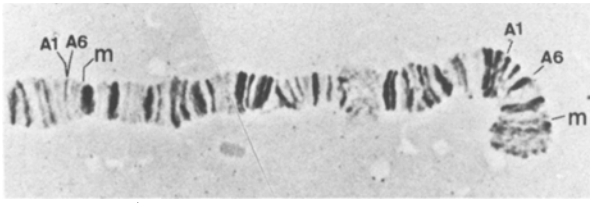

Figure 3. Proximal half (segment I) of the *D. subobscura* A (= X) chromosome with standard gene arrangement, where the *D. subobscura* A1 and A6 inversion break points and the *D. madeirensis* gene arrangement (m) are indicated.

sections shown in the figures and mentioned in the text are based on the standard salivary gland chromosome map of *D. subobscura*[3].

Figure 1 shows the A chromosome of F1 hybrids between *D. madeirensis* and strains A1 and A2 + 6 of *D. subobscura*. This figure shows unambiguously that the *D. madeirensis* gene arrangement is different from any of the *D. subobscura* arrangements. A1 and A6 inversions have the same distal break point, located at the 7D/8A subsections (figs 2, 3)[4], but they differ in the proximal one. This break point is located between subsections 2A and 2B in the A1 inversion and between 1C and 2A in the A6 inversion (figs 2, 3)[4]. The gene arrangement found in *D. madeirensis* differs from A1 and A6 inversions in both break points. The distal one is located between subsections 7C and 7D; in figure 2 it can be seen that the chromosomes of *D. madeirensis* show clearly the three bands of subsection 7D in the distal position, whereas in A1 and A6 *D. subobscura* chromosomes at least the first two bands of subsection 7D can be seen in the proximal position. The proximal break point is located possibly between subsections 1A and 1B, although in *D. subobscura* chromosomes the 1B/1C zone appears as a thick dark band, more apparent than in the *D. madeirensis* chromosomes. Figure 3 shows the proximal half of the *D. subobscura* standard X chromosome with the break points of A1 and A6 and *D. madeirensis* gene arrangements.

Thus, the gene arrangement of the X chromosome of *D. madeirensis* is different from any known arrangement of *D. subobscura,* both for segment I, that we have just analyzed, and for segment II, in which a small inversion is found in the distal end (fig. 1)[2]. Since all the other chromosomes of *D. madeirensis* are homosequential with the corresponding chromosomes of *D. subobscura* (Jst, U1 + 2, Est, O3)[2], the X chromosome is the only one that has undergone structural variation during the speciation process. In *D. guanche,* a closely related species, a greater structural variation in the X chromosome in comparison with the autosomes is also observed[5]. These results are in agreement with the expected higher incidence of rearrangements involving the X chromosome when related species are compared, as was proposed by Charlesworth et al.[6] in their model for the relative rates of evolution of sex chromosomes and autosomes.

1 Monclús, M., Z. zool. Syst. Evolut. Forsch. *22* (1984) 94.
2 Krimbas, C. B., and Loukas, M., Heredity *53* (1984) 469.
3 Kunze-Mühl, E., and Müller, E., Chromosoma *9* (1958) 559.
4 Jungen, H., Arch. Julius Klaus-Stift. VererbForsch. *43* (1968) 3.
5 Moltó, M. D., De Frutos, R., and Martinez-Sebastián, M. J., Genetica *75* (1987) 55.
6 Charlesworth, B., Coyne, J. A., and Barton, N. H., Am. Nat. *130* (1987) 113.

