Effect of Temperature on the Spectral Properties of Glutamic-Aspartic Transaminase and Pyridoxal Phosphate

In glutamic-aspartic transaminase (L-aspartate: 2-oxoglutarate aminotransferase E.C.2.6.1.1) changes in pH are connected with concomitant changes in the spectrum of the enzyme[1]. In the present report we have examined whether the increase or decrease of the temperature, which has a known effect on the activity of the transaminase[1], has any effect on its spectrum. The spectrum of the transaminase at pH 8.5 is characterized by a single absorption band with maximum at 362 nm, while at pH 5.5 it exhibits 2 absorption bands with maxima at 340 nm and 426 nm[2].

Spectroscopic studies were followed in a Beckman spectrophotometer with thermospacers and in a Cary recording spectrophotometer. The activity of the enzyme was measured by the method of CAMMARATA et al.[3]. The optical densities at different wavelengths and temperatures of the transaminase solutions alone or in the presence of ketosubstrates or aminosubstrates, as well as the optical densities of pyridoxal phosphate solutions under the same conditions, are shown in the Table.

It can be seen from the Table that a decrease in temperature (which is accompanied by a diminution of transaminase activity) increases the absorption of the transaminase in the visible area of the spectrum (maximum 426 nm) and decreases it in the UV-area (maximum 340 nm). Under the same conditions the solutions of free pyridoxal phosphate undergo a decrease in the absorption on their maximum at 388 nm and an increase on the maximum at 295 nm. The spectral changes observed in the solutions of free pyridoxal phosphate, when the temperature is varied, are not analogous to those of the transaminase. While in the former the optical density at 400 nm decreases at low temperatures, in the latter it increases (Table). Temperature dependence of the fluorescence efficiency and of the quantum yield for some vitamin B_6 compounds was previously reported[4].

Ketosubstrates, α-ketoglutarate and oxaloacetate, as well as dicarboxylic acids with 4 and 5 carbon atoms, combine with transaminase and form complexes with characteristic absorption spectra[1,2,5]. When the effect of the temperature on the transaminase spectrum in the presence of ketosubstrates was studied, it was found that under these conditions the sensitivity of the enzyme spectrum ceases. The optical density of both maxima (432 nm and 340 nm) remain stable for a wide range of temperatures. The observed stability of the transaminase spectrum in the presence of ketosubstrates is a linear function of their concentration. The sensitivity of the transaminase spectrum towards temperature changes decreases as the concentration of the ketosubstrates increases and finally it becomes insensitive and 'freezes' at a concentration of the compound close to its K_m value (Table). This stabilizing property of ketosubstrates against temperature changes possibly provides an explanation for their protective effect on the activity of the enzyme when added during the heating step in the process of the transaminase purification[1].

The behaviour of the transaminase spectrum towards temperature changes was also examined in enzyme solutions containing glutamate or aspartate. Aminosubstrates combine with transaminase and form products with a characteristic spectral maximum at 333 nm[2]. In contrast

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The effect of temperature variation on the absorption of glutamic-aspartic transaminase alone or of its reaction products with α-ketoglutarate, oxaloacetate, glutamate and aspartate. The effect on the absorption maxima of the spectrum of pyridoxal phosphate, under the same conditions, is also presented. In all experiments an enzyme solution containing 30 mg protein/ml (0.02 *M* phosphate buffer pH 5.5) and a pyridoxal phosphate solution of 50 μg/ml in the same buffer was used

Solution tested	Wave-length nm	Optical density at the temperatures shown below (°C) 2	20	40	50	60
(A) Transaminase						
1. alone	426	1.44	1.35	1.27	1.24	1.20
	340	1.25	1.30	1.33	1.35	1.38
2. in $2 \cdot 10^{-4} M$ α-ketoglutarate	432	1.46	1.38	1.32	1.30	1.28
	340	1.20	1.24	1.26	1.28	1.30
3. in $4 \cdot 10^{-4} M$ α-ketoglutarate	432	1.46	1.43	1.40	1.38	1.30
	340	1.19	1.23	1.24	1.26	1.27
4. in $8 \cdot 10^{-4} M$ α-ketoglutarate	432	1.48	1.48	1.48	1.48	1.47
	340	1.17	1.17	1.18	1.19	1.20
5. in $4 \cdot 10^{-3} M$ oxaloacetate	426	1.49	1.49	1.47	1.46	1.45
	340	1.26	1.27	1.28	1.29	1.31
6. in $4 \cdot 10^{-4} M$ L-glutamate	426	1.24	1.20	1.17	1.12	1.04
	333	1.36	1.40	1.42	1.48	1.52
7. in $4 \cdot 10^{-4} M$ L-aspartate	426	1.26	1.21	1.13	1.05	0.95
	333	1.29	1.33	1.38	1.47	1.59
(B) Pyridoxal phosphate	388	0.52	0.65	0.68	0.69	0.69
	295	0.85	0.61	0.52	0.45	0.43

