Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl16

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Version of record first published: 17 Oct 2011.

To cite this article: Milan Schara , Slavko Pečar & Erich Hecker (1984): EPR of Hydrophobic Interactions, Molecular Crystals and Liquid Crystals, 113:1, 123-129

To link to this article: http://dx.doi.org/10.1080/00268948408071677

Mol. Cryst. Liq. Cryst., 1984, Vol. 113, pp. 123-129
0026-8941/84/1134-0123/$15.00/0

EPR OF HYDROPHOBIC INTERACTIONS

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Abstract Molecules distributing between the membrane and solution are exposed to several different binding regions of the membranes. Binding of molecules to membranes, glass surfaces and a protein was compared. The specific structural differences are equally pronounced in the first two systems, although the protein binding is less dependent on the structural differences of the hydrophilic part of the molecule.

INTRODUCTION

Amphiphilic molecules bind to membranes in a wide range of tightness to several regions or domains where they can be dissolved, adhered or even incorporated into hydrophobic regions of the membrane bound proteins[1]. Several regions distinguished as receptor sites for a given type of molecules can emerge on the surface of the membrane. The most distinguished property is an increased binding strength with respect to the rest of the regions which might show specificity of binding.

Lipid bilayers and other lyotropic systems per se could in special situations show such binding site behaviour by a convenient phase separation to domains with a special composition. Proteins can extend these binding differences due to their hydrophobic properties.

MATERIALS AND METHODS

The spin labeled molecules methyl esters of dodecanoic and tetradecanoic acid MeFASL(m,n) labeled with an oxazolidine group, which is inserted at the position indicated by m and n, the numbers of CH_2 groups separating it from the terminal methyl group and from the carboxyl group, respectively, and the corresponding phorbol 13 acetate with spin labeled fatty acid at position 12 on PA (m,n), a biologically active substance, were synthesized[2].

Bovine serum albumin BSA was used as 5 and 1 % Tris HCl buffer solutions at pH 7.4. The albumin was labeled by the addition of the spin probes to the BSA solutions to the final concentrations of about 5×10^{-6} mol/l.

The spectra were measured in glass capillaries on Varian E–9 EPR spectrometer.

RESULTS AND DISCUSSION

Table I shows data on the hyperfine splitting $2\bar{A}_{\parallel}$ as well as on the empirical ratio R between the free and bound portion of the spin probe in the sample. Namely the BSA molecules do rotate slowly and the hyperfine splitting of the bound spin probes, which show an immobilized spectrum, decreases with the increasing temperature due to the viscosity decrease of the buffer solution and the corresponding increase in the rotational motion of the protein globule[3]. Besides to that, the spin probe spectra are containing the information on the adhered spin probe molecular mobility which is superimposed on the rotational motion of the globule. Therefore larger splittings mean stronger binding to the protein. One part of the molecules which are bound to BSA is strongly immobilized and their nitroxide groups are located deep in the protein interior. As found previously they can only hardly be reached by the penetrating ascorbate ions into the non denatured protein[3]. When the protein is unfolded the free mobility of the probe's chain can be observed and explained by a tight binding with only a part of the molecule to the unfolded portion of the protein.

TABLE I Hyperfine splitting and the ratio between the peak heights of the superimposed spectra pertaining to the free and BSA bound molecules (T = 20°C)

Spin probe	1 % BSA		5 % BSA	
	$2\bar{A}_{\parallel}$, mT	R	$2\bar{A}_{\parallel}$, mT	R
PA (4,5)	6.20	33.0	6.27	7.10
MeFASL (4,5)	6.31	77.5	6.03	8.71
PA (6,5)	6.40	10.5	6.37	1.43
MeFASL (6,5)	6.30	24.7	6.25	1.16

The binding isotherms in Figure 1. show the concentration of the free monomeric buffer dissolved molecules in relation to the total concentration of the added spin probe. Molecular aggregation at the critical micellar concentration (cmc) leads, to the disappearance of the EPR signal of the aggregated molecules, as their spectra are exchange broadened to the point beyond detection. For MeFASL (6,5) and PA (4,5) in the appropriate concentration range above their cmc there is a free component concentration decrease with the increased total concentration.

