Pergamon

Biochemical Pharmacology, Vol. 50, No. 7, pp. 1015–1020, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(95)00231-6

CORRELATION BETWEEN CATALYTIC ACTIVITY AND PROTEIN CONTENT FOR THE POLYMORPHICALLY EXPRESSED DIHYDROPYRIMIDINE DEHYDROGENASE IN HUMAN LYMPHOCYTES

PEDRO FERNANDEZ-SALGUERO,* FRANK J. GONZALEZ, MARIE-CHRISTINE ETIENNE,† GERARD MILANO† and SHIOKO KIMURA

Laboratory of Molecular Carcinogenesis, National Cancer Institute, Bethesda, MD 20892, U.S.A.; and †Laboratoire d' oncopharmacologie, Centre Antoine-Lacassagne, 36, Voie-Romaine, 06050 Nice, Cedex, France

(*Received* 30 *December* 1994; *accepted* 7 *April* 1995)

Abstract—A TLC procedure was developed to determine dihydropyrimidine dehydrogenase (DPD) activity in human peripheral lymphocytes. The assay, which used radiolabeled uracil as a substrate, was validated using recombinant pig DPD in which it was demonstrated to yield kinetic constants similar to those found by methods that rely on either spectroscopic determination of NADPH oxidation or HPLC. DPD activity was measured in a group of human lymphocyte extracts, including an extract from a subject that actually presented toxicity to 5-fluorouracil treatment. Measurements of DPD protein content using western immunoblots revealed a significant correlation with activity levels in human lymphocytes. Thus, this correlation could be used to determine not only the levels of expression of this enzyme, which is the cause of an inherited genetic deficiency in pyrimidine catabolism, but also to estimate the degree of sensitivity to pyrimidine-based cancer drugs such as 5-fluorouracil.

Key words: TLC, dihydropyrimidine dehydrogenase assay; 5-fluorouracil toxicity

DPD‡ (EC 1.3.1.2) is the rate-limiting enzyme in the catabolism of pyrimidines. The metabolism of uracil is the only pathway for the biosynthesis of β-alanine in mammals [1]. DPD is also involved in the catabolic inactivation of 5-FU, a pyrimidine analog widely used in the chemotherapeutic treatment of cancers related to the digestive tract (colorectal and gastric), breast, head, neck and ovary [2, 3]. A deficiency in DPD activity is responsible for an inborn error in pyrimidine metabolism called thymine uracilurea [4]. Several phenotypic traits have been associated with this deficiency, including convulsive disorders, high toxicity associated with low 5-FU metabolism after treatment of cancer patients with this chemical, microcephaly, some mental retardation, and minor dysmorphic features including hypertelorism, broad nose and lacunar teeth [4].

DPD deficiency was shown to be responsible for 5-FU toxicity that resulted in several deaths during cancer chemotherapy (reviewed in Ref. 3). Patients that experience 5-FU toxicity are believed to be genotypically heterozygotes or homozygous for a mutant *DPYD* gene. The frequency of heterozygotes in the population maybe as high as 3% [3]. Based on the Hardy–Weinberg equilibrium, the frequency of homozygous-deficient subjects could be as high as 1/1000. This deficiency is inherited as an autosomal recessive trait [5], and, for the first time, the molecular basis for DPD deficiency in humans has been determined in a Dutch family [6]. Biochemically, DPD deficiency results in almost complete lack of catalytic activity in human fibroblasts [7] and lymphocytes [8, 9]. In the present study, using a newly developed TLC method, and DPD protein quantification by western blots, we demonstrated a correlation between catalytic activity and DPD protein content in human lymphocytes. The method was characterized and validated using lymphocytes from several subjects and recombinant DPD produced in *Escherichia coli.*

MATERIALS AND METHODS

Chemicals

NADPH, phenylmethylsulfonyl fluoride (PMSF), EDTA, leupeptin and DTT were obtained from the Sigma Chemical Co. (St. Louis, MO) at either >98% purity or Sigma grade. [2-^{14}C]Uracil was purchased from DuPont-NEN (Boston, MA) (59.1 mCi/mmol; 98% pure as determined by the manufacturer by a TLC method). Ficoll-Paque ET (density = 1.077 g/mL) was obtained from Pharmacia LKB Biotechnology (Piscataway, NJ). TLC plates were purchased from J. T. Baker Inc. (Phillipsburg, NJ). All other chemicals were obtained from commercial sources and were of the best quality available.

