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MICROCALORIMETRIC INVESTIGATIONS OF THE BINDING OF FLAVONOIDS TO SYNTHETIC AND NATURAL MEMBRANES.

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ABSTRACT

The binding of flavonoids of different composition and structure to lipid model membranes and to erythrocyte stroma was investigated by microcalorimetric titration measurements. The affinity constants of the various liposome-flavonoid systems investigated vary from 0.3 to 9.6 10^{3} M^{-1}, depending on the chemical structure and the hydrophobicity of the ligands. The enthalpy and entropy terms exhibit large variations, indicating different binding mechanisms for the various flavonoids. The stoichiometry of the lipid - flavonoid complex is 2:1. With erythrocyte membranes the thermodynamics of flavonoid binding indicate that with decreasing hydrophobicity of the ligand, a different flavonoid-binding mechanism, probably to membrane proteins, becomes apparent.

INTRODUCTION

Flavonoids are benzo-γ-pyrone derivatives, occurring ubiquitously in plant cells. Havsteen (ref.1) summarizes in a recent review article the pharmacological and biochemical studies, reported on flavonoids. One of the most interesting aspects of biological flavonoid activities, is their effect on membrane properties (refs. 2,3). To our knowledge the thermodynamics of flavonoid binding to lipid and to natural membranes have not been investigated so far. In the present study, the energetics of the interaction of flavonoids of various composition and structure with liposomes and erythrocyte membranes have been investigated by microcalorimetry.

METHODS AND MATERIALS

Methods

The heats of reaction of the flavonoid binding processes were determined in an LKB batch microcalorimeter, equipped with an automatic microtitration assembly (ref. 4,5 . In some experiments, the amounts of membrane-bound flavonoid were determined spectroscopically (Beckmann Acta V spectrophotometer). The preparation of unilamellar liposomes was performed by the detergent-dialysis technique ("LIPOPREP" apparatus from Diachema).

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Materials

The soy phosphatidylocholine was from Diachema (high purity grade). The erythrocyte membranes (human erythrocyte stroma) were kindly provided by the Institute of Biochemistry. The flavonoids investigated were a generous gift from Zyma S.A., Nyon. All measurements were performed at 25°C, in Dulbecco buffer (pH 7.4).

RESULTS

Results obtained with model membranes (liposomes)

The thermodynamic parameters of flavonoid binding to unilamellar liposomes of soy-phosphatidylcholine were determined in microcalorimetric titration experiments. Figure 1 shows the titration or binding curves for a series of flavonoids, obtained by titration of the saturated flavonoid solutions (8.7 to 0.2 10^{-3}M) with liposome suspension (3.3 10^{-3}M).

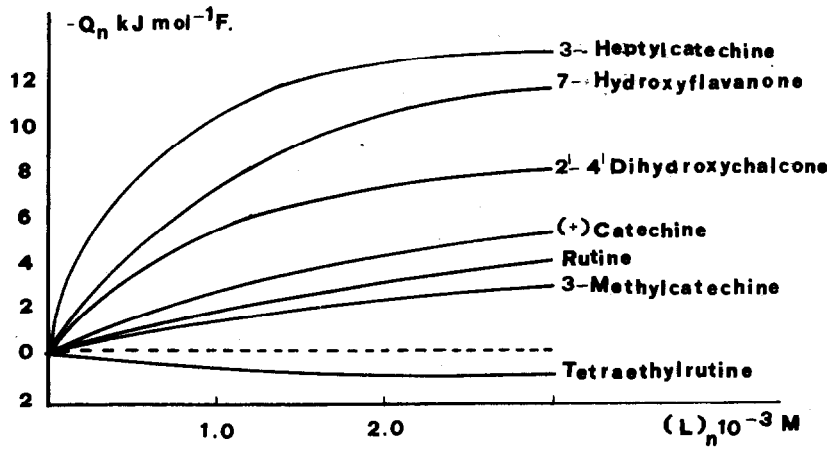

Fig.1. The incremental heats of reaction (Q_n) as a function of the concentration of free ligand (L_n) for the binding of various flavonoids to soy phosphatidylcholine liposomes. Q_n scale 2:1 reduced for 3-heptylcatechine.

