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Minireview

Photochemical labeling of membrane-associated and channel-forming domains of proteins directed by energy transfer

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Abstract

Singlet–singlet energy transfer reactions from excited tryptophan residues to photoactivatable probes possessing a suitable chromophore, generate reactive species in the vicinity of the protein, leading to its covalent labeling. This delayed labeling process can be used to map the membrane-surrounded regions of proteins with improved efficiency when it is applied with appropriate photoactivatable phospholipids. The same principle could also be applied to the labeling of channel-forming transmembrane domains of ion channels, provided that suitable photoactivatable permeant ions were available. Both applications will be discussed with regard to their potential and feasibility.

Key words: Energy transfer; Photoactivatable phospholipid; Photoactivatable permeant; Hydrophobic labeling; Photolabeling

1. Introduction

Ligand–receptor interaction studies are based on the specific recognition of a ligand (generally a small molecule) by a functional receptor (generally a protein) at the ligand binding site. Structural information on this binding site can be obtained through site-directed irreversible labeling studies by using chemically or photochemically reactive ligand analogs. These analogs are designed in such a manner that they will react covalently with the target proteins at their site of interaction. However, other protein domains which are not subjected to specific interaction with modulators might also play important roles. In particular, the membrane-associated domains of proteins often condition and modulate their function and the channel-forming transmembrane domains of ion channels are directly involved in signal transduction. How to probe such protein domains which have no specific interaction with ligands? A protein-mediated, energy transfer-directed photolabeling might constitute an answer to this question and is proposed successfully for the labeling of the membrane-surrounded regions of proteins and the channel-forming domains of ion channels.

2. Irreversible labeling induced by Trp-mediated energy transfer photoactivation

This labeling concept was originally developed [1] for a preferential photoactivation of a ligand analog when it is complexed with its receptor at a binding site, resulting in a site-specific covalent labeling reaction. The discrimination during the photoactivation step was based on the strong distance-dependence between the donor (Trp residue) and the acceptor (photosensitive ligand analog) in energy transfer reactions. The singlet–singlet energy transfer reaction requires the presence of the donor within or in close proximity to the binding site. The acceptor should have appropriate spectral characteristics: it should absorb as weakly as possible at the Trp excitation wavelength ($\lambda \sim 290$ nm) and exhibit the best spectral overlap with the Trp emission wavelength ($\lambda_{max} \sim 320$–340 nm). The main difficulty in the search of such photosensitive chromophores was to fulfill these two criteria at the same time.

Scheme 1 shows three candidate chromophores which have the appropriate spectral characteristics for Trp-mediated, energy transfer-induced photoactivation, leading to highly reactive species (carbenes and carbocations). This photoaffinity labeling strategy was first successfully demonstrated on the irreversible inactivation of acetylcholinesterase (AChE) by DDF (*para*-dimethylamino benzene diazonium fluoroborate), a competitive inhibitor of AChE. The labeling of AChE by DDF led to an increased specificity and efficiency when it was performed under Trp-mediated energy transfer conditions as compared to results obtained under the usual photoaffinity irradiation conditions [1]. The presence of several Trp residues, either within the active site of the enzyme or in close proximity to it, was suggested earlier by delayed fluorescence experiments [2] and recently confirmed by X-ray structural analysis of AChE [3]. A series of other successful site-directed labeling experiments

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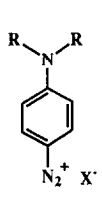
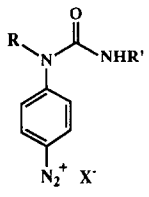
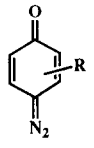
chromophores	 1	 2	 3
λ max (nm)	≈ 380	≈ 360	≈ 350
ε max ($l.mol^{-1}.cm^{-1}$)	> 35,000	> 25,000	> 15,000
ε 290nm ($l.mol^{-1}.cm^{-1}$)	< 1,000	< 1,000	≈ 2,000

Scheme 1. Spectral charateristics of three chromophores as acceptors for the Trp-mediated energy transfer reaction.

which are based on Trp-mediated energy transfer have since been described with Na^+,K^+ATPase [4], and the nicotinic [5] and muscarinic [6] acetylcholine receptors.