Isolation of lymphocytes from peripheral blood

Peripheral vein blood was obtained from donors by collecting a 10-mL aliquot into a heparinized syringe. The blood was diluted with 1 vol. of balanced salt solution [15 mM Tris–HCl buffer (pH 7.5) containing 0.01% glucose, 50 μM $CaCl_2$, 100 μM $MgCl_2$, 5.4 mM KCl and 125 mM NaCl] and centrifuged at 400 *g* for 40 min at 20° through a Ficoll-Paque ET discontinuous gra-

* Corresponding author: Dr. Pedro Fernandez-Salguero, Building 37, Room 3E-24, National Institutes of Health, Bethesda, MD 20892. Tel. (301) 496-7325; FAX (301) 496-8419.

‡ Abbreviations: DPD, dihydropyrimidine dehydrogenase; *DPYD*, dihydropyrimidine dehydrogenase gene; DTT, dithiothreitol; and 5-FU, 5-fluorouracil.

dient to isolate peripheral blood mononuclear cells from erythroid cells. The interphase of the gradient containing the lymphocytes was removed and washed twice with 4 vol. of balanced salt solution. The cells were then resuspended in 2 mL of 45 mM potassium phosphate buffer (pH 7.3), 20% glycerol, 10 mM EDTA, 1 mM DTT, 0.1 mM PMSF, and 2 μM leupeptin. The cell suspension was lysed on ice with two 20-sec bursts of a Heat Systems sonicator (model W 225-R at 20% of full power). The lysate was centrifuged at 100,000 *g* for 60 min at 4°. To avoid interference from glycerol during migration on the TLC plate, the supernatant was dialyzed for 18 hr at 4° against 1000 vol. of 45 mM potassium phosphate buffer (pH 7.3) containing 1 mM EDTA, 1 mM DTT, and 2.5 mM $MgCl_2$. The final protein was stored at –70° until used. This retains about 80–90% activity after 4 weeks of storage. Lymphocyte DPD was also shown by others to be stable after freezing [10].

Catalytic assay

DPD activity has been measured by a TLC protocol in which separation between the substrate ([2-^{14}C]uracil) and the product formed (5,6-dihydrouracil) can be achieved by migration on a silica gel TLC plate.

The reaction mixture contained 29 mM potassium phosphate buffer (pH 7.3), 1 mM EDTA, 1 mM DTT, 2.5 mM $MgCl_2$, 60 μM NADPH, 50 μM [2-^{14}C]uracil as substrate, and enzyme in a final volume of 100 μL. After starting the reaction by the addition of the substrate, the samples were incubated at 37° for 10 min for purified pig DPD or for 90 min for human lymphocyte DPD. The reaction was stopped by the addition of 1.5 vol. of ethanol, and the samples were dried under vacuum. The dry pellet was dissolved in 10 μL of distilled water and applied to a 20 × 20 cm silica gel IB TLC plate. The chromatography was developed using an *n*–butanol:glacial acetic acid:water (12:3:5, by vol.) solvent mixture until the front of the solvent reached the top of the plate. The TLC plate was removed from the chamber and air dried. To quantify the catalytic activity, the plates were exposed for 6–8 hr with Phosphor screens (Eastman Kodak Co., Rochester, NY) at room temperature, and the screens were read with a Phosphorimager (Molecular Dynamics, Sunnyvale, CA). The signal corresponding to the product was transformed into nanomoles of product by using a calibration curve constructed with known amounts of [2-^{14}C]uracil since the stoichiometry for the conversion to 5,6-dihydrouracil from uracil is 1:1 [11], and it has been reported previously that dihydrouracil is the only metabolite formed from uracil in human lymphocytes [12]. The catalytic activity is expressed as micromoles or nanomoles of product per minute and per milligram of protein for the pig purified DPD or human lymphocyte enzymes, respectively. DPD activity was measured from frozen lymphocyte preparations since it has been shown that the catalytic activity of frozen versus fresh lymphocyte preparations follows a linear relationship [12].

