lation, this confers a decided advantage to the porcine kidney enzyme, since binding of other metal ions does not require displacement of Zn^{2+} from this site. Hence, inhibition or activation would be achieved at lower concentrations of these metal ions, and this modulation may be more likely to have a physiological role.

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References

Basha, S. M. M., Horst, M. N., Bazer, F. W., & Roberts, R. M. (1978) *Arch. Biochem. Biophys. 185*, 174–184.
Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.
Bryce, G. F., & Rabin, B. R. (1964) *Biochem. J. 90*, 509–512.
Carpenter, F. H., & Vahl, J. M. (1973) *J. Biol. Chem. 248*, 294–304.
Delange, R. J., & Smith, E. L. (1971) *Enzymes, 3rd Ed. 3*, 81–118.
Good, N. E., Winget, G. D., Winter, W., Connolly, T. N., Izawa, S., & Singh, R. M. M. (1966) *Biochemistry 5*, 467–477.
Gornall, A. G., Bardawill, C. J., & David, M. M. (1949) *J. Biol. Chem. 177*, 751–766.
Himmelhoch, S. R. (1969) *Arch. Biochem. Biophys. 134*, 597–602.
Himmelhoch, S. R. (1970) *Methods Enzymol. 19*, 508–513.
Himmelhoch, S. R., & Peterson, E. A. (1968) *Biochemistry 7*, 2085–2092.
Lowry, O., Rosebrough, N., Farr, A., & Randall, R. (1951) *J. Biol. Chem. 193*, 265–275.
Melbye, S. W., & Carpenter, F. H. (1971) *J. Biol. Chem. 246*, 2459–2463.
O'Sullivan, W. J., & Smithers, G. W. (1979) *Methods Enzymol. 63*, 294–336.
Royer, G. P., & Andrews, J. P. (1973) *J. Biol. Chem. 248*, 1807–1812.
Shen, C., & Melius, P. (1977) *Prep. Biochem. 7*, 243–256.
Smith, E. L., & Spackman, D. H. (1955) *J. Biol. Chem. 212*, 271–299.
Spackman, D. H., Smith, E. L., & Brown, D. M. (1955) *J. Biol. Chem. 212*, 255–269.
Thompson, G. A., & Carpenter, F. H. (1976) *J. Biol. Chem. 251*, 1618–1624.
Vallee, B. L. (1960) *Enzymes, 2nd Ed. 3*, 225–276.

Fluorescence Studies on the Lipoprotein Complex of the Fatty Acid Synthetase from the Insect *Ceratitis capitata*†

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ABSTRACT: The fatty acid synthetase complex from the insect *Ceratitis capitata* forms a stable lipoprotein complex. The intrinsic fluorescence of the complex was studied by observing the emission spectra with different excitation wavelengths, both in the native complex and after treatment with sodium cholate and sodium dodecyl sulfate. The excitation spectrum of the native form also was recorded. The fluorescence behavior of the native enzyme showed two families of tryptophan residues. Cholate influenced the fluorescence, suggesting that phospholipids are the conformational support at this level. The two families of fluorescing tryptophan residues were similarly accessible to quenching by acrylamide. Thermal changes in the fluorescence characteristics were observed; warming caused a decrease in the quantum yield as well as a red shift in the emission maximum. The high fluorescence remaining after the thermal transition suggested that the lipid–protein interaction was affected but maintained shielding of the fluorophore by the lipids. Fluorescent probe molecules 1,6-diphenylhexa-1,3,5-triene (DPH) and dansylphosphatidylethanolamine (DPE) also were used. DPH uptake was temperature dependent, with a middle point consistent with the thermal conformation transition, indicating that internal lipids are nonrandomly distributed within the complex. DPE uptake did not reach the saturation of the complex, suggesting that its solubilization sites would be located on the lipoprotein surface.

Native fatty acid synthetase from *Ceratitis capitata* has been described as a lipoprotein (Gavilanes et al., 1979) which requires the lipid component for retaining its conformational properties (Gavilanes et al., 1978, 1979); thus, changes in the lipid content and composition resulted in modification of both secondary structure and enzymatic activity (Gavilanes et al., 1979, 1981). Triacylglycerols are the most abundant lipid class whereas a specific phosphatidylethanolamine dependence of the enzyme activity has been demonstrated (Gavilanes et al., 1979). An attempt to clarify the structural organization of the enzyme complex was carried out by a circular dichroism study of the native complex and after a series of sequential treatments with lipolytic and proteolytic enzymes; it obtained evidence for the shielding of certain polypeptide domains by the lipid environment (Gavilanes et al., 1981). Nevertheless, it is not clear from these experiments whether the phospholipids are interacting specifically with the polypeptide chains or if they are simply playing a structural role important for the complex conformation.

