforms without fatty acid-binding properties (see below). Competition binding assays on \([^{3}H]\)OA-FA-p34 showed that palmitic acid and stearic acid had poor competitive binding affinity versus \([^{3}H]\)oleic acid bound to FA-p34 (Fig. 5), whereas \(\alpha\)-linoleic acid, \(\gamma\)-linolenic acid, and arachidonic acid were good competitors with an IC\(_{50}\) of about 1 \(\mu\)M.

To study the role of free Ca\(^{2+}\) concentrations in \([^{3}H]\)oleic acid binding capacity, \([^{3}H]\)OA-FA-p34 levels were analyzed in the presence of increasing amounts of EDTA. For each EDTA concentration, the corresponding free Ca\(^{2+}\) concentration was calculated. Using a total and constant Ca\(^{2+}\) level of 150 \(\mu\)M and plotted versus the amounts of bound \([^{3}H]\)OA (Fig. 6). The \([^{3}H]\) oleic acid binding capacity of FA-p34 showed a plateau for values greater than 100 nM of free Ca\(^{2+}\), and about 70% of binding capacity was lost at a free Ca\(^{2+}\) concentration of 10 nM. An IC\(_{50}\) value of about 18 nM free Ca\(^{2+}\) was calculated.

**Reconstitution of FA-p34 from rMRP8 and rMRP14**—To reconstitute FA-p34 from purified FA-p34, which revealed a binding capacity of 31.35 pmol/mg protein, we used rMRP8 and rMRP14 as subunits. The reconstituted complex binds oleic acid with a K\(_d\) of 0.13 \(\mu\)M (Fig. 7A), suggesting that the rMRPs are very similar to the MRPs composing FA-p34.
A MRP8/14 Complex Binds Unsaturated Fatty Acids

FIG. 3. Expression studies of MRP8 and MRP14 in various samples. Cytosolic and particulate proteins of normal skin (lanes 1 and 2, respectively), from non-differentiated (20 μg each, lanes 3 and 4) and differentiating keratinocytes (10 μg each, lanes 5 and 6), psoriatic scales (10 μg each, lanes 7 and 8), partially purified FA-p34 from PMNL (fraction 16 of Fig. 8) (lane 9), and purified FA-p34 from psoriatic scales (1.6 μg, lane 10) were prepared as described under "Experimental Procedures" and separated by SDS-PAGE (15%) before subsequently blotted onto a nitrocellulose membrane. rMRP8 (1 μg, lane 11) and r-MRP14 (1 μg, lane 12) were used as standards. Detection of MRP8 and MRP14 was performed using mAbs 8–5C2 (A) and 8 36.48 (B), respectively, peroxidase-labeled goat anti-rabbit IgGFab' fragment, and diaminobenzidine and H2O2 as substrates.

FIG. 4. Saturation kinetics (A) and Scatchard analysis (B) of the binding of [3H]arachidonic acid to FA-p34. Specific binding (●) is defined as the total binding (□) minus the nonspecific binding (○). Scatchard analysis of the saturation kinetics represent bound (pmol)/ free (μM) versus bound (pmol). For conditions, see “Experimental Procedures.”

ders. The breadth of this peak and the shoulders compared with the thin and symmetric radioactive peak of FA-p34 suggest that several heterocomplexes, including FA-p34, were formed. Ligand binding was specific since a molar excess of unlabeled oleic acid almost abolished the peak. However, the oleic acid-binding capacity of this peak (50 μg) was lower compared with the 35 μg of purified FA-p34, analyzed under the same conditions (Fig. 1D), suggesting that reconstitution of the complex was only partial.

Measurement of FA-p34 from Polymorphonuclear Leukocytes—When cytosolic proteins of isolated human PMNL were

FIG. 5. Competitive binding studies on the [3H]oleic acid:FA-p34 complex. FA-p34 was incubated with 500 nM [3H]oleic acid; then, increasing concentrations of unlabeled FAs (10 nM to 100 μM) were used to compete with [3H]oleic acid. Bound radioligand was separated from free ligand by the charcoal-dextran technique (12). AA, arachidonic acid; LA, α-linoleic acid; LNA, γ-linolenic acid; PA, palmitic acid; SA, stearic acid.

FIG. 6. Influence of Ca2+ concentrations on the binding capacity of [3H]OA/FA-p34. FA-p34 was incubated with 500 nM [3H]oleic acid in the presence of increasing concentrations of EDTA (0–5 mM) to lower free calcium concentration in the mixture. Free Ca2+ concentrations were calculated for various reaction mixtures (x axis). Bound radioligand (in pmol/assay) was measured as in Fig. 5 (y axis).

analyzed by gel filtration using labeled oleic acid, a large radioactive peak of 34 kDa was detected. This peak was specific since it was almost abolished by an excess of unlabeled tracer (Fig. 8). This peak, composed essentially of MRPs, as analyzed by protein blotting (Fig. 3, A and B, lane 9), corresponded to FA-p34 from keratinocytes.