DPD exhibits a nonclassical ping–pong catalytic mechanism in which two substrates (thymine or uracil and NADPH) interact with two independent active sites [13]. Thus, both NADPH (not shown) and uracil concentrations were titrated. Catalytic activity determinations on different human lymphocyte samples were done using 60 μM NADPH and 50 μM uracil, which correspond to concentrations at which the enzyme is at V_{max}. Additionally, these conditions allow direct comparison with catalytic activities for human lymphocyte preparations obtained by others. DTT, EDTA and $MgCl_2$ were added to the reaction mixture at the standard concentrations previously reported for human lymphocyte DPD [14].

Protein concentration was determined using the bicinchoninic acid (BCA) procedure (Pierce Chemical Co., Rockford, IL), using bovine serum albumin as standard. All the protein determinations in this study were done within the linear range of the assay.

Western blot and quantification of the DPD content in human lymphocytes

SDS–PAGE gel electrophoresis was carried out using the method of Laemmli [15]. The gels were transferred to nitrocellulose membranes by semi-dry electroblotting for 90 min at 1.5 mA/cm^2. The membranes were blocked at room temperature by incubation in PBS solution containing 0.5% Tween 20 and 5% skim milk. After rinsing with PBS, the membranes were incubated for 2 hr at room temperature with rabbit anti-pig DPD polyclonal antibody (200-fold dilution in PBS). The blots were subsequently washed three times in PBS containing 0.5% Tween 20 and rinsed twice in PBS prior to incubation for 90 min with the alkaline phosphatase-labeled goat anti-rabbit IgG. The membranes were developed using the reagent 5′-bromo-4-chloro-3-indolyl-phosphate/nitro-blue tetrazolium (BCIP/NBT) (Kirkegaard & Perry Laboratories, Gaithersburg, MD).

The DPD protein content in human lymphocytes was quantified directly from the western blots by using a scanning densitometer (Molecular Dynamics). The signals obtained for the different subjects were used to calculate micrograms of lymphocyte DPD protein by reference to a calibration curve constructed with known amounts of pig DPD. This calibration curve shows linearity up to 1.0 μg of purified enzyme ($r = 0.995$, $P < 0.0005$).

Recombinant DPD was produced by expression in *E. coli* [16]. Briefly, DH-5α cells were transfected with the vector pSE420 containing the pig DPD cDNA. A single colony of transformed cells was used to produce a 250-mL culture that, following induction with isopropyl-β-*d*-thiogalactopyranoside, was harvested for isolation of DPD. The protein preparation was enriched in DPD content by ammonium sulfate fractionation prior to use, as described [16].

Statistical analyses

Simple linear correlations and Spearman rank correlations were done with the Stat View software program (Abacus Concepts Inc., Berkeley, CA).

RESULTS

The isolation of enriched DPD preparations from human lymphocytes by the Ficoll-Paque gradient and sonication method yields a catalytically active protein that reacts with the rabbit anti-DPD polyclonal antibody. This method yields between 1.5 and 2.6 μg of DPD protein (0.5 to 0.8 mg of total protein) per 10 mL of blood.

A mixture of *n*-butanol:glacial acetic acid:H_2O (12:3:5, by vol.) was found to adequately resolve the substrate (uracil) from the product (5,6-dihydrouracil). A typical

experiment for the linearity of the reaction with time using recombinant pig DPD shows the degree of resolution achieved (Fig. 1A). R_f values of 0.53 and 0.41 were obtained for the substrate and the product of the reaction, respectively; these R_f values were in the same range of resolution as those shown for a different TLC catalytic assay [17]. Some tailing of product to substrate was noted only under conditions of >40% conversion; by controlling the reaction time and the amount of DPD enzyme added to the assay, we kept the product conversion below 10–15% of the total substrate input. No detectable activity was obtained in the absence of added enzyme or in the absence of NADPH (results not shown). The catalytic assay, using [2-^{14}C]uracil as substrate, was found to be linear with the amount of lymphocyte DPD protein, at least up to 20 μg (Fig. 1B). Linearity was also found with the reaction time, at least up to 120 min (Fig. 1C). Linear regression analyses showed coefficients of r = 0.997 (P = 0.0001) and r = 0.989 (P = 0.0002) for the dependence on the amount of protein and with the reaction time, respectively.

