Epigenetics of dominance for enzyme activity

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We have isolated and purified two parental homodimers and a unique heterodimer of acid phosphatase [coded by *Acph*-$1^{1·05}$(*F*) and *Acph*-$1^{0·95}$(*S*)] from isogenic homozygotes and heterozygotes of *Drosophila malerkotliana.* *F* and *S* produce qualitatively different allozymes and the two alleles are expressed equally within and across all three genotypes and *F* and *S* play an equal role in the epigenetics of dominance. Subunit interaction in the heterodimer over a wide range of H^+ concentrations accounts for the epigenetics of dominance for enzyme activity.

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1. Introduction

Dominance is well understood for its role in conserving genetic variability and as overdominance (hybrid vigour) in plant and animal improvement. The epigenetics of dominance, however, remained largely unexplored till the late fifties, when at some enzyme loci, intragenic complementation (overdominance) was discovered (Fincham and Pateman 1957; Giles *et al* 1957) and explained through restoration of enzyme activity, resulting from subunit interaction in hybrid proteins supposedly formed in heterozygotes (Catcheside and Overton 1958; Fincham 1959). Subunit interaction has been reported at many loci controlling catalytic, structural or quasi-catalytic proteins and between polypeptides which were active or inactive as homomultimers (Zabin and Villarejo 1975; Kacser and Burns 1981; Hollacher and Place 1987; Stuber *et al* 1992; Xiau *et al* 1995; Stuber 1999). Using electrophoresis for isolating allozymes [different molecular forms of an enzyme coded by the same gene (Markert 1975)], free from contamination by other allozymes, enables us to evaluate quantitatively the contribution of heteromultimers in the epigenetics of dominance (Scandalios *et al* 1972; Trehan and Gill 1985). Superiority of the heterodimer over the better of the two parental homodimers at pH 5·0 was shown to account for half the heterosis for acid phosphatase activity in *Drosophila malerkotliana* (Trehan and Gill 1987). Based on the results for the same enzyme system in the same species at eight H^+ concentrations we now demonstrate that subunit interaction in the heterodimer solely accounts for the epigenetics of dominance observed at each pH.

2. Materials and methods

The present study has been carried on acid phosphatase (a dimeric enzyme) coded by two alleles, *Acph*-$1^{1·05}$(*F*) and *Acph*-$1^{0·95}$(*S*), in isogenic *D. malerkotliana* flies. Procedures for obtaining isogenic *F*/*F*, *S*/*S* and *F*/*S* flies, preparation of crude extracts, purification of allozymes by electrophoresis of crude extracts, assaying of enzyme activity (1-naphthol released μM/min) and determination of V_{max} and K_m are already reported (Wills and Nichols 1971; Trehan and Gill 1985). For assaying enzyme activity at eight different H^+ concentrations, ranging from 3·0 to 6·0, 0·05 M sodium barbiturate buffer, instead of acetate buffer was used. Activity of each enzyme was determined on three replications of each of the three independent preparations.

Keywords. Acid phosphatase; dominance; *Drosophila malerkotliana*; epigenetics; heterodimeric allozymes; subunit interaction

For the sake of clarification, we give below symbols and the sense in which these have been used in the text:

Symbols	Meaning
F/F, S/S, F/S	Enzymes/enzyme activity in crude extract obtained from *F/F*, *S/S* and *F/S* flies respectively
FF, SS, FS	Allozymes (FF homodimer, SS homodimer, FS heterodimer)/allozyme activity in purified preparations
F, S	Polypeptides
MP; MH	Mid-parent; Mid-homodimer/activity in MP/MH
P*	Activity in dominant parent
MH^{hom}	Mid-homodimer activity for FF and SS homodimers, purified from crude extracts of homozygotes
MH^{het}	Mid-homodimer activity for FF and SS homodimers purified from crude extract of heterozygote
MH^{exp}	Expected mid-homodimer activity for homodimers present in F/S enzyme

(i) Degree of dominance $= \dfrac{(F/S - MP)}{P^* - MP} \times 100$

(ii) Percent superiority of F/S enzymes activity over MP $= \dfrac{(F/S - MP)}{MP} \times 100$

