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Genetics

Purine-resistant *Drosophila melanogaster* result from mutations in the adenine phosphoribosyltransferase structural gene

(direct mutant selection/genetic mapping/cytogenetic mapping/isoelectric focusing)

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ABSTRACT **Mutants of *Drosophila melanogaster* selected for resistance to purine killing are deficient in adenine phosphoribosyltransferase (APRT; E.C. 2.4.2.7) activity. Genetic mapping and complementation analysis demonstrate that purine resistance, deficiency of APRT activity, and differences in the isoelectric point of APRT result from alterations at a single locus, *Aprt* (map position, 3:3.03). The level of APRT activity shows gene dose dependence in *Aprt* heterozygotes and in flies that are haploid for different *Aprt* alleles. *Drosophila* APRT is a dimer composed of apparently identical 23,000-dalton subunits. These results suggest that *Aprt* contains the structural gene for APRT.**

Present concepts of gene organization and control have been primarily derived from the genetic and biochemical analysis of induced mutations. The ability to isolate specific gene alterations through direct selection procedures has been important in directing the choice of particular gene loci for study. Despite the usefulness of the approach, direct mutant selection has seldom been possible in whole, complex eukaryotes.

We previously reported that mutations in *Drosophila melanogaster* that allow flies to survive the lethal effect of purine (7*H*-imidazo[4,5-*d*]pyrimidine) are correlated with a reduction in adenine phosphoribosyltransferase (APRT; E.C. 2.4.2.7) activity (1). To use this chemical selection technique to investigate gene organization and expression in *Drosophila*, it is essential to understand the mechanism by which mutations conferring resistance to purine killing concurrently result in deficiency of APRT activity.

APRT augments the synthesis of purine nucleotides by converting adenine and 5-phosphoribosyl-1-pyrophosphate into adenosine monophosphate. Studies of cultured eukaryotic cells selected for resistance to a variety of purine base analogues indicate that mutations within the structural gene for a specific purine base phosphoribosyltransferase enzyme can be the source of resistance to a particular analogue, because the defective enzyme will no longer convert the analogue to a toxic nucleotide (2–4). Evidence presented here shows that purine resistance and APRT deficiency in *Drosophila* result from mutations in the APRT structural gene.

METHODS

Nomenclature and Source of *Drosophila* Stocks. The purine-resistant mutants are designated with a numeral superscript of *Aprt* to indicate the order in which they were discovered. Alleles from the purine-sensitive wild-type strains *Ore-R* and *Canton-S* are designated $Aprt^A$ and $Aprt^B$, respectively, to indicate the relative acidic or basic isoelectric point of the APRT molecules produced by each strain. $Aprt^1$, $Aprt^2$, and $Aprt^3$ were originally designated *Pur*, Pur^r, and Pur^{r-1}, respectively. *Pur* was isolated by P. Duck (5) and given to us by V. Finnerty. Pur^r and Pur^{r-1} were generated by W. Gelbart and A. Chovnick.

The deletion $Df(3L)R^{+E}$ was the gift of J. J. Bonner (Indiana University). Translocation stocks *Dp(T(Y;3)H141)* and *Dp(T(Y;3)P3)* were obtained from D. Roberts (Genetics Laboratory, Oxford). All other stocks were obtained from the *Drosophila* Stock Center at Bowling Green State University or at the California Institute of Technology.

Culture of *Drosophila*, APRT Assay, and Selection of Purine-Resistant Mutations. The methods for culturing *Drosophila* and assay of APRT activity have been described (1). The APRT assay measures the rate of synthesis of [8-^{14}C]AMP from [8-^{14}C]-adenine and 5-phosphoribosyl-1-pyrophosphate.

Purine-resistant mutants $Aprt^4$ and $Aprt^5$ were generated by treating purine-sensitive *ru* $Aprt^B$ *h* male homozygotes with ethylmethane sulfonate (6). Mutagenized males were mated to homozygous ru^+ $Aprt^1$ h^+ females that were allowed to oviposit on 30 ml of standard culture medium for 48 hr. Adults then were removed and 10.5 mg of purine (Sigma) in 0.3 ml of distilled water was uniformly distributed on top of the culture medium to kill *ru* $Aprt^B$ *h*/ru^+ $Aprt^1$ h^+ heterozygotes. Purine-treated cultures were incubated at 25°C and surviving male flies were mated to purine-sensitive females carrying the multiply inverted third chromosome *In(3LR)CxF ru h D* to produce a balanced lethal stock of the mutant.