To further validate the assay system, kinetic studies were performed using purified and recombinant pig DPD and human lymphocyte samples. The results, shown in Fig. 2 and Table 1, revealed that the kinetic values obtained in this study by the TLC protocol for purified pig DPD are in good agreement with those previously reported [18]. The K_m values for the purified pig enzyme, determined by NADPH oxidation and either the recombinant or the purified pig DPD using the TLC assay, were almost identical at 2 μM. The calculated K_m for human lymphocytes using the TLC method ranged from 3.3 to 4.8 μM. These K_m values for the human lymphocyte enzyme are in close agreement with those obtained using human liver purified DPD assayed by HPLC [19]. Lymphocytes were isolated from eleven subjects, and the DPD activity was determined in the cell extracts. The results obtained are shown in Fig. 3. It can be observed that most of the subjects presented a catalytic activity that was statistically comparable between them (mean = 0.168 nmol product/min/mg lymphocyte protein; SD = 0.014) and with activities previously reported by others [12]. We found two subjects (2 and 3) with activities significantly above the mean, and two

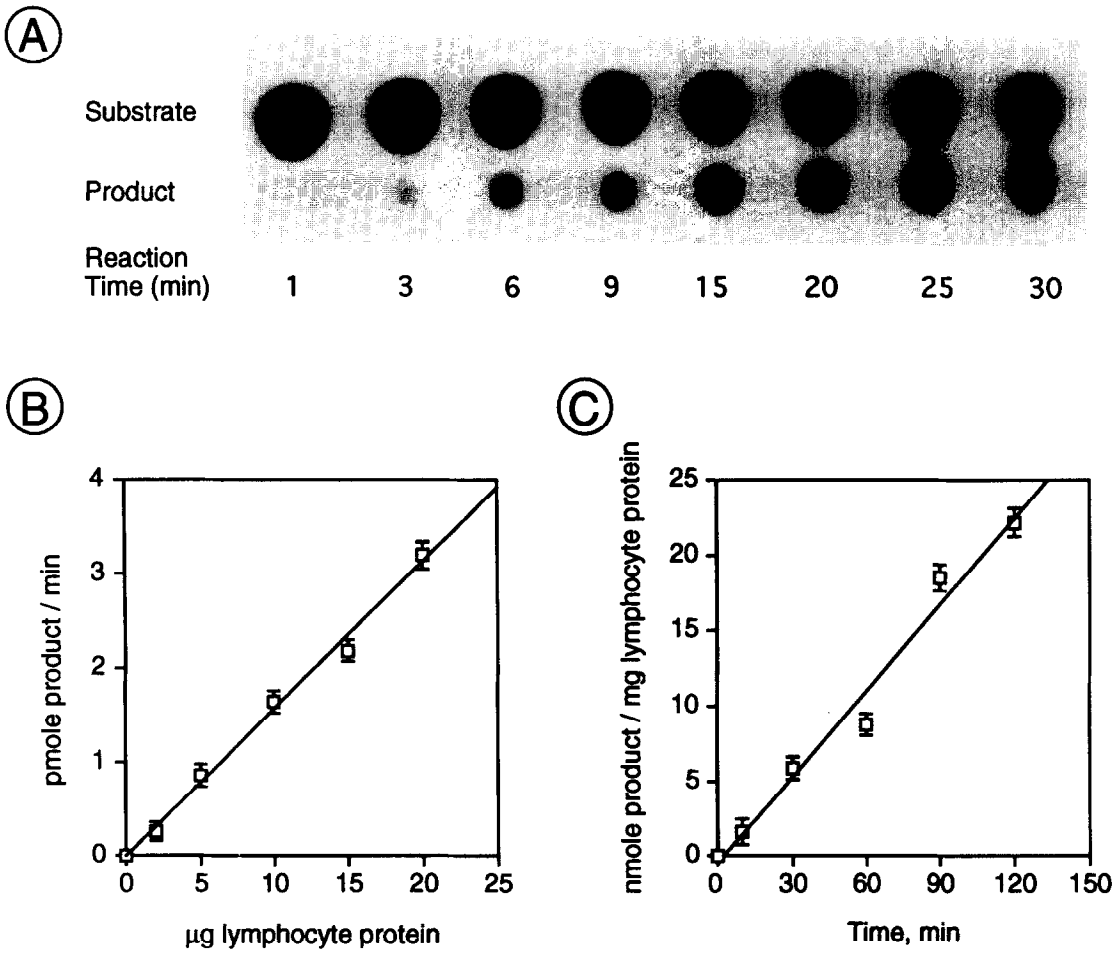

Fig. 1. (A). TLC assay of recombinant pig DPD activity as a function of time. The solvent conditions were *n*-butanol:glacial acetic acid:H_2O (12:3:5); 0.5 μg of recombinant enzyme was used. (B) Dependence of the catalytic activity on the amount of lymphocyte DPD protein. The reaction time used was 60 min. The data fit to a linear regression analysis with an r = 0.997 (P = 0.0001). (C) Dependence of the catalytic activity in human lymphocyte DPD preparations on the reaction time. The assay was carried out using 7.8 μg of total lymphocyte protein. The data show a linear correlation with an r = 0.989 (P = 0.0002). Values in panels B and C are means ± SD, N = 3.

