Vol. 83, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 14, 1978 Pages 998-1003

CIRCULAR DICHROISM STUDIES OF THE FATTY ACID SYNTHETASE COMPLEX FROM THE INSECT *CERATITIS CAPITATA*

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Received June 19, 1978

SUMMARY. Circular dichroism spectra of the native fatty acid synthetase complex from the insect *Ceratitis capitata* and of the lipidated and cholate- and SDS-treated enzyme have been obtained. Native enzyme has a calculated structure of 43% α-helix, 23% β structure and 31% random coil. Lipidation and cholate-treatment did not modify the structure of the enzyme complex whereas the SDS-treatment changed the native conformation into a structure based on 42.8% α-helix, 8.4% β structure and 18.8% random coil. These data are interpreted in terms of both the enzyme activity and the quaternary structure of the complex.

Fatty acid synthetase complex has been isolated and characterized from the larval stage of development of the insect *Ceratitis capitata* (1). The molecular weight (5.2×10^5) agrees with the values assigned to complexes from other sources (2-5). The native synthetase complex from the insect dissociated with 8M urea and 0.05% sodium dodecylsulphate into half-molecular subcomplexes (1) as determined by gel electrophoresis and Sephadex G-200 chromatography. Calculated frictional ratio (1.679) of the native dimer indicated a high assymetry of the molecule.

These results support the idea of two equal molecular weight polypeptides in agreement with the suggestions of Stoops *et al.* (6) and Buckner and Kolattukudy (7) for the fatty acid synthetase from different origins.

The study carried out in this paper is an initial attempt to evaluate the conformational properties of the fatty acid synthetase complex of the insect *Ceratitis capitata* analyzing circular dichroism data of the protein in the native state and after different treatments.

MATERIALS AND METHODS

Fatty acid synthetase complex was isolated and purified from larvae of *Ceratitis capitata* as previously described (1).

The enzyme activity was evaluated using (1-^{14}C)acetyl-CoA as

0006-291X/78/0833-0998$01.00/0

substrate as described (1).

Circular dichroism spectra of proteins were recorded with a Jobin Yvon, Mark III, dichrograph fitted with a 250 W xenon lamp at a 0.5 mm/sec. scanning speed. Millipore (5 μm pore diameter) filtered solutions of proteins were studied in 0.25 M sodium phosphate, pH 7.5, at 20°C, in the concentration range 0.2-0.4 mg/ml in 0.05 cm optical path cells. The concentration was determined using an $\varepsilon_1^{1\%}{}_{cm}$ of 1.00 at 278 nm. Circular dichroism results are the mean values of three determinations and are reported in terms of (θ), the mean residue ellipticity in units of degrees×cm^2/decimole. The mean residue weight was taken as 110.

Delipidation of the native fatty acid synthetase complex was carried out with chloroform-methanol by the method of Bligh and Dyer (8). The suspension was centrifuged in the refrigerated RC-2 Sorvall at 1 500×g for 10 min. The protein was dissolved in 0.25 M sodium phosphate buffer, pH 7.5, 2 mM EDTA and 1 mM mercaptoethanol by stirring for about 12 hours at 4°C. The solution was concentrated through ultrafiltration using Amicon membranes.

Either the native fatty acid synthetase complex or the delipidated material was incubated with either sodium dodecylsulphate (4%) or sodium cholate (1%) in sodium phosphate buffer, EDTA and mercapto - ethanol, pH 7.5, at 4°C. Times of incubation were usually 1 hour or 12 hours for the native enzyme or the delipidated material, respectively. If necessary, the extraction was further cleared by centrifugation at 1 500×g for 10 min.

RESULTS AND DISCUSSION

Figure 1 shows the circular dichroism curve of native fatty acid synthetase complex of the insect *Ceratitis capitata* in the 200-250 nm region, having a rather low ellipticity equal to approximately -16500 deg.cm^2/dmole at 208 nm. This experimental spectrum closely fits to the calculated values for a conformational variation of 43% α-helix, 23% β structure and 31% random coil, based on the experimentally obtained curves for the three standard conformations of poly-L-lysine according to the methos of Greenfield and Fasman (9). The α-helix has an extremum at 208 nm and its ellipticity is somewhat chain-length dependent (10,11); thus a large polypeptide chain can be attributed to the native complex.

