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Characterization of p40/GPR69A as a Peripheral Membrane Protein Related to the Lantibiotic Synthetase Component C

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The 40 kDa erythrocyte membrane protein p40/GPR69A, previously assigned to the G-protein-coupled receptor superfamily, was now identified by peptide-antibodies and characterized as a loosely associated peripheral membrane protein. This result is in striking contrast to the proposed seven-transmembrane protein structure and function and therefore we wish to correct our previous proposal. p40 is located at the cytoplasmic side of the membrane and is neither associated with the cytoskeleton nor lipid rafts. Refined sequence analysis revealed that p40 is related to the LanC family of bacterial membrane-associated proteins which are involved in the biosynthesis of antimicrobial peptides. Therefore, we rename p40 to LanC-like protein 1 (LANCL1) and suggest that it may play a similar role as a peptide-modifying enzyme component in eukaryotic cells.

Key Words: **erythrocyte; G-protein-coupled receptor; GPR69A; lanthionine synthetase; lantibiotic; peptide antibody; peripheral membrane protein; proteolysis; red blood cell.**

We have previously isolated and characterized the human erythrocyte membrane protein p40 (1), and its murine orthologue (2), which both contain seven hydrophobic domains with predicted α-helical transmembrane structure. Typically, seven-transmembrane proteins belong to the large superfamily of G-protein-coupled receptors (GPCR) (3). Although p40 did not show a significant similarity to any of the known GPCR subfamilies, several structural features of p40 were in accordance with GPCR characteristics. Analysis of the tissue- and cell-specific localization showed that p40 is mainly expressed in the brain and testis, in neurons, various epithelial cells, thymocytes, alveolar macrophages, and megakaryocytes, suggesting a function as a GPCR within the nervous and immune system (1, 2). Subsequently, human p40 was included in the GPCR superfamily and termed GPR69A by the Human Gene Nomenclature Committee. Updated methods for classifying GPCRs, however, failed to assert its membership (4). Recently, the sequences of orthologues from rat (Accession No. AJ131111), *Drosophila melanogaster* (Accession No. AAD38664), and *Arabidopsis thaliana* (Accession No. AAD20918) have been added to the GenBank database and many homologues from various plants appeared in the EST database, indicating that this protein plays a fundamental role in animals and plants.

The present data show now that p40 is not an integral, but a peripheral membrane protein, a finding that is incompatible with the proposed GPCR structure and function. Alternatively, refined sequence analyses reveal that p40 is composed of seven hydrophobic repeats, which are highly conserved in the eukaryotic homologues and the prokaryotic peptide-modifying enzymes LanC and LanM. This similarity to LanC suggests that p40 could play a role in the modification of human peptides.

MATERIALS AND METHODS

Reagents and cells. Human blood was obtained from healthy donors at the General Hospital Vienna. Red cells and ghosts were prepared routinely, as in (5), p40 was affinity-purified as described previously (1). Rabbit antisera were raised against the N- and C-terminus of p40, by immunizing with the octameric MAP peptides (Research Genetics, Huntsville, AL) AQRAFPNPYADYNKSLAEGY (H22 Ab) or ADLLVPTKARFPAFEL (R1 Ab), respectively. The antibodies were affinity-purified using either the MAP- or linear peptides coupled to activated CH-Sepharose (Pharmacia, Uppsala, Sweden), at 2 mg/ml gel, and acidic elution (0.1 M glycine–HCl, pH 2.5). Secondary antibodies conjugated with horseradish peroxidase, anti-mouse IgG (Promega, Madison, WI) and anti-rabbit IgG (Pierce, Rockford, IL), were used with the SuperSignal Chemiluminescent