Other information that may give insight as to the complex organization expected is from fluorescence data on this lipid–protein system. The intrinsic protein fluorescence arises from tyrosine and tryptophan residues, which act as nonperturbing hydrophobic probes of their immediate environment. Intrinsic fluorescence offers a sensitive tool for observing in-

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teractions between lipids and a wide series of peptide and proteins (Schneider & Edelhoch, 1972; Verdery & Nichols, 1974; Gwynne et al., 1975; Dufourq et al., 1975; Gennis et al., 1976; Epand et al., 1977; Dufourq & Faucon, 1977). On the other hand, the use of fluorescent probes has greatly facilitated studies on the structure and function of membrane dynamics (Azumi & McGlynn, 1962; Azzi, 1975; Radda, 1975; Schlessinger & Elson, 1979) and given valuable information on the nature of the microenvironment of the labeled sites and freedom of rotation of the labeling reagents (Stryer, 1968; Turner & Brand, 1968; Radda & Vanderkooi, 1972).

One of the widely used fluorescent probes is 1,6-diphenyl-1,3,5-hexatriene (DPH);[1] it incorporates spontaneously into the lipid components of the system and is a highly efficient fluidity probe of the lipid environment (Fuchs et al., 1975; Chen et al., 1977; Shinitzky & Barenholz, 1978; Rosenthal et al., 1978; Parola et al., 1979). Also, the use of fluorescence analogues of lipids has taken advantage of the high reactivity of the amino group of phosphatidylethanolamine (PE) to conjugate the dansyl residue to this natural phospholipid.

To gain further information on the internal structure of the fatty acid synthetase complex of the insect *Ceratitis capitata*, we have now examined the intrinsic fluorescence of the native enzyme as well as the fluorescence response of DPH and dansyl-PE under a series of conditions.

Materials and Methods

Fatty Acid Synthetase Complex. The enzyme complex was purified from the larval stage of the insect *Ceratitis capitata* as previously described (Municio et al., 1977). Protein homogeneity and conformation were checked by acrylamide gel electrophoresis, amino acid analysis, and circular dichroism studies; enzymatic activity was also verified. Lipid composition was determined in all enzyme preparations. All these results were coincident with those previously reported (Municio et al., 1977; Municio & Gavilanes, 1981).

Dansylphosphatidylethanolamine. Phosphatidylethanolamine was obtained from *C. capitata* larvae. Insects were homogenized in 0.1 M potassium buffer, pH 7.5, containing 2 mM EDTA and 1 mM 2-mercaptoethanol. The homogenate was centrifuged at 27000*g* for 15 min, and the clear supernatant was extracted according to the method of Bligh & Dyer (1959). The lipid fraction (100 mg) was chromatographed on 1-mm silica gel plates (20 × 20 cm) with chloroform/methanol/water (60:25:4 v/v/v) as the developing solvent. Identification of PE was done by comparison with a standard sample and through the positive reaction by spraying with the ninhydrin and Dittmer reagent (Dittmer & Lester, 1964). The PE band was eluted from the silica gel with 2:1 (v/v), 1:1, and 1:9 chloroform/methanol and finally with absolute methanol. Samples were concentrated under vacuum, and PE appeared as a single spot on the two-dimensional system chloroform/methanol/water (65:25:4 v/v) and butanol/acetic acid/water (60:20:20 v/v).

Dansylation of PE was carried out according to the general method of Grey (1967). To 1 mg of PE in 200 μL of 0.2 M sodium bicarbonate was added 200 μL of dansyl chloride (2.5 mg/mL) in acetone, and the reaction mixture was incubated at 45 °C for 30 min. The dansylation reaction was controlled by polyamide thin-layer chromatography with formic acid (1.5% v/v) as the solvent. Purification of dansyl-PE was performed by the silica gel procedure used for the phospholipid purification. Dansyl-PE was eluted from the silica gel with ethanol.

Other Materials. DPH was purchased from Aldrich (Belgium). Acrylamide, electrophoresis grade (Merck, West Germany), was recrystallized twice from chloroform. Dansyl chloride and sodium dodecyl sulfate ($NaDodSO_4$) were from Sigma (St. Louis, MO); $NaDodSO_4$ was recrystallized from ethanol. Thin-layer chromatography polyamide layers were obtained from Cheng-Chin Trading Co. (Taipei, Taiwan). Other products and solvents were of analytical grade.

Enzyme Activity. Fatty acid synthetase activity was determined as described (Gavilanes et al., 1979; Municio & Gavilanes, 1981).

Fluorescence Labeling. Stock 2×10^{-3} M DPH solution was prepared in tetrahydrofuran, and immediately prior to use, it was diluted 1:100 with vigorous mixing with 0.25 M potassium phosphate buffer, pH 7.5, containing 2 mM EDTA. Stirring was continued for 10 min at room temperature, and a clear and stable aqueous dispersion of 2×10^{-5} M DPH, void of fluorescence, was obtained. One volume of fatty acid synthetase complex solution (0.1 mg/mL) in the same phosphate buffer was incubated with 1 volume of the DPH dispersion with gentle shaking, at various temperatures and time intervals. The same procedure was used for the labeling of the fatty acid synthetase with dansyl-PE except that a 2×10^{-3} M dansyl-PE solution in ethanol was employed.