(iii) Percent recovery of $MH^{hom} = \dfrac{(MH^{hom})}{MP} \times 100$

(iv) Percent recovery of sum activities of all the three allozymes purified from *F/S* extract $= \dfrac{(FF + SS + FS)}{F/S} \times 100$

(v) Percent superiority of observed FS activity over MH^{het} on equimolar basis $= \dfrac{(FS - 2MH^{het})}{2MH^{het}} \times 100$

Note: The following formulae are based on the equal expression of *F* and *S* within and across all the three genotypes *F/F*, *S/S* and *F/S*. Random dimerization of F and S polypeptides would generate FF, FS and SS allozymes in 1 : 2 : 1 ratio.

(vi)(a) Expected activity of FF homodimer in F/S enzyme $= \frac{1}{4}$ F/F

(b) Expected activity of SS homodimer in F/S enzyme $= \frac{1}{4}$ S/S

(c) Expected activity of FS heterodimer in F/S enzyme $= F/S - \frac{1}{4}(F/F + S/S)$

$= F/S - \frac{1}{2}MP$

(d) $MH^{Exp} = \dfrac{\frac{1}{4}(FF + SS)}{2} = \frac{1}{4}MP$

(e) Percent superiority of expected FS allozyme over MH^{exp} on equimolar basis $= \dfrac{\{(F/S - \frac{1}{2}MP) - \frac{1}{2}MP\}}{\frac{1}{2}MP} \times 100$

$= \dfrac{(F/S - MP)}{\frac{1}{2}} \times 100$

For a multimeric enzyme with '*n*' polypeptide chains, percent superiority of expected activity of heteromultimer/s in F_1 over MP

$$= \frac{F_1 - MP}{\frac{2^{n-1} - 1}{2^{n-1}} MP} \times 100$$

(The coefficient, $2^{n-1} - 1$, is the portion of F_1 enzyme contributed by the heteromultimer/s. The percent superiority of expected activity of heteromultimer/s over MH^{exp} is greater than percent superiority of F_1 activity over MP by a factor equal to the reciprocal of the coefficient.)

(vii) Percent recovery of activity of FS heterodimer purified from F/S enzyme $= \dfrac{FS}{F/S - \frac{1}{2}MP} \times 100$

(viii) Percent recovery of $MH^{het} = \dfrac{MH^{het}}{\frac{1}{4}MP} \times 100$

K_m and V_{max} values were obtained from Lineweaver–Burk plots.

3. Results

3.1 *Enzyme activity in crude extract*

Optimum pH for enzyme activity is 4·4 for S/S and 5·0 for both F/F and F/S enzymes (table 1). Activity in S/S enzyme is significantly higher than that in F/F enzyme at all pHs, except at 3·0 where the former equals the latter. F/S enzyme activity is significantly higher than mid-parent value at each pH, positive dominance is thus indicated. At pH 4·0 and 4·4, F/S enzyme activity is significantly less but at the other six pHs it is more than that of the better parent. Moreover, the degree of dominance at pHs 4·0 and 4·4 is less and at the other six pHs, more than one (table 3, col. 2). Thus there is partial dominance at two and overdominance at six pHs. The degree of dominance, like that of the superiority of F/S

enzyme activity over mid parent value (table 3, col. 13), which is at its minimum at pH 4·4, increases with each rise or fall in pH. The degree of dominance and superiority of F/S are thus pH dependent, providing a good illustration of G × E interaction.

3.2 *Activities in purified allozymes*

Optimum pH for activity in FF homodimer, purified from *F/F* or *F/S* crude extract is 5·0 which is the same as that in F/F enzyme (table 1). Likewise, optimum pH for SS homodimer purified from *S/S* or *F/S* crude extract is 4·4, same as that for S/S enzyme. The optimum pH for a FS heterodimer, uniquely present in F/S enzyme is 4·7. For homodimers purified from homozygotes or heterozygotes, the activity of SS homodimer is significantly higher than that of FF homodimer at each pH. The FS heterodimer activity is significantly higher than both the mid-homodimer activity and the activity in the better homodimer on equimolar basis, at each pH.