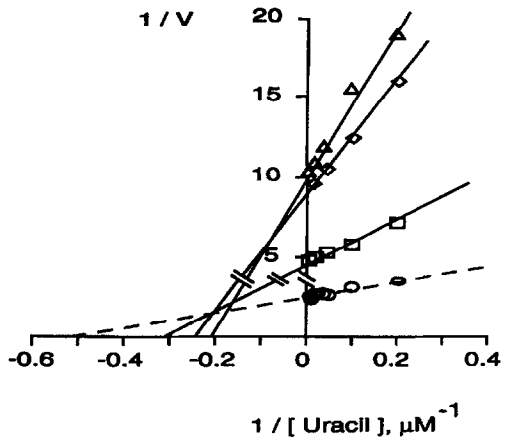

Fig. 2. Kinetic analysis of recombinant pig DPD and human lymphocyte DPD activity, using TLC. The amount of substrate was varied, and the activities obtained were plotted on a standard double-reciprocal Michaelis–Menten plot using recombinant (DPD) (○) and lymphocyte cell extract from a high activity (□) subject and two low activity (◇,△) subjects. These data were used to determine the K_m and V_{max} values shown in Table 1. Catalytic activities are expressed as micromoles and nanomoles of product per minute per milligram of protein for the recombinant DPD and for the lymphocyte DPD, respectively. The amounts of protein used for the recombinant and human lymphocyte DPD were 0.5 and 10 μg, respectively.

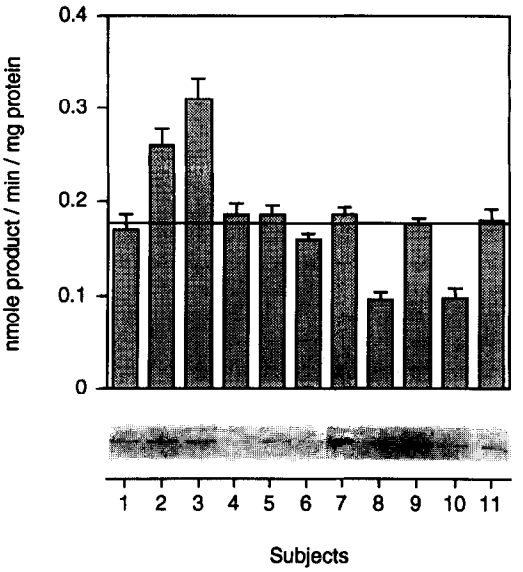

Fig. 3. DPD activity values obtained for eleven lymphocyte samples. Reactions were carried out using 7 μg of lymphocyte protein for 90 min at 37°. Results are averages of three determinations. Enzymatic activity is expressed as nanomoles per minute per milligram of total lymphocyte protein. The horizontal line stands for the reported average DPD activity for human lymphocytes as measured by HPLC. Western immunoblots were carried out on 15 μg of total lymphocyte protein using polyclonal antibody against pig DPD, except for sample 4 in which 4 μg of protein was used. The error bars indicate the standard deviation from the mean for every subject.

subjects (8 and 10) with about half the mean DPD activities. Subject 10 is actually a patient that exhibited toxicity from 5-FU treatment. The activity we obtained for this sample was very similar to that found with the HPLC assay method (90 pmol/min/mg protein, Etienne M-C and Milano G, unpublished results). We also found that another normal volunteer (subject 8) had low activity.