Ultraviolet Absorption. Spectra were recorded on a Cary 118 spectrophotometer, at 20 °C, in 1-cm optical path cells. Fatty acid synthetase samples were previously centrifuged at 27000*g* for 20 min and filtered through Millipore (0.45 μm). Residual light scattering was corrected by means of a linear plot of optical density vs. $1/\lambda^4$ in the 310–360-nm range.

Fluorescence. Measurements were made at different temperatures on a Fica 55 MK II spectrofluorometer fitted with a 450-W xenon lamp, with 2.5- and 7.5-nm slit widths for either excitation or emission beam, respectively. Excitation and emission spectra were corrected for both detector response and light source. Spectra were registered on a XY Ohnigrafic 200 at 10 nm/min. Spectra were obtained at least 3 times, with two or more enzyme preparations. Protein concentration was in the 30–50 μg/mL range as determined from the absorbance at 280 nm, a $E^{0.1\%}_{1cm}$ of 1.0 (Municio & Gavilanes, 1981) was used. Optical densities were below 0.05 at the exciting wavelength. Samples with higher absorbance were corrected for the inner-filter effect. The effective optical path for the instrument and cells used is 0.5 cm.

The excitation wavelength for the protein was 280 nm. The excitation and emission wavelengths for the fluorescent probes were respectively 365 and 425 nm.

Polarization measurements were performed with small Glan-Thompson prisms. The polarization of fluorescence was expressed as $P = (I_{vv} - fI_{vh})/(I_{vv} + fI_{vh})$, where I_{vv} and I_{vh} are the relative fluorescence intensities with the excitation and emission polarizers in parallel and perpendicular orientations, respectively, and f is the correction factor for photomultiplier sensitivity (Azumi & McGlynn, 1962).

Results

Fluorescence of the Native Fatty Acid Synthetase Complex. Intrinsic fluorescence of proteins has been extensively used to study structure–function relationships of these macromolecules. Generally, proteins have this type of fluorescence endowed by their tryptophan and tyrosine residues. The fluorescence emission spectrum of fatty acid synthetase from *Ceratitis*

[1] Abbreviations used: EDTA, ethylenediaminetetraacetic acid; PE, phosphatidylethanolamine; dansyl-PE (DPE), dansylphosphatidylethanolamine; dansyl (DAS), 5-(dimethylamino)naphthalene-1-sulfonyl; DPH, 1,6-diphenyl-1,3,5-hexatriene; $NaDodSO_4$, sodium dodecyl sulfate; P, fluorescence polarization.

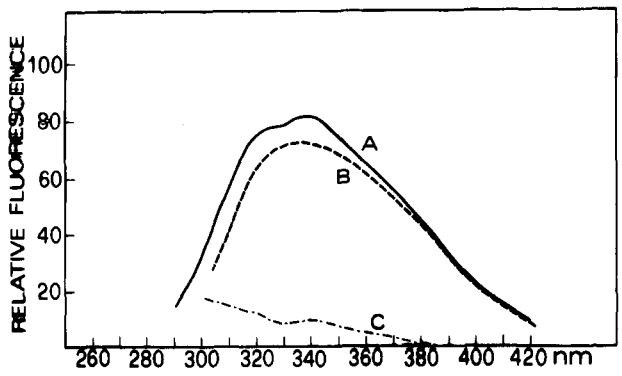

FIGURE 1: Fluorescence spectra of native fatty acid synthetase complex from *C. capitata*. Emission for excitation at 280 (A) and 295 nm (B) and their difference after normalization (C). Spectra were recorded in both 0.25 M sodium phosphate and 0.1 M sodium borate buffer, pH 7.5. Protein concentration 50 μg/mL; temperature 20 °C.

Table I: Tryptophan and Tyrosine Content of the Fatty Acid Synthetase Complex from *Ceratitis capitata*

method	M_{Tyr}/M_{Trp}
Beaven & Holiday (1952)	2.87
Edelhoch (1967)	3.05
Bencze & Schmid (1957)	2.91

