Intrinsic fluorescence of mitochondrial F_1-ATPase

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Summary. The emission maximum of the fluorescence spectrum of the mitochondrial F_1-ATPase is shifted from 305 to 334 nm when the excitation wavelength is altered from 270 to 300 nm. This indicates that both tyrosine and tryptophan contribute to the intrinsic fluorescence of the F_1-ATPase.

The mitochondrial F_1-ATPase is an essential part of the enzyme complex responsible for the synthesis of ATP by oxidative phosphorylation[3-6]. In order to have a better knowledge of the structure of this enzyme, we have studied its intrinsic fluorescence. The fluorescence spectrum of isolated pig heart mitochondrial F_1-ATPase reveals the presence of tryptophan, which has not been detected in beef heart or chloroplast F_1-ATPase by aminoacid analysis[7,8]. Further, the fluorescence due to tyrosine is particularly prominent. This may indicate that some tyrosine groups are exposed and that their fluorescence is not quenched as is the case for most proteins[9].

Fluorescence measurements were done either on a Farrand spectrofluorimeter or on a highly sensitive spectrofluorimeter, built by Motohiro Iwatsubo in Gif-sur-Yvette, CNRS, which has been previously described[10].

Isolated F_1-ATPase was prepared as described before[11] from pig heart mitochondria[12]; the heating step was omitted[13]. The purified enzyme had a sp.act. of 95-115 µmoles P_i formed per min per mg protein; the purity of each preparation was checked by gel electrophoresis[13]. F_1-ATPase was kept at 2 °C as a suspension in 2.3 M ammonium sulfate, 1 mM EDTA, 2 mM ATP, 10 mM Tris-sulfate, pH 7.5. Before use, the suspension was spun down in an Eppendorf bench centrifuge; the pellet was dissolved in a minimum volume of buffer containing 40 mM Tris-sulfate, 1 mM EDTA, pH 7.5. To remove free nucleotides, the enzyme was precipitated again by addition of ammonium sulfate to a concentration of 2.5 M; after centrifugation, the pellet was dissolved again in the same buffer. This step was repeated 3 times. Alternatively 40 mM HEPES-KOH, 1 mM EDTA, pH 7.7 was used instead of the Tris buffer.

The activity of the enzyme was checked before and after fluorescence measurements by spectrophotometric assay as previously described[13]. The activity was not modified by illumination in the presence or absence of nucleotides if the excitation wavelength was higher than 285 nm. Below this wavelength, the activity slowly decreased. For example, when the excitation wavelength was 270 nm, 5% of the activity was lost after a 10-min illumination of 0.2 mg F_1-ATPase in 1 ml Tris buffer.

The fluorescence spectrum of F_1-ATPase has an emission maximum which is dependent on the excitation wavelength (figure 1). There is a shift in this maximum from 305 nm to 334 nm when the excitation wavelength is increased from 275 nm to 300 nm. The fluorescence emission depends on the excitation wavelength in the following way: when excited at 285 nm, the fluorescence intensity is highest; at 290 nm, 2 emission maxima can be detected; at 300 nm, the contribution from the emission peak located between 305 and 310 nm becomes negligible and a small, but well defined peak centred on 334 nm appears. In figure 2, the intensity of the fluorescence emission at 310 and 334 nm, as a function of the excitation wavelength, has been compared. The ratio between the fluorescence emission at 334 nm (F_{334}) and the fluorescence emission at 310 nm (F_{310}) increases rapidly when the excitation wavelength is above 290 nm. The same ratio was obtained when the protein concentration was increased from 60 to 700 µg protein per ml.

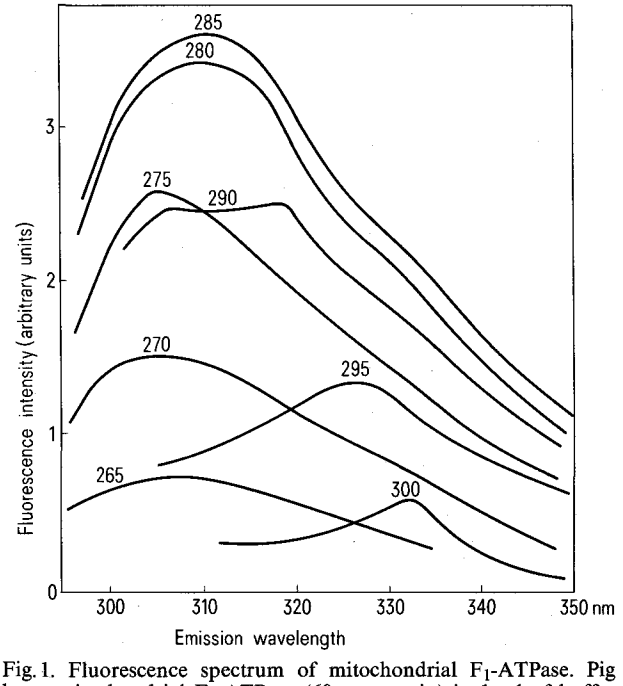

Fig. 1. Fluorescence spectrum of mitochondrial F_1-ATPase. Pig heart mitochondrial F_1-ATPase (60 µg protein) in 1 ml of buffer containing 40 mM Tris-sulfate, 1 mM EDTA, pH 7.5. The excitation wavelength was set at 265 nm. The spectrum was recorded in 30 sec. Then the beam light was closed by a shutter, the excitation wavelength was moved to 270 nm; the new spectrum was recorded and so on until 300 nm. The numbers written on each curve correspond to the value of the excitation wavelength.

