FLUORESCENCE OF D-GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE-SUBSTRATE COMPLEXES

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There is a well-known fluorescence of proteins at 340 mμ which is due to the excitation of ionizable tyrosines and tryptophans at 290 mμ (Shore and Pardee, 1956; Teale and Weber, 1957; Konev, 1957). In addition to this fluorescence Boross and Katchalski (1968) found that pig muscle D-glyceraldehyde-3-phosphate dehydrogenase (GAPD), yeast alcohol dehydrogenase, and pig muscle lactic dehydrogenase (LDH) when activated at 380 mμ exhibit a much weaker emission with a maximum at 460 mμ.

Independently from the work at Rehovoth we found the same fluorescence with GAPD from pig, rabbit and crayfish muscle as well as from yeast, with pig muscle LDH and with rabbit muscle α-glycerophosphate dehydrogenase (GDH). The results are shown in Fig.1. We find that a high protein concentration, at least 1 mg/ml is necessary to observe this fluorescence.

The newly found fluorescent band as well as that emitting at 340 mμ (Velick, 1958) was used for the study of the enzyme-substrate interaction in GAPD. Three times recrystallized preparations of GAPD were used which were obtained from mammalian (pig and rabbit muscle, Elődi and Szörényi, 1956) and crayfish muscle (Szörényi et al., 1956) and from yeast (Krebs, 1955). Since the muscle enzymes are crystallized with firmly bound NAD and

the yeast enzyme contains a bound nucleotide of unknown structure (Boross et al.,1960) all preparations were pretreated with charcoal.

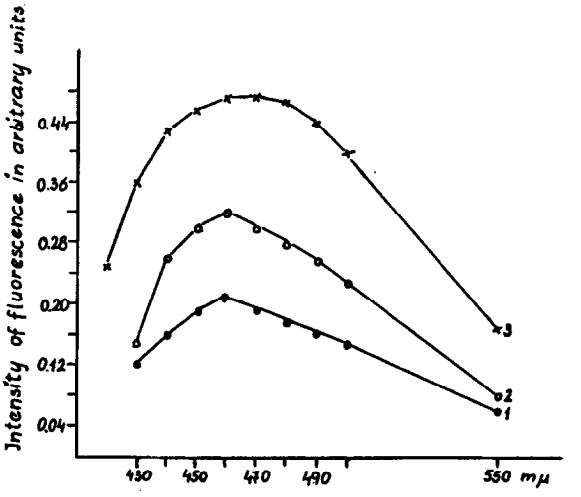

Fig.1. Fluorescence spectra of GAPD and GDH, activated at 380 mμ.
1: pig muscle GAPD; 2: charcoal treated pig muscle GAPD;
3: charcoal treated rabbit muscle GDH.
Charcoal treatment: 5 mg charcoal/mg protein + 20 mole-equivalents of mercaptoethanol were stirred for 10 minutes in cold then centrifuged and gel-filtered on a Sephadex G-50 column.
Solutions of 1 mg/ml in 0.1 M glycine buffer, pH 8.5 were measured with an Opton PMQ II spectrophotofluorimeter in silica cells of 1 cm light path at +4 °C.

Boross and Katchalski (1968) have found that by removing firmly bound NAD from native GAPD, the fluorescence at 460 mμ is increased. We have found that the addition of NAD to charcoal treated muscle GAPDs decreased the intensity of the fluorescence both at 340 mμ and 460 mμ, while the addition of phosphate or D-glyceraldehyde-3-phosphate (GAP) only decreased the fluorescence at 340 mμ (Fig.2.). The presence of NADH

decreased the fluorescence of the proteins at 340 mμ while at the same time the fluorescence of NADH at 460 mμ was diminished by the proteins* (Fig.2.).

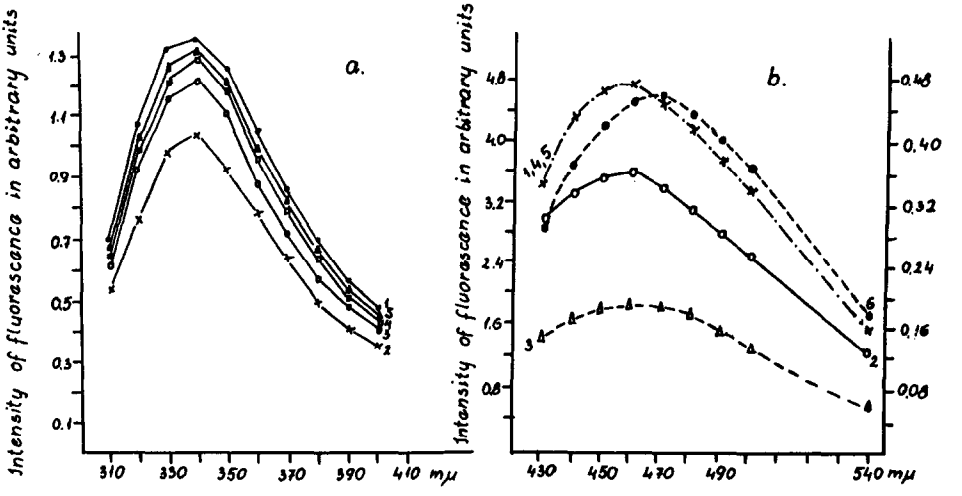

Fig.2. Fluorescence spectra of GAPD-substrate complexes.
1: charcoal treated pig muscle GAPD in 0.1 M glycine buffer, pH 8.5.
Additions, in 4 equivalents* 2: NAD (Boehringer); 3: NADH (Reanal); 4: GAP - prepared according to Szewczuk et al. (1961); 5: Na_2HPO_4 (reagent grade).
6: NADH alone
a./ Activation at 290 mμ. 52 ug/ml enzyme
b./ Activation at 380 mμ. 1 mg/ml enzyme
The lines drawn in full refer to the right ordinate, the dotted lines to the left ordinate.