4. Discussion

4.1 *Relative expression of* F *and* S *across homozygotes and within heterozygote*

The significantly higher activity of S/S enzyme over that of F/F enzyme at each pH (table 1) may result from higher catalytic efficiency of S/S enzyme or increased expression of *S* in *S/S* or both. The enzyme activity has been measured as 1-naphthol released μM/min, the specific enzyme activity has been measured as 1-naphthol released in μM/min/mg protein and V_{max} as 1-naphthol released in units of μM/min/mg protein at saturated substrate concentration.

At pH 5·0 activity (3·40); $1/K_m$ (M^{-1}) ($2·50 \times 10^3$) and V_{max}, maximum rate of formation of product (4·47 μM/min/mg protein), of S/S enzyme are higher than corresponding values (3·01; $1·47 \times 10^3$; 3·01) of F/F enzyme by 61%, 70% and 49%, respectively (Trehan and Gill 1987). We thus conclude that the catalytic efficiency of S/S enzyme is higher than that of F/F enzyme and must make a significant contribution towards the enhanced activity of S/S enzyme at pH 5·0 and by extrapolation at the remaining pHs also. A similar picture emerges from homodimers purified from extracts of homozygotes. At all the pHs, SS homodimer activity is significantly higher than that of FF homodimer (table 1). At pH 5·0, activity (3·503); $1/K_m$ ($2·50 \times 10^3$) and V_{max} (3·497) SS homodimer are higher than the corresponding values (1·706; $1·45 \times 10^3$; 1·704) of FF homodimer by 105%, 72% and 105% respectively (Trehan and Gill 1985). Higher V_{max} and $1/K_m$ values of SS homodimer point to its higher catalytic efficiency which probably is the factor primarily responsible for

Table 1. Acid phosphatase activities (μM 1-naphthol released/min) at different H^+ concentrations at 25°C in crude enzyme and purified allozyme preparation from *D. malerkotliana.*

					Allozymes						
	Enzymes				Homozygotes		Heterozygotes				Expected FS activity in F/S*
pH	F/F	S/S	F/S	MP	FF	SS	FF	SS	MH^{net}	FS	
1	2	3	4	5	6	7	8	9	10	11	12
3·0	0·922 ± 0·027	0·903 ± 0·021	1·707 ± 0·056	0·907	0·595 ± 0·029	0·782 ± 0·035	0·094 ± 0·003	0·114 ± 0·003	0·104	0·667 ± 0·023	0·252
3·5	1·020 ± 0·029	1·860 ± 0·029	2·581 ± 0·072	1·440	0·700 ± 0·037	1·556 ± 0·039	0·107 ± 0·003	0·225 ± 0·005	0·166	0·746 ± 0·015	1·861
4·0	1·330 ± 0·031	3·514 ± 0·044	3·378 ± 0·053	2·422	0·908 ± 0·034	2·870 ± 0·039	0·149 ± 0·004	0·419 ± 0·006	0·284	1·135 ± 0·020	2·167
4·4	1·780 ± 0·032	4·265 ± 0·027	4·061 ± 0·041	3·023	1·026 ± 0·033	3·862 ± 0·039	0·166 ± 0·004	0·587 ± 0·005	0·376	1·456 ± 0·016	2·549
4·7	2·010 ± 0·035	3·757 ± 0·043	4·400 ± 0·037	2·884	1·325 ± 0·037	3·635 ± 0·039	0·211 ± 0·004	0·552 ± 0·008	0·381	1·555 ± 0·009	2·958
5·0	2·200 ± 0·025	3·339 ± 0·049	4·592 ± 0·038	2·770	1·488 ± 0·033	3·057 ± 0·041	0·239 ± 0·004	0·461 ± 0·008	0·350	1·439 ± 0·016	3·207
5·5	1·444 ± 0·024	2·409 ± 0·059	3·947 ± 0·034	1·927	1·079 ± 0·036	2·105 ± 0·033	0·171 ± 0·033	0·306 ± 0·009	0·238	1·116 ± 0·017	2·984
6·0	0·797 ± 0·018	1·593 ± 0·502	3·226 ± 0·045	1·195	0·626 ± 0·031	1·448 ± 0·032	0·100 ± 0·002	0·190 ± 0·006	0·145	0·939 ± 0·018	2·626

*Formula given in §2.

its higher activity. This inference is assumed to be valid at all pHs.