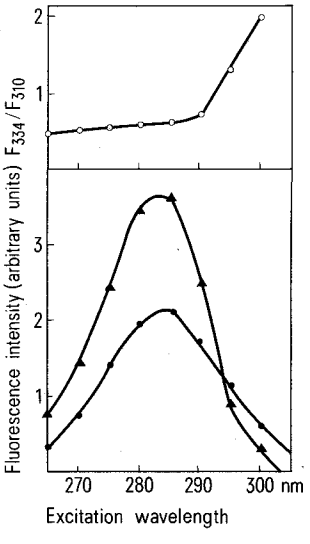

Fig. 2. Variations of the fluorescence intensity of F_1-ATPase at 310 and 334 nm with the excitation wavelength. Conditions are identical to figure 1. ▲—▲, fluorescence intensity at 310 nm; ●—●, fluorescence intensity at 334 nm.

The fluorescence spectra of pig heart mitochondrial F_1-ATPase indicate the presence of at least 2 aromatic species. The fluorescence intensity of tyrosine in solution is much lower than that of tryptophan when the excitation wavelength is higher than 290 nm, because above this wavelength only tryptophan absorbs[14]. The increase in the ratio of the fluorescence emission of F_1-ATPase at 334 nm to that at 310 nm, with an increase in the excitation wavelength, therefore indicates the presence of tryptophan. In most proteins, when tryptophan is present, its fluorescence masks that of tyrosine. The latter is normally detected with a low yield and with a maximum emission around 304 nm only in the absence of tryptophan. The tyrosine fluorescence in proteins is normally diminished by the quenching effect of hydrogen bonds formed between tyrosine and amino acid side chains[9] or peptide carbonyl groups[15]. Thus, the high fluorescence of tyrosine in F_1-ATPase may indicate the presence of tyrosine which is exposed and not quenched; the quenching normally induced by the vicinal peptide carbonyl groups would be abolished either by helical conformation or by location of these peptide linkages in a nonpolar environment[15]. Similar spectra showing an important contribution of the tyrosine in the total fluorescence spectra have been described for myelin and for the basic protein of myelin[16]. Myelin basic protein and F_1-ATPase are both proteins which can strongly interact with lipids in membranes, and are considered to sit on 1 face of the membrane[17,18]. It would be interesting to know if this high tyrosine fluorescence is a general characteristic of such membrane proteins, and if F_1-ATPase reassociated with liposomes keeps this property as does basic protein in myelin.

The presence of tryptophan has not been detected in beef heart F_1-ATPase by amino acid analysis[7] or in chloroplast F_1-ATPase. However, it is possible that a low content of tryptophan may have been overlooked in a protein such as F_1-ATPase, which has a high mol.wt (360,000 for beef heart ATPase according to Senior[4]), especially because tryptophan is susceptible to degradation during the hydrolysis of the protein that must precede the amino acid analysis. It is unlikely that the tryptophan fluorescence observed in F_1-ATPase is due to a contaminant protein, for, when the spectra were done with an F_1-ATPase preparation that contained contaminants (detectable in sodium dodecyl sulfate gel electrophoresis as 2 small protein bands of mol.wt around 80,000), the contribution of tryptophan in the spectra was not increased as compared to spectra obtained with a preparation containing no visible contaminant.

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Imbalance in the activities of alkaline phosphatase and Na^+-K^+-ATPase in the brain of experimentally induced phenylketonuric squirrels *(Funambulus palmarum)*[1]

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Summary. Phenylketonuric squirrels have shown marked inhibition of alkaline phosphatase in the olfactory lobes and cerebral hemispheres, whereas the Na^+-K^+-ATPase remained less altered. In the pathogenesis of phenylketonuria inhibition of alkaline phosphatase at the level of 'Blood-Brain Barrier' (BBB), leads transport system to impaired functioning.

In phenylketonuria, the massive increase and accumulation of phenylalanine and its metabolites has been believed to cause permanent brain damage in men. The intricacies of altered brain functions in phenylketonuria are sill unsolved. Previous reports have, however, shown that the uptake of other amino acids into the brain is inhibited if the concentration of a single amino acid increases in the plasma[2,3]. It is far from clear how the entry of amino acids into the brain is repressed. An inhibition of enzymes mediating transport of amino acids, has, however, been presumed to be one of the causative factors. In order to evaluate this supposition, the author has investigated the effects on alkaline phosphatase and Na^+-K^+-ATPase in the brain of experimentally produced phenylketonuria in squirrels.

Materials and methods. 1. The 1st series of experiments were performed on experimentally induced phenylketonuric squirrels, produced by the method of Antonas et al.[4] i.e. 4 days treatment of 300 mg/kg p-chlorophenylalanine + 200 mg/kg L-phenylalanine. In all cases, control groups received injections of equal volumes of 0.9% saline.
2. The 2nd series of experiments was carried out by directly injecting intracerebrally and bilaterally, under ether anaesthesia 8.0, 32.0 and 64.0 µg of L-phenylalanine in a total volume of 4 µl. Control groups received 4 µl of 0.9% saline. After 1 h the animals were killed by fracturing the neck and