Genet. Res., Camb. (1974), **24**, *pp.* 73–80
With 1 text-figure
Printed in Great Britain

Regulation of enzyme activities in *Drosophila*

II. Characterization of enzyme responses in aneuploid flies

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(*Received* 28 *March* 1974)

SUMMARY

To test whether large changes in the enzyme levels of segmentally aneuploid *Drosophila melanogaster* can be ascribed to changes in kinetic properties of the enzymes affected, comparisons have been made with regard to the heat stability, substrate concentration dependency, and the presence of heat-stable inhibitors or activators within the extracts of aneuploid and control flies. By these criteria, no differences were found between controls and the α-GPDH activity of flies trisomic for chromosome II segments 27D–31E, 35A–40, 41–45F, and 57B–60F and no differences were evident between the IDH properties of 70CD–71B aneuploids and their controls. The enzyme changes observed in these aneuploids are more likely associated with changes in the rates of accumulation of the enzyme molecules. The IDH of flies trisomic for the 27D–31E region was more heat-stable than that of controls while the α-GPDH of flies trisomic for the 21A–25CD region displayed an apparent Michaelis constant for α-glycerophosphate lower than that of controls. The possible bases for these latter qualitative distinctions are discussed.

1. INTRODUCTION

A search has been conducted for regulatory genes in *D. melanogaster* by measuring enzyme activity levels in flies aneuploid for discrete autosomal regions (Rawls & Lucchesi, 1974). It was found that the levels of X-linked glucose-6-phosphate dehydrogenase (G6PD) and autosomally linked α-glycerophosphate dehydrogenase (α-GPDH) and NADP-dependent isocitrate dehydrogenase (IDH) were often specifically altered in crude extracts of such flies. Since it is unlikely that all of these chromosome regions contain structural genes for the affected enzymes, it was proposed that most of the enzyme responses resulted from changes in the dosage of regulatory elements located within the effective autosome segments.

In order to better understand the natures of these chromosome segments and their functional relationships to the enzymes that they modulate, we initiated studies to characterize the altered enzyme activities. For certain aneuploid flies displaying α-GPDH (E. C. 1.1.1.8) or IDH (E.C. 1.1.1.42) levels differing from

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control levels by at least 25%, thermal lability, substrate affinity, and extract mixing experiments were performed. The results show that most of the affected enzymes do not undergo discernible qualitative changes and therefore appear to represent increases or decreases in the net amount of enzyme accumulated in aneuploid individuals.

2. MATERIALS AND METHODS

(i) *Genetic techniques and fly culture*

Flies aneuploid and euploid for the autosomal regions studied were produced using translocations of the *Y* chromosome with chromosomes II or III. These rearrangements are described in detail elsewhere (Lindsley *et al.* 1972). The specific crosses employed and the autosome segments studied are presented in Table 1.

Table 1. *Genetic crosses*

Region aneuploid	Maternal genotype	Paternal genotype	Male progeny phenotypes: Controls	Male progeny phenotypes: Aneuploids
21A–25CD	*X·Y/X·Y;SM1/Sco*	*X·Y/T(Y;2)D6/cn bw*	$y^+B^S Sco$	$yB^S Sco$
27D–31E	*X/X;cn bw*	*X/T(Y;2)B231/cn bw*	$B^S cn^+ bw^+$	$B^S cn\ bw$
35A–40	*X/X;cn bw*	*X/T(Y;2)J54/cn bw*	$B^S cn^+ bw^+$	$B^S cn\ bw$
41–45F	$\widehat{XX}$*/T(Y;2)B177/SM1*	*X·Y/T(Y;2)L23/SM1*	$y^+B^S Cy$	$y^+B^+ Cy$
57B–60F	*X·Y/X·Y;SM1/Sco*	*X·Y/T(Y;2)L107/cn bw*	$y^+B^S Sco$	$y^+B^+ Sco$
70CD–71B	$\widehat{XX}$*/T(Y;3)A60/TM6*	*X·Y/T(Y;3)91/TM6*	$y^+B^S Ubx$	$yB^S Ubx$
70CD–71B (monosomic)	$\widehat{XX}$*/T(Y;3)R91/TM6*	*X·Y/T(Y;3)A60/TM6*	$y^+B^S Ubx$	$y^+B^+ Ubx$