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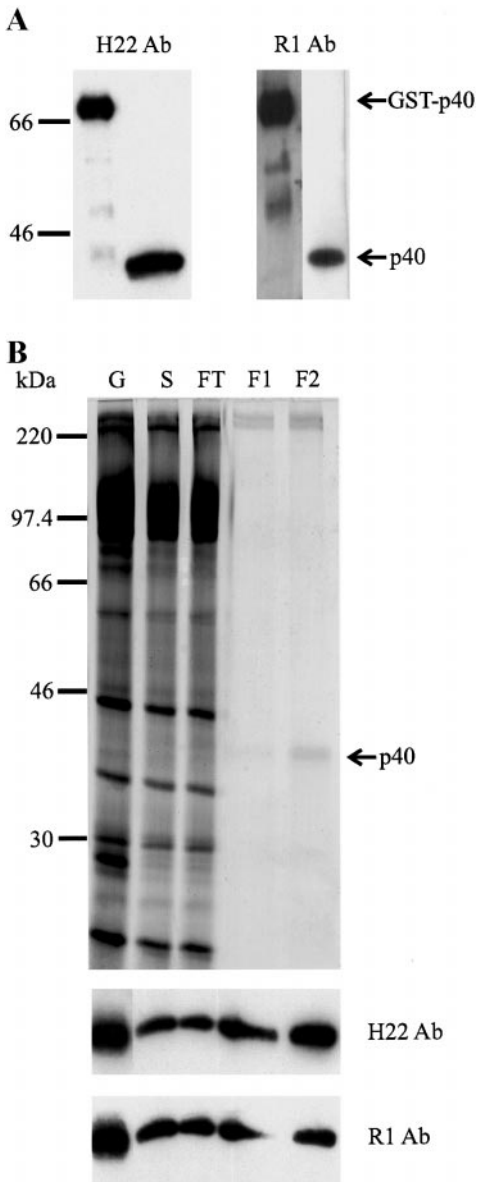

FIG. 1. Immunochemical identification of p40. (A) The affinity-purified peptide-antibodies H22 Ab (N-terminus) and R1 Ab (C-terminus) were tested with purified p40 and GST-p40 fusion protein by Western blotting. (B) p40 was isolated from a Triton X-100 extract of red cell ghosts by H22 Ab-Sepharose affinity chromatography and identified by silver staining (upper panel) and Western blotting using both antibodies (lower panels). Ab, antibody; GST, glutathione *S*-transferase; G, ghosts; S, supernatant; FT, flow-through, F1, F2, acidic elution fractions.

Substrate (Pierce). The monoclonal antibody against stomatin was described (5). A variety of other reagents were purchased commercially: monoclonal antibodies against band 3 and actin (Sigma, St. Louis, MO), human tissue samples/Protein Medleys (Clontech, Palo Alto, CA), Proteinase K (Roche Diagnostics, Mannheim, Germany). Recombinant GST-p40 purified from *E. coli* was a kind gift of Katharina Ronacher. Chemicals were reagent grade (Merck, Darmstadt, Germany).

Immunochemical identification. Proteins were identified by the standard methods SDS–PAGE/silver staining and Western blot analysis, as described (5), except that the SuperSignal (Pierce) detection was used for Western blotting. In one experiment, p40 was isolated by immunoaffinity chromatography (5) and identified by Western blotting.

Solubilization studies. Red cell ghosts were incubated with various buffers or detergent solutions, as indicated in the results, the incubation mixture was centrifuged (10,000*g*, 10 min, 4°C) and the pellet and supernatant fractions were dissolved in electrophoresis sample buffer. Aliquots of the fractions were analysed by Western blotting. In one experiment, washed, intact red cells were lysed with either hypotonic buffer (5 mM Na phosphate, pH 8.0), or 0.1% saponin in PBS, or by a freeze/thaw cycle (6). The lysed cells were separated from the supernatant ("cytosol") by centrifugation (28,000*g*, 20 min, 4°C) and were washed with cold lysis buffer, PBS, or buffered KCl solution (6), respectively. The primary and secondary red cell ghost pellets, the cytosol fractions and the wash solutions were analysed by Western blotting. p40 was precipitated from the cytosol at 40% ammonium sulphate saturation prior to analysis, in order to prevent high background due to hemoglobin.