For homodimers, purified from *F/S* extract, activity of SS homodimer is again significantly higher than that of FF homodimer (table 1). At pH 5·0, activity (0·513); $1/K_m$ ($2{\cdot}44 \times 10^3$) and V_{max} (0·583) of SS homodimer are higher than the corresponding values (0·345; $1{\cdot}47 \times 10^3$; 0·359) of FF homodimer by 49%, 66% and 62% respectively. Here again the higher catalytic efficiency of SS homodimer accounts for the higher activity of SS homodimer to a large extent at pH 5·0 and by extrapolation at other pHs. Summing up, significantly higher enzyme activity in S/S results primarily from its higher catalytic efficiency.

Are *F* and *S* expressed equally or unequally across *F/F* and *S/S* and within *F/S*? Under conditions of equal expression, F and S polypeptides and FF and SS allozymes are formed in equal amounts across *F/F* and *S/S* and within *F/S* (table 2; cols 5 and 7). Enzyme activities of FF and SS homodimers are equal or unequal (depending upon their catalytic efficiencies) across homozygotes and within the heterozygotes (table 2, col 8). Relative SS (or FF) activity across the two homozygotes equals that in the heterozygote (table 2, col. 9). It may also be noted that relative activity of FF or SS is a function of catalytic efficiencies of FF and SS allozymes. Under conditions of unequal expression, F and S polypeptides and FF and SS allozymes are produced in unequal amounts across *F/F* and *S/S* and also within *F/S*; the inequality at the level of allozymes is more than that at the level of polypeptides in *F/S* (table 2, cols 5 and 7). In equality in activities of FF and SS allozymes may be further affected if catalytic efficiencies of FF and SS allozymes are different (table 2, col. 8). Relative SS or FF activity across *F/F* and *S/S* is not equal to that within *F/S* (table 2, col. 9) in contrast to situation under equal allelic expression relative SS or FF activity is a function of both relative catalytic efficiencies and quantities of FF and SS.

Our results show that relative activity of SS allozyme across homozygotes is almost equal to that in heterozygote at all the eight pHs (table 3, cols 3, 4). Mean relative SS activity (2·44) in homozygotes is again almost equal to that (2·24) in the heterozygote. The relative ratio between the two values is 1·00 : 0·92. This small discrepancy, at its face value pointing to a lower rather than higher expressions of *S* in *F/S* is treated as an experimental error. We thus conclude that *F* and *S* are expressed equally across the homozygotes and also within the heterozygote. If so, the mean relative allozyme activities reflect their catalytic efficiencies. We can thus conclude that catalytic efficiency of SS allozyme is 2·34 times that of FF allozyme and is the sole factor for higher activity of S/S enzyme in homozygotes.

Equal allelic expression at *Acph*-1 locus in *D. malerkotliana* contrasts with the situation at certain other loci

Table 2. Relative activities* of SS homodimers in homozygotes (*F/F* and *S/S*) and within heterozygote (F/S under conditions of equal and unequal expression of F and S).

