EFFECT OF PHOSPHOLIPIDS ON THE LENGTH OF THE HELICAL SEGMENTS IN THE FATTY ACID SYNTHETASE COMPLEX FROM *CERATITIS CAPITATA*

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1. Introduction

Lipid–protein interactions are essential in the structure of biological membranes and plasma lipoproteins. The lipids, since they are flexible molecules, will follow the shape of the proteins to a certain extent creating a closely packed hydrophobic barrier. Thus, the function of the lipid–protein systems is in part dependent on that hydrophobicity. Among the different lipid classes, phospholipids play an important role on the enzymic and regulatory properties of many enzymes [1–3]; interactions between phospholipids and apoproteins seem fundamental to the binding of neutral lipids by the plasma lipoproteins [4,5]. These facts assign to phospholipids the central role in the study of the lipid–protein interactions.

The alignement of the phospholipids to the polypeptide chains is accompanied by structural changes, many of them should become available from circular dichroism (CD) measurements [6,7]. The detailed analysis of such data lead to a quantitative understanding of the α-helix content of the protein. From these studies, the structural role of phospholipids on lipoproteins could be referred to an increase in the α-helix content of the polypeptide chains [6,7]. According to this idea, an amphipatic helical theory of lipid binding was developed [8], that has been refined by testing a series of model lipid-binding peptides as well as fragments of native apolipoproteins [7,9–11]. They formulated a number of rules concerning the properties of the lipid-binding peptide region, that can be summarized in that a high hydrophobical peptide having a critical length of ~20 residues spontaneously associates with phospholipids achieving α-helix conformation [12]. From a structural point of view, the above data would indicate that the role of phospholipids is to generate α-helical structure in lipoproteins with the corresponding functional influence on the system.

Fatty acid synthetase complex from the insect *Ceratitis capitata* is a soluble lipoprotein with a 1:1 (w/w) lipid/protein ratio [13]. CD studies on this enzyme complex indicate a high content in α-helix structure [13,14]. Complete delipidation results in a significant decrease of the ellipticity in the peptide bond wavelength range as well as of the enzymic activity [15]. Moreover, the synthetase complex is dependent on phospholipids for the catalytic activity [13]. Then, if the enzyme could be prepared lacking mainly phospholipids, it could be used for studying the variations in both protein structure and enzymic activity. Moreover, such partially delipidated protein should be an excellent model system to study the phospholipid–protein interactions in soluble lipoproteins other than those of plasma.

Here, we examine the consequences of the lipid–protein interactions on the structure of the insect fatty acid synthetase, based on the results obtained for a phospholipidless enzyme. These results are a new contribution to the above theory of the amphipatic helix for the lipid binding to proteins.

2. Materials and methods

Fatty acid synthetase complex was isolated and purified from larvae of the insect *C. capitata* as in [16]. The enzyme activity was evaluated using labelled acetyl-CoA as substrate as in [17].

Circular dichroism spectra of the enzyme complex have been recorded in a Jobin Yvon, Mark III, dichrograph fitted with a 250 W xenon lamp at a 0.2 nm/s scanning speed. Millipore (5 μm pore diam.) filtered

solutions of the enzyme were studied at 20°C. The protein concentration was determined using an $E_{0.1\%,1\ cm}$ of 1.00 at 280 nm; this concentration was always over 0.2–0.4 mg/ml. Cells of 0.05 cm optical path were used and the instrument was operated at a sensitivity of 1×10^{-5} ΔA/mm. Circular dichroism results are the mean values of 5 determinations for different enzyme preparations and they are reported in terms of θ_{mrw}, the mean residue ellipticity in units of degrees × cm^2 × $dmol^{-1}$.

Quantitative determination of total phosphoglycerides, free fatty acids, diacylglycerols and triacylglycerols was done as in [18] by using pentadecanoic acid as internal standard. Phosphoglyceride, free fatty acid, diacylglycerol and triacylglycerol bands from the one-dimensional thin-layer chromatography plates were scraped off and directly methanolysed with boron trifluoride in methanol as in [19]. Gas-liquid chromatographic analysis were performed on a Hewlett-Packard, mod.5750, equipped with a flame ionization detector and an integrator, mod.3370A. Operating temperature was 170°C and the column was 20% DEGS on chromosorb W/AW (80–100 mesh).