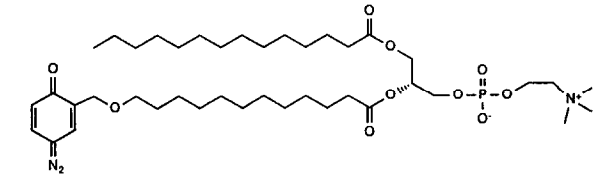
3. Labeling of membrane-associated domains of proteins

The hydrophobic photolabeling approach has been developed to enhance the structural information on membrane-associated domains of proteins [7–9]. Photoactivatable hydrophobic reagents fall in to three main classes: simple hydrophobic, amphipatic and phospholipid probes. When these reagents are activated by light, they generate reactive species which react irreversibly with the membrane constituents. The use of radioactive probes allows further structural analysis by identification of the labeled membrane–peptide fragments and can eventually reach the molecular level by characterization of the labeled amino acid residues. The photoactivatable phospholipid probes introduced by Khorana and coworkers [10] have the advantage of closely resembling the natural membrane components and were initially expected to allow depth-dependent labeling studies. Evidently, a correlation between the position of the photosensitive moiety on the phospholipid chain and the depth of anchoring of the protein in the membrane could not only afford valuable structural information but also be very useful in studying dynamic processes such as insertion and translocation of proteins through the lipid bilayer.

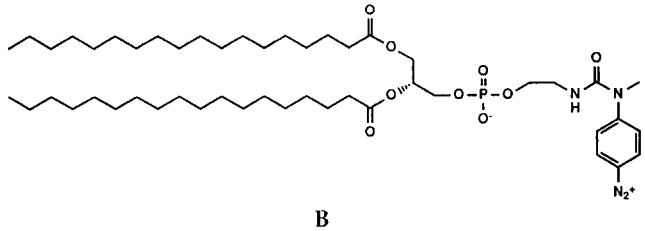
However, a series of pitfalls emerged from these studies [7–10]. These can be summarized as follows: (i) the distribution of labeling on the neighbouring phospholipids revealed no depth-dependence when the photoactivatable phospholipids were used; (ii) a preferential reaction with nucleophilic amino acids was observed during the labeling of proteins; (iii) the coupling yields of the probes to the target proteins vs. the surrounding lipids are generally low. To elude these difficulties, new strategies are necessary. First, transmembrane probes have been suggested [11–13] to best achieve the depth-dependent labeling. They should achieve the required rigid transmembrane insertion and avoid a 'U-shaped' incorporation in to the membrane. Alternatively, it has been shown that the addition of a large proportion of cholesterol to the membranes (up to 33% molar concentration) increased sufficiently the rigidity of the probe to achieve a depth-dependent labeling [14]. Second, the observed preferential coupling to nucleophilic amino acids is a matter of intrinsic chemical reactivity of the photogenerated species. As a general rule, carbenes or carbocations should be preferred to nitrenes as labeling tools, although recently described fluoro arylazido derivatives [15] have also been demonstrated to be powerful photoaffinity reagents. Finally, the low yields of labeled proteins reflect a low statistical distribution of the protein in the membrane. To overcome this problem, we propose to use protein-mediated, energy transfer photoactivation.

4. New photoactivatable phospholipids for efficient probing of membrane-associated protein domains

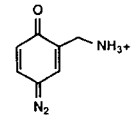
The labeling methodology which uses Trp-mediated energy transfer, allows a preferential photoactivation of



A



B



C

Scheme 2. Photoactivatable probes for Trp-mediated energy transfer-directed photolabeling. Probes A and B are phospholipid analogs designed to label the membrane-associated protein domains in the membrane core and at the membrane–water interface, respectively; probe C is a permeant designed for the labeling of cationic ion channels.