0014-4754/89/030310-03$1.50 + 0.20/0

Single amino acid substitutions in *sn*-glycerol-3-phosphate dehydrogenase allozymes from *Drosophila virilis*

H. Tominaga, K. Arai[a] and S. Narise

Biological Laboratory and [a]Department of Chemistry, Faculty of Science, Josai University, Sakado, Saitama 350-02 (Japan)
Received 19 September 1988; accepted 2 November 1988

Summary. The amino acid sequence was compared among the three allelic variants (allozymes) of *sn*-glycerol-3-phosphate dehydrogenase in *D. virilis,* which are detected by one-dimensional electrophoresis. The αGPDH^f variant was different from the αGPDH^m by only one substitution of 68-lysine for asparagine; αGPDH^s differed from αGPDH^m by substitution of 127-glycine for arginine. No electrophoretically 'silent' substitutions were found in a total of 352 amino acid residues.
Key words. Drosophila virilis; *sn*-glycerol-3-phosphate dehydrogenase; allozymes; single amino acid substitution.

Glycerol-3-phosphate dehydrogenase (*sn*-glycerol-3-phosphate: NAD^+ 2-oxidoreductase, EC 1.1.1.8) in adult *Drosophila* functions in flight muscle metabolism[1]. *Drosophila virilis* in Japanese populations has three alleles at the αGPDH structural locus, one of which (*αGPDH^m*) comprises more than 95% of the genes[2]. Biochemical studies of the allozymes (αGPDH^s, αGPDH^m, and αGPDH^f) produced by homozygotes for the three alleles revealed that although no significant differences in Michaelis constants for NADH, G-3-P and NAD^+ were detectable, subtle but significant differences were observed in thermostability at 35 °C and inhibition by excess dihydroxyacetone phosphate, one of the substrates. The αGPDH^f allozyme was most thermolabile, and the αGPDH^s form was most susceptible to the inhibition[3]. We take an interest in the relation between functional differences between allozymes produced by the *αGpdh* locus and their structural differences. We have already determined the primary structure of αGPDH^m[4]. Therefore, we have undertaken complete sequence analysis of the αGPDH^s and αGPDH^f forms. The present paper demonstrates that the three allozymes differ from each other by a single amino acid substitution only.

The two *D. virilis* allozyme strains, αGPDH^s and αGPDH^f, were used. These strains are homozygous for each of these alleles at the NAD^+-dependent glycerol-3-phosphate dehydrogenase locus. Each allozyme was purified from frozen 1-2-day-old flies according to the methods described before[4]. Since the αGPDH^f enzyme was partly leaked from a hydroxyapatite gel column at pH 7.4, this pH being the same as that for preparation of αGPDH^s, the pH was lowered to 6.8 for αGPDH^f. Final preparations gave a single band on polyacrylamide gel electrophoresis.

Methods for analysis of the enzyme protein sequence were fundamentally the same as those used for αGPDH^m allozyme[4]. The carboxymethylated purified αGPDH^s and αGPDH^f were cleaved at specific methionine residues with cyanogen bromide. After separation on a column of Sephadex G-75 superfine, single peptides were isolated by reverse-phase HPLC. The amino acid sequences of the peptides were determined by Edman degradation and carboxypeptidase treatment. Edman degradation was automatically or sometimes manually performed. Phenylthiohydantoin derivatives of amino acids were analyzed by reverse-phase HPLC. Large peptides were digested with trypsin, chymotrypsin, and *S. aureus* V8 proteinase and then used for sequence analysis. Amino acid compositions of the isolated peptides were obtained using a JEOL JLC-200A amino acid analyzer.

The complete amino acid sequences of αGPDH^s and αGPDH^f were determined and compared with the sequence of αGPDH^m, which has already been described[4]. Cyanogen bromide fragments were entirely identical among the three forms except for the CB3 fragment. CB3 is the largest of the cyanogen bromide fragments, spanning 45-isoleucine through 149-methionine. Figure 1 shows typical HPLC patterns of the CB3 tryptic subdigests from the two allozymes. These chromatographic peaks were compared with αGPDH^m on the basis of retention time and amino acid composition of the peptides. As seen in figure 1, there are a few peaks unique to each allozyme. Peak 6′ instead of peak 6 appeared for αGPDH^f. αGPDH^s had two unique peaks (10′ and 11′). but lacked three peaks (10, 11 and 12) common to αGPDH^m and αGPDH^f. The remaining peaks were common to all three allozymes, which indicated that large parts of the CB3 fragment are identical.