Chromosome region descriptions refer to the chromosome map of Bridges (1935). Except as noted, all aneuploid genotypes are trisomic for the chromosome region. Symbols: *X·Y* ($Y^S X \cdot Y^L$,*In(1)EN,y*), $\widehat{XX}$ (either *C(1)RM,y* or *C(1)M3*, $y^2\ bb$), *SM1* (either *In(2LR)SM1*, al^2 *Cy* $cn^2\ sp^2$ *or In(2L+2R)Cy*, *Cy* cn^2), *TM6* (*In(3LR)TM6,*Ubx^{67b} *e*). All other symbols and mutations are described in Lindsley & Grell (1968). All translocations are described by Lindsley *et al.* (1972).

Fly culture and sample collection procedures are described by Rawls & Lucchesi (1974). All samples used for enzyme studies consisted of mixtures of adult males ranging in age from two to five days after emergence from their pupal cases.

(ii) *Extract preparations and enzyme assays*

For studies on α-GPDH, adults were homogenized in Dounce all-glass tissue grinders in 0·1 M potassium phosphate buffer (pH 7·1) containing 10 mM EDTA (ethylenediaminetetraacetate) and 0·5 mM dithiothreitol (Cleland's reagent) at a live weight to volume ratio of 10 mg/ml. For analogous studies on IDH, Tris-HCl replaced the phosphate buffer, EDTA was omitted and the live weight to volume ratio was 30:1. Twenty minutes after homogenization, samples were centrifuged for 40 min at 12000 ***g*** in a Sorvall RC2-B centrifuge; the resultant clear supernatant was drawn and used as an enzyme source. Throughout preparation and until assay all extracts were maintained at 0–5°.

Enzyme activities were observed by monitoring NAD^+ or $NADP^+$ reduction spectrophotometrically at 340 namometers. α-GPDH activity was measured by the oxidation of α-glycerophosphate while IDH activity was measured by the NADP-dependent oxidation of isocitrate. The assay conditions and substrate systems utilized have been described previously (Lucchesi & Rawls, 1973). A unit of enzyme activity is defined as the reduction of 1 μmole of NAD^+ per minute at 29°.

Protein determinations were performed on 0·05 ml aliquots of extract using the procedure of Lowry, Rosebrough, Farr & Randall (1951). Bovine serum albumin served as a protein standard.

(iii) *Substrate kinetic studies*

The velocities of the α-GPDH reaction with respect to varying α-glycerophosphate or NAD^+ concentrations and of IDH with respect to varying isocitrate or $NADP^+$ concentrations were measured as changes in optical density at 340 nm from 5 through 20 sec after the addition of crude extract to substrate solutions. All studies with α-GPDH were carried out in 0·1 M glycine-NaOH buffer, pH 9·5. The influence of varying α-glycerophosphate levels was monitored in the presence of 4·5 mM NAD^+ while NAD^+ concentrations were assayed in the presence of 30 mM α-glycerophosphate. For similar studies on IDH, the buffer was 0·1 M Tris-HCl, pH 8·5, 20 mM $MgCl_2$, and either 0·78 mM $NADP^+$ or 5·8 mM isocitrate. Determinations were performed on aneuploid and control samples using identical substrate solutions and conditions and apparent Michaelis constants were computed and compared using the method of Cleland (1967).

(iv) *Heat stability studies*

Enzyme heat stabilities were determined simultaneously on aneuploid and control extracts by placing 0·3 ml aliquots into a circulating water bath for which the preset temperature had been maintained for 20 min prior to beginning the experiment. At various time intervals incubation was terminated by placing the samples on ice. Treated and untreated aliquots were then assayed for α-GPDH or IDH activity.