Proteolytic degradation. Red cells were mixed with two volumes PBS or lysis buffer and the intact or disrupted red cells were incubated for 15 min at 37°C, with or without proteinase K (0.1 mg/ml final concentration). Subsequently, the intact red cells were washed three times with PBS, containing 1 mM phenyl methyl sulphonyl fluoride (PMSF), and lysed with five volumes of lysis buffer, 1 mM PMSF. The disrupted red cells were also mixed with five volumes of lysis buffer, 1 mM PMSF. Red cell ghosts from both incubation mixtures were then isolated by centrifugation (10,000*g*, 5 min, 4°C), washed once with lysis buffer, 1 mM PMSF, and analysed by Western blotting. In another experiment, red cell ghosts were prepared by hypotonic lysis, purified by five washing steps, and incubated for varying times at 37°C, with or without proteinase K (0.1 mg/ml) in lysis buffer. Aliquots of the incubation mixtures were heated with electrophoresis buffer and analysed by Western blotting.

Sequence analyses. The human p40 amino acid sequence (1) was compared to the sequences in the GenBank database. Twenty representative sequences were identified in the database searches using Psi-BLAST (7). Blocks conserved between these representative sequences were identified and aligned using MACAW (8). A global multiple alignment piled up with CLUSTALW (9), was used to calculate and draw a rooted phylogenetic tree with PHYLIP (10).

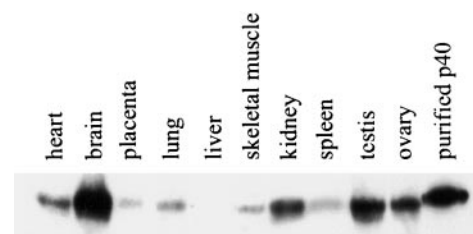

FIG. 2. Tissue-specific expression of p40. Ten human tissues (Clontech Protein Medleys) were applied to an SDS electrophoresis gel at 0.1 mg total protein per lane, along with 150 ng purified erythrocyte p40, and identified by H22 Ab Western blotting. The highest expression is seen in brain, followed by testis, ovary and kidney.

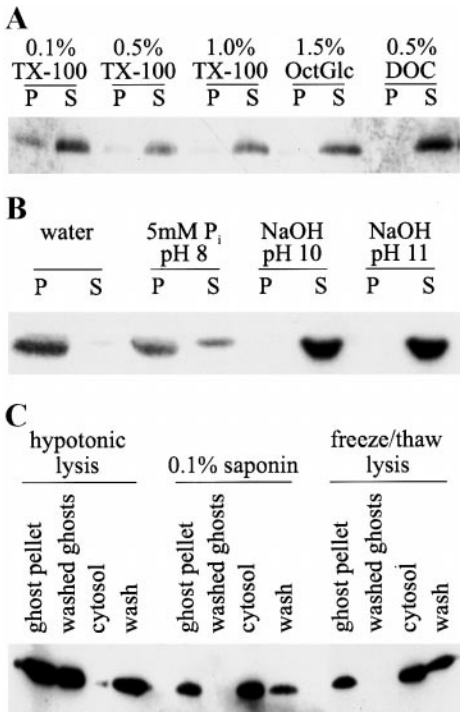

FIG. 3. Western blot analysis of p40 solubilization. Red cell ghosts were treated with (A) detergent or (B) alkaline solutions and pelleted. Pellets and supernatants were analysed by Western blotting. (C) Red cells were lysed by addition of hypotonic buffer, saponin in PBS, or by freezing/thawing, and the mixtures were separated by centrifugation into the ghost pellet and cytosol. The ghosts were washed with the corresponding buffer and separated again into the washed ghosts pellet and wash fraction. Aliquots of all fractions were analysed by Western blotting. TX-100, Triton X-100; OctGlc, *n*-octyl glucoside; DOC, Na desoxycholate; PBS, phosphate buffered saline; Pi, phosphate buffer; P, pellet; S, supernatant.

