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Fatty acid acylation of lens fiber plasma membrane proteins

MP26 and α-crystallin are palmitoylated

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We describe in this report the fatty acylation of some of the main polypeptides from the eye lens fibers. MP26, the major lens fiber plasma membrane protein, and probably MP22, its natural degradation product, are palmitoylated in a post-translational process. This is also the case for α-crystallin, a major cytoplasmic structural protein shown to interact directly with the plasma membrane. Furthermore, a 65 kDa non-identified polypeptide and a high molecular weight component are also modified in the same way.

Fatty acylation; Lens fiber; MP26; α-crystallin

1. INTRODUCTION

Fatty acylation of proteins, i.e. the covalent binding of fatty acid molecules, was discovered and extensively investigated for a few years [1–3]. Intrinsic as well as extrinsic membrane polypeptides have been shown to be acylated (for a recent review, see [4]) in two distinct enzymatic processes. Although their role(s) is/are not well known, such modifications are expected to change the physical properties of the proteic entities, and in this way, modify their potential interactions with the other constituents of the cell, particularly those from the plasma membrane.

Two major types of fatty acylation have been previously described: one involves the covalent attachment of myristic acid (myristylation) to the protein, through an amide linkage, in a co-translational process. The other one is a post-translational event, involving palmitic, oleic or stearic acids, and occurs through an ester or thio-ester linkage.

In a recent work, Hertzberg et al. [5] described the fatty acylation of the 43 kDa gap junctional protein from heart, suggesting that fatty acylation may be important for gap junction formation and stabilization. We now describe palmitoylation of MP26, the major membrane protein from eye lens fibers, shown to be implicated in gap junction formation in these cells [6]. Furthermore, α-crystallin, a cytoplasmic protein is also palmitoylated.

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2. MATERIALS AND METHODS

2.1. *Radiolabelling and sample preparation*

One-year-old rats were sacrificed and their lenses immediately dissected under sterile conditions. Each complete lens (with its capsule and its epithelium) was incubated for 6 h in 0.5 ml of Eagle's minimum essential medium (modified with Earles salt and 2 g/l $NaHCO_3$) (Gibco) supplemented with 10% dialysed and delipidated [7] fetal calf serum (FCS; Eurobio, Paris) and 50 μl of penicillin (10000 IU/ml)/streptomycin (10000 UG/ml) solution (Gibco). The incubation was performed at 37°C in the presence of either 100 μCi of [^{3}H]amino acid mixture or 200 μCi of [^{3}H]myristic acid resuspended in ethanol, or 500 μCi of [^{3}H]palmitic acid (Amersham). The lenses were then removed from the incubation mixture, and their epithelium torn loose from the cortex with fine forceps under a dissection microscope. The most external cortical fibers were extracted in PBS, pH 7.3, by mechanical agitation in a vortex. They were washed twice in 1 mM $NaHCO_3$, with a mild potter homogenization at each step. The resulting pellets were resuspended in 50 μl of electrophoresis sample buffer according to Laemmli [8]. Verification of the radioactivity incorporation into the fibers was performed by counting 5 μl of the fibers dissolved in sample buffer by liquid scintillation in a 1 N intertechnique SL4000 counter.

2.2. *PAGE-SDS, immunoblotting and autoradiography*

PAGE-SDS of the different fractions was performed in 12.5% polyacrylamide gels according to Laemmli [8]. Gels were stained with Coomassie blue, incubated for fluorography in Enhancer (Amersham) for 30 min, dried, and placed in cassettes with Hyperfilm MP (Amersham) for 4 weeks at −70°C. In some cases, they were incubated in 1 M hydroxylamine for 18 h before being processed as described above. Immunoblotting was performed as previously described [9], and MP26 detected with a specific polyclonal antibody previously characterized [6]. After revelation with alkaline phosphatase the resulting immunoblots were autoradiographed with Hyperfilm-^{3}H (Amersham) for 4 weeks, to facilitate correlation between immunoreactive bands and radiolabelling.

3. RESULTS

In order to measure the rate of fatty acid incorpora-

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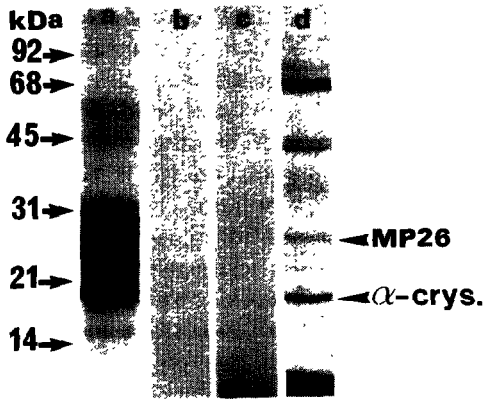

Fig.1. Radiolabelling of whole lenses was performed either with [^{3}H]amino acid mixture, or [^{3}H]myristate, or [^{3}H]palmitate. A plasma membrane fraction of lens external fibers was prepared after the incubation. (a) PAGE-SDS of this fraction in the case of palmitate labelling (the patterns are the same after the incubation with amino acids or myristate). (b–d) Autoradiograms obtained after amino acids (b), myristate (c), and palmitate radiolabelling (d). Low molecular weight markers from BioRad were used.

