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SUBUNIT COOPERATIVITY IN THE ACTION OF LACTATE DEHYDROGENASE

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SUMMARY

Interspecific hybrids of pig heart lactate dehydrogenase (L-lactate:NAD^+ oxidoreductase, EC 1.1.1.27) isozyme($(PH)_4$) and chicken heart lactate dehydrogenase isozyme($(CH)_4$) were obtained by freezing and thawing. Heat inactivation profiles of the interspecific hybrids revealed:

1. At 70°, the pig heart lactate dehydrogenase ($(PH)_4$) is appreciably less stable than the chicken heart lactate dehydrogenase ($(CH)_4$).

2. The hybrid isozyme ($(CH)_3(PH)_1$) is completely inactivated along with the inactivation of the pig heart isozyme ($(PH)_4$).

To explain these observations, four types of models ("active monomer" model, "active dimer" model, "active trimer" model and "active tetramer" model) were tested. The theoretical heat inactivation profiles deduced from the "active dimer" model gives the best fit to the experimental heat inactivation profiles. This fact suggests that two protomers act in cooperation as an active dimer in a tetrameric molecule of lactate dehydrogenase.

INTRODUCTION

Increasing evidence that many enzyme molecules are composed of more than one polypeptide chain has brought about interest in problems concerning the interaction and cooperation of the individual subunits. Lactate dehydrogenase (L-lactate: NAD^+ oxidoreductase, EC 11.1.27) is a tetramer of molecular weight about 145 000[1–3]. Reassociation *in vitro* of the tour subunits of lactate dehydrogenases from a wide variety of related and unrelated organisms produces functional hybrid molecules[4–10]. Two different subunits, A and B, associate to yield five tetramers, conveniently designated as Hyb-1(A_4), Hyb-2(A_3B_1), Hyb-3(A_2B_2) Hyb-4(A_1B_3) and Hyb-5(B_4). The dissociation and recombination of these subunits are accomplished by the reversible denaturation of the enzyme molecules by urea[5] or guanidine–HCl[8] or by freezing and thawing the isozymes in the presence of specific hybridization-promoting ions[6].

Abbreviations: PMS, phenozine methosulfate; PH, pig heart lactate dehydrogenase subunit, CH, chicken heart lactate dehydrogenase subunit.

MILLAR[1] has shown that appreciable dissociation of the tetrameric lactate dehydrogenase molecule is observed at concentrations below 1.5 mg/ml and that the dilute enzyme in NaCl solution will dissociate into a 5.5-S dimer with one half of the molecular weight of the tetramer. This observation implies an active dimer form of lactate dehydrogenase molecule. However, contrary to this idea, BERNFELD *et al.*[11] found that the tetramer dissociates to nonactive subunits at high dilution (1.5 μg/ml). A similar result was obtained by HATHAWAY AND CRIDDLE[12], who observed that the sedimentation of lactate dehydrogenase molecule at a protein concentration of 0.3 mg/ml was dependent upon existing pyruvate concentration and explained the substrate inhibition of the action of lactate dehydrogenase as dissociation of the active tetramer into inactive dimers taking place upon increasing the substrate concentration.

The purpose of this work is to give some light to this controversy concerning the active oligomeric form of the molecule lactate dehydrogenase. By analysing the heat inactivation profiles of interspecific hybrid isozymes of the heat sensitive pig heart isozyme $(PH)_4$ and the heat resistant chicken heart isozyme $(CH)_4$, it is suggested that two protomers cooperate in the action of lactate dehydrogenase.

MATERIALS AND METHODS

Enzyme sources

Chicken lactate dehydrogenase-1 was isolated by DEAE-Sephadex column chromatography from a crystalline preparation of chicken heart lactate dehydrogenase purchased from Sigma Chemical Co. The enzyme was eluted from the column with a Tris–HCl gradient 10–50 mM at pH 7.2. Pig lactate dehydrogenase-1 was purified from a crystalline preparation of pig heart lactate dehydrogenase which is a product of Boehringer, Mannheim. Purification of pig lactate dehydrogenase-1 was also performed with DEAE-Sephadex column in 10 mM phosphate buffer at pH 7.2 with a NaCl gradient 0.1–0.2 M. Both purified isozymes migrated as a single band in polyacrylamide disc electrophoresis.