capitata excited at 280 and 295 nm is shown in Figure 1. The emission spectrum of the enzyme upon excitation at 280 nm shows two maxima, at 340 and 325 nm (Figure 1, spectrum A), in accordance with the predominance of indole fluorescence. Although the resolution of protein emission into the contributions from distinctly emitting species remains an intriguing problem, the existence of these maxima may be related at least with two well-defined tryptophan populations. The tryptophan emission maxima at 340 and 325 nm in the synthetase complex is considerably blue shifted when compared to the emission maxima of free tryptophan in aqueous solution (λ_{max} 352 nm). One of these maxima (325 nm) is also significantly more blue shifted than the proposed maximum (330–332 nm) for tryptophan fluorescence in a model for the intrinsic fluorescence of water-soluble proteins (Burnstein et al., 1973). Thus, although the position of the emission maximum is not always a reliable measure of the environmental exposure, the fluorescence of the native synthetase appears characteristic of two groups of trypotphan residues in a buried, hydrophobic environment. On excitation at 295 nm, in which absorption due to tyrosine is less than 10% (Teale, 1960), the emission band is not resolved, showing only a broad maximum band centered at 335 nm (Figure 1, spectrum B). Taking into account that fluorescence above 380 nm is only due to tryptophan residues, both spectra, excitation at 280 and 295 nm, are normalized on the basis of the fluorescence value in the range 380–400 nm. The difference spectrum (Figure 1, spectrum C) calculated after normalization reveals a weak contribution of tyrosine centered at 310 nm and tryptophan residues in multiple environments. Thus, fluorescence in the fatty acid synthetase molecule is dominated by the tryptophan emission, although the spectrophotometric determinations of the tyrosine/tryptophan ratio give a value of 2.9, based on the methods outlined in Table I.

The quenching of the intrinsic fluorescence of the fatty acid synthetase complex by acrylamide was studied in an attempt to resolve the heterogeneity in the tryptophan fluorescence. Acrylamide is an efficient collisional quencher of indole derivatives, and it is able to permeate a protein matrix to interact with tryptophan residues (Eftink & Ghiron, 1975, 1976). Native fatty acid synthetase has been titrated with acrylamide

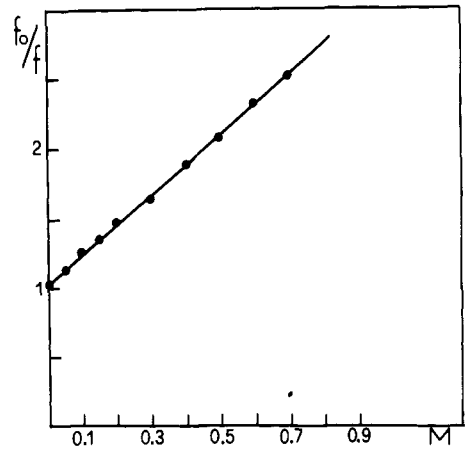

FIGURE 2: Stern–Volmer plot for acrylamide quenching of fluorescence in the fatty acid synthetase at 20 °C. Points are the experimental intensities at 340 nm with excitation at 280 nm. The straight line is the best-fit curve by the least-squares method.

to a final concentration of 0.6 M. For consideration of only tryptophan emission, samples were excited at 295 nm. In all cases, an inner filter correction was applied, as described under Materials and Methods. Figure 2 is a Stern–Volmer plot for acrylamide quenching of tryptophan fluorescence in the synthetase complex. Data were analyzed in terms of the Stern–Volmer law for collisional quenching:

$$F_0/F = 1 + Kq(Q) \quad (1)$$

where F_0 and F are the fluorescence intensities at 340 nm in the absence and the presence of acrylamide, respectively, at concentration Q and Kq is the quenching constant. The value of Kq calculated according to eq 1 is 2.19 M^{-1}. This result is consistent with the presence of tryptophan residues buried in nonpolar environments (Eftink & Ghiron, 1976). The acrylamide quenching is accompanied by a blue shift in the emission band and the absence of resolution of the spectrum into the tryptophan contributions in the presence of 0.6 M acrylamide, showing only one maximum at 325 nm. This blue shift is interpreted as due to the less quenched tryptophan residues located in more buried environments (contribution at 325 nm) than those contributing to the 340-nm maximum band. These quenching data support the view that the observed fluorescence from fatty acid synthetase arises from at least two different populations of tryptophan residues, located in environments of different polarity. The absence of a red shift provides evidence that acrylamide does not denature the protein since denaturation results in higher exposure of tryptophan residues.

Lipid–Protein Interactions and the Intrinsic Fluorescence of Fatty Acid Synthetase Complex. It is known that the enzymatic activity of the purified synthetase from *Ceratitis capitata* is highly dependent on its lipid content (Gavilanes et al., 1979, 1981). The effect of sodium cholate and sodium dodecyl sulfate was investigated to gain more information on the nature of the tryptophan and tyrosine environments in this lipid–protein system. The emission spectra of the enzyme were registered at a series of $NaDodSO_4$ and sodium cholate concentrations. The $NaDodSO_4$-treated protein at 0.1% (w/v) detergent concentration shows maxima at 365, 345, and 310 nm (Figure 3, spectrum A). The cholate-treated protein at 2% (w/v) detergent concentration shows band maxima at 360 and 345 nm as well as a shoulder at 310 nm (Figure 3, spectrum B). In both cases, the two populations of tryptophan residues were mainfest. However, the red-shift emission exhibited by the detergent-treated enzyme complex indicates a

