Hypothesis

Membrane protein-lipid hydrogen bonding: evidence from protein kinase C, diglyceride, and tumor promotors

Hans Brockerhoff

New York State Office of Mental Retardation and Developmental Disabilities, Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY 10314, USA

Received 14 March 1986

Membrane-bound proteins owe their retention and conformation in the lipid bilayer to hydrophobic peptide domains. Additional fixation, by protein-lipid hydrogen bonding, has been suggested, and recent reports on protein kinase C activation by diacylglycerol (DG) provide an unambiguous model for such bonding. The *sn*-1,2-diacylglycerol appears to donate a hydrogen bond from the *sn*-3 hydroxyl to the enzyme and to receive two hydrogen bonds, in the *sn*-1 and *sn*-2 ester CO groups, from the enzyme. This arrangement is confirmed in phorbol ester, a competitive inhibitor of DG for the kinase. This tumor promotor has a nearly identical spatial arrangement of hydrogen bond donor (9α-OH) and acceptors (12 and 13 ester CO); so have two other tumor promotors, teleocidin and aplysiatoxin. There are reasons to believe that protein kinase C is not the only protein that is bound to membrane lipids by hydrogen bonding, and such bonding will have to be considered in membrane-associated events such as fusion, cross-membrane transport, or anesthesia.

Lipid-protein bonding *Hydrogen bonding* *Diglyceride* *Protein kinase C* *Phorbol ester* *Aplysiatoxin* *Teleocidin* *Glucose-6-phosphatase*

The ubiquitous phosphorylating enzyme, protein kinase C, binds to lipid bilayers with an affinity greatly enhanced by the combined presence of anionic lipid (especially phosphatidylserine) and diacylglycerol (DG) [1–4]. This uncharged lipid also increases the affinity of a third co-factor, Ca^{2+}, for the enzyme, bringing the calcium requirement down to physiological levels (~1 μM) [3,4]. The structure of the diacylglycerol is very critical: the length and degree of saturation of the fatty esters are not very important [1,5–7], but ether bonds in *sn*-1 and *sn*-2 positions of the glycerol are not acceptable [7] and the *sn*-3 OH group is irreplaceable [6]. Also, the lipid-enzyme association is stereospecific: only *sn*-1,2-DG, but not its stereoisomer, *sn*-2,3-DG, is active [8,9]. Therefore, there must be 3-point fixation of the lipid to the protein, and since diacylglycerol has only three possible bonding sites there is no room for ambiguity: the C=O oxygens in position *sn*-1 and *sn*-2 receive hydrogen-bonding protons from the DG-receiving site of the enzyme, and the *sn*-3 OH group of DG donates a hydrogen bond to a receiving atom, probably a C=O oxygen, on the enzyme (fig.1b).

More evidence, if it is needed, for the triple hydrogen bonding from the lipid effector to the kinase is supplied by certain tumor promotors. Phorbol diesters, in particular, compete with diglyceride for binding to the kinase [10–15]. They might then show the same spatial bonding pattern of one hydrogen bond donating and two accepting groups (though it is not implied that every competitor must necessarily bond to all three sites). A space-filling model of 4β-phorbol-12-tetradecanoate-13-acetate (TPA, fig.1d) shows that the two