Both the NAD^+ and NADH (approx. 98%), phenazine methosulfate (PMS) and nitro blue tetrazolium were products of Sigma Chemical Co.. All other chemicals were reageant grade and were obtained from Nakarai Chemicals Co., Kyoto.

Hybridization procedure

Hybridization was accomplished by freezing and thawing a mixture of chicken lactate dehydrogenase-1 and pig lactate dehydrogenase-1 in the following manner. Before use the purified enzyme solutions were dialysed for 24 h against 0.1 M phosphate buffer solution (pH 7.0). The dialysates were centrifuged for 5 min at 4° at 10 000 × *g* and the resulting supernatant solutions were diluted with 0.1 M phosphate buffer solution (pH 7.0) and concentrated to a desired concentation with collodion bag by vacuum dialysis. Protein concentration of the enzyme solution was determined with an ultraviolet spectrophotometer (Shimadzu, QV-50) by measuring the extinction coefficient at 280 nm. The extinction coefficients used were assumed 1.36 and 1.37 ml/mg·cm for chicken lactate dehydrogenase-1 and pig lactate dehydrogenase-1, respectively[2,3]. Isozyme solutions containing equal enzyme activities were mixed at 4° and dialysed against 300 vol. of 0.9 M NaCl solution in 0.1 M

phosphate buffer (pH 7.0). Usually total enzyme concentration was adjusted to between 0.1 and 0.5 mg per ml. After freezing, the enzyme mixture was thawed at room temperature and kept at 4°.

Detection of hybridization

The interspecific hybrids of lactate dehydrogenase isozymes were detected by polyacrylamide disc electrophoresis. The polyacrylamide gels were prepared according to the method of DAVIS[13]. The running of the sample was performed at pH 8.6 in Tris–HCl buffer system at 4° with a electric field gradient of about 20 V/cm. The location of lactate dehydrogenase activity in polyacrylamide gels were detected by incubating the gels in the dark at 37° in the following medium: 90 ml, 0.05 M Tris–HCl (pH 7.4), 8 ml 0.2 M NaCN, 6 ml 2 M sodium lactate, 8 ml $6.5 \cdot 10^{-4}$ M PMS, 60 mg NAD^+, 15 mg nitro blue tetrazolium.

Purification of interspecific hybrids

Interspecific hybrids obtained by hybridization of pig lactate dehydrogenase-1 and chicken lactate dehydrogenase-1 were purified by the following way. The mixture of hybrids was desalted by dialysis for 12 h at 4° against two changes of 1000 vol. of 10 mM phosphate buffer solution (pH 7.2). After desalting, the mixture solution was placed on a column of DEAE-Sephadex. The column was eluted with 0–0.25 M NaCl gradient in 10 mM phosphate buffer at pH 7.2. The enzyme was eluted in five peaks as shown in Fig. 1a. The mobilities of the five peaks fraction on polyacrylamide gels were in the order of elution from the DEAE-Sephadex column. The first peak was the chicken lactate dehydrogenase-1 and the last peak was the most negatively charged isozyme, pig lactate dehydrogenase-1. The intermediate three peaks, conveniently named Hyb-2, Hyb-3 and Hyb-4 in order of elution, are interspecific hybrids of chicken lactate dehydrogenase-1 and pig lactate dehydrogenase-1, *i.e.* $(CH)_3(PH)_1$, $(CH)_2(PH)_2$ and $(CH)_1(PH)_3$, respectively. For a further purification, the fractions for Hyb-3, Hyb-4 and pig lactate dehydrogenase-1 were collected and placed on a DEAE-Sephadex column. The isozymes were eluted with an NaCl gradient 0.1–0.2 M in 10 mM phosphate buffer (pH 7.2). The elution pattern of the second purification is shown Fig. 1b. Finally the enzyme solution was concentrated to an appropriate concentration with a collodion bag by vacuum dialysis and stored at 4°. The purified hybrid isozymes migrated as single bands on polyacrylamide gel.

Enzyme assays

Enzyme activity of the lactate dehydrogenase solution was determined spectrophotometrically with a Shimadzu ultraviolet spectrophotometer (QV-50) by measuring the decrease in absorbance at 340 nm resulting from the oxidation of NADH to NAD^+ accompanying the reduction of pyruvate to lactate by lactate dehydrogenase. The assay method is the same as that described in the Biochemica Catalogue.