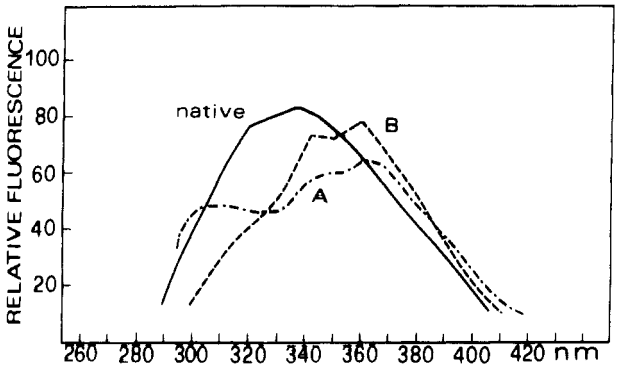

FIGURE 3: Fluorescence emission spectra of the native fatty acid synthetase and in the presence of 0.1% (w/v) $NaDodSO_4$ (A) and 2% (w/v) sodium cholate (B). Excitation at 280 nm. Spectra were recorded at 20 °C and 50 μg/mL protein concentration in 0.25 M sodium phosphate buffer, pH 7.5.

local increase in polarity for both families, higher for the $NaDodSO_4$ than for the cholate treatment.

Sodium cholate does not affect the quaternary structure of the enzyme (Gavilanes et al., 1979); however, $NaDodSO_4$ dissociates the complex. It is known from previous circular dichroism studies that the enzyme in the presence of 0.1% $NaDodSO_4$ (w/v) has the same α-helical content as the native synthetase. This has been attributed to the conformational transitions $\beta \rightarrow$ aperiodic $\rightarrow \alpha$ (Gavilanes et al., 1978). The midpoint of this process takes place at 0.001% $NaDodSO_4$ (w/v). Plots of either F_{310}/F_{360} or F_{340}/F_{360} values vs. $NaDodSO_4$ concentration show the existence of a conformational transition at that detergent concentration. Thus, all of the $NaDodSO_4$-induced transitions at the different structural levels are concomitant processes, and the structural quenching observed for tryptophan residues in the detergent-treated protein could be related to the exposure of those residues in the dissociated polypeptide chains. In the presence of sodium cholate, an exchange between detergent and phospholipids has been observed (Gavilanes et al., 1979). Fluorescence data at increasing concentrations of cholate show a conformational transition with a midpoint at 0.5% (w/v) concentration in coincidence with that for the decrease of the enzymatic activity induced by cholate (Gavilanes et al., 1979). On the basis of the observed red-shift emission, phospholipids provide a more hydrophobic environment for tryptophan residues than cholate (Figure 3). Although the overall secondary structure is not modified by cholate (Gavilanes et al., 1979), the exchange of detergent–phospholipids results in a modification of the tertiary structure of the complex. These data support the view that phospholipids are essential for the native conformation, creating specific hydrophobic interactions which could not be substituted in this lipid–protein system even by molecules with similar hydrophobicity.

Extrinsic Fluorescence Studies. Fatty acid synthetase from *C. capitata* has a lipid/protein ratio of 1.0 (w/w), with 80% of triacylglycerols as the main component and 2% of phosphatidylethanolamine as the essential lipid class for the catalytic activity (Gavilanes et al., 1979, 1981). 1,6-Diphenylhexa-1,3,5-triene (DPH) is a hydrophobic fluorescent probe commonly used in the studies of the nonpolar regions of lipid structures. Schroeder & Goh (1979) found that 80% of DPH was located in the lipid core of serum VLDL and triacylglycerols are the main component (92%) of this lipid core. Similar results were obtained by Sklar et al. (1980) from resonance energy transfer data for that serum lipoprotein. Thus, DPH could be a good extrinsic fluorescent probe essentially for the triacylglycerols of the fatty acid synthetase

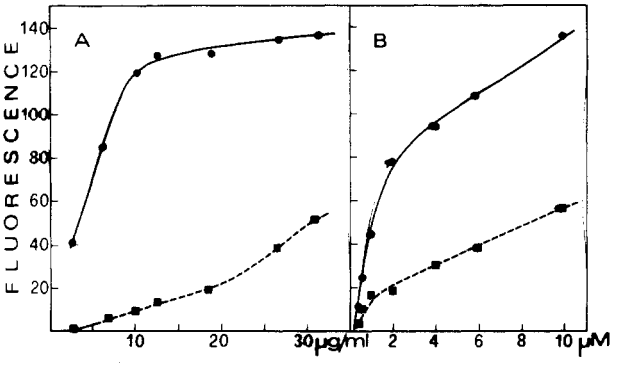

FIGURE 4: DPH (●) and DPE (■) uptake by the fatty acid synthetase. Fluorescence was measured at 20 °C after 37 °C/30-min incubation. Fluorescence intensity values are obtained for emission at 425 nm and excitation at 365 nm. (A) Fluorescence vs. protein concentration at 10 μM probe concentration and (B) fluorescence vs. probe concentration at 30 μg/mL protein concentration.