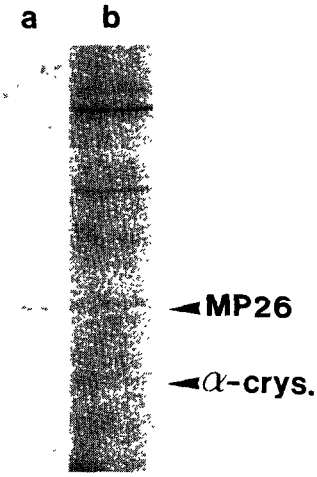

Fig.2. (a) Immunoblot of fig.1, lane a, with a polyclonal specific antibody against MP26, and (b) corresponding autoradiogram.

tion in our system, liquid scintillation counting was performed with sample buffer-solubilized membrane fractions. We could determine that 1% of the palmitate and 0.5% of the myristate initially present in the incubation vials were recovered in the membrane-cytoskeleton fractions. The electrophoretic pattern of the fraction incubated with palmitate is presented in fig.1, lane a. A similar pattern was obtained after amino acids or myristate labelling (not shown). The corresponding autoradiograms are presented in lanes b, c, and d for amino acids, myristate and palmitate, respectively. Protein biosynthesis cannot be detected in our system, as can be seen in lane b, where no labelled bands were present. Myristate incorporation into the membranes was confirmed in lane c where labelling probably due to free fatty acid molecules was observed in the front of the gel. However, no radioactive polypeptidic bands were detected in this fraction, implicating that myristylation does not occur. In contrast, the incubation with [^{3}H]palmitate led to the fatty acylation of at least 6 distinct polypeptides (lane d): one, of high molecular mass, is not separated in these conditions, and the others are of 65, 45, 26, 22–23, and 20 kDa. Incubation of the gels in 1 M hydroxylamine prior to fluorography led to a complete loss of radioactivity, indicating the thio-ester nature of the link between the polypeptides and the palmitate molecules. The palmitoylated polypeptide migrating at 26 kDa was identified as MP26, the major lens fiber plasma membrane protein, by immunoblotting with a specific antibody against this polypeptide: the immunolabelling obtained on the palmitate-labelled fraction (fig.2, lane a), coincides exactly with the 26 kDa radioactive band in the corresponding autoradiogram (lane b), implicating that MP26 is palmitoylated in these experimental conditions.

4. DISCUSSION

We have investigated the palmitoylation of plasma membrane and cytoskeleton polypeptides in cortical lens fibers. Protein biosynthesis cannot be detected in these cells. This may be due either to a lack of penetration of radiolabelled amino acids into the fibers, or to metabolic inactivity in these cells we worked with. Protein biosynthesis in the lens was shown to occur essentially in the elongation zone [10] where the epithelial cells differentiate into fibers and to be reduced in the cells present deeper in the cortex. The elongation zone was removed from the cortex together with the capsule and the epithelium after the incubation, and protein biosynthesis can be detected in the cells from this region. This should mean that the present results were obtained with fibers from a deeper region of the cortex, where protein biosynthesis is reduced, if not absent. However, we do not rule out the possibility that the amino acids were not incorporated into the cells. The absence of detectable myristylation in this work is significant, since myristate penetration into the cells occurred correctly. Myristylation was shown to be a co-translational process [4], and the absence of both protein synthesis and myristylation in our system is in good accordance with this data.

In contrast, palmitoylation was shown to be a post-translational event [4] as can be confirmed from our present results. We could identify one of the radioactive bands as MP26, the major protein of lens fiber plasma membrane, shown to be a component of gap junctions in the lens [6]. The 43 kDa heart gap junction constituent has recently been shown to be palmitoylated [5].

Three cysteine residues are present in MP26. According to the model of Gorin et al. [11], Cys-88 would be a good candidate for the palmitoylation site, since it is close to the surface of the bilayer. The electrophoretic pattern of lens fiber plasma membrane has been well documented [12]. Interestingly, MP26 undergoes a natural proteolytic degradation yielding a stable membrane-associated product of 22 kDa called MP22. Since Cys-88 is present in MP22 [11], it is likely that this polypeptide will also be a substrate for the palmitoylating enzyme. This should explain the presence of the 22–23 kDa radioactive band in the gel. Whether the proteolytic degradation and the palmitoylation event can be related one to another is under investigation.

The fatty acylation of a 20 kDa component is also demonstrated in this work. As precedently evoked, the lens fiber plasma membrane electrophoretic pattern is well known, and a major well-defined component of 20 kDa was identified as α-crystallin, a cytoplasmic structural protein shown to have direct interaction with the plasma membrane [13,14]. The covalent attachment of a hydrophobic aliphaatic chain may be of crucial importance for anchoring the protein onto the membrane, and indirectly, for lens transparency.

Of more difficult interpretation is the fatty acylation of 45 and 65 kDa components. We had previously shown that molecular aggregates of MP26/MP22 family could be detected by immunoblotting [6], one of them with a 45 kDa molecular mass. This can be one explanation for the 45 kDa radioactive band, but we cannot rule out the presence of an acylated non-identified component with this molecular mass. We did not directly identify the 65 kDa acylated component. However, a 70 kDa minor membrane component (MP70) was recently isolated, and shown to participate in gap junction formation in the lens [15]. When membrane extraction is performed, a 64 kDa degradation product is mainly recovered [16]. Whether the 65 kDa radioactive band can be attributed to this product is under investigation.

The acylation ratio, i.e. the amount of radioactivity incorporated per mass of protein, varies dramatically from one component to another. MP26 seems to be poorly acylated when compared, for example, with the 65 kDa component, which gives a heavy band on the autoradiogram but is practically not detected by Coomassie blue staining. This non-stoichiometric acylation of MP26 can reflect two types of cellular events: one should be that only a subpopulation of MP26 is acylated in vivo, with a specific role in the formation of membrane domains, and, for example, in gap junction assemblies. The other explanation can be related with the incubation conditions in this work: fatty acylation can be a chronologically well programmed event, dependent on cell differentiation and aging. We do not know what the level of natural acylation of MP26 in the fibers we worked with is, i.e. whether the sites of acylation are still accessible to radiolabelling with palmitate or blocked by a previous intrinsic acylation. This problem is under investigation.

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