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SECONDARY STRUCTURES OF RAT LIPOLYTIC ENZYMES: CIRCULAR DICHROISM STUDIES AND RELATION TO HYDROPHOBIC MOMENTS

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To explore the secondary structures of lingual and pancreatic lipases, circular dichroism measurements were performed. Maximum average ellipticities were used to calculate the percentage of α-helices, β-sheets, and random coils. Lingual lipase had an ellipticity of -20235 ± 140 deg cm^2/dmol(mean$\pm$SE) at 220nm suggesting 60% α-helix, 20% β-sheet and 20% random coil structure, but the mean ellipticity for pancreatic lipase was -14093 ± 82 deg cm^2/dmol(mean $\pm$SE) at 210nm suggesting a 34.8% α-helical, 25% β-sheet and 40% random coil secondary structure. An α-helical stretch of residues with a large hydrophobic moment ("globular" α-helix by hydrophobic moment plot) from amino acids 382 through 389 at the COOH-terminal end of lingual lipase was noted. This sequence, absent in pancreatic lipase, may account for the avid binding of lingual lipase to fat emulsion particles.

Lipolytic enzymes are unique in that they undergo "activation" at oil-water interfaces in contrast to conventional esterases which act on soluble substrates (1). In addition to active sites, lipolytic enzymes contain hydrophobic domains which are important in binding to oil-water interfaces. After the enzyme has bound to the substrate, the active site is optimally

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positioned for hydrolysis. The primary structures encoded for by the genes of these enzymes determine the possible secondary structures for these proteins. Computer predictions of possible secondary structures based on the primary sequence are useful, but are in agreement with direct biophysical measurements of secondary structure by x-ray diffraction or circular dichroism only 50-60% of the time (2,3). X-ray diffraction methods require crystallization of the protein to be studied, while circular dichroism measurements can be determined on an enzyme in solution. Since rat lingual and pancreatic lipases have not yet been crystallized, we have performed, for the first time, circular dichroism measurements on the purified enzymes in solution and compared our findings with computer-predicted secondary structures based on the primary sequences found in the literature.

Materials and Methods

Rat lingual lipase and pancreatic lipase were purified to homogeneity as previously described (4-6). Lingual lipase and pancreatic lipase gave single protein bands of M_r = 50,000 and M_r=34,000, respectively, after silver staining (4-6).

Circular dichroism measurements:

Circular dichroism experiments were performed in the Laboratory of Chemical Physics at the National Institutes of Health. Circular dichroism spectra were obtained using a Jasco 500A spectropolarimeter equipped with a thermostatted cell holder on 100-200 µg of enzyme dialyzed against either phosphate buffer pH 6.0 or buffer alone [pH 6.0 is the typical pH found in both the stomach and duodenum during the first hour after a meal (7)]. The cell path length varied between 0.497 and 0.502 mm. Temperature was maintained at 24°C and sample spectra and blanks were obtained from wavelengths 270 to 200 nm. All measurements were repeated at least 10 times on one aliquot of each purified enzyme or blank. The data were collected at one circular dichroism session under identical conditions. The raw data (ellipticity) were transferred via a microcomputer interface into a data acquisition, LDACS, and processing system, APR, developed at the Laboratory of Chemical Physics, National Institutes of Health, Bethesda, MD and processed (8) using the MLAB program developed by the Computer Systems Laboratory at NIH. Mean residue ellipticities at each wavelength were calculated (9,10) according to the following equation after correction for the appropriate buffer blanks:

$$[\theta] = \frac{\theta_{obs} \times 113}{10 \times l \times c}$$

where θ_{obs} is the observed ellipticity, 113 is the mean residue molecular weight (derived from amino acid composition), l is the

pathlength in centimeters, and c is the protein concentration in g/ml. The results were then further processed through an elliptic smoothing algorithm (8) prior to printing and plotting.

Computer predictions of protein secondary structure:
We analyzed the secondary structures of porcine pancreatic lipase based on tryptic peptide hydrolysis (11) and rat lingual lipase based on cDNA cloning (12) by the computer programs SEARCH, ALIGN, RELATE, CHOFAS (13). We also used the DELPHI program in order to predict areas of possible α-helices, β-sheets, and 3-10 helices (3). We then used a program (14) to predict the hydrophobic moments of the helices found. Programs were run on a VAX-11/780 mainframe computer in the Laboratory of Mathematical Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD.