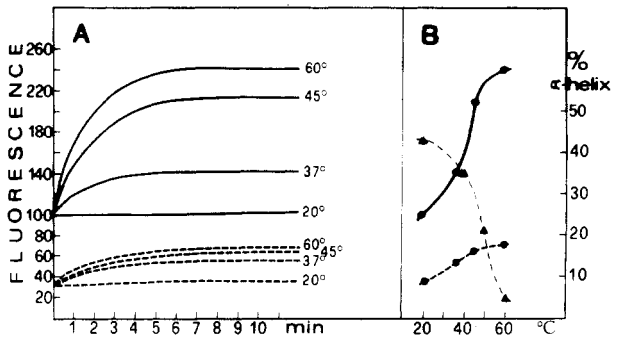

FIGURE 5: Fluorescence intensity values for emission at 435 nm and excitation at 365 nm. (A) Time course of the DPH (—) and DPE (---) uptake by the fatty acid synthetase at different temperatures; 30 μg/mL and 10 μM protein and fluorescent probe concentration, respectively. (B) F_{425} maximum values for DPH (–●–) and DPE (--●--) uptake by the fatty acid synthetase and α-helix content of the enzyme vs. temperature (▲).

complex. The specific role of phospholipids on both the conformation and the enzymatic activity of the synthetase complex was investigated by using dansylphosphatidylethanolamine (DPE) as the fluorescent probe. Since DPE is incorporated preferentially into the boundary lipids of membrane-bound proteins (Gupte et al., 1979), the dansyl group is able to monitor the structural changes in the polar head groups.

The results of the uptake of these probes (Figure 4) indicate that the DPH–synthetase system reaches a position of saturation that is affected by both probe and protein concentration. This system was saturated with 10 μM DPH at 15 μg/mL protein concentration. At each protein concentration, the amount of probe taken up by the lipoprotein depends only on the DPH concentration. This suggests that the amount of DPH taken up by the synthetase complex depends on the number of DPH vesicles of a size suitable for solubilization sites on the enzyme complex; the size factor is probe concentration dependent. However, DPE does not reach this situation at the same concentration range. These results would indicate that the DPE solubilization is mainly dependent on a solubilizing hydrophobic environment whereas DPH is also dependent on a size factor.

In Figure 5A, the DPH and DPE uptake curves at 20, 25, 37, 45, and 60 °C are given. They show that the incorporation of both probes is temperature dependent. The presence of both probes in the lipoprotein system of the synthetase complex results in a rise of fluorescence, with an apparent first-order rate of incorporation being in equilibrium after 10 min at each

Table II: Incorporation Rates for DPH and DPE to the Fatty Acid Synthetase Complex of *Ceratitis capitata* at Different Temperatures

temp (°C)	DPH (s^{-1})	DPE (s^{-1})
37	5.5×10^{-3}	6.3×10^{-3}
45	8.2×10^{-3}	6.2×10^{-3}
60	10.0×10^{-3}	6.3×10^{-3}

temperature. Thus, when a given amount of probe and synthetase complex was mixed, the probe uptake increased with the temperature of the incubation mixture. The incorporation rate values at different temperatures are given in Table II. However, from 20 to 60 °C incubation temperature, the DPH and DPE incorporations increase respectively 2.4 and 1.7 times. At 20 °C the probe uptake, for both DPH and DPE, is independent of time. The hydrophobic character of the lipid components of the complex diminishes the quenching effect for probe fluorophores, and it accounts for the fluorescence intensity at this temperature. Lentz et al. (1976) did also obtain an increase of the DPH uptake by phosphatidylcholine liposomes. They observed, however, that DPH incorporation does not follow first-order kinetics, detecting at least two processes.

Thermal denaturation studies showed a decrease in both α-helical content and enzymatic activity over the 20–60 °C temperature range (Gavilanes et al., 1981). When fluorescence intensity, as an index of the probe uptake at each temperature, vs. temperature (Figure 5B) is plotted, a thermal transition can be observed for the probe incorporation. Changes in the DPH incorporation in phosphatidylcholine liposomes with temperature have been attributed to the gel to liquid crystalline phase transition (Lentz et al., 1976). Thus, thermal denaturation of the synthetase and phase transition for the lipid bulk solubilizing DPH in the complex could occur at the same temperature range.

Excitation at 280 and 295 nm for the DPH–synthetase system shows an emission maximum centered at 445 nm. This maximum corresponds to the emission band of DPH. These results indicate the existence of energy transfer between indole donors and DPH. Similar studies on the DPE–synthetase complex do not reveal the existence of any emission band at this wavelength range. Jonas (1974) has estimated a range of distances from 23 to 66 Å for the energy transfer between indole donors and DPH in serum lipoproteins. Thus the above results indicate that the distance between tryptophan residues in the synthetase complex and DPH molecules is less than 66 Å for many of the mentioned chromophoric pairs. On the basis of the absence of energy transfer for the DPE–synthetase system, the results can be interpreted in terms of a closer proximity between DPH and indole donors than between DPE and the same kind of residues.