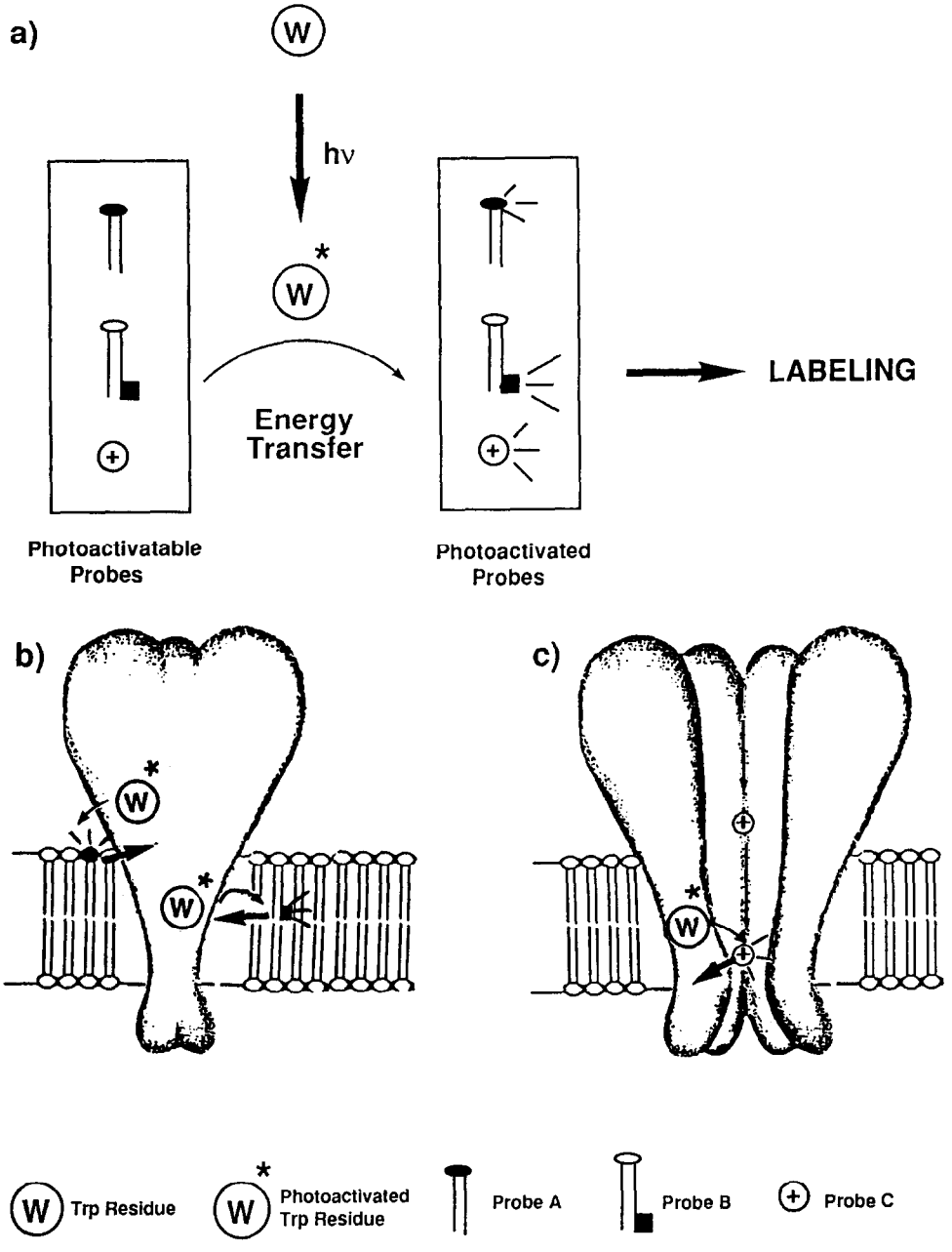


Fig. 1. (a) Principle of Trp-mediated energy transfer photolabeling. (b) Photolabeling of membrane-associated domains of proteins in the membrane core by probe A and at the membrane–water interface by probe B. (c) Photolabeling of channel-forming regions of ion channel proteins by probe C.