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

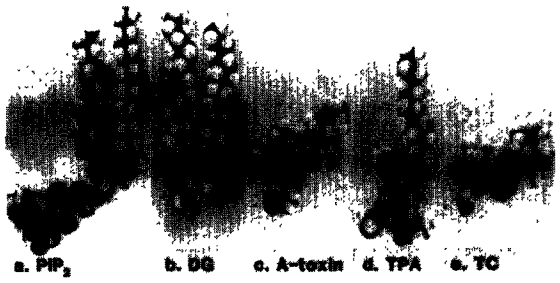

Fig.1. Space-filling molecular models, positioned so as to bring out the congruity of the hydrogen bond donating and receiving groups involved in bonding to the diglyceride (DG)-receiving site of protein kinase C. All molecules were initially constructed as skeletal Dreiding models for verification of conformation and assessment of steric hindrance and strain effects, following published, sterically explicit structures [32–34]. The numbers refer to the carbon atoms to which the oxygen atoms in question are bound. The triangles (which have no physical meaning) serve to demonstrate the possibility of congruity between the hydrogen bonding patterns of the five molecules. (a) PIP_2, phosphatidylinositol bisphosphate, precursor to DG; (b) DG, diglyceride; (c) A-toxin, aplysiatoxin; (d) TPA, phorbol-12-tetradecanoate-13-acetate. The dimethylcyclopropane group of this molecule could not be constructed with the available space-filling model set; a Dreiding molecular model showed that this group projects to the back of the model as shown here and does in no way interfere with the face shown. Note the steric restriction of the hydrogen bond donor [9] to bonding in directions between 5 and 9 o'clock only, and the protective hydrophobic domain in its back. As for the possibility of 4β-OH rather than 9α-OH being the required hydrogen bond donor, see the text. (e) TC, teleocidin. The O= atom corresponding to position *sn*-1 in DG is replaced here by a tertiary amino group [13], a hydrogen bond acceptor by virtue of its lone electron pair.

ester =O oxygens on C-12 and 13 and the 9α-OH group are superposable on the DG structure, not only in their position but also in the direction of the hydrogen bonds: coming in from the viewer onto the center of the =O atoms, pointing west (the hook) from the proton of the 9-OH group. Even the positioning of hydrogen atoms in the active oxygen triangle is similar. The models (fig. 1b,d) also offer a suggestion as to why phorbol diester has a much higher affinity, maybe 1000-fold, for the kinase than the proper effector, DG; in DG, which has a mobile, primary hydroxyl, the *sn*-3-OH···protein hydrogen bond may have to compete with hydrogen bonding to and from water, and the O atom of the *sn*-3-OH may be susceptible to enzymic phosphorylation (possibly the mechanism of DG deactivation). In the phorbol diester, however, the 9-OH group sits in a hydrophobic pocket, where it can rotate only from 5–9 o'clock (fig.1d) with its back safely protected by a hydrophobic niche.

It must be mentioned that the possibility cannot be discarded that the 4β-OH (not visible in our projection) rather than the 9α-OH is the H-donor used in the DG-receiving site; it appears that inversion in position 4, or methylation of 4-OH, destroys the kinase-activating faculty of phorbol ester [16]. On the other hand, deoxygenation at 4 is reported to leave the biological activity intact [17]; it is likely, therefore, that the 4-OH has some steric influence on phorbol-kinase bonding but does not itself bond to the DG-receiving site. In a model, the ester =O oxygens and the 4α-OH can (in the back of the model of fig.1d) form a triangular pattern resembling that involving 9α-OH, except for the bulky intrusion of a dimethylcyclopropane group into the triangle of oxygens. This steric hindrance, and a lack of complete congruity with the effector sites of the remaining molecules of fig.1, argue for 9-12-13 rather than 4-12-13 constituting that site. A final decision cannot yet be made, and is not necessary for the argument: either configuration allows for the two-acceptor–one-donor hydrogen bond arrangement.

In two other, tumor-promoting, kinase-activating compounds there is no chance for spatial duplicity. Aplysiatoxin [18,19] (fig.1c) proffers the same familiar arrangement of one OH and two =O groups. Teleocidin [18–21] (fig.1e) possesses only one CO (corresponding to the *sn*-2-CO of DG); a tertiary amino group in position 13 stands in for the other =O, with the lone electron pair accepting the hydrogen bond; and the OH group is in its expected place.