Purification of APRT. *Drosophila* APRT was purified to apparent homogeneity from $Aprt^A$ and $Aprt^B$ adult homozygotes. All steps were performed at 0–2°C. Fifty grams of *Drosophila* were homogenized in 300 ml of buffer A (20 mM Tris·HCl/10 mM $MgSO_4$/0.1 mM Na_2EDTA, pH 7.2. The crude homogenate was filtered and centrifuged at 48,000 × *g* (g_{max}) for 30 min. The resulting lipid- and soft-pellet-free supernatant was brought to 70% saturation with ultrapure ammonium sulfate (wt/vol, ignoring second-order volume changes) by the addition of cold salt with constant stirring for 30 min. The centrifugation was repeated and the supernatant was brought to complete saturation with ammonium sulfate. The centrifugation was again repeated and the resulting pellets were resuspended in a minimal volume of buffer A that was dialyzed for 2 hr against 500 ml of 55% ammonium sulfate-saturated buffer A. The dialyzed protein then was loaded onto a 1.5 × 15 cm column of octyl agarose (Pharmacia) equilibrated in the dialysis buffer and the column was eluted with 15 ml of dialysis buffer followed by elution with 30% ammonium sulfate-saturated buffer A. Fractions containing the majority of APRT activity were pooled and dialyzed for 4 hr against 2 liters of buffer A containing 10% glycerol.

The last dialyzed fraction was loaded on top of a 10-ml col-

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Abbreviation: APRT, adenine phosphoribosyltransferase.

umn of 5'-AMP linked to agarose by hexane attachment at C8 (P-L Biochemicals), which was equilibrated in buffer A containing 10% glycerol; the column was eluted with 20 ml of equilibration buffer. The flow then was reversed and the column was eluted with 10 mM ammonium bicarbonate (pH 6.9) until no additional 280 nm-absorbing material could be detected in the eluant (0.005 A_{max}). The top outlet of the AMP column then was connected to the bottom outlet of a 1.5 × 20 cm column of blue agarose (Sigma) equilibrated in the ammonium bicarbonate buffer. Elution of the AMP column then was continued with the ammonium bicarbonate buffer containing 0.1 mM 5-phosphoribosyl-1-pyrophosphate and the purified APRT was collected in 2.5-ml fractions of this eluant.

Electrophoresis and Isoelectric Focusing of APRT. Nondenaturing isoelectric focusing of APRT was performed in photopolymerized acrylamide slab gels as described (7), except that APRT activity was visualized by incubating the gel for 15 min at 25°C with the standard APRT assay mixture (see legend to Table 1), 0.2 ml per 10 × 12.5 cm gel. The subunit molecular mass of *Drosophila* APRT was estimated by electrophoresis of purified APRT by using the denaturing system of Laemmli (8). Two-dimensional analysis of APRT purified from $Aprt^A$ and $Aprt^B$ homozygotes was conducted as described by O'Farrell (9). First-dimension gels were focused at 25°C for 3.5 hr under a constant power of 4 W per 8.8-cm^3 gel. Gels were stained with a recently modified silver-staining procedure (Robert Switzer, personal communication). Ampholyte was obtained from Bio-Rad.

RESULTS

We previously reported that *Aprt* resides on chromosome 3 (1). To determine the location of *Aprt* on chromosome 3, recombination mapping was conducted with a series of recessive and dominant mutations (Table 1 and Fig. 1). The procedures employed in the genetic mapping of *Aprt* were designed to map the locus of purine resistance. Stocks carrying the recombinant chromosomes were also constructed and assayed for APRT activity. The purine-resistant recombinant flies were deficient in APRT activity. These data place $Aprt^1$ ≈0.1 centimorgan to the right of *R* on chromosome 3.