To determine if DPD activities correlate with protein levels, western immunoblots were performed using antibody against pig DPD [16, 18]. The blots, shown in Fig. 3, were quantified by scanning densitometry and plotted against DPD specific activities (Fig. 4). A statistically significant correlation was found between activity and protein, although there were two subjects (1 and 6) with levels of protein of about half the levels found in four other subjects with similar activities. The two samples with higher activity (2 and 3) had levels of protein that were not significantly above the mean average samples.

DISCUSSION

Another TLC method was reported to determine the catalytic activity of DPD [17]. This method involves multiple steps requiring identification of the product on the TLC plate (by UV or staining), recovery of the gel-containing product, and quantification of ^3H by scintillation counting. Additionally, in this former method, only rat liver DPD was used and the suitability of the procedure is not evaluated for practical analysis of human lymphocyte DPD. In this respect, only HPLC was reported to be useful in measuring DPD activity in human lymphocytes [14].

The TLC method presented in this report has been used to measure DPD activity in human lymphocytes. Since dihydrouracil is the only metabolite formed from

Table 1. Comparison of the kinetic parameters of human lymphocyte DPD activity with liver purified human and pig and recombinant pig enzymes

Assay method		K_m	V_{max}
HPLC method	HPLC-human purified*	4.9	0.6
NADPH oxidation	Pig purified*	1.98	0.33
TLC (this study)	Recombinant pig	2.0	0.38
	Human lymphocytes	3.3–4.8	0.09–0.3
	Pig purified	2.0	0.35

Values for V_{max} are expressed as μmol/min/mg of purified DPD and nmol/min/mg of lymphocyte protein. K_m values are expressed as μM. Kinetic parameters were calculated from data displayed in Fig. 2.

* The values for human [19] and pig [18] have been published.

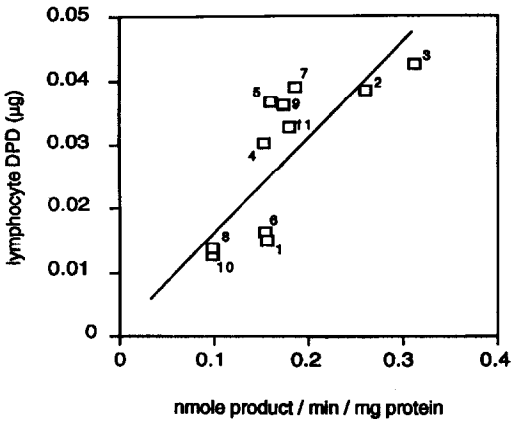

Fig. 4. Correlation between DPD activity and immunoquantified DPD protein. The values were plotted from data derived from Fig. 3. Linear regression of the data shows $r = 0.778$ ($P = 0.008$). By using a Spearman rank correlation, $\sigma = 0.927$ ($P = 0.054$) was obtained.

uracil in human lymphocytes [12], this method is a single-step analysis that allows direct quantification of the product and the substrate of the catalytic reaction after exposure of the TLC plate to X-ray film or to Phosphorimager screens due to the ^{14}C-labeling of both molecules. We have obtained accurate and highly reproducible quantification not only with the recombinant DPD enzyme, but also with the DPD protein extracted from peripheral human lymphocytes. The sensitivity of the method has been confirmed by the accurate determination of DPD activity in crude lymphocyte extracts from DPD-deficient human patients having activity more than 3 orders of magnitude lower than that of the human purified enzyme.

Using an HPLC method, DPD activities of 189 [10] and 211 pmol/min/mg protein [3] were found for normal subjects. As shown in Fig. 3, these values are very similar to those obtained in the present study. Furthermore, subject 10, identified as having a lower level of activity by the HPLC method (90 pmol/min/mg protein), was also found to have a lower catalytic activity by this TLC method (100 pmol/min/mg protein). Interestingly, subjects 8 and 10 presented a catalytic activity in the same range as that reported for heterozygous family members of patients with a complete deficiency in DPD activity [6]. Additionally, considering that DPD is the major enzyme involved in the metabolism of 5-FU, this deficiency in catalytic activity may be responsible for the observed toxic effects of this chemotherapeutic agent (as actually seen in subject 10). Genetic characterization of the DPD deficiency in this subject is currently under study.