Heat inactivation

Heat inactivation profiles of isozymes were obtained by two different methods. One method is as follows. An aliquot of isozyme solution was diluted to a concen-

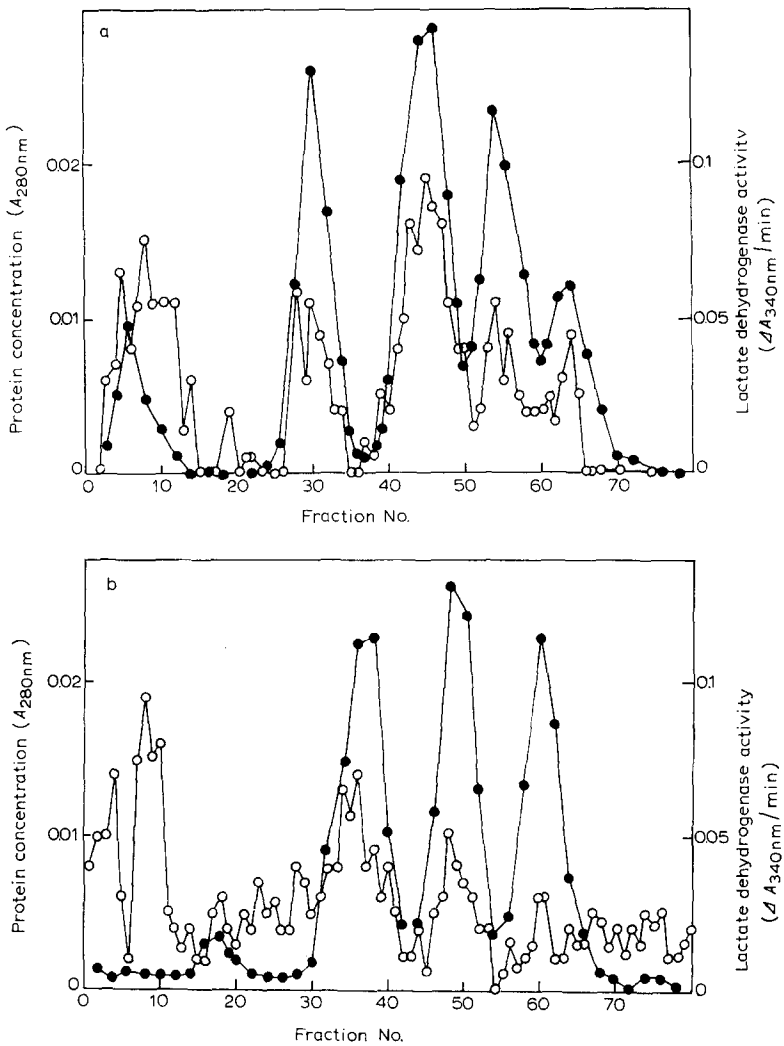

Fig. 1. (a) DEAE-Sephadex column chromatography of interspecific hybrids of chicken heart lactate dehydrogenase-1 and pig heart lactate dehydrogenase-1 placed on a column (2.5 cm × 20 cm). The enzyme was eluted with a linear gradient of 0–0.2 M NaCl in 10 mM phosphate (pH 7.2). Each fraction (4 ml) was measured for absorbance at 280 nm (○—○) and for lactate dehydrogenase activity (●—●). (b) DEAE-Sephadex column chromatography of interspecific hybrids ($(CH)_2(PH)_2$), ($(CH)_1(PH)_3$) and pig heart lactate dehydrogenase-1 placed on a column (2.5 cm × 20 cm). The enzyme was eluted with a linear gradient of 0.1–0.2 M NaCl in 10 mM phosphate (pH 7.2). Each fraction (4 ml) was measured for absorbance at 280 nm (○—○) and for lactate dehydrogenase activity (●—●).

tration of between 2 and 10 μg/ml in test tube with albumin buffer*. About 0.5 ml of the solution was incubated in a thermobath at 70° for various time intervals. Immediately after the incubation, the solution was cooled to 0° in an ice bath and

* This buffer contains dithiothreitol (0.1 mM) and albumin from egg (1 mg/ml) in 0.1 M phosphate (pH 7.0).

stored at 4°. The enzyme activity of the heat treated enzyme solution was measured as described above. The other method used to observe the heat inactivation profiles of the isozymes was to inactivate the isozymes which are placed in polyacrylamide gels after separation of the isozyme mixture. After migration, the gel tubes were incubated in a thermobath at 70° for various time intervals before visualization of the lactate dehydrogenase activity.