Sequence analysis of the tryptic peptides of CB3 (fig. 2 and table) revealed that the substitution of 68-lysine for asparagine in αGPDH^f produced peptide 6′ instead of peptides 5 and 6. Meanwhile, in αGPDH^s, the change of 127-glycine to arginine produced peptides 10′ and 11′ instead of 12. However, the two small peptides, 121-glycine to 124-lysine and 125-alanine to 127-arginine, which might be released by breakage at position 124-lysine could not be detected, perhaps because of the small amount present. These results show that αGPDH^f and αGPDH^s each differ from αGPDH^m by only a single substitution. These differences cause a charge increase of + 1 unit in order of αGPDH^f, αGPDH^m, and αGPDH^s. This difference is in accordance with the difference in their relative mobilities in gel electrophoresis[3].

The mutations observed are explicable by a single third base alteration in the codon AAA or AAG to AAT or AAC in the DNA sequence in αGPDH^f and a single first base alteration in GGA or GGG to AGA or AGG in αGPDH^s. Although an electrophoretically silent substitution has been found among ADH allozymes from *D. melanogaster*[5], such a substitution did not occur in αGPDH allozymes from *D. virilis*. These findings indicate that a single amino acid replacement in an enzyme protein can affect the thermostability or kinetics (i.e., inhibition) of the αGPDH enzyme. Also, it was re-