RESULTS

Immunochemical Identification and Tissue-Specific Expression of p40

Antibodies against the N- and C-terminus of p40 were used to identify the protein in various preparations and tissues. Purified p40 and recombinant GST-p40 fusion protein gave strong signals with both antibodies, at 40 kDa and 70 kDa, respectively (Fig. 1A). Additionally, p40 was H22 Ab-affinity purified from the Triton X-100 soluble red cell membrane fraction and was identified by SDS–PAGE/silver staining and Western blotting, using either H22 or R1 Ab (Fig. 1B). These results prove that both antibodies react specifically with red cell membrane p40.

Western blot analysis of various human tissues showed that p40 is mainly expressed in brain, testis, ovary, and kidney (Fig. 2), in accordance with the Northern blot and mRNA dot blot results previously described (1).

p40 Is a Peripheral Membrane Protein

When we treated red cell ghosts with non-ionic detergents, p40 was completely solubilized (Fig. 3A), indicating that this protein is neither associated with the cytoskeleton nor the lipid rafts. On the other hand, when we treated ghosts with various basic solutions, p40 was also solubilized (Fig. 3B), showing that it is not an integral membrane protein, as previously believed, but a peripheral protein. Even the red cell lysis buffer (5 mM Na phosphate, pH 8.0) could partially extract p40 from the membranes. Water was not efficient in solubilizing p40 (Fig. 3B). This indicates that p40 is a rather weakly associated peripheral membrane protein. The true solubility of p40 in alkaline buffer was further tested by ultracentrifugation. p40 was not pelleted after 16 h at 100,000*g* (data not shown), indicating that it is not associated with micro- or nanovesicles.

When red cells were hypotonically lysed, p40 was mainly bound to the membranes and only a small amount was found in the cytosol (Fig. 3C). However, when red cells were lysed with saponin in PBS or disrupted by a freeze/thaw cycle, p40 was completely soluble in the isotonic buffers (Fig. 3C).

Proteolysis of p40

When intact erythrocytes were incubated with proteinase K in PBS, p40 was not digested (Fig. 4A), as

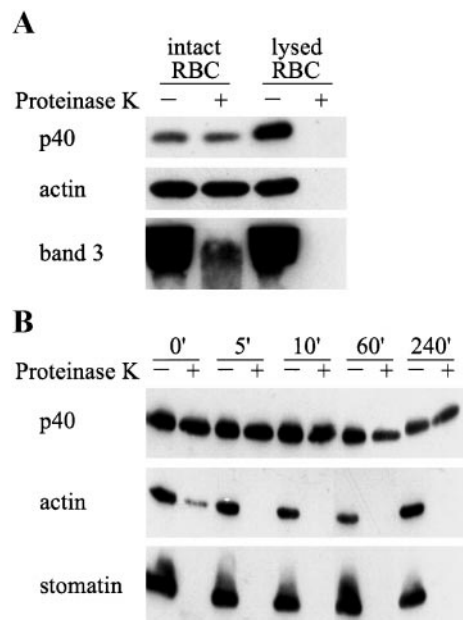

FIG. 4. Western blot analysis of p40 proteolysis. (A) Intact or lysed red cells were incubated with or without proteinase K and analysed by Western blotting. (B) Washed red cell ghosts were incubated with or without proteinase K in lysis buffer. At the indicated intervals, aliquots were taken and analysed by Western blotting. RBC, red blood cell.