From fluorescence polarization studies, at saturating concentration of DPH, an intrinsic microviscosity (η) value of 5.4 P is obtained at 20 °C according to the equation $\eta = 2p/(0.46 - p)$, where p is the degree of polarization of fluorescence (Rossenen et al., 1979; Hildebrand & Nicolau, 1979). However, the polarization of the DPE emission was insignificant under the conditions of assay. These results reflect higher motional freedom for DPE in its solubilization sites than for DPH.

Discussion

From these results it can be concluded that the fluorescence properties of the fatty acid synthetase complex are consistent with the presence of two families of tryptophan residues in environments buried within the complex matrix. This behavior has been exhibited by all spectra carried out under different conditions, even in the dissociated enzyme, suggesting the existence of two different polypeptide domains in the complex. Since the enzyme complex contains abundant residues of tryptophan (1.28 molar percentage), the constancy in the difference between both degrees of exposure for tryptophan fluorophores could be related to the presence in the complex of two types of subunits. On the basis of this idea and considering that the subunits of the complex have similar molecular weights (Municio et al., 1977), the different fluorescence properties for both subunits would indicate either two different polypeptide chains or different lipid environments for each subunit. In view of these facts, the fatty acid synthetase from *C. capitata* should be a nonsymmetrical structure.

Fatty acid synthetase from the insect larvae is a soluble lipoprotein with a specific and constant lipid composition that is essential not only for the enzymatic activity but also for the tertiary organization of the complex (Gavilanes et al., 1979). From the detergent-treated protein, it is possible to conclude a specific role for phospholipids in the conformational support of the enzyme through their polar and hydrophobic interactions. Phospholipid exchange by detergent results in the tertiary structure modification, even in the presence of cholate, which does not affect either the secondary or the quaternary structures.

Extrinsic fluorescence studies indicate that for each DPH concentration a saturating value is achieved, indicating the existence of specific sites for the solubilization of DPH dependent on a size factor. This is in agreement with the hypothesis of Shinitzky & Inbar (1974) on the DPH–biological membrane system. When the lipid composition of the synthetase complex is taken into account, a concentration of 15 μg/mL, which accomplishes saturation for 10 μM DPH, implies an acyl chain concentration of 27 μM; thus, the saturation of the system at 20 °C accomplished an acyl chain/DPH ratio of about 2–3.

When the different results obtained on the uptake of DPH and DPE as well as their difference in shape and hydrophobicity are considered, we can conclude that lipids are nonrandomly distributed within the enzyme complex matrix. The buried character of tryptophan residues in the enzyme complex and the energy transfer between indole groups and DPH suggest a buried location for the solubilization sites of this probe. Since DPH is mainly distributed within the triacylglycerols, these molecules would constitute a lipid core in the synthetase complex. On the other hand, the nonobserved energy transfer between indole donors and DPE, the buried character of tryptophan residues, and the polar heads of DPE would suggest a main surface location for the solubilization sites of this probe. The specific role of phosphatidylethanolamine on the enzymatic activity (Gavilanes et al., 1979) indicates that phospholipids are nonrandomly distributed in the complex, with phosphatidylethanolamine being involved in the conformation of the active site, which according to the above reasoning would have a surface location.

Since the dispersions of DPH are practically void of fluorescence, the increase of fluorescence intensity in the labeled synthetase is ascribed to a process of solubilization of the fluorophore. The temperature-dependent rates of DPH incorporation into the enzyme are higher than those obtained for the uptake of adipocyte membranes that also follows apparent first-order kinetics (Hubbart & Garratt, 1979). Thus, the increase in solubilization which occurs over the same temperature range of thermal denaturation of the protein could

be related to this process but at higher rates than in membranes. The phase transition for the lipid-solubilizing DPH and thermal denaturation of the enzyme could then be concomitant processes.

The intrinsic microviscosity value obtained from the DPH-labeled synthetase in native conditions is similar to those obtained for serum HDL and LDL (Jonas, 1977), despite the higher triacylglycerol ratio in the synthetase. This fact would indicate an important order for the lipid core of the enzyme complex independent of its high content in triacylglycerols.

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References

Azumi, T., & McGlynn, S. P. (1962) *J. Chem. Phys. 37*, 2413–2420.

Azzi, A. (1975) *Q. Rev. Biophys. 8*, 237–316.

Beaven, G. H., & Holiday, E. R. (1952) *Adv. Protein Chem. 7*, 319–382.

Bencze, W. L., & Schmid, K. (1957) *Anal. Chem. 29*, 1193.

Bligh, E. G., & Dyer, W. J. (1959) *Can. J. Biochem. Physiol. 39*, 911–917.