Allelic expression	Genotype	Allele	Polypeptide	Frequency of polypeptide/ allele	Dimers	Frequency of dimers	Enzyme activity	Relative enzyme activity*
1	2	3	4	5	6	7	8	9
Equal	*F/F*	*F*	F	0·5	FF	0·5	0·5y**	$\frac{0.5y'}{0.5y} = \frac{y'}{y}$
	S/S	*S*	S	0·5	SS	0·5	0·5y′	
	F/S	*F*	F	0·5	FF	0·25	0·25y	$\frac{0.25y'}{0.2y} = \frac{y'}{y}$
			S		FS	0·50		
		S		0·5	SS	0·25	0·25yx	
Unequal	*F/S*	*F*	F	0·5 – x***	FF	0·5 – x	(0·5 – x)y	$\frac{(0.5+x)y'^{a}}{(0.5-x)y}$
	S/S	*S*	S	0·5 + x	SS	0·5 + x	(0·5 + x)y′	
	F/S	*F*	F	0·5 – x	FF	$(0{\cdot}5 - x)^2$	$(0{\cdot}5 - x)^2y$	$\frac{(0.5+x)^2 y'^{a}}{(0.5-x)^2 y}$
		FS	S		FS	$2(0{\cdot}25 - x^2)$		
		SS		0·5 + x	SS	$(0{\cdot}5 + x)^2$	$(0{\cdot}5 + x)^2y'$	

*Relative SS activity in homozygotes = SS activity in S/S, FF activity in FF; relative SS activity in the heterozygote = SS activity in F/S, FF activity in F/S.
**y and y′ are relative catalytic efficiencies of Ff and Ss allozymes $y' > y \gg y'a$.
***x is deviation from 0·3, $0 < x < 0{\cdot}5$.
[a]If (0·5 + x)y′ (0·5 – x) is taken as 1 then relative activity in the heterozygote become (0·5 + x)2X(0·5 – X)y = 0·5 + x.
Note: 1 : 1 relationship, 1 : 0·5 + x relationship between relative activities in homozygotes and within heterozygote respectively, are not influenced by relative catalytic efficiencies of FF and SS allozymes.

in other organisms, where the two alleles are expressed differentially either only in the heterozygote (Endo 1971; Efron 1973; Schwartz 1976) or in homozygotes as well as in heterozygote (Kiddy *et al* 1965; Salcedo *et al* 1978).

4.2 *Epigenetics of dominance*

Dominance, partial at pH 4·0 and 4·4 and over dominance at other pHs, expressed for acid phosphatase activity, may result from increased level of expression of *F* and *S* in *F*/*S* over than in *F*/*F* and *S*/*S* or higher catalytic efficiency of the heterodimer or both.

4.3 *Absence of increased allelic expression in F/S*

If *F* and *S* are expressed equally within and across all the three genotypes, activity of each homodimer, purified from *F*/*S* extract is expected to be 25% of the activity of the same homodimer, purified from the extracts of the appropriate homozygote. On the average, FF activity from heterozygote is 16% of that of from the homozygote. For the SS allozyme, this value is 14·6% (table 3, cols 5, 6). In other words, mean activity of the homodimer from the heterozygote 61% $(\frac{1}{2}(\frac{16\cdot0+14\cdot6}{25})\times100)$ of the expected value. Thus, the level of expression of either *F* or *S* in F/S is certainly not greater than that in the corresponding homozygote. Is it equal?

After electrophoresis of an *F*/*F* or *S*/*S* extract, the entire enzyme forms only one band, whereas after electrophoresis of the same amount of *F*/*S* extract, the enzyme is distributed over three bands, each homodimer band containing only one-fourth of total enzyme. The percentage recovery of allozyme activity is perhaps proportional to the quantity of allozyme present in the band. This does appear to be the case. Mean recovery of the mid-homodimer activity in homodimers purified from *F*/*F* and *S*/*S* extract is 81% while that of the sum of activities of all the three allozymes from *F*/*S* extract is just 47% (table 3, cols 7, 8). In other words the recovery of activity in allozymes purified from the *F*/*S* extract is 58% of that in allozymes purified from *F*/*F* and *S*/*S* extracts. Lower (61%) than expected activity of homodimers from the heterozygote may, thus, be related directly to its lower (58%) recovery of activity. We, may therefore, conclude that activity of each homodimer purified from *F*/*S* extract is equivalent to 25% of that of the same homodimer, purified from appropriate homozygote. In other words, *F* and *S* in *F*/*S* are expressed at the same level as they are in the two homozygotes. Combining this conclusion with that reached in the last section we now state that *F* and *S* are equally expressed within and across all the three genotypes. This conclusion is consistent with the isogenic background of the three genotypes. Increased allelic expression in *F*/*S* is thus eliminated as a factor in the epigenetics of dominance.