*The fluorescence at 340 mμ of rabbit muscle GDH (Telegdi, 1964) decreases if we add α-glycerophosphate (GP), GAP or NADH to the enzyme. On the other hand, fluorescence intensity increases or remains unchanged when dihydroxiacetonphosphate (DHAP) or NAD is added (Telegdi and Keleti, 1968).

The presence of substrates had a similar effect on the fluorescence of yeast GAPD at 340 mμ. However, above pH 8.5, the binding of NAD increased about three times the intensity of the light emitted at 460 mμ and the activation maximum was shifted to 360 mμ. The increase in intensity was found to be proportional to the amount of NAD bound to the enzyme and this permitted the determination of the number of coenzyme binding sites**. In this way we determined the binding of 3.5 moles NAD per mole of yeast GAPD in agreement with Kirschner et al. (1966) and in contrast to Stockel (1959) who found 2 binding sites.

The intensity of fluorescence of yeast GAPD at 460 mμ is independent of pH, between pH 7 and 9.5. In contrast, the fluorescence of the enzyme-NAD complex depends on pH and the titration curve has an apparent pK at about pH 8 (Fig.3.).

The fluorescence of NADH at 460 mu is only slightly decreased by yeast GAPD.***

Carboxymethylation of 1 SH-group/subunit in the active center of muscle GAPD abolishes the characteristic absorption of the enzyme-NAD complex (Racker, 1954) but the coenzyme is still bound to the muscle enzyme although the dissociation constant is higher (Friedrich, 1965). We observed

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**The fluorescence of rabbit muscle GDH at 460 mμ did not change when GAP, GP or DHAP was added to the enzyme, however, NAD increased the intensity of fluorescence about 3 to 4 times (Telegdi and Keleti, 1968).

***In contrast to GAPDs the fluorescence of NADH at 460 mμ is increased 3 to 4 times by GDH (Telegdi and Keleti, 1968).

the same peculiar phenomenon as Boross and Katchalski(1968) when the fluorescence of carboxymethylated muscle GAPDs was measured at 460 mμ in the presence of NAD.While fluorescence of charcoal treated native protein is decreased by the addition of coenzyme,fluorescence of charcoal treated carboxymethylated muscle GAPD is increased 2 to 3 times.We have also found that the intensity of this fluorescence increases further (up to a value which is 10 to 15 times higher than the original) if the samples are continously illuminated at 340-380 mμ.Iodoacetate treatment of yeast GAPD does not affect the increased fluorescence of the enzyme-NAD complex at 460 mμ,i.e. the coenzyme is bound to carboxymethylated yeast GAPD.

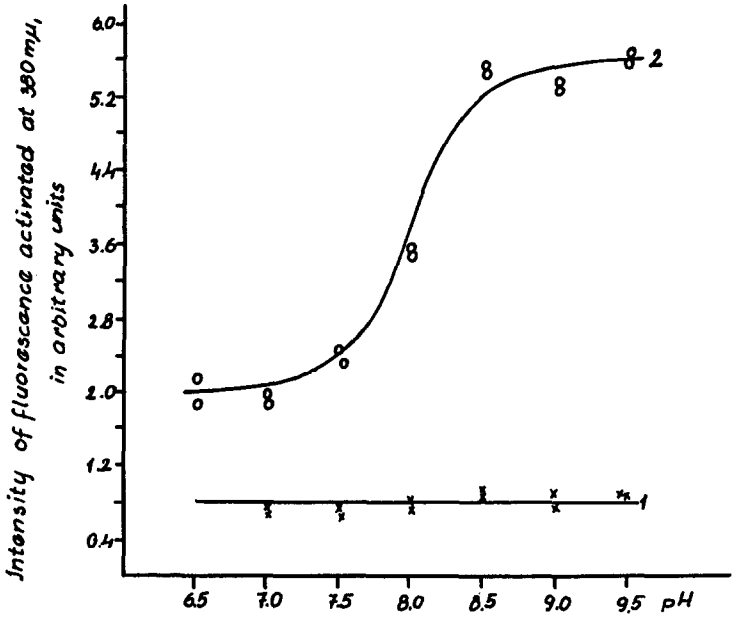

Fig.3.pH dependence of the fluorescence at 460 mμ of yeast GAPD
1:charcoal treated yeast GAPD. 1 mg/ml in 0.1 M glycine buffer
2:the same as in 1 + 4 equivalents of NAD

We do not know yet the group(s) of the proteins

which emit light at 460 mμ when excited at 380 mμ.It is suggested that these groups are common in some dehydrogenases even though they are apparently in a different position in yeast GAPD and in muscle GAPD,since the interaction of the two enzymes with NAD results in opposite effects.

Changes in the intensity of protein fluorescence at 340 mμ are usually ascribed to alterations of steric structure,by assuming that buried tyrosines and tryptophans become unmasked and ionized and thus excitable at 280 mμ (Joly, 1965).Our results obtained with GAPD and with GAPD-substrate complexes confirm previous observations that binding of substrates induces changes in the conformation of this enzyme (Elődi and Szabolcsi,1959;Dévényi et al.,1960;Keleti and Batke,1965;Bolotina et al.,1966;Závodszky et al.,1966;Listowski et al.,1965).

It seems that the newly found fluorescence emitted at 460 mu can be usefully applied for a study of enzyme-substrate complexes.

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