RESULTS

Heat inactivation

The time course of heat inactivation in a test tube of the native chicken lactate dehydrogenase-1 and pig lactate dehydrogenase-1 and the hybrid isozymes Hyb-2 ($(CH)_3(PH)_1$), Hyb-3 ($(CH)_2(PH)_1$) and Hyb-4 ($(CH)_1(PH)_3$) are presented in Fig. 2. It is seen from this figure that chicken lactate dehydrogenase-1 is appreciably more heat-resistant than pig lactate dehydrogenase-1. The heat sensitivity of the interspecific hybrids is in the order of the number of heat-sensitive protomers (PH) contained in each hybrid. After 10 min heat treatment at 70°, along with the complete inactivation of pig lactate dehydrogenase-1 ($(PH)_4$), the enzyme activity of Hyb-4 ($(CH)_1(PH)_3$) was completely lost, while the remaining activities of Hyb-3 ($(CH)_2(PH)_2$) and Hyb-2 ($(CH)_3(PH)_1$) were both about 1/3 of that of chicken lactate dehydrogenase-1. The heat inactivation curves for Hyb-2 and Hyb-3 have two components, *i.e.* a fast decreasing component and a slowly decreasing component. The remaining activities of Hyb-2 and Hyb-3 rapidly decrease with increasing incubation time until a critical point at which Hyb-4 and pig lactate dehydrogenase-1 are completely inactivated and thereafter Hyb-2 and Hyb-3 are slowly inactivated along with the inactivation of chicken lactate dehydrogenase-1.

A similar result was obtained from the heat inactivation profiles of hybrid

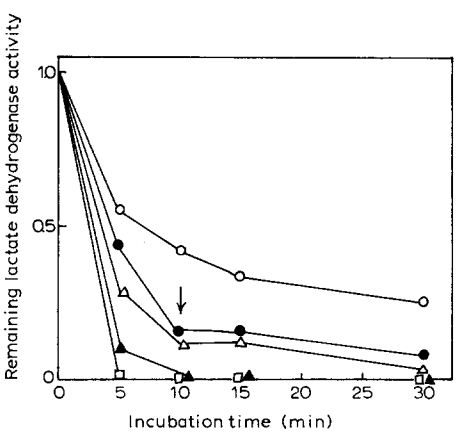

Fig. 2. Heat inactivation curves for interspecific hybrids of chicken heart lactate dehydrogenase-1 and pig heart lactate dehydrogenase-1. ○—○, chicken heart lactate dehydrogenase-1 ($(CH)_4$); ●—●,; Hyb-2 ($(CH)_3$ $(PH)_1$); △—△, Hyb-3 ($(CH)_2$ $(PH)_2$); ▲—▲, Hyb-4 ($(CH)_1$ $(PH)_3$); □—□, pig heart lactate dehydrogenase-1 ($(PH)_4$). Remaining lactate dehydrogenase activity was plotted against heat inactivation time. The arrow shows a sharp inflection of the curves for Hyb-2 and Hyb -3 occurs at this point. The value of the remaining activity is a mean value from three separate measurements.

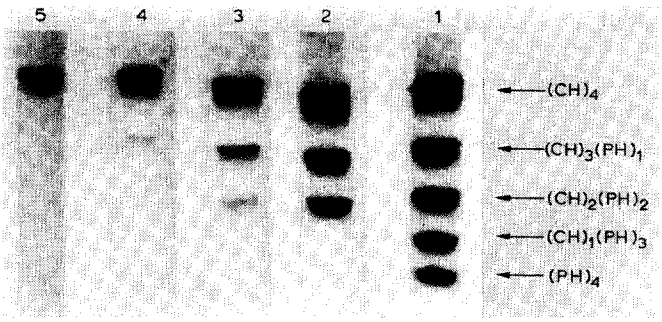

Fig. 3. Heat inactivation profiles of interspecific hybrids of chicken heart lactate dehydrogenase-1 and pig heart lactate dehydrogenase-1. Heat treatment was performed before visualization of lactate dehydrogenase activity by incubating at 70°. Time intervals for heat treatment were, right to left, 1, no heat treatment; 2, 2 min; 3, 5 min; 4, 7 min; and 5, 10 min.