To determine the cytogenetic location of *Aprt*, strains carrying *Dp(T(Y;3)H141)* and *Dp(T(Y;3)P3)* were made homozygous for *ru* and $Aprt^1$ on chromosome 3. These duplications contain translocations of a portion of chromosome 3 (Fig. 1) onto a freely assorting Y chromosome. Therefore, males of these strains are triploid for a portion of chromosome 3. Male flies homozygous for *ru* $Aprt^1$ and carrying *Dp(T(Y;3)H141)* are ru^+ and have an intermediate level of APRT activity. However, males homozygous for *ru* $Aprt^1$ and carrying *Dp(T(Y;3)P3)* are *ru* and deficient in APRT activity. These results indicate that *Aprt* and *ru* must lie within the region 61E1–62D7. Flies deficient for a small terminal fragment of the left arm of chromosome 3 were constructed by crossing the segmental aneuploid stock *Df(T(Y;3)R132)* to *ru* $Aprt^1$ homozygotes. The former strain carries an acentric terminal fragment of the left arm of chromosome 3 inserted onto a centromere-containing fragment of the Y chromosome, which is marked with B^S. The remainder of this broken third chromosome (Fig. 1) contains the centro-

Table 1. Recombination mapping of *Aprt* on chromosome 3

Sex	Genotype of parental cross	Phenotype of recombinant F_1 survivors, %*			Total survivors, no.
		R	ru^+	h^+	
♂ ♀	mwh^+ ve^+ R^+ $Aprt^1$ *h*/mwh^+ ve^+ R^+ $Aprt^1$ *h* × *mwh* ve *R* $Aprt^A$ h^+/*mwh* ve^+ R^+ $Aprt^1$ *h*	0.13	—	NS	4,012
♂ ♀	*ru* $Aprt^1$ *h*/*ru* $Aprt^1$ *h* × *ru* $Aprt^1$ *h*/ru^+ $Aprt^A$ h^+	—	0.7	26.5	431
♂ ♀	*ru* $Aprt^4$ *h*/*ru* $Aprt^4$ *h* × *ru* $Aprt^4$ *h*/ru^+ $Aprt^A$ h^+	—	1.1	22.8	561
♂ ♀	*ru* $Aprt^5$ *h*/*ru* $Aprt^5$ *h* × *ru* $Aprt^5$ *h*/ru^+ $Aprt^A$ h^+	—	0.4	37.5	245

* Progeny were reared on purine-containing media to confine survival to purine-resistant homozygotes. No double recombinants were obtained in these experiments. Of the five *R* recombinants three were fertile in a backcross to *mwh* and *ve h* homozygotes, which gave the genotype of the *R* recombinant chromosomes. All three *R* recombinant chromosomes had the same genotype, mwh^+ ve *R* $Aprt^1$ *h*. Recombination between $Aprt^1$ and *h* was not scored (NS).