3. Results and discussion

When the fatty acid synthetase from *C. capitata* is incubated at room temperature and low ionic strength (0.01 M sodium phosphate buffer (pH 7.5)) a marked decrease on enzymic activity is observed. Table 1 shows the time-course of enzymic activity at this phosphate buffer concentration, as well as at 0.1 M phosphate buffer (pH 7.5) as control. After 10 h incubation at low ionic strength, the remaining activity is 2% of the value at zero time, whereas the control exhibits 87% activity for the same incubation time. When the native enzyme complex was chromatographied through Sephadex G-25 medium (1.6 × 50 cm) equilibrated in the 0.01 M phosphate buffer, the synthetase is inactivated to the same extent as after the 10 h treatment at low ionic strength. Taking into account the dependence of the enzymic activity on the lipid content [15], the above inactivations could be related to a loss of lipids. Certainly both of these treatments, enzyme aging and gel filtration at low ionic strength, released lipids from the native lipoprotein. Table 2 shows the lipid class content of both, native and partially delipidated enzyme, through both phosphate buffer and gel filtration treatment. Differerences in the lipid composition can be mainly related to the phospholipid content. Thus, the lack of phospholipids in the molecule results in the inactivation of the complex.

Table 1
Time-course (min) of the fatty acid synthetase activity at two concentrations of phosphate buffer (pH 7.5)

Buffer	0	30	60	120	300	600
0.1 M	2.60	2.60	2.60	2.60	2.35	2.26
0.01 M	2.60	2.23	1.92	1.13	0.31	0.05

Activity values are given as nmol synthesized fatty acid/mg protein

Table 2
Lipid composition of native and partially delipidated fatty acid synthetase complex

Enzyme	PG	FFA	DG	TG
Native	5.0	2.8	10.0	82.2
Non-active[a]	0	2.0	8.7	72.4

[a] Values are referred to the total lipid amount in the native enzyme

Values are given as percentages of phosphoglycerides (PG), free fatty acids (FFA), diacylglycerols (DG) and triacylglycerols (TG) (mean values of three different batches of enzyme preparations)

This selective delipidation, ionic strength-dependent, is accomplished neither with organic solvents, acetone or chloroform/methanol, nor by lipolytic treatments. However, in the presence of 1% (w/v) sodium cholate, a selective exchange cholate-phospholipids has been observed for the insect synthetase [13]; since the secondary structure of the protein is retained throughout this treatment, it cannot be used for the understanding of the structural role of phospholipids. Thus, only the partially delipidated enzyme by low ionic strength treatment (named non-active fatty acid synthetase) can be employed to study the specific structural role of phosphoglycerides.

In addition to the enzymic activity, the effect of phospholipids has been considered on secondary structure. Fig.1 shows the circular dichroism spectra of the native synthetase complex (A) and the non-active enzyme form (B). Protein conformation analysis from the CD data can be performed by two general methods. One of them uses synthetic polypeptides as

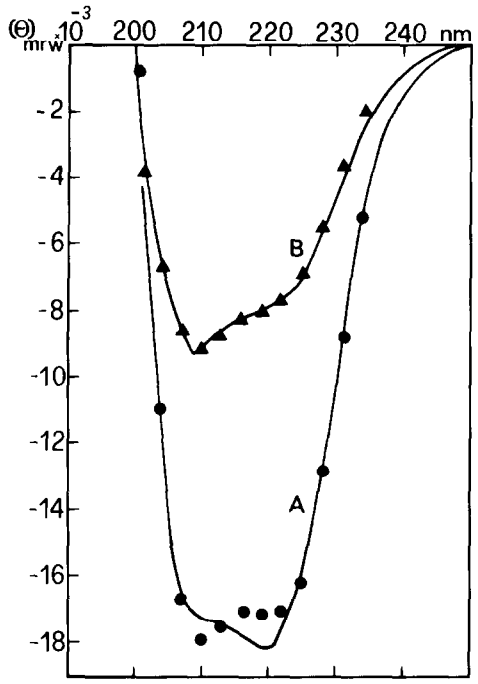

Fig.1. Circular dichroism spectra of the fatty acid synthetase complex from the insect *C. capitata* in its native form (A) and the partially delipidated, non-active, form (B): (•) theoretical values for the native enzyme based on the secondary structure estimations given in table 3; (▲) theoretical values for the non-active enzyme on the basis of its secondary structure estimation as given in table 3.