(v) *Extract mixing studies*

Crude and/or denatured extracts were mixed and maintained on ice for thirty minutes prior to assay for α-GPDH or IDH activity. Denatured extracts were prepared by boiling crude extracts for eight minutes and clearing the resultant suspension by centrifugation at 12000 *g* for forty minutes.

3. RESULTS

If the altered enzyme activities associated with aneuploidy of specific autosomal segments were the results of changes in the concentration of molecules that inhibit or activate the enzyme, it should be possible to detect the presence of such

molecules by experiments in which normal extracts are mixed with aneuploid extracts. In Table 2 are presented the results of such experiments carried out using certain aneuploid extracts containing altered levels of α-GPDH. Since diluting control enzyme 1:1 with boiled aneuploid extract never yielded α-GPDH activity

Table 2. *α-GPDH activities in mixtures of segmental hyperploid and control extracts*

Enzyme source	Aneuploid regions studied		
	27D–31E	35A–40	41–45F
Control flies	0·73	0·70	0·65
Hyperploid flies	0·53	0·52	0·55
Control + hyperploid mixture*	—	—	0·60
Control + boiled hyperploid mixture*	0·36	0·37	0·34
Boiled control + hyperploid mixture*	0·26	0·26	0·26

Enzyme activities are expressed as units per ml of enzyme source. Each experiment was performed using a single extract of hyperploid flies and a single extract of control flies.

* Obtained by mixing the hyperploid and control extracts in equal portions. Aliquots of control or hyperploid extract were sometimes boiled prior to mixing.

Table 3. *IDH activity in mixtures of segmental aneuploid and control extracts*

Enzyme source	Aneuploid regions studied		
	27D–31E (trisomic)	70CD–71B (trisomic)	70CD–71B (monsomic)
Control flies	0·32	0·27	0·37
Aneuploid flies	0·44	0·35	0·27
Control + aneuploid mixture*	0·39	0·30	0·31
Control + boiled aneuploid mixture*	0·17	0·13	0·20
Boiled control + aneuploid mixture*	0·21	0·18	—

Enzyme activities are expressed as units per ml of enzyme source. Each experiment was performed using a single extract of aneuploid flies and a single extract from control flies.

* Obtained by mixing the aneuploid and the control extracts in equal portions. Aliquots of control of aneuploid extract were sometimes boiled prior to mixing.

of less than 50% of the undiluted control value, it does not appear that the depressed aneuploid enzyme levels are due to the presence of heat-stable inhibitors. In addition, the failure of aneuploid enzyme to exceed 50% of its undiluted value when diluted 1:1 with boiled control extract indicates that control extracts do not contain additional amounts of heat-stable activating substances. Similar conclusions are possible with respect to the role of specific activating or inhibitory elements in the altered IDH activities of aneuploid flies (Table 3).

The stability of enzyme activity in the extracts of aneuploid and control flies was studied under conditions of elevated temperature and the data were plotted as the logarithm of residual activity after varying periods of incubation. For those chromosome II segments listed in Table 1, the rates of α-GPDH inactivation at 55° in aneuploid and control extracts were consistently indistinguishable. Although similar results were obtained for the IDH activities of flies aneuploid and euploid