Burnstein, E. A., Vedenkina, N. S., & Ikowa, M. N. (1973) *Photochem. Photobiol. 16*, 2325–2329.

Chen, L. A., Dale, R. E., Roth, S., & Brand, L. (1977) *J. Biol. Chem. 252*, 2163–2169.

Dittmer, J. C., & Lester, R. L. (1964) *J. Lipid Res. 5*, 126–127.

Dufourq, J., & Faucon, J. F. (1977) *Biochim. Biophys. Acta 467*, 1–11.

Dufourq, J., Faucon, J. F., Lussan, C., & Bernon, R. (1975) *FEBS Lett. 51*, 112–116.

Edelhoch, H. (1967) *Biochemistry 6*, 1948–1954.

Eftink, M. R., & Ghiron, C. A. (1975) *Proc. Natl. Acad. Sci. U.S.A. 9*, 3290–3294.

Eftink, M. R., & Ghiron, C. A. (1976) *Biochemistry 15*, 672–680.

Epand, R. M., Jones, A. J. S., & Schreier, S. (1977) *Biochim. Biophys. Acta 491*, 296–304.

Fuchs, P., Parola, A., Robbins, P. W., & Blout, E. R. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 3351–3354.

Gavilanes, J. G., Lizarbe, M. A., Municio, A. M., & Oñaderra, M. (1978) *Biochem. Biophys. Res. Commun. 83*, 998–1002.

Gavilanes, J. G., Lizarbe, M. A., Municio, A. M., Ramos, J. A., & Relaño, E. (1979) *J. Biol. Chem. 254*, 4015–4021.

Gavilanes, J. G., Lizarbe, M. A., Municio, A. M., & Oñaderra, M. (1981) *Biochim. Biophys. Acta 668*, 246–256.

Gennis, R. B., Sinensky, M., & Strominger, J. L. (1976) *J. Biol. Chem. 251*, 1270–1276.

Grey, T. (1967) *Methods Enzymol. 11*, 139.

Gupte, S. S., Lane, L. K., Johnson, J. D., Wallick, E. T., & Schwartz, A. (1979) *J. Biol. Chem. 254*, 5099–5103.

Gwynne, J., Palumbo, G., Brewer, H. B., & Edelhoch, H. (1975) *J. Biol. Chem. 250*, 7300–7306.

Hildebrand, K., & Nicolau, C. (1979) *Biochim. Biophys. Acta 553*, 365–377.

Hubbart, R. E., & Garratt, C. J. (1979) *Biochem. Soc. Trans. 7*, 993–995.

Jonas, A. (1977) *Biochim. Biophys. Acta 486*, 10–22.

Lentz, B. R., Barenholz, Y., & Thompson, T. E. (1976) *Biochemistry 15*, 4521–4528.

Municio, A. M., & Gavilanes, J. G. (1981) *Methods Enzymol. 71*, 127–133.

Municio, A. M., Lizarbe, M. A., Relaño, E., & Ramos, J. A. (1977) *Biochim. Biophys. Acta 481*, 175–188.

Parola, A. H., Robbins, P. W., & Blout, E. R. (1979) *Exp. Cell Res. 118*, 205–214.

Radda, G. K. (1975) *Methods Membr. Biol. 4*, 97–188.

Radda, G. K., & Vanderkooi, J. (1972) *Biochim. Biophys. Acta 265*, 509–549.

Rosenthal, S. L., Parola, A. H., Blout, E. R., & Davidson, R. L. (1978) *Exp. Cell Res. 118*, 205–214.

Rossenen, M., Verlaemst, R., Caster, H., Lievens, M. J., Van Tornout, P., & Herbert, P. N. (1979) *Eur. J. Biochem. 96*, 357–362.

Schlessinger, J., & Elson, E. L. (1979) in *Biophysical Methods* (Ehrenstein, G., & Lecar, H., Eds.) Academic Press, New York.

Schneider, A. B., & Edelhoch, H. (1972) *J. Biol. Chem. 241*, 4986–4991.

Schroeder, F., & Goh, E. H. (1979) *J. Biol. Chem. 254*, 2464–2470.

Shinitzky, M., & Inbar, M. (1974) *J. Mol. Biol. 85*, 603–615.

Shinitzky, M., & Barenholz, Y. (1978) *Biochim. Biophys. Acta 515*, 367–394.

Sklar, L. A., Doody, M. C., Gotto, A. M., Jr., & Pownall, H. J. (1980) *Biochemistry 19*, 1294–1301.

Stryer, L. (1968) *Science (Washington, D.C.) 162*, 526–533.

Teale, F. W. J. (1960) *Biochem. J. 76*, 381–388.

Turner, D. C., & Brand, L. (1968) *Biochemistry 7*, 3381–3390.

Verdery, R. B., & Nichols, A. V. (1974) *Biochem. Biophys. Res. Commun. 51*, 1271–1278.