model compounds for the three conformations [20], whereas the other one computes the reference spectra from CD of proteins of known three-dimensional structure [21,22]. Helical synthetic polypeptides of high M_r are unlike the short helical segments in a protein, whose CD is chainlength-dependent [23]. Thus, to account for the chainlength dependence, the helical reference value should be:

$$\theta_H^n = \theta_H^\infty (1 - k/n)$$

where: n is the average number of residues per helical segment; k is a wavelength-dependent constant; and the superscript ∞ refer to a helix of infinite length [21]. Analysis of the CD spectra according to this method [21] gave the results shown in table 3. The native synthetase has its helical structure arranged in long segments of which the av. no. residues/segment is 40 according to the computarized fit analysis. The obtained n value for the native enzyme is equivalent

Table 3
Secondary structure of native and partially delipidated fatty acid synthetase complex

Enzyme	α-Helix	β-Structure	Aperiodic structure	n
Native	45	16	39	40
Non-active	30	25	45	7

Percentages are evaluated as in [21] by computer fit

to an infinite length of the helix, because the dependence of the ellipticity with the chainlength of the helical structure reaches a plateau at $n = \sim 40$ [21]. This fact is corroborated using the other method for protein conformation analysis [20] which consider helical synthetic polypeptides of high M_r, infinite length, as reference. The secondary structure of the native enzyme according to this method is 43% α-helix, 26% β-form and 31% aperiodic structure, also by computerized fit analysis, whose values are in agreement with the calculated above.

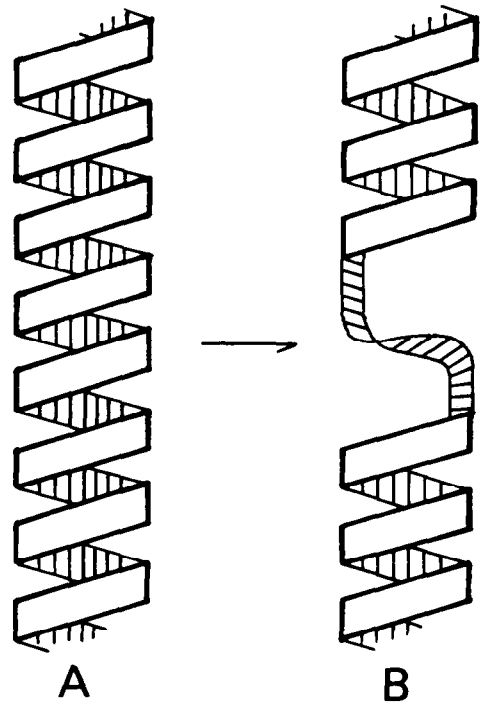

Fig.2. Proposed model for the structural role of phospholipids in the fatty acid synthetase from insect: (A) helical region in the native enzyme; (B) the same region after removal of phospholipids; the non-helical part would be the region stabilized by phosphoglycerides in the native enzyme.

From the results in table 3, it can be observed that the structural variation of the non-active enzyme indicates a small decrease in the α-helix content (30% *vs* 45% in the native enzyme), but it appears an important decrease of the *n* value (from an infinite length to $n = 7$ for the non-active enzyme). Thus, partial delipidation of the insect enzyme results essentially in a lower av. no. residues/helical segment. The released molecules of phospholipids should be involved in the maintenance of the helical conformation of small regions of the polypeptyde chains. Thus, phosphoglycerides do not increase notably the α-helix content in lipoproteins but they would increase the length of helical segments through a stabilizing effect on regions between segments in helical conformation (fig.2). This length increase is consistent with the arrangement of an amphipatic helix in which, the polar residues would be distributed on one face of the helix directed toward the aqueous phase whereas the non-polar residues situated on the opposite face would be buried into the hydrophobic core of the lipoprotein, according to the molecular theory of the lipid-binding regions of native apolipoproteins [8,12]. According to this theory, phospholipids will create α-helix. Our results accumulate experimental evidence supporting effectively the increase in the α-helical content of proteins upon association with phospholipids. However, this increase is mainly referred in our case to an increase in the length of the segments having α-helix conformation. Thus, theories for the lipid–protein interactions should consider phospholipids not only as molecules supporting α-helix but also as increasing factors the length of the helix. This could explain many activation effects observed for phosphoglycerides on lipid-dependent proteins, where a small number of molecules implies an important modification of the function of the protein, stabilizing small regions upon the active sites of the molecule. Fatty acid synthetase from *C. capitata* has been accounted for these ideas.

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