a probe positioned in close proximity to a protein. Consequently, an increased coupling yield of the probe to the neighbouring protein is expected. Considering the energy transfer requirements for probes as acceptors, two of the chromophores shown in Scheme 1 have been selected as candidates to be incorporated in to the phospholipids (Scheme 2).

Probe A, a diazocyclohexadienyl phospholipid compound, should allow efficient labeling of proteins in the middle of the membrane core by using the cholesterol-enriched membrane strategy [14]. The diazocyclohexadienyl moiety is neutral and should allow the incorporation of such a lipid probe into the membrane. The high chemical reactivity of the photogenerated carbene [16] should guarantee a non-discriminative labeling of all amino acid residues present in the vicinity of the probe.

Probe B, a phospholipid analog bearing a positively charged *para*-acylamidobenzene diazonium at the polar head, was synthesized to efficiently probe the proteins at the lipid–water interface through Trp-mediated energy transfer. Several aryldiazonium derivatives have been used as photosensitive analogs of cholinergic quaternary

ammonium ions [1,5,6,17], therefore probe B might best substitute for phosphocholine lipids. Finally, the hyperreactivity of the photogenerated aryl cations [18] ensures an efficient labeling of its entire environment.

Both probes A and B should therefore fulfill the physico-chemical criteria necessary to achieve efficient labeling of membrane-associated regions of proteins within the membrane core or at the membrane–water interface. Incorporation of radioactive atoms has been foreseen for future labeling experiments and has been integrated in the synthetic schemes for both probes. Fig. 1 illustrates the principle of the energy transfer-directed labeling using these two probes. The comparison of the yield of labeled protein by energy transfer-mediated photoactivation with that achieved by a usual photoactivation process requires controled irradiation conditions. All the previous comparative quantification experiments [1,5,6] have shown markedly improved coupling yields by energy transfer photoactivation.

5. Labeling of channel-forming transmembrane domains

The channel-forming transmembrane domains are an essential functional feature for ion channel receptors such as transmitter-gated ion channels. Based on the ligand–receptor interactions, photosensitive channel blockers have been used to irreversibly label the channel entrances. For instance, chlorpromazine [19] and the aryldiazirine derivative, TID [20], have been used to label the ion channel entrance of the nicotinic receptor, indicating involvement of the putative M2 transmembrane segments from all the pentameric receptor subunits in the ion channel formation.

How to probe an ionic channel along its entire depth? This can theoretically be achieved by using photosensitive permeants. Several major difficulties emerge immediately from this approach: (i) the photosensitive molecules have to be small in size and carry an appropriate charge in order to mimic the ions and to permit efficient permeation through the channel; (ii) these probes are designed as substituents for ions and therefore they will be used at high concentration; (iii) little specific recognition exists between the protein and the permeants.

A protein-mediated energy transfer excitation process will theoretically allow the activation of photosensitive molecules when they are in proximity to the protein. The photosensitive permeant ions would be most efficiently activated when they are passing through the channel. These labeling experiments would enable us to map an ion channel along its depth, as illustrated in Fig. 1. These probes should have the absorption characteristics mentioned above. Probe C shown in Scheme 2 was synthesized as a candidate permeant to probe cationic ion channels through Trp-mediated labeling. Although this concept looks attractive, especially for ion channel proteins having several Trp-residues in their membrane part, it has been overtaken by the site-directed mutagenesis approach. The latter approach can rapidly yield much information, provided that functional expression of the gene(s) coding for the channel-forming protein(s) has been established in an appropriate system.