4.4 *Catalytic efficiency of F/S* enzyme

At pH 5·0 activity (4·56); $1/K_m$ ($2\cdot78 \times 10^3$) and V_{max} (5·53) of *F*/*S* enzyme is higher than corresponding values (2·11, $1\cdot47 \times 10^3$, 3·01) of *F*/*F* enzyme by 117%, 89%, and 84% and higher than those (3·40, $2\cdot50 \times 10^3$; and 4·47) of *S*/*S* enzyme by 34%, 11% and 24% respectively (Trehan and Gill 1987). Catalytic efficiency of F/S enzyme, as indicated by its higher $1/K_m$ and V_{max} values is, quite high and may be high enough to account for the entire expression of dominance at pH 5·0 and this may be true at other pHs also.

Table 3. Analysis of the data for *D. malerkotliana* acid phosphatase activities given in table 1.

				Activity ratios		Percentage recoveries of activities of allozymes purified from						
		SS relative activity		FF from F/S / FF from F/F	SS from F/S / SS from S/S		Heterozygotes			FS superiority (%)		F/S superi-ority over MP (%)
pH	Degree of domi-nance	Homo-zygotes	Hetero-zygotes			Homo-zygotes MH	(FF + SS + FS)	FS	MH	Obs.	Expt.	
1	2	3	4	5	6	7	8	9	10	11	12	13
3·0	200·00	1·31	1·21	0·158	0·146	75·91	51·23*	53·27*	45·87*	220·67	176·41	88·20*
0·5	32·72	2·22	2·11	0·153	0·145	78·33	41·77	40·09	46·11	124·70	158·47	79·25
4·0	0·87	3·16	2·81	0·164	0·146	77·99	50·41	52·38	46·90	99·82	78·94	39·47
4·4	0·84	3·76	3·54	0·162	0·152	80·85	54·40	57·12	49·75	93·62	68·61	34·34
4·7	1·74	2·74	2·62	1·159	0·152	85·99	52·68	52·56	52·84	104·07	105·13	52·57
5·0	3·20	2·05	1·93	0·161	0·151	82·04	46·58	44·87	50·54	105·57	131·55	65·78
5·5	4·18	1·95	1·79	0·159	0·146	82·62	40·36	37·40	49·40	134·45	209·65	104·83
6·0	5·10	2·31	1·90	0·160	0·131	86·78	38·10	35·76	48·54	223·79	339·93	169·96

*Formulae given in §2.

4.5 *Catalytic efficiency of F/S enzyme*

Of the three constituents of F/S enzyme, the two homodimers, FF and SS, constituting half of the F/S enzyme will generate only 0·5 MP activity and thus are not expected to contribute towards dominance. FS heterodimer, constituting the remaining half of the F/S enzyme then, becomes the obvious candidate for the expression of dominance. Our data supports this inference. At each pH, FS allozyme activity is more than twice the combined activity of both FF and SS homodimers (table 1, cols 8, 9 and 11). Mean combined activity of the FF and SS homodimers is 0·514 while that of the FS heterodimer is 1·132. In other words, FS contributes 2·2 times more activity than the two homodimers put together. We thus conclude that FS heterodimer is primarily responsible for the epigenetics of dominance for acid phosphatase activity in *F*/*S*. More evidence to support this contention is presented below.

Expected activity of FS heterodimer in F/S enzyme at each pH is computed by subtracting combined expected activity of FF and SS homodimers from the activity of FS enzyme. If FS heterodimer accounts for the entire dominance effect, FS purified from F/S extract should exhibit activity equal to its expected value. Comparison of the observed and expected activities of FS (table 1, cols 11, 12) reveals that at each pH, observed activity is much less than the expected activity. In fact, the mean observed activity (1.13) is 46% of the mean expected activity (2·45). Does this mean that FS heterodimer accounts for only half of the dominance effect, attributing the other half to a second unknown factor? This was the conclusion reached by Trehan and Gill (1987). However, when we explore the reasons for lower recovery of FS activity after purification, we find that FS heterodimer solely accounts for the epigenetics of dominance.