The binding curves represented in figure 1 were evaluated by iterative least squares regression procedures to obtain the affinity constants (K_a), the molar free energies ($\Delta G^o = -RT \ln K_a$) and the molar reaction enthalpies (ΔH^o). The molar entropy terms were then computed from: $\Delta G^o - \Delta H^o = T \Delta S^o$. Table 1 summarizes the results obtained, and shows the following points of interest: 1) The affinity constants decrease by a factor 30 in the order of flavonoids, given in table 1. 2) The enthalpy and entropy terms show large and irregular variations. The

binding reactions of all ligands investigated are enthalpy-controlled, with the exception of the chalcon-derivative and of tetra-ethyl rutine, which are bound to liposomes by an entropy-controlled mecnanism ($\Delta H^{o} < T\Delta S^{o}$).

TABLE 1

Thermodynamic parameters of the interaction of flavonoids with lipid membranes (liposomes).

Flavonoid	K_a 10^3 $l\ mol^{-1}$	$-\Delta G^{o}$ $kJ\ mol^{-1}$	$-\Delta H^{o}$ $kJ\ mol^{-1}$	ΔS^{o} $J\ mol^{-1}deg^{-1}$
3-Heptylcatechine	9.6	22.7	32.9	-34.2
2'-4' Dihydroxychalcone	3.3	20.1	9.0	37.1
7-Hydroxyflavanone	0.8	16.6	16.8	0.7
3-Methylcatechine	0.7	16.2	15.3	3.1
Tetra Ethylrutine	0.4	14.8	+2.3	57.5
Mono-and Tri Ethylrutine	-	-	0	-
Rutine	0.3	14.1	9.0	17.2
(+) Catechine	0.3	14.1	16.5	0

The stoichiometry of the flavonoid-lipid complex formation was investigated in reciprocal titration experiments. The results obtained with (+) catechine and 3-methylcatechine, show in good agreement that the stoichiometry factor n is 1.92 $\pm$ 0.2. Two moles of lipid thus bind one mole of flavonoid.

Results obtained with erythrocyte membranes

The interaction of (+) catechine, 3-methyl and 3-heptylcatechine with erythrocyte membranes (human erythrocyte stroma, HES) has been investigated by microcalorimetric and spectroscopic measurements.

TABLE 2

Thermodynamic parameters of the interaction of flavonoids with HES

Membrane suspension	Flavonoid	K_a $l\ mol^{-1}$ (*	Q_r $kJ\ mol^{-1}$ F.
HES	(+) Catechine	0.7 (0.03)	2.1 (16.5)
"	3-Methylcat.	0.2 (0.07)	3.5 (15.3)
"	3-Heptylcat.	1.0 (1.0)	40.6 (33.0)

(* The K_a terms were computed from spectroscopic data, and are given in relative units, referring to K_a for 3-heptylcatechine = 1.0.( ) Data from table 1, K_a in relative units.

The main differences between the results obtained with lipid- and with natural membranes are the following : 1) The molar heats of reaction (Q_r) are dra-

stically reduced for (+) catechine, to a lesser degree for 3-methylcatechine, and remain approx. unchanged for 3-heptylcatechine. 2) The relative affinity constants (K_a) are 23 and 3 fold increased for (+) catechine and 3-methylcatechine, respectively.

DISCUSSION

From the investigations of flavonoid-liposome systems, the following information is obtained : The lipid affinity of flavonoids is increased by introduction of apolar side chains (3-methylcatechine,3-heptylcatechine), by opening of the rigid γ-pyronring (chalcon derivative),and by reduction of the number of phenolic groups (7-hydroxyflavanon). Increasing hydrophobicity (or flexibility) of the ligand molecule enhances its liposome-affinity,indicating an interaction with the lipid region of the phospholipid bilayer. Introduction of a glycosidic residue (rutine) and/ or ethylation of the phenolic groups (tetra-ethylrutine) do not affect the K_a values. (+) catechine, rutine and its tetraethyl derivative thus seem to bind to the polar surface of liposomes. The entropy terms of the ligand-binding reactions seem to indicate that different structural effects are induced in the bilayer structure by the various flavonoids. In erythrocyte membranes, the interaction of 3-heptylcatechine is -by its heat of reaction- comparable to the interaction with liposomes. This ligand thus appears to bind preferentially to lipid regions of the membrane. With decreasing hydrophobicity of the ligands, the changes in the thermodynamic parameters indicate the appearance of a different binding mechanism, probably to membrane proteins.

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