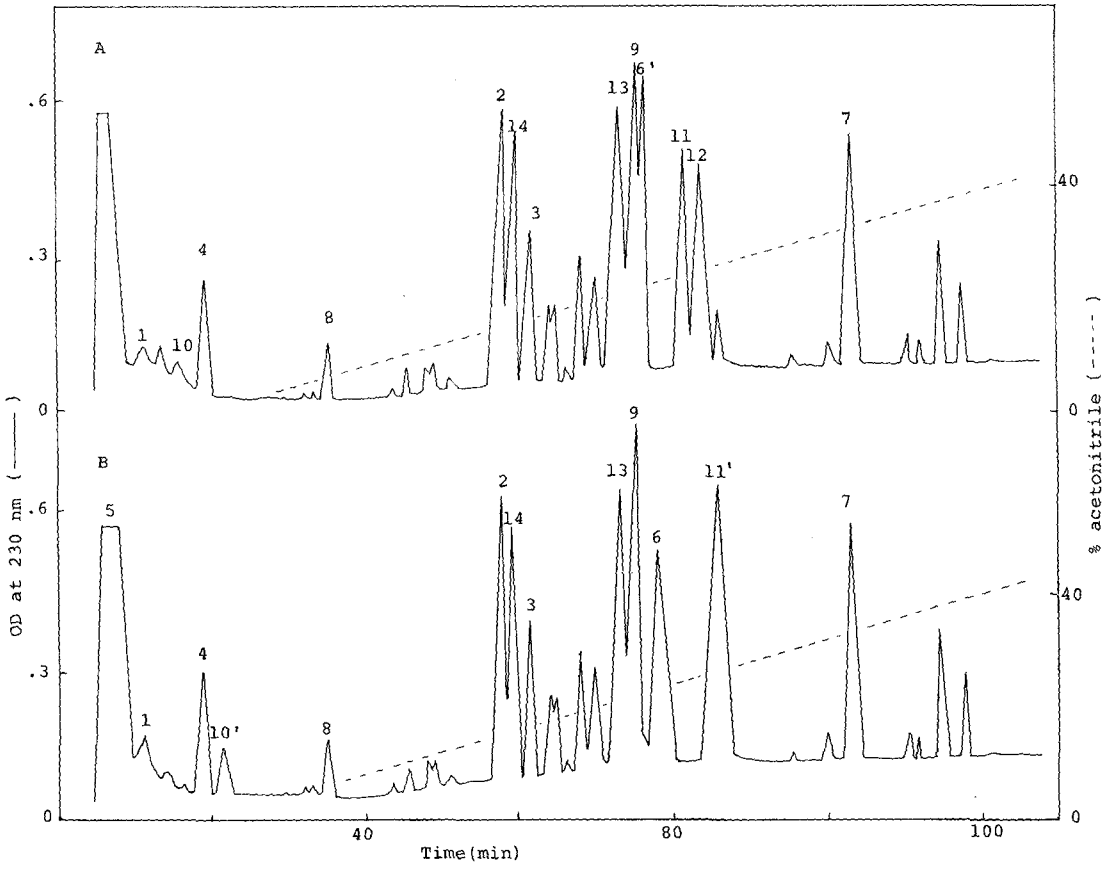

Figure 1. Separation of tryptic peptides of CB3 fragments. Tryptic peptides were separated by HPLC on Inertsil 300 C8 column (4.6 × 100 mm) in 0.1% trifluoroacetic acid with a gradient of acetonitrile. *A* αGPDH^f; *B* αGPDH^s. Each peptide was subsequently purified by HPLC on a Capcell pak C18 (4.6 × 250 mm) in 0.01 M HCOOH/NH_3, pH 9.0, with a gradient of acetonitrile and then used for sequence analysis. The single peptides were numbered 1 through 14 corresponding to those of αGPDH^m described before. Peptide number with a prime mark is specific to each allozyme.

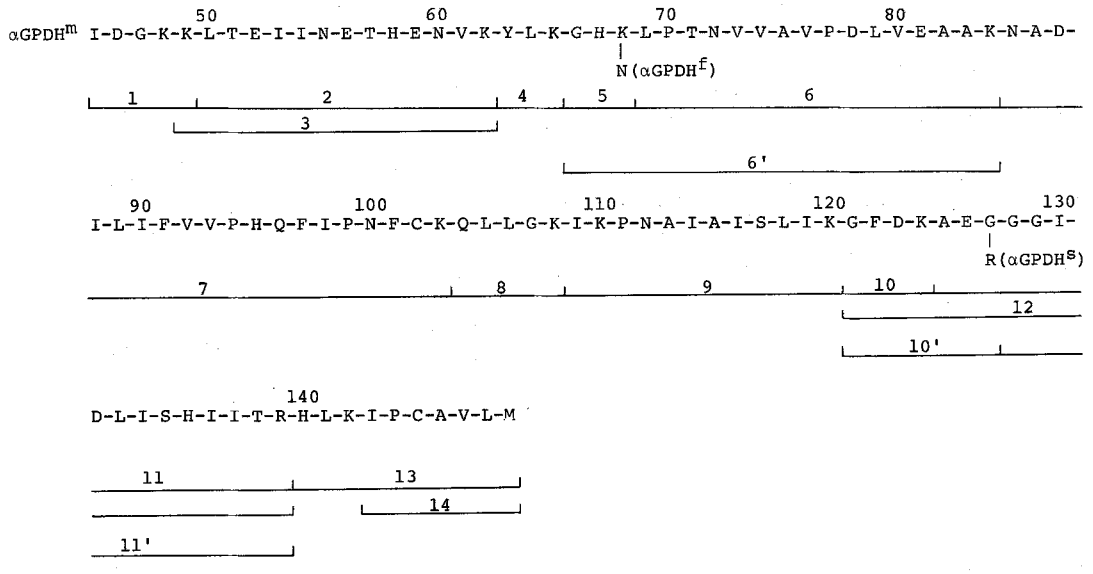

Figure 2. Summary of the data used to deduce the sequence of CB3 fragments from αGPDH^f and αGPDH^s. The sequence of αGPDH^m was taken from fig. 1 of Arai et al.[4]. Peptide number corresponds to peak number in fig. 1.

Amino acid compositions for peptides from CB3 fragment exhibiting changes between αGPDH^f or αGPDH^s and αGPDH^m.