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Consensus            -ly-G--Gi-l-l---       s-f-G--g--------       d-i-G--g----ll--
LANCL1_MOUSE  [ 59].TGYTGWAGIAVLYLHL.[29].TFLCGDAGPLAVAAVL.[29].EMLYGRIGYIFALLFV
LANCL1_HUMAN  [ 59].TGYTGWAGIAVLYLHL.[29].TFLCGDAGPLAVAAVL.[29].EMLYGRIGYIYALLFV
LANCL1_DROME  [ 54].DLYVGNAGIAFMFWKL.[38].SFLCGNAGIYAVSAAI.[35].EVLVGRAGYLSGCYWL
LANCL1_ARATH  [ 33].TLYSGTLGAAFLLFRA.[30].TFLCGRAGVCGLGAVA.[28].ELLYGRVGYLWACLFI
SPAC_BACSU    [ 51].ALSHGIPGICMLYGEL.[32].SMFSGAAGIGLAAICL.[39].DVIEGVSGIANYLLLF
NISC_LACLA    [ 37].TLSTGLPGIILMLAEL.[32].SLYSGAAGIALSILHL.[39].DVIEGLSGILSYLLLI
EPIC_STAEP    [ 77].TLSHGYPGIILFLSAS.[30].SLFSGLSGIGFALDIA.[38].DIIQGFSGIGRYLLNR
ECIC_STAEP    [ 34].SLITGYPGIAMTLFSI.[32].SLFEGAFGVIFSLLIC.[43].DIVSGCSGTLSVLLLA
PEPC_STAEP    [ 34].SLITGYPGIAISLFAI.[32].SLFEGAFGTIFSLLVC.[43].DIISGCAGTLSLLLLA
MUTC_STRMU    [ 42].SLSSGITSLLIFYQEY.[30].SLFDGLAGVGFAINYV.[40].DVISGNAGVARYLMER
SCF1_STRCO    [ 78].TLGSGSPGLALAFAGA.[30].GVFKGPGALAFAVLLA.[40].EVVRGLTGVGRYLLAR
MRSM_BACSP    [711].SLYEGVGGISLFLAYL.[30].GAFGGIASYLYLLDHL.[31].DILTGVAGTAVILINL
SACM1_STAAU   {626}.DLYSGRTGPFLALLLS.[33].LLMSGIGAFSGMSGII.[39].DMISGSSGAIIMRYKI
CYLM_ENTFA    {692}.NMYDGLPGIFIFYVAL.[29].SAFFGKGSLIYPLLVD.[33].DWIHGHNSIIKVLLLL
LCN2_LACLA    {579}.DIYKGISGIGLALLEY.[34].GFYVGLIGEYSFLRKY.[32].DVIAGEAGIIIYISNL
MUTM_STRMU    {565}.TIYKGIAGIKLAFLLS.[30].SFYEGTFGSQLPSYKE.[36].EIIEQYADYLVISLQK
SCNM_STRPY    {583}.SLYEGLSGIAISLSEA.[33].SFFVGKLGIYSALKRI.[37].EITALRNSDIKIDNLT
LASM_LACSA    {586}.PGLYNSVGFLLSLWRY.[42].SVFSGLGGLLYCSCYL.[34].EYLNGISGLLLLFQNL
LCNDR2_LACLA  {606}.CSFHDSGGIITLLEHH.[29].SIISGSESLEFLYTHR.[19].DVFLGKLGLYLILASY
ORF6_BUTFI    {569}.SLYSGISGIGLMYYTE.[34].SYFCGLTGIYSFIKKV.[29].DSLSGVHSAVIYYYGD