In presence of selfaggregation, binding to the protein and of the monomeric ligand molecules and at the constant total concentration of the protein c_p^o in the buffer solution, according to Appendix A the concentration of the free monomeric ligand molecules c_L can be written

$$c_L = \frac{u + v + c_p^o - (2 + m)\, c_{pLm} + c_L^o}{(n^2 + 1 - m)} \qquad (1)$$

Here m and n are the numbers of protein bound ligands and the aggregation number of the ligands, $u = (\mu_p^o - \mu_{pLm}^o + m\mu_L^o)/kT$ and $v = (n\mu_{Ln}^o - n^2\mu_L^o)/kT$ where μ_p^o, μ_{pLm}^o, μ_L^o and μ_{Ln}^o are the standard free energies for the protein, ligand bound protein, ligand, and ligand selfaggregate. As long as the total ligand concentration c_L^o increases at constant u, v, m and n then c_L increases linearly with c_L^o. When m or n also increase then c_L starts to get saturated—the

c_L^o (c_L^o) curve (Figure 1) changes the inclination which can get also negative. This happens if the system changes in so far that c_{pLm} increases. It probably has to do with a cooperative effect, as more protein gets involved in the ligand bindings, due to the longer exposure of its binding sites for the amphiphile.

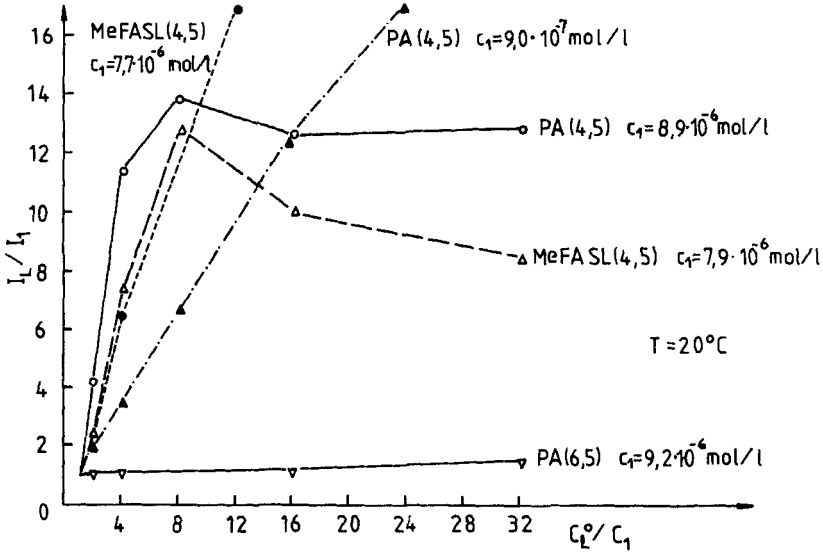

FIGURE 1. The free component intensity I_L plotted against the total concentration of the spin probe c_L^o. Both quantities are normalized with respect to the corresponding starting intensities I_1 and concentrations c_1.

The inclination of the binding isotherms from Figure 1 can be written

$$\frac{d\,c_L}{d\,c_L^o} = \frac{\partial\,c_L}{\partial\,c_{pLm}} \frac{d\,c_{pLm}}{d\,c_L^o} + \frac{\partial\,c_L}{\partial\,c_L^o} \sim \frac{-(1+m)}{n^2+1-m} \qquad (2)$$

and the condition for a negative inclination is $(n^2 + 1) > m$. Therefore the membrane – solution partition coefficient, obtained when the measurements are performed at higher concentrations of the

amphiphiles, are exposed to large variations, as in addition to the problems of molecular aggregation[4] the proteins can be also involved in binding.