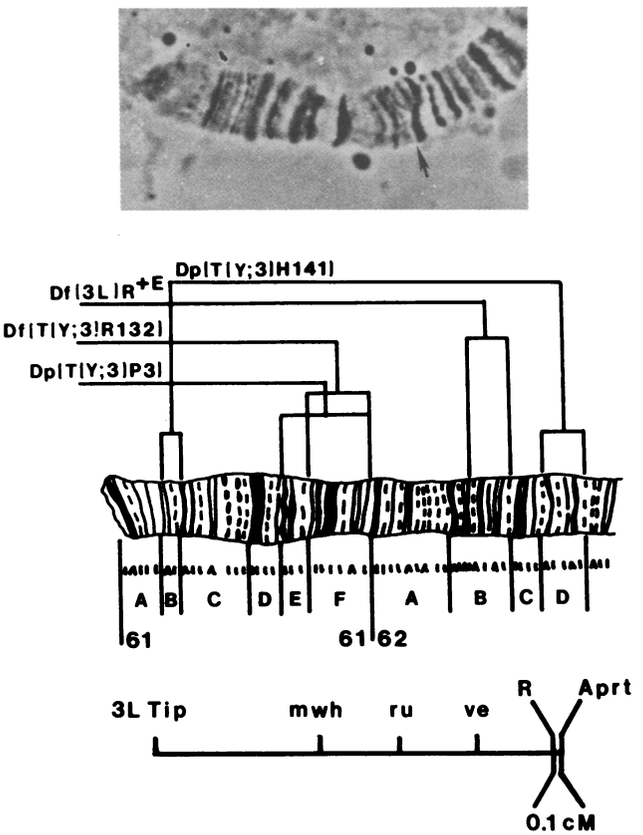

FIG. 1. (*Top*) The left terminal end of chromosome 3 in a salivary gland squash from mid-third instar larvae carrying $Df(3L)R^{+E}$ and $Aprt^1$. The arrow indicates the location of the deletion in the deficiency homologue. (*Middle*) Cytological map of the left terminal arm of chromosome 3 (10) and the breakpoints of the translocations and deficiencies used in the cytogenetic mapping of *Aprt* as presented by the Roberts (11). The vertical brackets indicate: (*i*) the approximate left (61B1–3) and right (62D1–7) breakpoints for the interstitial translocation *Dp(T(Y;3)H141)*; (*ii*) the deleted region in $Df(3L)R^{+E}$; (*iii*) the approximate breakpoint (61F1–7) of the terminal deficiency translocation *Df(T(Y;3)R132*; and (*iv*) the approximate breakpoint (61E1–F7) of the terminal duplication translocation *Dp(T(Y;3)P3)*. The right breakpoint of *Df(T(Y;3)R132)* and *Dp(T(Y;3)P3)* cannot be identical, as drawn by the Roberts (11), because ru^+ is not carried by the deficiency homologue in *Df(T(Y;3)R132* and is not present in the *Dp(T(Y;3)P3)*-translocated terminal fragment of chromosome 3. (*Bottom*) The linkage order of the loci as determined in this study with the exception of *h*, which lies to the extreme right of *Aprt*. The map position of the loci are: (*i*) *mwh* 3:1.24, *ru* 3:1.54, *ve* 3:1.7, data from the Roberts (11); (*ii*) *R* 3:2.9, *h* 3:28.04, our revision based on data from the Roberts (11) and Lindsley and Grell (10); and (*iii*) *Aprt* 3:3.03, based on the position of $Aprt^1$ with respect to *R* derived from data in Table 1. cM, centimorgan.

mere. The homologous third chromosome in this attached *X* strain is multiply inverted and marked with *Ubx*. The B^+ Ubx^+ progeny of this cross were *ru* but had an intermediate level of APRT activity. Therefore, *Aprt* must lie to the right of the breakpoint at 61F1–7 in *Df(T(Y;3)R132)* and to the right of *ru*. The $Df(3L)R^{+E}$ chromosome carries a recessive lethal deletion for bands 62B7–12 (Fig. 1), which appears to include the *R* locus (J. J. Bonner, personal communication). Because *Aprt* maps within 0.1 centimorgan of *R*, flies carrying *Aprt* alleles in *trans* to this deficiency were assayed for APRT activity (Table 2). These flies show a dose-dependent response in that the deficiency homologue does not contribute to the level of APRT activity, which is approximately half the level of activity observed in the respective *Aprt* parental homozygotes. These data show that $Df(3L)R^{+E}$ includes all or part of *Aprt*, which must lie in the region 62B7–12.

The results of complementation analysis involving all of the *Aprt* alleles and $Df(3L)R^{+E}$ (Table 2) demonstrate an absence of complementation and show an allele dose dependence for APRT activity. Dose effects of this kind resulting from mutations at a single locus have been widely reported in *Drosophila* and provide strong evidence of structural gene alterations (12–15). Although the genetic data suggest that *Aprt* contains a structural gene for the enzyme APRT, we have conducted a more detailed analysis of *Aprt* and its product to confirm this interpretation.

Gel filtration of APRT indicates that under nondenaturing conditions APRT has an apparent molecular mass of 38,000 daltons (Fig. 2). Electrophoresis of APRT under denaturing conditions (Fig. 3) provides a molecular mass of 23,000 daltons and suggests, together with the gel filtration data, that APRT is active as a dimer. In this respect, *Drosophila* APRT resembles APRT from mammals, including rat, hamster, and man. The APRT from these animals is also a dimer composed of ≈20,000-dalton subunits (16, 17).

Two-dimensional electrophoresis of APRT purified from $Aprt^A$ and $Aprt^B$ homozygotes demonstrates that each variant produces APRT molecules of similar molecular mass but with a different isoelectric point (Fig. 4). APRT purified from $Aprt^A$ homozygotes on three separate occasions produced single bands in first-dimension gels that were consistently resolved into one major and one minor spot in the second-dimension gel, as shown in Fig. 4*A* and Fig. 5*A*. In addition, we frequently detected other minor silver-staining bands with isoelectric points more acidic than the major silver-staining band in first-dimension gels containing purified APRT from either variant ($Aprt^A$ shown in Fig. 5*A*, arrowheads).