The enzyme activity of the purified complex from the insect was highly dependent of its lipid content and the treatment of the complex with phospholipase A_2 eliminated the activity (12). It agrees with the

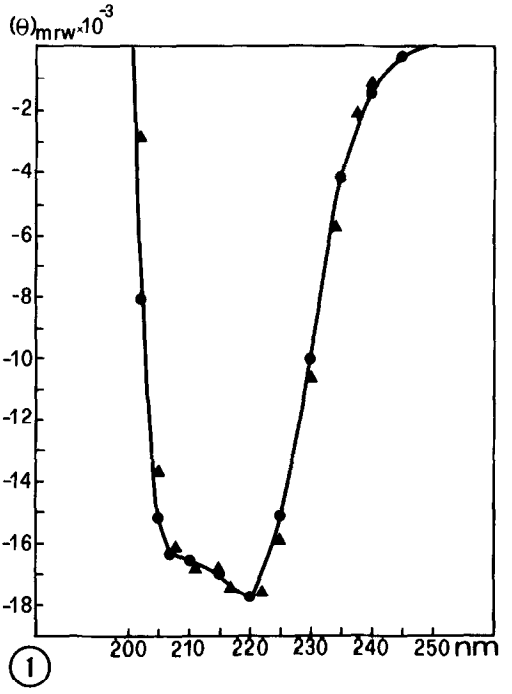

Figure 1.Circular dichroism curve of the native fatty acid synthetase complex (—●—) and calculated data (▲) of α-helix (43%), β structure (23%) and random coil (31%).

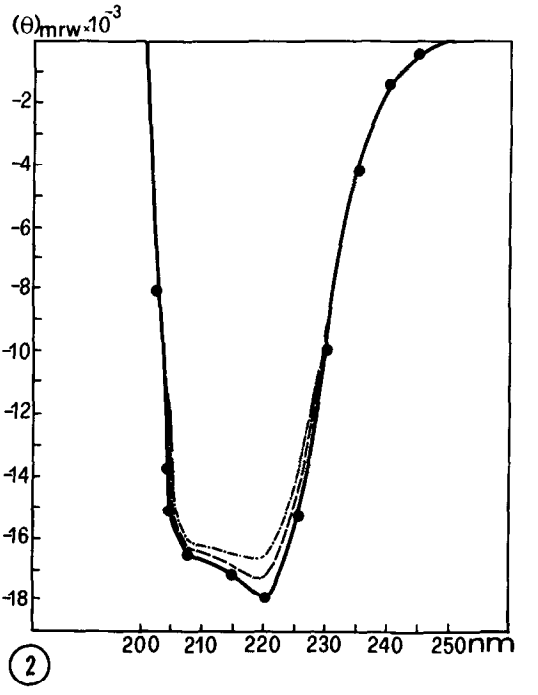

Figure 2.Circular dichroism curves of the native fatty acid synthetase complex (—●—) and after treatment with total lipids (----) and sodium cholate (-·-·-).

variations of the enzyme activity induced by cholate treatment and the incorporation of exogenous natural lipids into the complex as summarized in Table 1. The activity of the enzyme incubated in the presence of a wide range of cholate concentrations decreased progressively to reach the virtual inactivation of the enzyme over 1.0 % concentration. On the other hand, the enzyme activity of the native purified complex was enhanced by increasing the lipid/protein ratio with total lipids from the larval insect; over a lipid/protein ratio of 0.8 resulted in a maximum value of fatty acid synthetase activity.

Figure 2 shows the circular dichroism curves of native fatty acid synthetase and after treatment of the protein complex with either 1% sodium cholate or exogenous total lipids of the insect to reach a lipid/protein ratio of 1.0.

The practical identity of the three curves can be regarded as an indication of the retaining of the native conformation of the fatty acid synthetase complex after both types of treatments. Thus, neither the addition of total lipids to the enzyme complex nor their removal

by cholate caused significant changes in the native organization although the activity varied in a wide range (Table 1). Therefore, these data established that the absence of activity induced by sodium cholate can not parallel a denaturation of the lipoprotein; the exposure of the enzyme complex to cholate could promote the dissociation of some lipids, such as phosphoglycerides, that located on the lipoprotein surface, influence the catalytic activity but it could not eliminate the non polar protection of protein helices by other lipids from competitive interactions with water. It agrees with both the slight enhancement of the enzyme activity when total lipids were added to the native enzyme and the total loss of activity after incubation of the complex with phospholipase A_2 (12).

TABLE 1. Modification of the activity of the fatty acid synthetase complex from the larval insect *Ceratitis capitata*.
Protein solution (0.2 ml) and detergent solution (0.1 ml) were mixed to give 0.1 mg/ml of proteins and the detergent concentrations given below. Lipids were added to the final ratios of the table.

SDS		CHOLATE		LIPIDS	
%	% activity	%	% activity	lipid/protein	% activity
Control	100	Control	100	Control	100
0.0005	68.5	0.1	77	0.08	108
0.001	45.0	0.25	42	0.16	112
0.005	17.0	0.5	18	0.42	121
0.01	11.5	1.0	4	0.83	124
0.05	6.5	2.0	3	1.60	124
0.1	2.1	4.0	-	2.50	124

The circular dichroism spectrum of the delipidated enzyme by extracting with mild solvents (figure 3) allows to estimate a 3% of αhelix for the conformation of this protein (9). The complete removal of lipids resulted in the disorganization of the complex as judged from both the total enzyme inactivation and the changes in the α-helix content.