The influence of the specific groups can be substantial when molecular distributions are concerned. For a membrane where the molecules are assumed to distribute homogeneously within the lipid bilayer the partition coefficient $K = c_m / c_s$ can be defined as the ratio between the concentrations of the concerned type of molecules in the membrane c_m and the buffer solution c_s. If the molecules are distributed between the glass surface adherent and the buffer dissolved portion then an adsorption coefficient

$$\alpha = \frac{N_g}{S_g} / \frac{N_s}{V_s}$$

defined as the ratio between the surface density to the buffer concentration of the molecules. Here N, S and V stand for the number of molecules, glass surface area and buffer solution volume, the indices g and s are for glass and solution.

For erythrocytes the ratio of the partition coefficients for PA (4,5) and MeFASL (4,5) is 5 and the ratio for the glass adsorption coefficients is 5.9 as it was determined previously[5]. These data are in agreement with the ratio of the partition coefficients, which were derived by the methods described by Rekker[6] where the contribution of the particular groups and their mutual inductions were considered, for the same pair of molecules and $K_{PA(4,5)} / K_{MeFASL(4,5)} = 5.1$.

The fairly comparable partition coefficient ratious derived for this pair of molecules in such diverse systems as the membrane, glass surface and the calculated value for octanol–water indicate that there must be a similar type of binding difference in all the three systems. It was shown previously[7] that the CH_2 groups adjacent to the phorbol moiety stay in the hydrophilic environment when the PA molecules are bound to erythrocyte membranes and the rest of the hydrophobic tail dips into the lipid bilayer. If it is assumed that the molecules adhere to the glass surface primarily in all trans conformations of the hydrocarbon tails then again both molecules differ essentially in their

head group binding or to be more precise in their free energy differences for both environments. This difference between the phorbol – and methyl – ester head group is about 4 kJ/mol.

This difference must be substantially smaller for the binding of these molecules to albumin as here the ratio R_{MeFASL}/R_{PA} is only about 2.3 when the smaller BSA concentration results of Table I are used. Here the involvement of the head groups should be different and it might depend on sterical differences between the phorbol and methyl group.

It was shown that the membrane – solution partition coefficients could depend in addition to the structural features of the concerned molecules and their concentrations also on the properties of the membrane domains and the involvement of their components in the process of binding.

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APPENDIX A

The free energy for the system composed from N_o solvent molecules, N_L monomeric amphiphiles, N_{Ln} lipid aggregates with the aggregation number n, N_p protein molecules and N_{pLm} lipid protein aggregates with the aggregation number m lipide or ligand molecules bound, can be written:

$$\phi = \sum_i \left(\mu_i^o N_i + \frac{N_i}{kT} \ln \frac{N_i}{N_t}\right) \tag{1A}$$

Here μ_i^o are the standard free energies per molecule for the molecules identified with the corresponding indices as shown above and N_t is the sum of all molecules in the sample.

Using the relations

$$N_L^o = N_L + nN_{Ln} + mN_{pLm} \tag{2A}$$

and

$$N_p^o = N_p + N_{pLm} \tag{3A}$$

as constraints in the free energy minimum determination from

$$\phi - \lambda_1 N_L^o - \lambda_2 N_p^o = 0 \tag{4A}$$

where λ_1 and λ_2 are the Lagrange multipliers and solving the system of equations $\partial\phi/\partial N_i = 0$ where i = L, Ln, p, and pLm and using the expression $c_i = N_i/N_t$ for the concentrations the following relation between the free component and the total added ligand is

$$c_L = (u + v + c_p^o - (2 + m)\, c_{pLm} + c_L^o)/(n^2 + 1 - m) \tag{5A}$$

here $u = (\mu_p^o - \mu_{pLm}^o + m\mu_L^o)/kT$ and

$v = (n\mu_{Ln}^o - n^2\mu_L^o)/kT$.