We believe that all or most of the silver-staining material seen in these gels is APRT because: (*i*) the protein purified from $Aprt^A$ homozygotes has the same isoelectric point as the activity of purified APRT from this strain (Fig. 5 *B* and *C*); (*ii*) all of the protein in fractions of APRT purified from either variant, regardless of isoelectric point, has a molecular mass of ≈23,000 daltons (Fig. 3, lane 2, and Fig. 4), including the faint more acidic bands seen in first-dimension gels as presented in Fig. 5*A* (molecular mass data not shown); and (*iii*) the intensity of the minor silver-staining bands increases with consecutive freeze-thawing of the protein sample, whereas the apparent molecular mass of these proteins remains unchanged (data not shown). Relatively moderate methods of handling protein, such as freezing a lyophilized sample (not done with APRT), can induce charge heterogeneity in a protein (9). Artifactual charge heterogeneity, as reported by O'Farrell (9), results in a series of spots in second-

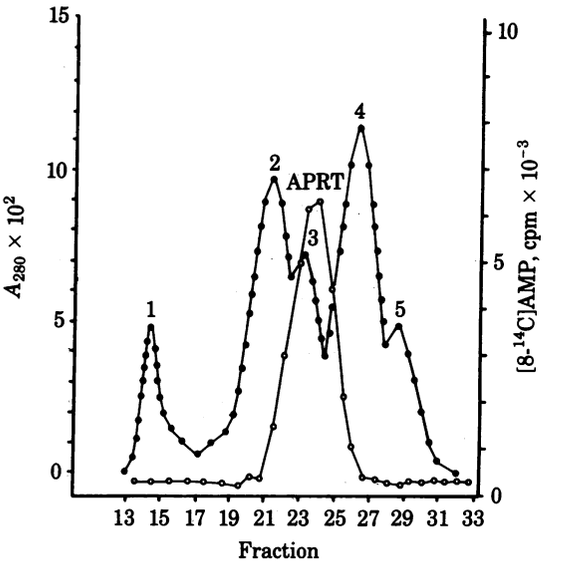

FIG. 2. The native molecular mass of APRT was determined by gel filtration on Sephacryl S200 SF. A 0.9 × 58 cm column of gel was packed in column buffer (50 mM Tris·HCl/10 mM $MgSO_4$/0.1 mM NaEDTA/0.3 M $(NH_4)_2SO_4$, pH 7.5) and then was calibrated with standard proteins and blue dextran 2000. Samples including ammonium sulfate-precipitated APRT were suspended in column buffer containing 10% sucrose and were developed in column buffer at a flow rate of 0.2 ml/min at 2°C. The molecular mass of APRT is based on the mean K_{av} from three separate chromatographic runs that did not vary by >2%. The elution pattern of standard proteins and blue dextran 2000 (●) is reconstructed from separate chromatographic runs: peak 1, blue dextran 2000 (2×10^6 daltons); peak 2, bovine serum albumin (67,000 daltons); peak 3, ovalbumin (43,000 daltons); peak 4, chymotrypsinogen A (25,000 daltons); and peak 5, ribonuclease A (13,700 daltons). The elution profile of APRT (○) represents the cpm of [8-^{14}C]AMP formed in the APRT assay after 5 min of incubation by 10 μl of each eluant fraction from the gel filtration of ammonium sulfate-precipitated APRT.

Table 2. Complementation analysis of *Aprt* alleles and $Df(3L)R^{+E}$

	$Aprt^A$	$Aprt^B$	$Aprt^1$	$Aprt^2$	$Aprt^3$	$Aprt^4$	$Aprt^5$	DfR^{+E}
$Aprt^A$	100.0	68.0	61.0	57.0	50.0	44.0	45.0	43.0
$Aprt^B$		45.0	23.9	26.5	24.9	17.4	21.2	23.0
$Aprt^1$			2.0	7.5	1.5	1.5	1.0	1.0
$Aprt^2$				9.5	5.0	5.0	7.5	4.0
$Aprt^3$					0.5	0.0	0.5	0.5
$Aprt^4$						0.5	1.5	0.0
$Aprt^5$							0.5	0.5

Values represent the percentage of APRT activity in flies of each genotype compared to $Aprt^A$ homozygotes. Each datum point is based on the average specific activity of APRT from at least two independent determinations. Assay mixtures contained: 60 mM Tris·HCl, 0.1 mM Na_2EDTA, 26.0 mM $MgCl_2$, 2.0 mM dithiothreitol, 6.25 mM sodium phosphoribosyl pyrophosphate, 76 μM [8-^{14}C]adenine, and 10 μl of *Drosophila* homogenate in a total volume of 35 μl. Homogenates were prepared by extraction of 10 newly eclosed adults (5 males and 5 females) in 100 μl of distilled H_2O in a glass tissue grinder at 4°C. All other procedures of the assay have been described (1).