isozymes placed in polyacrylamide gels (Fig. 3). After 2 min heat treatment, both the enzyme activities of Hyb-4 ($(CH)_1(PH)_3$) and pig lactate dehydrogenase-1 ($(PH)_4$) were lost. After 10 min heat treatment, only chicken lactate dehydrogenase-1 ($(CH)_4$) showed enzyme activity.

MODELS AND DISCUSSION

The time course of heat inactivation of the inter-specific isozymes is quite different from that expected from the assumption that a single protomer acts as an active enzyme molecule. If this is the case, the remaining activity of a hybrid isozyme after complete inactivation of the pig heart protomer should be proportional to the number of the heat-resistant chicken heart protomers (CH) contained in each hybrid. However, the oberved result was quite different from this expectation.

The fact that Hyb-4 ($(CH)_1(PH)_3$) lost enzyme activity accompanying the inactivation of pig lactate dehydrogenase-1 ($(PH)_4$) suggests that more than one protomer acts as an active enzyme unit in a tetrameric lactate dehydrogenase molecule. Thus, three different models were tested for the interpretation of the observed result. The models are based on the following assumptions; (1) The four protomers of a tetrameric lactate dehydrogenase molecule are inactivated independently of each other. (2) The component protomers of an active oligomeric enzyme are fixed to one active oligomer. (3) More than one protomers in the tetrameric lactate dehydrogenase molecule acts in cooperation as an active oligomer. Inactivation of one protomer of an active oligomer results in the inactivation of the whole active oligomer. (4) All the hybrid isozymes composed of active protomers A and B have the same catalytic activity.

In the following discussion, the time course of heat inactivation of hybrids containing protomers A and B is considered.

The probability R is defined as that by which a tetrameric lactate dehydrogenase molecule remains active after heat treatment. R is a function of the inactivation probabilities of protomers A and B, which are defined as a and b, respectively. To deduce the relation of R to a and b, it is first necessary to know the relation of the

TABLE I

THE NUMERICAL FACTORS FOR THE CALCULATION OF THE REMAINING ACTIVITY OF MODEL HYBRIDS

$r(n_A+n_B)$, remaining activity of a tetrameric lactate dehydrogenase molecule which contains n_A+n_B inactivated protomers; $P_A(n_A)$, the probability by which a tetrameric lactate dehydrogenase molecule contains n_A inactive protomer A's; $P_B(n_B)$, the probability by which a tetrameric lactate dehydrogenase molecule contains n_B inactive protomer B; a, inactivation probability of protomer A; b, inactivation probability of protomer B.