The kinase-DG interaction presents a clear case of protein-lipid hydrogen bonding (regardless of a possible future revision of tumor promotor-enzyme bond assignments). For some other cases, e.g. the interaction of steroids with their receptors, the existence of such bonding is also incontestable;

but protein-lipid hydrogen bonding is rarely conceived as a feature common to all membrane architecture. Evidence has been presented [22] for one membrane enzyme, glucose-6-phosphatase: its activity, and thus its conformation, depends on the composition of the membrane 'hydrogen belts' [23–25], i.e. those strata of the membrane containing hydrogen bond acceptors (CO of phospholipids) and donors (OH of cholesterol, sphingosine, proteins) (fig.2). The evidence collected here for the kinase C is more direct, and conclusive. Admittedly, free diacylglycerol is only a minor and transient component of the lipid bilayer; but as a common moiety of nearly all phospholipids it is the most prominent of all its building blocks. Fig.1a shows the precursor of free DG [26–28], phosphatidylinositol bisphosphate (PIP_2): in this lipid, as in all phosphoglycerides, the *sn*-3 OH is blocked, but the *sn*-1 and *sn*-2 =O atoms are already available for hydrogen bonding from a membrane protein (and it might be speculated that a hydrogen-bonded PIP_2-protein kinase complex is the direct precursor of the DG-protein kinase complex). It does not seem reasonable to expect that glucose-6-phosphatase and protein kinase C are the only existing proteins that can engage in hydrogen bonding to membrane

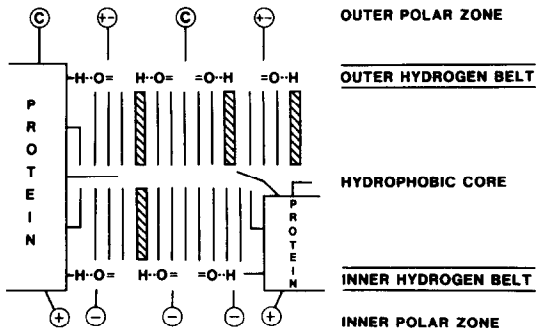

Fig.2. Schematic model of a plasma membrane. The isolated lines represent aliphatic chains of lipids or hydrophobic peptides. The shaded rectangles are cholesterol; C, carbohydrates; + and −, charged lipid headgroups and amino acids. Besides lipid-lipid bonding in the hydrogen belts, hydrophobic lipid-protein bonding in the hydrophobic core, and ionic lipid-protein bonding in the polar zones, the model proposes hydrogen bonding from proteins to lipids taking place in the hydrogen belts (from [23]).

lipids; it would seem more likely that many, even most, membrane-associated proteins make use not only of hydrophobic bonding but also of hydrogen bonding to maintain their place in the bilayer. This has been suggested in fig.2. A thermodynamic argument can also be made for such bonding. The hydrophobic bonding energy between an average fatty acid ester and a completely hydrophobic peptide is around 12 kcal/mol [29]. Addition of a hydrogen bond between peptide and fatty ester CO would add another 3–6 kcal (the $-\Delta G$ of an O-H···O bond [30,31]), i.e. such hydrogen bonding might contribute up to 33% of the energy keeping the protein in the membrane. Even more drastic, a bobbing of the protein vertical to the bilayer with an amplitude of one average bond length would make a difference of 0.8 kcal/mol fatty ester (the value of $-\Delta G$ for hydrophilic-lipophilic transfer of one CH_2 group [29]); but if such bobbing would require the breaking of hydrogen bonds to the fatty esters, the bonding energy lost could be 6-times larger. In view of such a thermodynamic advantage it would be odd if protein-lipid hydrogen bonding were not made use of in the hydrogen belts of membranes.

REFERENCES

[1] Nishizuka, Y. (1984) Nature 308, 693–698.
[2] Kaibuchi, K., Takai, Y. and Nishizuka, Y. (1981) J. Biol. Chem. 256, 7146–7149.
[3] Takai, Y., Kishimoto, A., Kikkawa, U., Mori, T. and Nishizuka, Y. (1979) Biochem. Biophys. Res. Commun. 91, 1218–1224.
[4] Kishimoto, A., Takai, Y., Mori, T., Kikkawa, U. and Nishizuka, Y. (1980) J. Biol. Chem. 255, 2273–2276.
[5] Lapetina, E.G., Reep, B., Ganong, B.R. and Bell, R.M. (1985) J. Biol. Chem. 260, 1358–1361.
[6] Davis, R.J., Ganong, B.R., Bell, R.M. and Czech, M.P. (1985) J. Biol. Chem. 260, 5315–5322.
[7] Cabot, M.C. and Jaken, S. (1984) Biochem. Biophys. Res. Commun. 125, 163–169.
[8] Rando, R.R. and Young, N. (1984) Biochem. Biophys. Res. Commun. 122, 818–823.
[9] Boni, L.T. and Rando, R.R. (1985) J. Biol. Chem. 260, 10819–10825.
[10] Kikkawa, U., Takai, Y., Tanaka, Y., Miyake, R. and Nishizuka, Y. (1983) J. Biol. Chem. 258, 11442–11445.
[11] Sharkey, N.A., Leach, K.L. and Blumberg, P.M. (1984) Proc. Natl. Acad. Sci. USA 81, 607–610.