to the stabilizing influence of ketosubstrates, the aminosubstrates seem to exert an opposite effect. The increase in temperature of a transaminase solution containing aminosubstrates causes additional augmentation in the absorption maximum at 333 nm on the spectrum of the enzyme. In this respect elevation of temperature has the same effect as an additional amount of aminosubstrate (Table). This increase in absorption at 333 nm upon heating may explain the instability and inactivation of transaminase when treated with aminosubstrates during the heating step in the process of enzyme purification[1].

Some general conclusions can be drawn from these observations. Transaminase, when stored at low temperatures around or below zero, acquires a conformation similar to the one the enzyme takes in the presence of ketosubstrates. This conformation is expressed on the enzyme spectrum with a high absorption on its maximum in the area of 430 nm. In both cases the transaminase is stable and protected from inactivation. In contrast, high temperatures or the presence of aminosubstrates induce a conformation of the transaminase molecule which is expressed by an increase in the absorption at 340 nm (Table) At this configuration the transaminase is unstable and rapidly inactivated.

If the described spectral behaviour of transaminase is valid for other vitamin B_6 enzymes, then one might predict their stability towards temperature and propose optimal conditions to protect them from inactivation[6].

Zusammenfassung. Ein Zusammenhang zwischen optischen Eigenschaften und Stabilität der Transaminasen wird nachgewiesen. Temperaturabhängigkeit und Art der Partnerverbindung im Enzymsubstrat-Komplex (Amino- oder α-Ketoverbindung) einerseits und Konformation anderseits sind analog.

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Malate Dehydrogenase Isoenzymes in the Pancreatic Islets of Obese-Hyperglycemic Mice

It has been shown for a number of species that the pancreatic islets are characterized by a considerable malate dehydrogenase (MDH) activity[1,2]. It has also recently been demonstrated that the MDH in many cells can be segregated into different molecular forms, which have tentatively been assigned different physiological roles. Kaplan[3] suggested that a soluble MDH in the cytoplasm catalyses the formation of malate, which after the entrance into the mitochondria is oxidized by a mitochondrial MDH. The oxaloacetate produced may then be released into the cytoplasm, thus enabling the cycle to be repeated. The possibility of using disc electrophoresis[4,5] on polyacrylamide gels for the separation of enzymes from the small amount of tissue represented by the pancreatic islets of mammals[6] was taken advantage of in the present attempt at a further characterization of the MDH in mammalian B-cells with respect to molecular heterogeneity.

Material and methods. Adult American obese-hyperglycemic mice[7] of both sexes were used. Fresh pancreatic islets were isolated from the surrounding exocrine parenchyma as described by Hellerström[8] and homogenized in a 5% (v/v) aqueous solution of Triton-X-100 by means of a lucite micro-homogenizer. In addition, homogenates of exocrine pancreas and liver were similarly prepared. Electrophoresis was carried out as described by Ornstein[4] and Davis[5] at 3 °C (2.5 mA/gel; running time about 90 min) and the gels were subsequently incubated at 37 °C in the substrate-reagent medium given by Laycock et al.[9]. After extraction of the enzyme from the unstained central part of the gel[6], the activity of the different isoenzymes was fluorophotometrically assayed with oxaloacetate as substrate[10]. The total MDH activity was, in addition, assayed on crude tissue homogenates, the protein concentration of which was determined as described by Lowry et al.[11].

Results. The islets displayed an enzyme activity of at least the same magnitude as the exocrine pancreas (Table I). It appears from Figure 1 that 2 distinct MDH isoenzymes could be separated from the islet homogenates, as well as from the exocrine parenchyma and the liver. In all the tissues the more rapidly migrating fraction contained the highest activity towards malate, as estimated from the staining intensity. In an experiment, in which equal volumes of homogenate of all the 3 tissues were mixed prior to electrophoresis, the running distances

Table I. Malate dehydrogenase activity in the endocrine and exocrine pancreas and the liver of obese-hyperglycemic mice

Islets (6)	Acinar tissue (6)	Liver (5)
143.4 ± 18.1	116.1 ± 32.1	296.4 ± 37.7

1.1 m*M* of oxaloacetate was used as substrate and the figures denote moles of $NADH_2$ oxidized per kg protein and per hour. Mean values ± S.E.M. The number of animals studied is given in parentheses.

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