Hybrid	*Protomer A*		*Protomer B*		*Remaining enzyme activity*		
	No. of inactive protomer A (n_A)	*Probability ($P_A(n_A)$)*	*No. of inactive protomer B (n_B)*	*Probability ($P_A(n_A)$)*	*Dimer model ($r(n_A+n_B)$)*	*Trimer model ($r(n_A+n_B)$)*	*Tetramer model ($r(n_A+n_B)$)*
A_4	0	$(1-a)^4$	0	1	1	1	1
	1	$4(1-a)^3a$	0	1	$\frac{1}{2}$	$\frac{1}{4}$	0
	2	$6(1-a)^2a^2$	0	1	$\frac{1}{6}$	0	0
	3	$4(1-a)a^3$	0	1	0	0	0
	4	a^4	0	1	0	0	0
A_3B_1	0	$(1-a)^3$	0	$1-b$	1	1	1
			1	b	$\frac{1}{2}$	$\frac{1}{4}$	0
	1	$3(1-a)^2a$	0	$1-b$	$\frac{1}{2}$	$\frac{1}{4}$	0
			1	b	$\frac{1}{6}$	0	0
	2	$3(1-a)a^2$	0	$1-b$	$\frac{1}{6}$	0	0
			1	b	0	0	0
	3	a^3	0	$1-b$	0	0	0
			1	b	0	0	0
A_2B_2	0	$(1-a)^2$	0	$(1-b)^2$	1	1	1
			1	$2(1-b)b$	$\frac{1}{2}$	$\frac{1}{4}$	0
			2	b^2	$\frac{1}{6}$	0	0
	1	$2(1-a)a$	0	$(1-b)^2$	$\frac{1}{2}$	$\frac{1}{4}$	0
			1	$2(1-b)b$	$\frac{1}{6}$	0	0
			2	b^2	0	0	0
	2	a^2	0	$(1-b)^2$	$\frac{1}{6}$	0	0
			1	$2(1-b)b$	0	0	0
			2	b^2	0	0	0
A_1B_3	0	$1-a$	0	$(1-b)^3$	1	1	1
	1	a					
	0	$1-a$	1	$3(1-b)^2b$	$\frac{1}{2}$	$\frac{1}{4}$	0
	1	a			$\frac{1}{6}$	0	0
	0	$1-a$	2	$3(1-b)b^2$	$\frac{1}{6}$	0	0
	1	a			0	0	0
	0	$1-a$	3	b^3	0	0	0
	1	a			0	0	0
B_4	0	$(1-b)^4$	0	1	1	1	1
	1	$4(1-b)^3a$	0	1	$\frac{1}{2}$	$\frac{1}{4}$	0
	2	$6(1-b)^2b^2$	0	1	$\frac{1}{6}$	0	0
	3	$4(1-b)b^3$	0	1	0	0	0
	4	b^4	0	1	0	0	0

numbers of inactivated component protomers and the remaining activity of the heat-treated tetrameric lactate dehydrogenase molecule.

(1) "Active dimer" model. In this model, a tetrameric lactate dehydrogenase molecule is composed of two active dimers. Inactivation of one protomer results in the inactivation of the dimer in which the inactivated protomer is contained. Therefore the remaining activity of the tetrameric lactate dehydrogenase molecule is 1/2

of that before heat treatment. If two protomers are inactivated, the tetrameric lactate dehydrogenase molecule has enzyme activity only when the both inactivated protomers are contained in the same dimer. Since the probability that the two inactive protomers are contained in the same dimer is 1/3, the remaining activity of a tetramer containing two inactive protomers is 1/6 of an intact tetramer.

(2) "Active trimer" model. In this model, three protomers act in cooperation as an active trimer. Inactivation of one protomer in a tetrameric lactate dehydrogenase molecule results no loss of enzyme activity if the inactivated protomer is not contained in the active trimer. The probability of the occurence of this case is 1/4. Therefore, the remaining activity of the tetrameric lactate dehydrogenase molecule containing one inactive protomer is 1/4. If two protomers are inactivated, there remains no active trimer and the tetrameric lactate dehydrogenase molecule is completely inactivated.

(3) "Active tetramer" model. In the "active tetramer" model, all the protomers composing a tetramer cooperate for lactate dehydrogenase activity. Inactivation of one protomer causes the inactivation of the whole tetrameric molecule.

By basing upon the above considerations, the probability R is simply formulated as

$$R = \sum_{n_A, n_B} r(n_A + n_B)\, P_A(n_A) P_B(n_B)$$

where n_A = the number of inactive protomer A's, n_B = the number of inactive protomer B's, $P_A(n_A)$ = the probability n_A inactive protomer A's are contained in a tetrameric lactate dehydrogenase molecule, $P_B(n_B)$ = the probability n_B inactive

TABLE II

THE REMAINING ACTIVITY OF HYBRIDS OF PROTOMERS A AND B AS A FUNCTION OF INACTIVATION PROBABILITIES OF PROTOMERS A AND B

The remaining activity R is formulated by substituting the factors in Table I into the relation $R = \sum_{n_A, n_B} r(n_A+n_B)\, P_A(n_A)\, P_B(n_B)$.