[12] Ashendel, C.L., Staller, J.M. and Boutwell, R.K. (1983) Biochem. Biophys. Res. Commun. 111, 340–345.
[13] Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, U. and Nishizuka, Y. (1982) J. Biol. Chem. 257, 7847–7851.
[14] Niedel, J.E., Kuhn, L.J. and Vandenbark, G.R. (1983) Proc. Natl. Acad. Sci. USA 80, 36–40.
[15] Wolf, M., Cuatrecasas, P. and Sahyoun, N.J. (1985) J. Biol. Chem. 260, 15718–15722.
[16] DeRiemer, S.A., Greengard, P. and Kaczmarek, L.K. (1985) J. Neurosci. 5, 2672–2676.
[17] Ashendel, C.L. (1985) Biochim. Biophys. Acta 822, 219–242.
[18] Horowitz, A.D., Fujiki, H., Weinstein, I.B., Jeffrey, A., Okin, E., Moore, R.E. and Sugimura, T. (1983) Cancer Res. 43, 1529–1535.
[19] Fujiki, H., Tanaka, Y., Miyake, R., Kikkawa, U., Nishizuka, Y. and Sugimura, T. (1984) Biochem. Biophys. Res. Commun. 120, 339–343.
[20] Fujiki, H., Mori, M., Nakayasu, M., Terada, M., Sugimura, T. and Moore, R.E. (1981) Proc. Natl. Acad. Sci. USA 78, 3872–3876.
[21] Fujiki, H., Suganuma, M., Hakii, H., Nakayasu, M., Endo, Y., Shudo, K., Irie, K., Koshimizu, K. and Sugimura, T. (1985) Proc. Jap. Acad. 61, Ser.B, 45–47.
[22] Chauhan, V.P.S., Ramsammy, L.S. and Brockerhoff, H. (1984) Biochim. Biophys. Acta 772, 239–243.
[23] Brockerhoff, H. (1974) Lipids 9, 645–650.
[24] Brockerhoff, H. (1977) in: Bioorganic Chemistry (Van Tamelen, E.E. ed.) vol.III, pp.1–20, Academic Press, New York.
[25] Brockerhoff, H. (1982) Lipids 17, 1001–1003.
[26] Berridge, M.J. (1983) Biochem. J. 212, 849–858.
[27] Michell, R.H. (1975) Biochim. Biophys. Acta 415, 81–147.
[28] Streb, H., Irvine, R.F., Berridge, M.J. and Schulz, I. (1983) Nature 306, 67–69.
[29] Tanford, C. (1980) in: The Hydrophobic Effect: Formation of Micelles and Biological Membranes, 2nd edn, p.9, Wiley, New York.
[30] Pauling, L. (1960) The Nature of the Chemical Bond, Cornell University Press, Ithaca, NY.
[31] Pimentel, G.C. and McClellan, A.C. (1982) The Hydrogen Bond, Freeman, San Francisco.
[32] Sugimura, T. (1982) Gann 73, 499–507.
[33] Moore, R.E. (1974) in: Cellular Interactions by Environmental Tumor Promoters (Fujiki, H. et al. eds) pp.49–57, Jap. Sci. Soc. Press, Tokyo/VNU Science Press, Utrecht.
[34] Endo, Y., Shudo, K. and Okamoto, T. (1982) Chem. Pharm. Bull. 30, 3457–3460.