Consensus            g-AHG--G----l---       -WG-G--Gi---l---       -lCHG--G-l--ll--
LANCL1_MOUSE  .[50].GAAHGLAGIYYYLMQP.[42].HWCHGAPGVIYMLIQA.[30].GLCHGAAGNAYAFLAL
LANCL1_HUMAN  .[50].GAAHGLAGIYYYLMQP.[42].HWCHGAPGVIYMLIQA.[30].GLCHGSAGNAYAFLTL
LANCL1_DROME  .[48].GAAHGLCAILHMLLDS.[51].HWCHGAPGAVYVLAKA.[30].GICHGVAGNGYVFLLL
LANCL1_ARATH  .[46].GAAHGLAGIMHVLMDV.[40].HWCHGAPGIALTLGKA.[29].GICHGISGNAYVFLAL
SPAC_BACSU    .[57].GLAHGIPGPICVLSSA.[57].AWCYGRPGVCLALVKA.[30].TICHGYSGIGQILLAV
NISC_LACLA    .[52].GLAHGLAGVGCILAYA.[57].AWCYGGPGISLLYLYG.[30].MICHGYSGLIEICSLF
EPIC_STAEP    .[51].GLAHGILGPLSLTALS.[50].GWCYGDTGIMNTLLLS.[31].TFCHGLASHLTIIHQA
ECIC_STAEP    .[43].GYAHGMPGVINILCNS.[46].AWCYGMPSVAYTIYNI.[33].TLCHGFSGIVIISLIM
PEPC_STAEP    .[43].GYAHGIPGIINTLCNS.[46].AWCYGLPSVAYTIFNV.[33].TLCHGFSGVVMISLLM
MUTC_STRMU    .[47].GLAHGLLGPTTIMSLY.[51].GWCYGDNGIYSTLFLM.[31].TFCHGFAGKANFFLLQ
SCF1_STRCO    .[54].GLSHGVAGPLALLALA.[57].AWCYGAPGVSRALQLA.[33].ALCHGWAGALHQLGRL
MRSM_BACSP    .[43].GFAHGASGIIWALYEI.[47].AWCNGAAGIGLSRILI.[29].SLCHGDLGNLDILMYA
SACM1_STAAU   .[30].GLAHGISQIMYFFAII.[44].SWCNGLSGLLVSYYEG.[27].ILCHGSLGVYDSLKYV
CYLM_ENTFA    .[29].GFGHGIYSYVHLLSKF.[25].SWCKGTVGELLATIEL.[20].CLCHGNAGTLEGLIQL
LCN2_LACLA    .[28].SYAHGNSGIATAFVHG.[41].QWCHGASGQAIARMEW.[39].CLCHGILGNLLILNTY
MUTM_STRMU    .[19].SFAHGNSGCMTALLIS.[41].NWCHGSTGALTSRLLW.[39].SLCHGIMGNLIALNEY
SCNM_STRPY    .[27].SLAHGNLGVEIALLYL.[39].NWCHGSTGVLVARLAQ.[39].SLCHGTSGNLLALTYY
LASM_LACSA    .[35].GIGHGISGKLIGMSVL.[31].SWCRGTLGILDMLLIN.[34].SMCHGLGGIINFITSV
LCNDR2_LACLA  .[24].GIAHGELGYLWTIFRI.[29].GWCNGLSGILMILSEM.[28].SVCHGASGVLQTLLFV
ORF6_BUTFI    .[34].SFAHGYTGLMTSLLCM.[42].YWCHGSCGIMQARLFW.[ 5]

Consensus            gl--G--G-ll-ll--
LANCL1_MOUSE  .[35].SLFEGMAGTIYFLADL.[13]
LANCL1_HUMAN  .[35].SLFEGMAGTIYFLADL.[13]
LANCL1_DROME  .[36].SLYEGVAGTVCYLVDL.[15]
LANCL1_ARATH  .[40].SLFEGVAGMAYLFLDM.[13]
SPAC_BACSU    .[49].GLLDGAVGVGLGVLNM.[14]
NISC_LACLA    .[33].GFLEGISGCILVLSKF.[26]
EPIC_STAEP    .[48].GILEGELGVLLALLDY.[18]
ECIC_STAEP    .[38].GLLNGNSGILLTMLSF.[16]
PEPC_STAEP    .[38].GLLNGNAGILLTLLSY.[17]
MUTC_STRMU    .[52].GLLSGTVGVLLVLMEY.[19]
SCF1_STRCO    .[46].GYLDGAAGVALALDSY.[16]
MRSM_BACSP    .[39].SLMMGYAGIGLGLLKI.[20]
SACM1_STAAU   .[40].GLMTGKTGALLHLCKL.[14]
CYLM_ENTFA    .[40].GFFVGISGVGYELLRN.[13]
LCN2_LACLA    .[42].GLMTGISGILYGLIRQ.[15]
MUTM_STRMU    .[40].GLMTGLAGILYGIIKI.[16]
SCNM_STRPY    .[43].GLMTGVSGILFSTVKY.[14]
LASM_LACSA    .[35].SYMLSTTGLVDSILRY.[16]
LCNDR2_LACLA  .[40].GYFQGWSGFTDSALLL.[40]
ORF6_BUTFI
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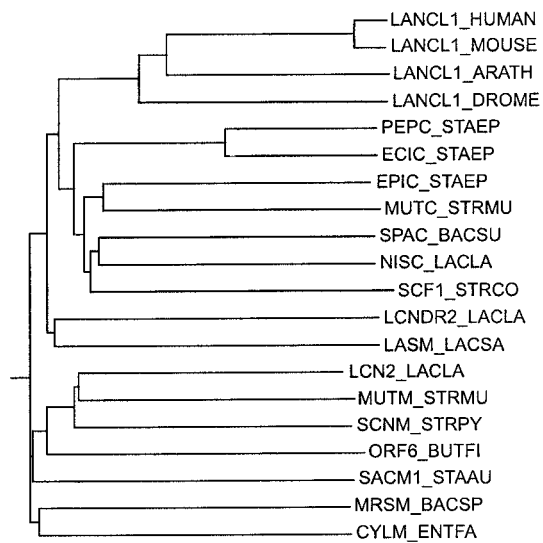