Results

Lingual lipase demonstrated an ellipticity of -20235$\pm$ 140 deg cm^2/dmol (mean$\pm$SE) at 220 nm. The data curve (see Figure 1) corresponded most closely to a protein exhibiting a secondary structure of 60% α-helix, 20% β-sheet and 20% random coil. Pancreatic lipase was found to have an ellipticity of -14093$\pm$82 deg cm^2/dmol (mean$\pm$SE) at 210 nm predictive of a 34.8% α-helix, 25% β-sheet and 40% random coil secondary structure (see Figure 2).

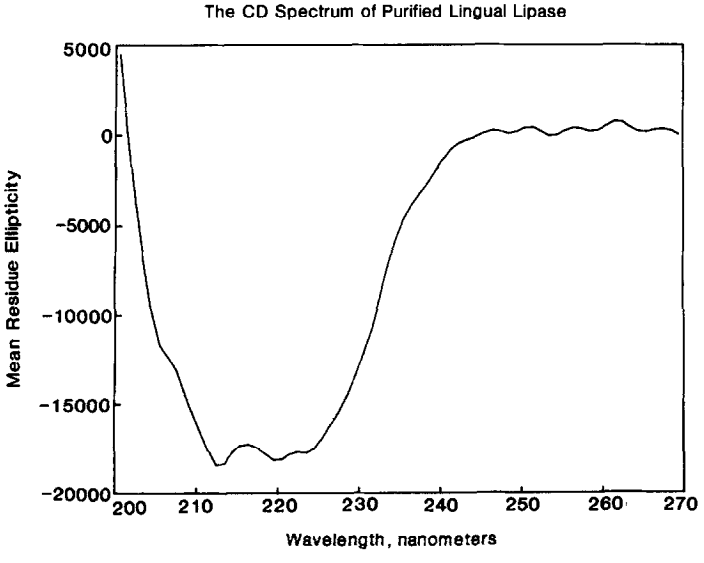


Figure 1. Mean residue ellipticity of purified rat lingual lipase as a function of wavelength. The curve best fits a 60% α-helical, 20% β-sheet and 20% random coil secondary structure.