Lower recovery of enzyme activity after purification holds good not only for FS but for FF and SS allozymes too and whether an allozyme is purified from *F*/*F*, *S*/*S* or *F*/*S* extract (table 3, cols 7–10). Specifically, mean recovery of the sum of activities in all three allozymes, purified from *F*/*S* extract, is 47% (table 3, col. 8). Recalling that mean recovery of FS activity is 46%, we conclude that lower recovery of FS activity after purification is a reflection of loss of allozyme activity during its purification. What happens during purification? Do we lose a portion of the allozyme? Or do we lose the unknown factor? If the first alternative is accepted, superiority of observed activity of FS heterodimer over mid-homodimer (calculated using observed activities of FF, SS and FS allozymes purified from F/S extract) is equal to the superiority of expected activity of FS heterodimer over mid-homodimer^{exp} (calculated using expected activities of FF, SS and FS allozymes in FS extract). However, if the second alternative is valid, observed superiority of the FS heterodimer should be less than its expected superiority. Why? Because expected activity of FS is calculated by subtracting the expected FF and SS activities from F/S activity and should therefore, include not only FS heterodimer activity but also that contributed by the unknown factor. Our results show that the mean observed FS superiority value (129·3) is almost the same as mean expected superiority (136·8) of FS (table 3, cols 11, 12). Compared at each pH, observed superiority value is higher than expected superiority value at pHs 3·0, 4·0 and 4·4 equal to 4·7 and at pH lower than at pHs 3·5, 5·0, 5·5 and 6·0 (table 3, cols 11, 12). Discrepancy between the observed and expected superiority values at each pH is traceable directly to that between recoveries of activities of FS heterodimer and mid-homodimer^{het} activities, used for calculating observed FS superiority. Recovery of activity in heterodimer at pH 3·0, 4·0 and 4·4 is higher, at pH 4·7 equal to and at pHs 3·5, 5·0, 5·5 and 6·0 lower than that of mid homodimer (table 3, cols 9, 10). In view of this explanation, we regard observed superiority of FS heterodimer at each pH equal to its expected superiority. We therefore, conclude that lower recovery of FS heterodimer activity after purification results from loss of a portion of FS allozyme. We may, therefore, treat expected and observed activities of FS allozyme as equal and conclude that dominance at each pH is entirely a consequence of activity of FS allozyme.

Comparison of formula (vi) with formula (ii) shows that percent superiority of expected FS heterodimer activity in F/S enzyme over mid-homodimer^{exp} activity is twice the percent superiority of F/S enzyme over mid-parent. Comparison of values given in columns 12 and 13 of table 3, shows that it is so. If dominance expression is entirely dependent on FS heterodimer activity, as demonstrated above, percent superiority of observed FS heterodimer activity over mid-homodimer^{het} should also be twice of F/S enzyme over mid-parent activity. Comparison of values in columns 11 and 13 of table 3, shows that observed superiority of FS is 1·3 to 2·7 times the superiority of F/S enzyme. Mean observed FS superiority is 1·7 times the mean F/S superiority. We demonstrated above that observed superiority of FS heterodimer is equal to its expected superiority. If expected superiority of FS heterodimer at each pH is twice that of F/S enzyme, the observed superiority of *FS* allozyme may also be regarded as twice the superiority of FS enzyme over MP. We, therefore, conclude that FS heterodimer accounts for the entire expression of dominance.

Summing up, FS heterodimer, satisfies the requirements to qualify as the sole factor accounting for the whole spectrum of dominance expression in *F*/*S*. Its higher activity and superiority over mid-homodimer^{het} activity are equivalent to those expected as required. Its

superiority, over MP^{het} is twice that of F/S enzyme again as required. We can, therefore, conclude that FS heterodimer is solely accountable for epigenetics of dominance.