Figure 4 shows the circular dichroism spectra of both the native fatty acid synthetase complex and the inactive enzyme by incubating the native complex with 4% sodium dodecylsulphate. Upon denaturation, both the magnitudes and the positions of the extrema were shifted and the experimental curve fits a conformation having 42.8% α-helix, 8.4% β structure and 48.8% random coil (9).

Thus, the helix calculated values for the native and SDS-denaturated enzyme are very similar, whereas a partial transition from the β structure, found in the native state as in many other globular proteins into a disordered conformation took place. A similar qualitative change was observed when the delipidated enzyme was incubated with sodium dodecylsulphate (figure 3).

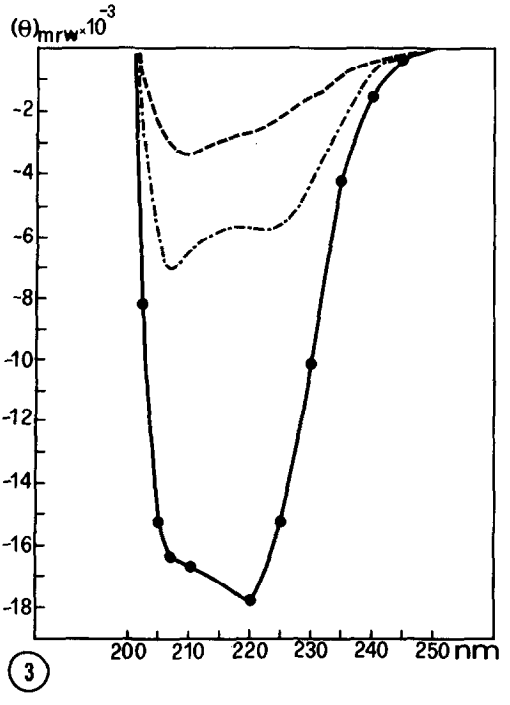

Figure 3.Circular dichroism curves of the native fatty acid synthetase complex (—●—), delipidated enzyme (----) and delipidated enzyme added of 4% SDS (-·-·-).

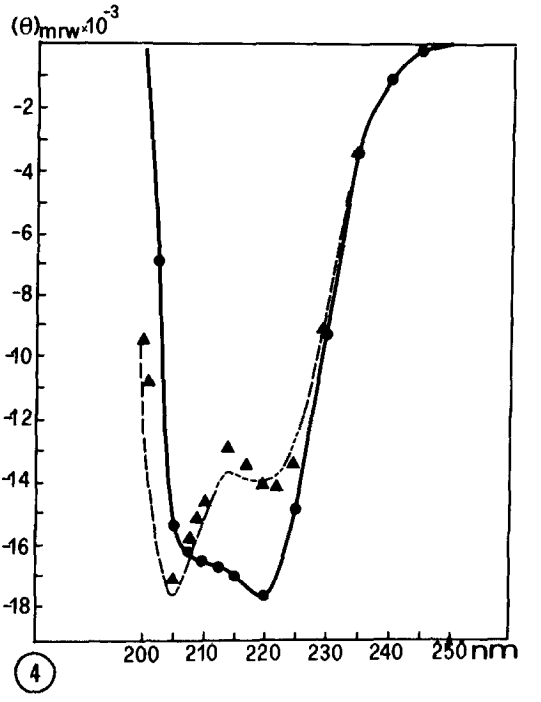

Figure 4.Circular dichroism curves of the native fatty acid synthetase complex (—●—) and after treatment with 4% SDS.

Jirgensons (13) indicated that some nonhelical proteins in the native state can be partially converted to the α-helical conformation by treatment with anionic detergents. The magnitude of the conformation transitions β→R→α induced by detergents (14-16) is highly dependent on the protein compactness and rigidity. Bearing all the above in mind, if the native enzyme had a compact structure with seven polypeptide subunits, the SDS-denaturation would follow a higher α-helix content but this variation cannot be supported by the circular dichroism spectrum (figure 4). On the contrary, whether the enzyme complex had only two more flexible polypeptide chains, the α-helical confor-

mation would be easier preserved when treated with the detergent. These results add new data in support of a quaternary structure of the fatty acid synthetase of the insect based on two assymetrical subunits (1).

Attempts can be made to correlate our circular dichroism results on the effects of the different classes of activity modu - lators with the enzyme activities and conformational data of the isolated subunits of the complex.

This research has been granted by the "Comisión Asesora de Investigación Científica y Técnica" (n°1794)

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