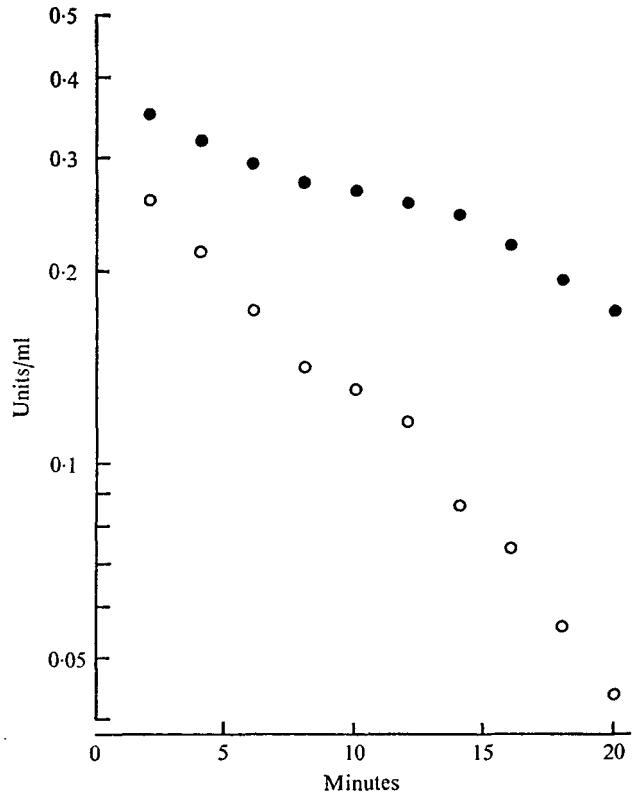

Fig. 1. Inactivation of IDH activity at 49° in the extracts of flies trisomic for the 27D–31E region (○) and their euploid controls (●). Protein concentration in the aneuploid extract was 1·4 mg/ml while the control value was 1·3 mg/ml.

for the 70CD–71B region, IDH activity in the extracts of flies hyperploid for the 27D–31E chromosome segment was noticeably more stable than the enzyme of controls (Fig. 1).

Enzyme activity in the extracts of aneuploid and control flies was measured at different concentrations of substrate or coenzyme. Both α-GPDH and IDH displayed typical Michaelis–Menton kinetics when either substrate or coenzyme concentrations were varied. Apparent Michaelis constants of α-GPDH are presented in Table 4 and show that only flies hyperploid for the 21A-25CD region differed significantly from their controls with respect to α-glycerophosphate kinetics. No

Table. 4. *Apparent Michaelis constants of α-GPDH from segmentally aneuploid and control flies*

Region aneuploid	Genotype	Apparent K_m α-glycero-phosphate	Apparent K_m NAD+
21A–25CD	Hyperploid	0·89 ± 0·07 (6)*	0·175 ± 0·015 (6)
	Control	1·20 ± 0·05 (6)	0·200 ± 0·014 (6)
27D–31E	Hyperploid	1·60 ± 0·10 (7)	0·146 ± 0·007 (6)
	Control	1·26 ± 0·07 (6)	0·155 ± 0·004 (6)
35A–40	Hyperploid	1·42 ± 0·12 (7)	0·160 ± 0·012 (6)
	Control	1·32 ± 0·13 (6)	0·163 ± 0·014 (6)
41–45F	Hyperploid	1·01 ± 0·03 (6)	0·126 ± 0·012 (6)
	Control	1·18 ± 0·08 (6)	0·136 ± 0·013 (6)
57B–60F	Hyperploid	1·14 ± 0·15 (5)	0·186 ± 0·023 (6)
	Control	1·03 ± 0·04 (6)	0·192 ± 0·023 (6)

Values are expressed as substrate molarity $\times 10^3 \pm$ S.E. In parentheses are the number of substrate concentrations at which the reaction velocity was measured.

* Aneuploid value differing from its control value at the 1 % level of significance.

Table 5. *Apparent Michaelis constants of IDH from segmentally aneuploid and control flies*

Region aneuploid	Genotype	Apparent K_m Isocitrate	Apparent K_m NADP+
27D–31E	Hyperploid	3·00 ± 0·19 (6)	1·60 ± 0·12 (6)
	Control	2·60 ± 0·20 (6)	1·61 ± 0·08 (6)
70CD–71B	Hyperploid	2·88 ± 0·17 (6)	1·44 ± 0·05 (5)
	Control	2·48 ± 0·19 (7)	1·33 ± 0·11 (6)
70CD–71B	Hypoploid	3·33 ± 0·09 (7)	1·27 ± 0·08 (7)
	Control	3·17 ± 0·28 (8)	1·38 ± 0·13 (7)

Values are expressed as substrate molarity $\times 10^5 \pm$ S.E. In parentheses are the number of substrate concentrations at which the reaction velocity was measured.

differences were detected between the apparent Michaelis constants of IDH from aneuploid and control sources (Table 5).