Trehan and Gill (1987) purified FF and SS homodimers and FS heterodimer from F/S extract. At pH 5·0 activity of FS heterodimer (0·739) on equimolar basis is 114% and 44% higher than that of FF homodimer (0·345) and SS homodimer (0·513), respectively; $1/K_m$ of FS heterodimer ($2 \cdot 78 \times 10^3$) is 89% and 14% higher than that of FF homodimer ($1 \cdot 47 \times 10^3$) and SS homodimer ($2 \cdot 44 \times 10^3$) respectively and V_{max} of FS heterodimer (0·838) is 133% and 44% higher than that of FF homodimer (0·359) and SS homodimer (0·583) respectively. Higher activity of FS heterodimer is thus a reflection of its higher catalytic efficiency as indicated by its higher $1/K_m$ and V_{max} values at pH 5·0 and by its extrapolation at other pHs also.

4.6 *Subunit interaction: molecular basis of dominance*

Higher catalytic efficiency of the FS heterodimer is a reflection of its superior conformation, probably resulting from interaction between its two subunits, F and S polypeptides (Catcheside and Overton 1958; Fincham 1959; Kapuler and Bernstein 1963; Crick and Orgal 1964; McGavin 1968; Schwartz 1975). Because the latter are functional as homodimers, interaction between them is important for adaptation (Fincham 1972; Mitton and Grant 1984; Hall and Wills 1987). To account for the entire dominance effect, when an enzyme is a dimer, trimer or tetramer, required superiority of activity of heteromultimer/s over that of mid-homodimer is 2·00, 1·33 or 1·14 times respectively, that of activity of the F/S enzyme over that of mid-parent. In a dimeric enzyme required superiority of the heterodimer (2·0) is a tall order (Hall and Wills 1987) FS, a heterodimer of acid phosphatase in *D. malerkotliana* apparently has achieved this distinction. With an increase in the number of polypeptide chains in an enzyme, required superiority of the heteromultimers decreases exponentially and seems within reach. Hence we propose that subunit interaction in heteromultimers is an adequate molecular basis for epigenetics of dominance, for enzyme activity for oligomeric enzymes.

4.7 *Role of parental alleles in epigenetics of dominance*

Dominance for enzyme activity was exhibited at each pH (table 1). At pH 3·0, activities of F/F and S/S enzymes are equivalent and equidistant from the activity of F/S enzyme, no allele is therefore, dominant over the other. Thus, epigenetics of dominance is not dependent on dominance-recessiveness of the parent alleles. From pH 3·5 to 6·0, activity of S/S enzyme is different from that of F/F enzyme and the former is closer to activity of F/S enzyme than the latter; *S* is thus, dominant over *F*. An allele, therefore, becomes dominant when phenotypic values of the heterozygote is closer to that of the homozygote for this allele than to that of the homozygote for the other allele. Thus, dominance-recessiveness is simply a convenient term for describing the relationship amongst the phenotypes, generated by the three genotypes and may not be regarded as an attribute of genes (Kacser and Burns 1981).

Extrapolation of results obtained from *in vitro* studies to *in vivo* conditions deserves caution as we used a synthetic substrate (Na-1-naphthyl phosphate). Moreover, *in vivo* enzymes do not act in isolation but are kinetically linked to one another through their substrates and products (Kacser and Burns 1981). However, as desired for extrapolation (Singh *et al* 1974), we used wild type alleles and determined enzyme activity directly after preparing crude extracts in relation to pH, an important component of the *in vivo* environment. Moreover, the pH range used almost certainly includes the physiological pH. Dominance is, thus, likely to be manifested in *in vivo* conditions. If exhibited, heterodimer superiority and not increased allelic expression shall account for the degree of dominance expressed.

5. Conclusions

The two parental alleles (*F* and *S*) are expressed equally within and across all three genotypes, *F/F*, *S/S* and *F/S*. Epigenetics of dominance is explainable entirely by the superior catalytic efficiency of the heterodimer and there is no evidence for any increased allelic expression in the heterozygote. Subunit interaction is an adequate molecular basis for partial, complete and overdominance. Dominance may be expressed without one parental allele being ‘dominant’ and the other ‘recessive’. In other words, dominance and recessiveness is not an attribute of genes by themselves.

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