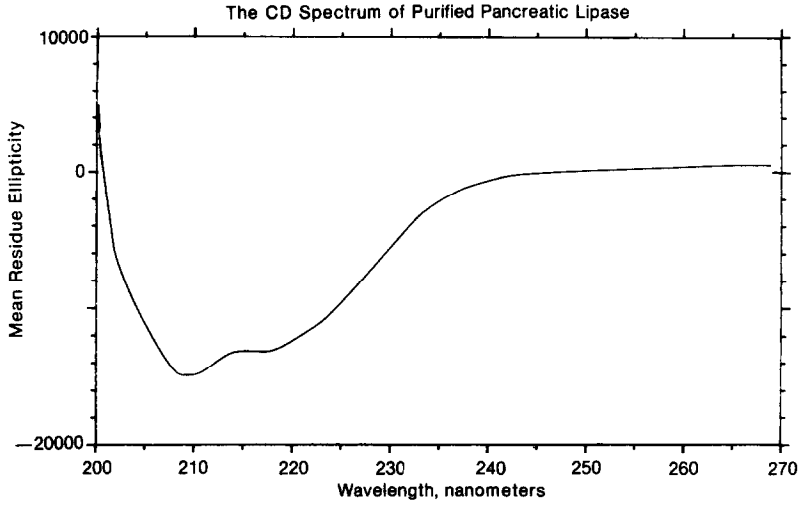


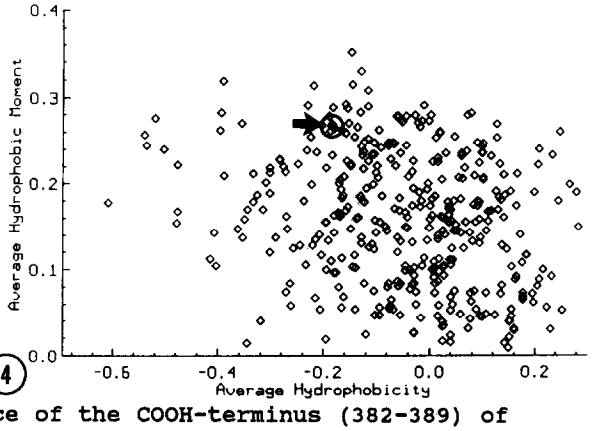
Figure 2. Mean residue ellipticity of purified rat pancreatic lipase as a function of wavelength. The data fits closely a 34.8% α-helix, 25% β-sheet, 40% random coil secondary structure.

Both lingual lipase and porcine pancreatic lipase had hydrophobic NH_2-terminal signal sequences which were predicted to be α-helical in nature by the computer. In addition, lingual lipase possessed a unique stretch of α-helical residues at its COOH-terminal (amino acids 382-389) (Figure 3) with a very strong amphipathic signal (>3.0). Hydrophobic moment plots demonstrated

GLN-GLU-VAL-TYR-ASN-GLU-MET-ILE

382 389

(3)



(4)

Figure 3. Amino acid sequence of the COOH-terminus (382-389) of rat lingual lipase.

Figure 4. Average hydrophobic moments of rat lingual lipase as a function of the average hydrophobicity using the method of Eisenberg. Each diamond represents an 11-residue α-helix, the ordinate giving the mean hydrophobic moment per residue $\langle\mu_H\rangle$ and the abscissa giving the mean hydrophobicity per residue <H>. The encircled black diamond corresponds to the α-helix beginning with residue 382. All hydrophobic moments of lingual lipase fall within the boundaries of those found in globular proteins.

that this was a "globular"-type α-helix (see Figure 4). Pancreatic lipase did not have such an area at its COOH-terminus.

Discussion

Lingual lipase and pancreatic cholesterol esterase are relatively hydrophobic proteins; protocols for the purification of these two enzymes require detergent solubilization in order to use chromatographic separation procedures (4,5,15). On the other hand, pancreatic lipase may be purified either by homogenation of pancreatic tissue in rats (6) or by collection of pancreatic juice in large animals (11). Although the amino acid composition and primary amino acid sequence of a protein provide some information about its nature, direct biophysical measurements of secondary structure by X-ray diffraction or circular dichroism are often most revealing.

Lingual lipase was the most α-helical (60%) lipolytic enzyme as determined by circular dichroism measurements (rat cholesterol esterase is only 28.9% α-helical (16). As the primary amino acid sequence of both lingual lipase (by molecular cloning) and pancreatic lipase (by tryptic hydrolysis) have been established, we used the DELPHI computer program to search for α-helical areas. Although lingual lipase and pancreatic lipase contain α-helical "signal" sequences at the NH_2-terminus (which are expected for most secretory proteins), lingual lipase contains a unique 8 amino acid amphipathic α-helical area with a large hydrophobic moment (14) at the COOH-terminal end of the molecule a finding not seen in pancreatic lipase. This α-helical region may be responsible for the interaction of the enzyme with lipids and is different from the serine-containing hexapeptide "interfacial" site reported in mid-portion of the primary sequence of porcine pancreatic lipase (11), rat lingual lipase (12), lipoprotein lipase in several species (17-19), rat hepatic

lipase (20) and human lecithin-cholesterol acyltransferase (LCAT) (21). It is interesting to note that lingual lipase is quite unrelated to pancreatic lipase in primary sequence, but very similar to human gastric lipase (78% homology)(22); the SEARCH program gives the next highest scores for similarity with cytochrome c oxidase (EC 1.9.3.1), but there is no overall similarity to pancreatic lipase or LCAT using the RELATE and ALIGN programs (the primary sequence of cholesterol esterase is not yet reported so no direct comparison may be made). The unique strongly amphipathic sequence at the COOH-end of rat lingual lipase may afford additional stability to the enzyme when binding to triglyceride/phospholipid emulsion particles, an ability that pancreatic lipase lacks unless its cofactor, colipase, is present (23).

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