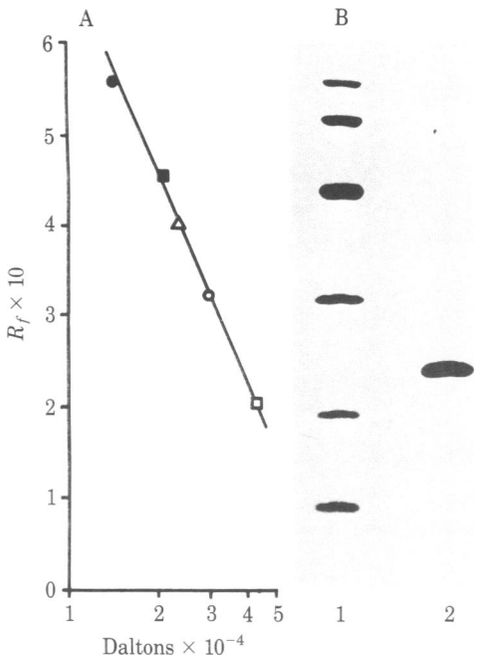

FIG. 3. Denaturing polyacrylamide gel electrophoresis and silver staining of purified *Drosophila* APRT and standard proteins. The semilogarithmic plot (*A*) is derived from the migration of standard proteins in the gel (*B*). Only those standard proteins with an R_f that produces a linear function of R_f vs. log protein molecular mass are plotted. Lane 1 of the gel contained in descending order: phosphorylase b (94,000 daltons); bovine serum albumin (67,000 daltons); ovalbumin (□) (43,000 daltons); carbonic anhydrase (○) (30,000 daltons); trypsin inhibitor (■) (20,100 daltons); and α-lactalbumin (●) (14,400 daltons). Lane 2 contained *Drosophila* APRT (△) purified from $Aprt^A$ homozygotes.

dimension gels that are of identical molecular mass but of more acidic isoelectric point and decreased staining intensity than the major protein from which they are derived. Because this description matches the behavior of the APRT protein in this gel system and because APRT purified from $Aprt^B$ homozygotes does not contain a minor protein spot equivalent to that seen in the $Aprt^A$ protein (Fig. 4), we suspect that the minor protein spot seen in preparations of APRT from $Aprt^A$ may be due to artifactual charge heterogeneity. These results, when considered with the gel filtration data, suggest that *Drosophila* APRT is a dimer composed of a single kind of polypeptide, as appears to be the case for human APRT (16).

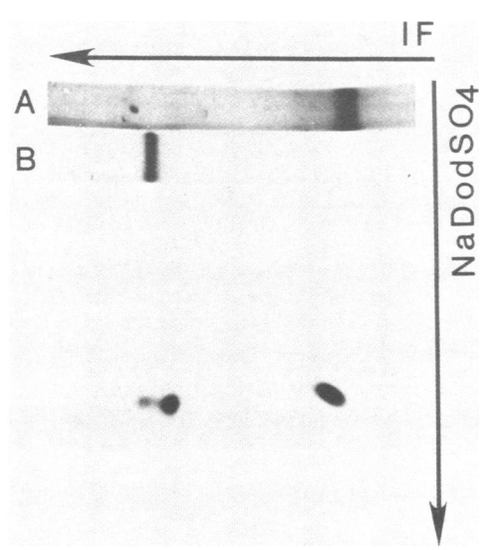

FIG. 4. Two-dimensional electrophoresis of purified *Drosophila* APRT, which employs denaturing conditions in both dimensions, and silver staining of proteins. First-dimension gels containing purified APRT from $Aprt^B$ and $Aprt^A$ homozygotes are shown in *A* and *B*, respectively, which are aligned with each other according to pH above an equivalent two-dimension gel containing both purified enzymes. The isoelectric points of APRT from $Aprt^A$ and $Aprt^B$ variants are 6.0 and 6.3, respectively. IF, isoelectric focusing.