Amino acid	5	6	6′	10	10′	11	11′	12
Asp/Asn		2.3 (1/1)	3.0 (1/2)	1.0 (1/0)	1.1 (1/0)	1.1 (1/0)	1.1 (1/0)	2.0 (2/0)
Thr		0.9 (1)	0.9 (1)			0.9 (1)	0.8 (1)	0.9 (1)
Ser						0.9 (1)	0.7 (1)	0.8 (1)
Glu/Gln		1.0 (1/0)	1.0 (1/0)		1.0 (1/0)	1.1 (1.0)		1.0 (1/0)
Pro		2.1 (2)	2.0 (2)					
Gly	1.1 (1)		1.0 (1)	1.0 (1)	1.0 (1)	3.3 (3)	2.2 (2)	4.3 (4)
Ala		3.4 (3)	3.0 (3)		1.0 (1)	1.0 (1)		1.0 (1)
Val		2.9 (4)	3.3 (4)					
Ile						3.1 (4)	2.7 (4)	3.1 (4)
Leu		2.0 (2)	2.0 (2)			1.0 (1)	0.9 (1)	1.0 (1)
Phe				1.0 (1)	1.0 (1)			1.0 (1)
His	1.0 (1)		1.0 (1)			1.0 (1)	0.9 (1)	1.0 (1)
Lys	1.0 (1)	1.0 (1)	1.0 (1)	1.0 (1)	1.0 (1)			1.0 (1)
Arg					1.0 (1)	1.0 (1)	1.0 (1)	1.0 (1)
Total	3	16	19	4	7	15	12	19
Yield (%)	24.3	27.9	47.3	12.9	50.7	19.6	20.2	54.0

Values are molar ratios after hydrolysis for 24 h with integers from the sequence analysis in parentheses. Peptide number corresponds to peak number in fig. 1.

cently reported that a thermostable variant of the ADH^F allozyme has a single amino acid replacement[6].

Secondary structures of the three αGPDH allozymes were predicted from their amino acid sequences by the methods of Chow and Fasman[7,8]. They were almost the same as each other, and both of the substitutions in αGPDH^f and αGPDH^s (residues 68 and 127, respectively) occurred with regions of irregular conformation; the former slightly enhanced a tendency to form turn, while the latter reduced it. It has been found for the tertiary structure of glycerol-3-phosphate dehydrogenase of rabbit muscle[9], whose amino acid sequence is 63% identical to that of the same enzyme in *D. virilis*[4], that residue 68 is located in a turn between βC and βD parallel sheets in the NAD-binding domain and residue 127 is also in a turn within the catalytic domain. Furthermore, structural analysis of lysozymes of wild-type and various temperature-sensitive mutants of bacteriophage T4 demonstrated that when a single amino acid located in an irregular loop on the surface of the protein was substituted by other amino acids, these mutant proteins showed a different thermostability[10]. It is probable that single amino acid substitutions in the αGPDH allozymes contribute to differences in thermostability and substrate inhibition of the allozyme proteins.

1 Bewley, B. C., and Miller, S., Isozymes: Curr. Top. biol. med. Res. *3* (1979) 23.
2 Ohba, S., in: Population Genetics, p. 99. UP Biology Series, Tokyo University Press, Tokyo 1977.
3 Narise, S., Biochim. biophys. Acta *615* (1980) 289.
4 Arai, K., Tominaga, H., Yokote, Y., and Narise, S., Biochim. biophys. Acta *953* (1988) 6.
5 Thatcher, D. R., Biochem. J. *187* (1980) 875.
6 Collet, C., J. molec. Evol. *27* (1988) 142.
7 Chow, P. Y., and Fasman, G. D., Biochemistry *13* (1974) 211.
8 Chow, P. Y., and Fasman, G. D., Biochemistry *13* (1974) 222.
9 Otto, J., Agros, P., and Rossmann, M. G., Eur. J. Biochem. *109* (1980) 325.
10 Alber, T., Dao-pin, S., Wilson, K., Wozniak, J. K., Cook, S. P., and Mattews, B. W., Nature *330* (1987) 41.

0014-4754/89/030312-03$1.50 + 0.20/0