DISCUSSION

Properties of FA-p34—In this report, we describe the purification and characterization of a heterocomplex of 34 kDa (FA-p34) from human keratinocytes, formed by the non-covalent association of the two well known calcium-binding proteins MRP8 and MRP14. FA-p34 is capable of specifically binding unsaturated fatty acids with high affinity and differs structurally from common FABPs of 15 kDa (reviewed in Ref. 4). Arachidonic acid binds to FA-p34 in a saturating and reversible manner to a single class of binding sites with a calculated Kd of 0.13 μM, a value in the range described for FABPs (4). Fatty acid-competition studies on the [3H]OA/FA-p34 complex show that oleic, α-linoleic, γ-linolenic, and arachidonic acids have similar IC50 values of 1 μM, whereas palmitic and stearic acid, both saturated fatty acids implicated mainly in energy delivery and structural functions, or retinoic acid are poor competitors. Interestingly, the binding capacity of FA-p34 was found to be dependent on the free Ca2+ concentration. About 100 nM of free Ca2+ were necessary to obtain full fatty acid-binding capacity, whereas at 18 nM the binding property diminished about 50%.
Such a range in \( \text{Ca}^{2+} \) concentrations is currently thought to be physiological. Taking in account that keratinocytes do not express detectable amounts of other FABPs than the epidermal type (29, 30), which has no or low affinity for arachidonic and \( \gamma \)-linolenic acid (6), our data suggest that these fatty acids might be transported in a differentiating keratinocytes in a calcium-dependent way by the highly expressed FA-p34. Whether more specific, yet unknown carriers for unsaturated fatty acids might co-exist in keratinocytes remains to be determined.

The Subunits of the FA-p34 Heteromer—Partial amino acid sequencing and protein blotting with specific mAbs revealed that FA-p34 is composed of the subunits MRP8 and MRP14. However, the stoichiometry of the subunits in FA-p34 remains to be determined. Recently, the migration inhibitory factor-related proteins (reviewed in Ref. 9) MRP8 and MRP14 have been isolated and molecularly cloned from human neutrophils (27). Both proteins are members of the S100 family of proteins that contain two calcium-binding domains (reviewed in Refs. 10 and 28). MRP8 and MRP14 are found predominantly as a non-covalently associated hetero- or homodimer, and higher molecular weight forms have been detected (15, 18). Whether ionic domains, which by complementary affinity domain association allow the formation of homo- or heteromers (10). We hypothesize that the specific juxtaposition of the hydrophobic properties. S100 proteins contain two hydrophobic and two ionic domains, which by complementary affinity domain association allow the formation of homo- or heteromers (10). We hypothesize that the specific juxtaposition of the hydrophobic domains of MRP8 and MRP14 in a yet undefined stoichiometry allows the formation of a fatty acid-binding site.

Postulated Functions of FA-p34—FA-p34 levels are increased in keratinocytes that were induced to differentiate by extracellular calcium and in psoriatic skin, which displays higher than normal calcium concentrations and an alteration of the normal calcium gradient that programs keratinocytes’ terminal differentiation (32). These findings reinforce our in vitro observations that increased \( \text{Ca}^{2+} \) concentrations preserve fatty acid-binding properties of FA-p34. Moreover, the high FA-p34 levels found in psoriatic skin might also correlate with high metabolism of unsaturated fatty acids in this disease compared with normal skin (33).
FA-p34 is not solely a cytosolic complex, and its presence in particulate fractions, from where it can be released by high salt buffers, suggests that FA-p34 is associated with membrane components. This is especially the case in psoriatic scales, were almost 90% of total FA-p34 is membrane associated.

Although no definite function has been assigned to MRPs and MRP14, their expression in myeloid cells (up to 45% of total cytosolic protein of neutrophils) (34–36) as well as in epithelial cells of inflammatory (11–14) has suggested a role for MRPs in inflammation and differentiation (37). Preliminary data show that high levels of FA-p34 are also found in PMNL, suggesting that this complex is not specific for keratinocytes. Since unsaturated fatty acids play an important role for MRPs in inflammation and differentiation (37), our findings make FA-p34 a good candidate for mediating effects of unsaturated fatty acids in a calcium-dependent way.

Acknowledgments—Recombinant MRP8 and MRP14 were a generous gift from Dr. A. Suter (Novartis, Basel). Drs. J. A. Cox and M. Rossier are gratefully acknowledged for valuable discussions and help in Ca2+ level measurements and calculations. Dr. R. W. James is thanked for reading the manuscript. We also thank Dr. R. Schmidt for helpful discussions.

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Georges Siegenthaler, Karen Roulin, Dominique Chatellard-Gruaz, Raymonde Hotz, Jean Hilaire Saurat, Ulf Hellman and Gerry Hagens

J. Biol. Chem. 1997, 272:9371-9377. doi: 10.1074/jbc.272.14.9371

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