Model	*Hybrid*	*Remaining activity*
Dimer model	A_4	$(1-a)^4 + 2(1-a)^3a + (1-a)^2a^2$
	A_3B_1	$(1-a)^3(1-b) + \frac{1}{2}(1-a)^3b + {}^3/_2(1-a)^2a(1-b) + \frac{1}{2}(1-a)^2ab + \frac{1}{2}(1-a)a^2(1-b)$
	A_2B_2	$(1-a)^2(1-b)^2 + (1-a)^2(1-b)b + {}^1/_6(1-a)^2b^2 + (1-a)a(1-b)^2 + \frac{2}{3}(1-a)a(1-b)b + \frac{1}{6}a^2(1-b)^2$
	A_1B_3	$(1-b)^3(1-a) + \frac{1}{2}(1-b)^3a + {}^3/_2(1-b)^2b(1-a) + \frac{1}{2}(1-b)^2ab + \frac{1}{2}(1-b)b^2(1-a)$
	B_4	$(1-b)^4 + 2(1-b)^3b + (1-b)^2b^2$
Trimer model	A_4	$(1-a)^3$
	A_3B_1	$(1-a)^3(1-b) + \frac{1}{4}(1-a)^3b + \frac{3}{4}(1-a)^2a(1-b)$
	A_2B_2	$(1-a)^2(1-b)^2 + \frac{1}{2}(1-a)^2(1-b)b + \frac{1}{2}(1-a)a(1-b)^2$
	A_1B_3	$(1-b)^3(1-a) + \frac{1}{4}(1-b)^3a + \frac{3}{4}(1-b)^2b(1-a)$
	B_4	$(1-b)^3$
Tetramer model	A_4	$(1-a)^4$
	A_3B_1	$(1-a)^3(1-b)$
	A_2B_2	$(1-a)^2(1-b)^2$
	A_1B_3	$(1-b)^3(1-a)$
	B_4	$(1-b)^4$