4. DISCUSSION

In a previous study (Rawls & Lucchesi, 1974), flies aneuploid for any of several autosomal regions were found to display altered levels of enzyme activity in crude extracts and it was suggested that these changes result from dosage effects of regulatory sites located within the aneuploid segments. Fluctuations in enzymatic activity might reflect either changes in the net quantity of enzyme molecules present, or differential inhibition or activation of an otherwise constant amount of enzyme. Quantitative differences in the total amount of enzyme present are more likely to result from effects on enzyme synthesis or degradation while the inhibition

and activation of enzymatic activity are operative at the level of interaction of the enzyme molecule with its substrate. Distinction between these two alternatives is essential to the understanding of how putative regulatory genes function.

In order to test whether large (i.e. equal to or greater than 25%) changes in aneuploid enzyme levels can be ascribed to changes in the kinetic properties of the enzyme, comparisons have been made with regard to the heat stability, substrate concentration dependency, and the presence of heat-stable effectors of enzyme activity in the extracts of aneuploid and control flies. In particular, no inhibitory or kinetic effects on α-GPDH activity were found in flies hyperploid for the chromosome II segments 27D–31E, 35A–40, 41–45F and 57B–60F and no differences were evident between the IDH activities of controls and flies aneuploid for the chromosome III segment 70CD–71B. Such negative evidence is consistent with the premise that the number of enzyme molecules, and therefore synthesis and/or degradation of the enzyme, is altered in these segmentally trisomic flies.

The enhanced heat stability of IDH activity in the extracts of flies hyperploid for the 27D–31E segment and the reduced apparent Michaelis constant for α-glycerophosphate of α-GPDH from flies hyperploid for the 21A–25CD segment imply that these enzymes in these flies are qualitatively modified as compared to controls. The altered stability of IDH in 27D–31E trisomics is specific for that enzyme since the α-GPDH activity of the same flies was no less heat-labile than controls. The altered inactivation kinetics of IDH in these aneuploids suggests that the increased enzyme activity accompanying three doses of the 27D–31E region does not reflect a structural gene dosage response since it is difficult to causally relate increased enzyme synthesis and increased heat stability of the mature enzyme.

Altered α-GPDH kinetics with respect to substrate concentration might be interpretable by taking into consideration the isozymic nature of the enzyme in *Drosophila*. In adults, several electrophoretically distinguishable forms of α-GPDH exist which are apparently all products of a common genetic locus (Wright & Shaw, 1969) but have been shown to possess different apparent Michaelis constants (Bewley, Rawls & Lucchesi, 1974). It is therefore possible that changes in the relative proportions of these isozymes will be reflected as changes in the apparent Michaelis constant of crude extracts. Accordingly, the decreased value for α-glycerophosphate exhibited by flies trisomic for the 21A–25CD segment might reflect a specific decrease in amount of isozyme GPDH-1 which has the largest apparent Michaelis constant for α-glycerophosphate of all the α-GPDH isozymes (Bewley *et al.* 1974). Further studies of flies trisomic for the 21A–25CD segment may provide insight into the genetic control of the origin and distribution of α-GPDH isozymes.

These studies represent a portion of the dissertation submitted by the senior author to the Department of Zoology, University of North Carolina, in partial fulfilment of the requirements for the degree of Doctor of Philosophy. This investigation was supported by Research Grant GM-15691, Genetics Training Grant GM-685, and a Research Career Development Award (to J.C.L.) KO4-GM-13,277 from the National Institute of General Medical Sciences.

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