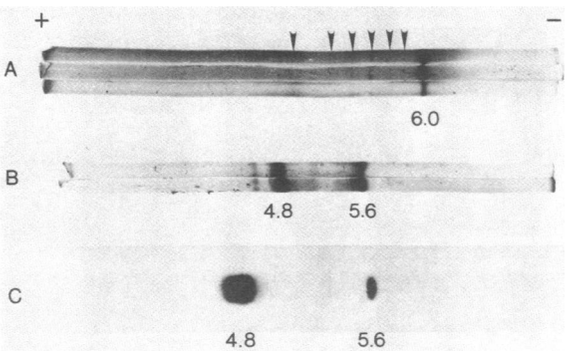

FIG. 5. *Drosophila* APRT purified from $Aprt^A$ homozygotes was subjected to isoelectric focusing under the following conditions: (*A*) denaturing conditions according to O'Farrell (9) and the gels silver stained for protein; (*B*) nondenaturing conditions (described in *Methods*) and the gels silver stained for protein; and (*C*) nondenaturing conditions as in *B* in a gel fluorographed for APRT activity. The pH gradient was from 4 (+) to 7 (−) in gels from each panel and the pH of gel slices that correspond to the location of protein bands or APRT activity is presented below each panel. Arrowheads, see text.

Nondenaturing isoelectric focusing of APRT activity exposes a complex pattern with at least one acidic and one more basic form of enzyme activity in each homozygous variant strain (Fig. 6). These patterns are caused only by APRT activity because $Aprt^1$ homozygotes produced no activity pattern (Fig. 6*B*, lane 3) nor did $Aprt^A$ homozygotes when 5-phosphoribosyl-1-pyrophosphate was omitted from the gel reaction mixture (data not shown). The complex pattern of APRT activity is probably due to conformational or quarternary structural differences, or both (18). That the complex pattern of APRT activity in each homozygous variant results from conformational or quarternary structural differences (or both) among APRT molecules is indicated by isoelectric focusing of purified APRT under both native and denaturing conditions (Fig. 5). This analysis shows that the isoelectric points of APRT activity (Fig. 5*C*) correspond to the isoelectric points of purified APRT protein (Fig. 5*B*). However, APRT produces a single major silver-staining band at a more basic isoelectric point under denaturing conditions designed to produce a single conformation for a given polypeptide (Fig. 5*A*).

We suspect that the most acidic bands of APRT activity (Fig. 6, pH 4.8–5.0) may be produced by multimeric forms of the enzyme, whereas the more basic bands of activity (pH 5.6 and pH 5.8) are produced by monomers. Our reasons are: (*i*) there is no band of APRT activity at an intermediate pH between the more basic bands (pH 5.6 and pH 5.8) in heterozygotes, as would be expected if heterodimers were formed (Fig. 6*C*, lanes 1 and 4), and (*ii*) the more acidic band of APRT activity (pH 4.8–5.0) appears to span this entire pH range in heterozygotes, suggesting that it contains both homodimers of each type and heterodimers (Fig. 6*C*, lanes 1 and 4).

It is possible that APRT is active as both a dimer and a monomer under the conditions of native isoelectric focusing. A portion of rat APRT purified by nondenaturing isoelectric focusing is active as a 20,000-dalton protein (19); yet, when this protein is analyzed without prior purification by isoelectric focusing, it appears to be active as a 40,000-dalton dimer (17). Although there is no physical evidence supporting this hypothesis for