expected of a cytoplasmically oriented peripheral membrane protein. The control protein actin was not degraded, band 3 protein was cleaved at extracellular sites (Fig. 4A). On the other hand, the incubation of disrupted red cells with proteinase K led to the complete degradation of p40 and the control proteins (Fig. 4A).

Interestingly, when washed ghosts, prepared by hypotonic lysis, were incubated with proteinase K in lysis buffer, p40 was highly resistant for 4 h. In contrast, the control proteins actin and stomatin were rapidly degraded (Fig. 4B). This result suggests that low salt conditions induce a protease-resistant conformation in p40.

p40 Is Similar to the Bacterial Lantibiotic Synthetase C Components (LanC)

Searching the GenBank database for homologues of mammalian p40, we found related eukaryotic proteins from *D. melanogaster* and *A. thaliana* with unknown function, but also two families of prokaryotic proteins, LanC and LanM, which are involved in the biosynthesis of lantibiotic peptides (11). Interestingly, these proteins have seven repetitive hydrophobic domains containing a GxxG motif (Fig. 5), as described for p40 (1). It seems evident that LanC, LanM and p40 are members of the same superfamily of proteins and therefore we rename p40/GPR69A to LanC-like protein 1 (LANCL1). In addition to the general repeat structure of the seven hydrophobic domains, several residues appear invariable in single repeats of all analysed species: there is an acidic residue in repeat 3, His in repeat 4, Trp and Cys in repeat 5, and Cys and His in repeat 6 (Fig. 5). It is likely that these residues are essential for the function of the LanC-related proteins. A rooted phylogenetic tree based on a global multiple alignment indicates the relationship between the members of this superfamily (Fig. 5, inset). A tree calculated from the alignment shown in Fig. 5 does not suggest a significantly different family organization (data not shown).

DISCUSSION

Previously, the immunochemical and proteinchemical characterization of p40 was hampered, because specific antibodies were not available. Only recently we were able to produce two specific peptide-antisera, which identified p40 in red cells and various human tissues (Figs. 1 and 2), in accordance with the published protein isolation and tissue-specific mRNA expression data (1). Unexpectedly, we found that p40 can be extracted from red cell membranes by alkaline solutions (Fig. 3), thereby classifying it as a peripheral membrane protein. This result is in striking contrast to the proposed GPCR structure and function, which has been suggested by the presence of seven hydrophobic domains with predicted helical secondary structure and other similarities to the known features of GPCRs (1).