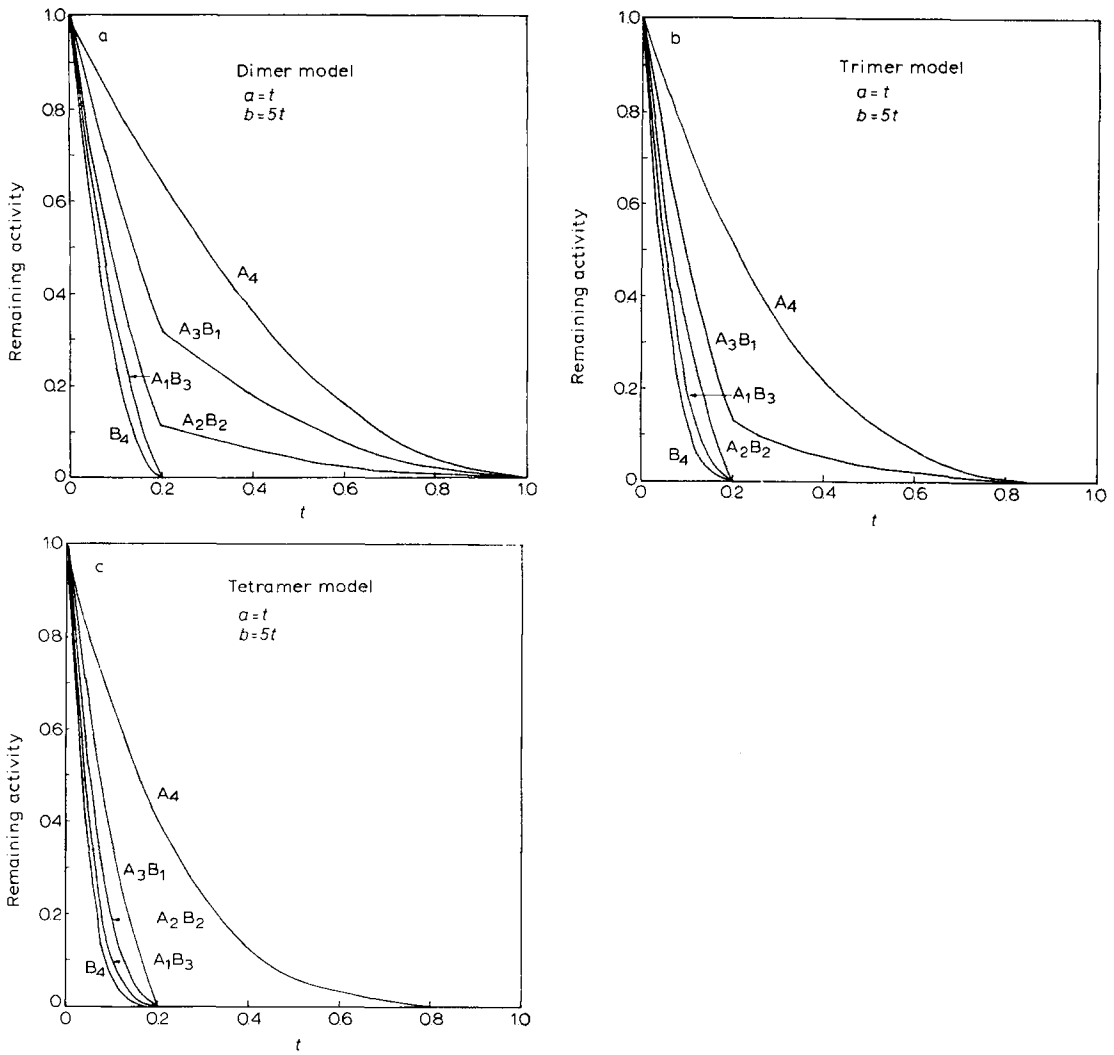

Fig. 4. (a) Theoretical heat inactivation curves for "active dimer model". Remaining activity is plotted against heat inactivation time (t). The curves were calculated by assuming the inactivation probabilities of protomers A and B as t and $5t$, respectively. (b) Theoretical heat inactivation curves for "active trimer model". Remaining activity is plotted against heat inactivation time (t). The curves were calculated by assuming the inactivation probabilities of protomers A and B are t and $5t$, respectively. (c) Theoretical heat inactivation curves for "active tetramer model". Remaining activity is plotted against heat inactivation time (t). The curves were calculated by assuming the inactivation probabilities of protomers A and B are t and $5t$, respectively.

protomer B's are contained in a tetrameric lactate dehydrogenase molecule, $r(n_A+n_B)$ = the remaining activity of a tetrameric lactate dehydrogenase molecule which contains (n_A+n_B) inactive protomers. The above numerical factors and R are listed in Table I and Table II.

To obtain qualitative curves for the three models, the inactivation probabilities a and b are assumed for simplicity's sake to be proportional to the duration time

of heat inactivation. Under this assumption, for the case $a = t$ and $b = 5t$, the remaining activities of the five model hybrids were calculated as shown in Fig. 4.

The model curves for the "active dimer" model reveal; (1) The inactivation of the heat sensitive hybrid B_4 parallels the inactivation of the hybrid A_1B_4. (2) With increasing heat inactivation time there is a first rapid decrease of the remaining activities of A_3B_1 and A_2B_2 until a critical time when A_1B_3 and B_4 are completely inactivated. At this point a sharp inflexion of the curves for A_3B_1 and A_2B_2 occurs, and thereafter the remaining activities of A_3B_1 and A_2B_2 decrease moderately.

These features are characteristic of the "active dimer" model. The sharp inflection which appears in the model curves means that, after this point, the remaining activity depends only on the activity of the heat resistant protomer A. This inflexion point is also observed in the experimental curves (see Fig. 2). As seen from Fig. 4b and Fig. 4c, in the "active trimer" and "active tetramer" models, the hybrid A_2B_2 should be inactivated in parallel with the inactivation of the hybrid B_4. This is not the case in the experimental results.

The heat inactivation profile in polyacrylamide gel also supports the "active dimer" model. With increasing heat inactivation time, Hyb-4 ($(CH)_1(PH)_3$) is inactivated along with the inactivation of pig lactate dehydrogenase-1 ($(PH)_4$).

Since we assumed no dimer–dimer interaction exists between two dimers, the 'active dimer' model is applicable for the case in which the lactate dehydrogenase molecule is in the equilibrium state of dimer and tetramer. The remaining activity formula is therefore useful in the same form for the analysis of inactivation process of the lactate dehydrogenase molecule after its dissociation to dimer.

The treatment of heat inactivation profiles described in this report is generally applicable for the investigation of active oligomeric forms of enzyme molecules which have subunit structure. The conditions for application are: (1) Each component protomer is inactivated independently of each other. (2) The different types of protomers differ in sensitivity to heat or some other inactivation reagent.

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