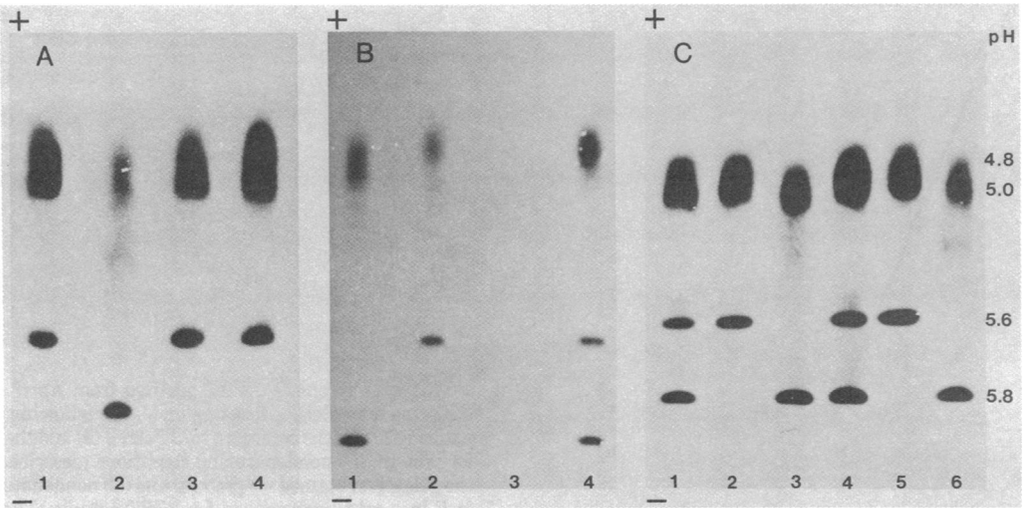

FIG. 6. Three separate nondenaturing isoelectric focusing autoradiograms of APRT activity. The pH gradient is from 4 (+) to 7 (−) in each gel and the pH of gel slices corresponding to APRT activity is shown for the gel in *C*. Lanes contained APRT from flies of the following genotypes. (*A*) Lane 1, flies carrying the chromosome in which $Df(3L)R^{+E}$ was generated ($Aprt^A$ homozygote); lane 2, $Df(3L)R^{+E}/Aprt^B$; lane 3, $Df(3L)R^{+E}/Aprt^A$; and lane 4, male flies homozygous for *ru* $Aprt^1$ on the third chromosome and carrying *Dp(T(Y;3)H141*. (*B*) Lane 1, flies homozygous for chromosome 3 derived from $Aprt^B$ homozygotes but in which chromosomes 1 and 4 were derived from $Aprt^A$ homozygotes; lane 2, flies homozygous for chromosome 3 derived from $Aprt^A$ homozygotes but in which chromosome 2 was derived from $Aprt^B$ homozygotes; lane 3, females of the *Dp(T(Y;3)H141* strain that are homozygous for *ru* $Aprt^1$ on chromosome 3 but do not carry the translocation; and lane 4, $Aprt^A/Aprt^B$. (*C*) Lanes 1 and 4, $Aprt^A/Aprt^B$; lanes 2 and 5, $Aprt^A/Aprt^A$; and lanes 3 and 6, $Aprt^B/Aprt^B$.

Drosophila APRT, the more basic bands of activity (pH 5.6 and pH 5.8) must represent either monomers or homodimers because these activity bands have the same isoelectric point in either heterozygotes or the respective homozygote (Fig. 6*C*). In any case, the isoelectric point forms of APRT activity characteristic of each homozygous variant assort together in a simple Mendelian fashion with chromosome 3 (Fig. 6*B*, lanes 1 and 2). A locus specifying the $Aprt^A$ isoelectric point forms of APRT activity resides on the third chromosome translocation in *Dp(T(Y;3)H141)* (Fig. 6*A*, lane 4) and the expression of these same variant forms of APRT activity have been extinguished as the result of deletion in the $Df(3L)R^{+E}$ chromosome (Fig. 6*A*, lanes 1–3). This analysis demonstrates that physical alterations of APRT that result in the difference of isoelectric point between the variant APRT molecules are caused by genetic variation at or near the *Aprt* locus.

DISCUSSION

The comparative analysis of purified APRT from the purine-sensitive $Aprt^A$ and $Aprt^B$ variants by two-dimensional electrophoresis shows that each variant produces APRT molecules with a similar molecular mass but different isoelectric point (Fig. 4). This result indicates that *Drosophila* APRT is composed of a single polypeptide encoded by a single structural gene. The mutants and natural variants of *Drosophila* affecting purine sensitivity, specific activity of APRT, or isoelectric point of APRT show codominant expression of APRT activity in heterozygotes and are therefore confined to a single complementation group. Genetic and cytogenetic mapping support the results of complementation tests because these same mutants and variants appear to arise from alterations at a single locus designated *Aprt* (Table 1 and Fig. 6). The deficiency $Df(3L)R^{+E}$ fails to complement any of the alleles of *Aprt* and does not contribute to the level of APRT activity in the deficiency heterozygotes (Table 2). In addition, this deficiency appears to have caused the loss of an $Aprt^A$ allele as well as the closely linked *R* allele (Figs. 1 and 6).

Our primary reason for investigating *Aprt* is that mutations causing a deficiency of APRT activity can be readily isolated in whole *Drosophila* by selection for purine resistance. Results presented here demonstrate that *Aprt* contains the structural gene for APRT and suggest that purine selection of mutations in *Aprt* can be used to study the organization and control of this gene in intact *D. melanogaster*.

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