The analysis of p40 solubility and proteolysis showed that this protein is cytoplasmically oriented and neither associated with the cytoskeleton nor the detergent-insoluble lipid domains. p40 solubility is reminiscent of the erythrocyte band 6 properties (12) by showing tight membrane binding in hypotonic buffers and solubilization in isotonic solutions. Band 6 is a glycolytic enzyme (glyceraldehyde-3-phosphate dehydrogenase), which binds to the N-terminal cytoplasmic domain of band 3 (13). The binding partner of p40 is currently unknown, however, its identification will probably shed light on the function of p40. Interestingly, p40 was highly resistant to proteolysis in washed ghosts under hypotonic conditions, but readily digested in hemolysate (Figs. 3 and 4) suggesting that this protein may undergo a structural change during the hypotonic lysis and subsequent washing steps, probably due to the low ionic strength. An ionic strength-dependent structural change has been described for the band 3 protein (14).

Database searches revealed the similarity of eukaryotic p40 to two prokaryotic protein families, collectively

FIG. 5. Multiple alignment of representative LanC-like domains, each containing seven hydrophobic sequence repeats around the GxxG consensus motif. Numbers in square brackets indicate the lengths of unaligned regions not displayed. Consensus residues are indicated on top of the alignment, in uppercase if the weighted (19) frequency of the most frequent residue is 80% or more, in lowercase for the interval between 40 and 80% weighted frequency. Glycine residues which are part of the GxxG-motif are highlighted as white text on black background, as are completely conserved residue positions. Grey shading indicates residues with apolar and/or aromatic sidechains in columns where they are found in 15 or more of the 20 representative sequences. The alignment contains four eukaryotic sequences and 16 sequences from prokaryots (seven LanC, nine LanM). ORF6_BUTFI is incomplete at its C-terminus, missing two of the expected seven repeats. The proteins aligned are (name and gi accession numbers in parentheses): LANCL1_MOUSE (mouse LANCL1, 3492793), LANCL1_HUMAN (human LANCL1, 5174445), LANCL1_DROME (*Drosophila melanogaster* LANCL1, 5052668), LANCL1_ARATH (*Arabidopsis thaliana* LANCL1, 4454471), SPAC_BACSU (*Bacillus subtilis* SpaC, 417797), NISC_LACLA (*Lactococcus lactis* NisC, 417367), EPIC_STAEP (*Staphylococcus epidermidis* EpiC, 1169542), ECIC_STAEP (*S. epidermidis* EciC, 2708730), PEPC_STAEP (*S. epidermidis* PepC, 2126592), MUTC_STRMU (*Streptococcus mutans* MutC, 5918761), SCF1_STRCO (*Streptomyces coelicolor* SCF1, 5869949), MRSM_BACSP (*Bacillus sp.* MrsM, 6318175), SACM1_STAAU (*Staphylococcus aureus* SacM1, 5690276), CYLM_ENTFA (*Enterococcus faecalis* CylM, 1075688), LCN2_LACLA (*L. lactis* LCN2, 585385), MUTM_STRMU (*S. mutans* MutM, 2853236), SCNM_STRPY (*Streptococcus pyogenes* ScnM, 2502070), LASM_LACSA (*Lactobacillus sakei* LasM, 1150480), LCNDR2_LACLA (*L. lactis* LCNDR2, 3582217), ORF6_BUTFI (*Butyrivibrio fibrisolvens* unknown protein, 3201688). A rooted phylogenetic tree based on a global multiple alignment is shown in the lower right corner (inset).

known as LanC and LanM (Fig. 5). These proteins are components of a multimeric membrane-associated lanthionine synthetase complex, consisting of the enzyme subunits LanB and LanC (or LanM), the ABC transporter LanT, and regulatory factors (11, 15, 16). This complex is necessary for the production and export of lantibiotics (11), uniquely modified lanthionine-containing antimicrobial peptides. While these are produced by Gram-positive bacteria, functionally similar defense peptides exist in higher organisms, like plants, insects and vertebrates, and play an essential role in the innate immune system (17, 18). In the light of these data, it is conceivable that p40 / LANCL1 may have a function similar to LanC and LanM as a peptide-modifying enzyme.

ACKNOWLEDGMENT

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