6. Conclusion

The proposed methodology combines 'regio-selective' photoactivation with a highly reactive photogenerated species within areas in contact with proteins. The protein-mediated energy transfer reaction is designed to achieve photoactivation which is targeted to the probe in closest proximity to the protein. Photoactivatable phospholipid probes with appropriate chromophores have been synthesized for this purpose. They should prove to be original tools for mapping the membrane-associated protein domains and studying protein translocation and insertion processes. Moreover, this photoactivation should increase the labeling efficiency not only of the target protein but also of the annular lipids [21], offering a unique way to characterize them.

References

[1] Goeldner, M. and Hirth, C. (1980) Proc. Natl. Acad. Sci. USA 77, 6439–6442.
[2] Shinitzky, M., Dudai, Y. and Silman, I. (1973) FEBS Lett. 30, 125–128.
[3] Sussman, J.L., Harel, M., Frolow, F., Oefner, C., Goldman, A., Toker, L. and Silman, I. (1991) Science 253, 872–879.
[4] Goeldner, M.P., Hirth, C.G., Rossi, B., Ponzio, G. and Lazdunski,M. (1983) Biochemistry 22, 4685–4690.
[5] Langenbuch-Cachat, J., Bon, C., Mulle, C., Goeldner, M., Hirth, C. and Changeux, J.P. (1988) Biochemistry, 27, 2337–2345.
[6] Ilien, B. and Hirth, C. (1989) Eur. J. Biochem. 183, 331–337.
[7] Brunner, J., (1981) Trends Biochem. Sci., 6, 44–46; Bayley, H. (1983) in: Photogenerated Reagents in Biochemistry and Molecular Biology (Work, T.S. and Burdon, R.H. eds.) pp. 138–163, Elsevier, Amsterdam.
[8] Bisson, R., Montecucco, C., (1985) in: Progress in Protein–Lipid Interactions (Watts, A. and Depont, J. eds.) Ch. 7, pp. 259–287, Elsevier, Amsterdam.
[9] Brunner, J., (1989) Methods Enzymol. 172, 628–687.
[10] Chakrabarti, P. and Khorana, H.G. (1975) Biochemistry, 14, 5021–5033.
[11] Diyizou, Y.L., genevois, A., Lazrak, T., Wolff, G., Nakatani, Y., Ourisson, G. (1987) Tetrahedron Lett. 28, 5743–5746.
[12] Delphino, J. M., Schrieber, S.L. and Richards, F.M. (1993) J. Am. Chem. Soc. 115, 3458–3474.
[13] Yamamoto, M., Warnock, W., Milon, A., Nakatani, Y. and Ourisson, G. (1993) Angew. Chem. Int. Ed. Engl. 32, 259–261.
[14] Fredriksen, S.B., Dollé, V;, Masakuni, Y., Nakatani, Y., Goeldner, M. and Ourisson, G. (1994) Angew. Chem. Int. Ed. Engl. (in press).
[15] Schuster, G.B. and Platz, M.S. (1992) Adv. Photochem. 17, 69–143.

[16] Arnold, B.R., Scaiano, J.C., Bucher, G.F. and Sander, W.W. (1992) J. Org. Chem. 57, 6469–6474.
[17] Harel, M., Schalk, I., Ehret-Sabatier, L., Bouet, F., Goeldner, M., Hirth, C., Axelsen, P.H, Silman, I. and Sussman (1993) Proc. Natl. Acad. Sci. USA 90, 9031–9035.
[18] Scaiano, J.C. and Nguyen (1983) J. Photochem. 23, 269–276.
[19] Giraudat, J., Dennis, M;, Heidmann, T., Haumont, P.Y., Lederer; F. and Changeux, J.P. (1987) Biochemistry 26, 2410–2418.
[20] White, B.H. and Cohen, J.B. (1992) J. Biol. Chem. 267, 15770–15783.
[21] Barrantes, F.J. (1989) Crit. Rev. Biochem. Mol. Biol. 24, 437–478.