On the other hand, the fact that subjects with similar levels of protein exhibit different (higher) levels of DPD activity suggests that high activity variants may be due to amino acid changes in the protein that result in enzymes having levels of activity above the mean of the normal population. A linear correlation coefficient of $r = 0.778$ ($P = 0.008$) was obtained between catalytic activity and DPD protein content in these human lymphocyte samples that agrees with the level of correlation found between DPD activity and 5-fluorouracil systemic clearance in humans ($r = 0.71$) [14]. When a Spearman rank correlation was applied, $\sigma = 0.927$ ($P = 0.054$) was obtained, further indicating the existence of statistically significant correlation between both variables.

The results presented in this report can be summarized as follows: (i) a simple and reproducible method to obtain crude preparations of DPD protein and a specific assay for DPD activity have been developed that require only small amounts of human lymphocytes for accurate determinations; (ii) the enzymatic assay was validated using recombinant pig DPD and lymphocyte extracts, and the K_m and V_{max} values obtained were found to be in close agreement with those reported in the literature for purified pig and human DPD and for human lymphocyte DPD; (iii) a correlation between enzymatic activity and DPD protein content has been demonstrated; and (iv) in our sample set two subjects were found, who had about one-half of the activity and protein content with respect to subjects having activities within the normal median range of the population. We have reported a close relationship between DPD activity and DPD protein content in human fibroblasts for a family having a sibling who lacks DPD activity [6]. Since other relatives in that family with an intermediate level of DPD activity and an intermediate DPD protein content were genotyped as heterozygous for an inactivating mutation in the *DPYD* gene, we suggest that the two subjects found here having intermediate DPD activity (8 and 10) could also be heterozygous for the *DPYD* gene. In addition, it has been shown that a diminished DPD activity in cancer patients leads to 5-FU toxicity [3, 4]. Thus, we suggest that the relationship between catalytic activity and DPD protein content can be useful in the analysis of 5-FU-derived toxicity and genetic alterations in the *DPYD* gene in cancer patients.

Acknowledgements—We wish to thank Dr. Klaus D. Schnackerz for the purified pig DPD enzyme and for the anti-pig DPD antibody.

REFERENCES

1. Wasternack C, Degradation of pyrimidine analogs—Pathways and mutual influences. *Pharmacol Ther* **8**: 629–651, 1980.
2. Spiers ASD, Kasimis BS and Janis MG, High dose intravenous infusions of 5-fluorouracil for refractory solid tumors—The hi-FU regimen. *Clin Oncol* **6**: 63–69, 1980.
3. Milano G and Etienne MC, Potential importance of dihydropyrimidine dehydrogenase (DPD) in cancer therapy. *Pharmacogenetics* **4**: 301–306, 1994.
4. Brockstedt M, Jacobs C, Smit LME, van Gennip A and Berger R, A new case of dihydropyrimidine dehydrogenase deficiency. *J Inherited Metab Dis* **3**: 121–124, 1990.
5. Diasio RB, Beavers TL and Carpenter JT, Familial deficiency of dihydropyrimidine dehydrogenase. Biochemical basis for familial pyrimidinemia and severe 5-fluorouracil-induced toxicity. *J Clin Invest* **81**: 47–51, 1988.
6. Meinsma R, Fernandez-Salguero P, Van Kuilenburg ABP, Van Gennip AH and Gonzalez FJ, Human polymorphism in drug metabolism: Mutation in the dihydropyrimidine dehydrogenase gene results in exon skipping and thymine uraciluria. *DNA Cell Biol* **14**: 1–6, 1995.
7. Bakkeren JAJM, De Abreu RA, Sengers RCA, Gabreëls FJM, Maas JM and Reiner WO, Elevated urine, blood and cerebrospinal fluid levels of uracil and thymine in a child with dihydrothymine dehydrogenase deficiency. *Clin Chim Acta* **140**: 247–256, 1984.
8. Berger R, Stoker-de Vries SA, Wadman SK, Duran M,

Beemer FA, de Bree PK, Weits-Binnerts JJ, Penders TJ and van der Woude JK, Dihydropyrimidine dehydrogenase deficiency leading to thymine-uraciluria. An inborn error of pyrimidine metabolism. *Clin Chim Acta* **141:** 227–234, 1984.

9. Piper AA, Tattersal MHN and Fox RM, The activity of thymine metabolizing enzymes during the cell cycle of a human lymphocyte cell line LA7-007 synchronized by centrifugal elutriation. *Biochim Biophys Acta* **633:** 400–409, 1980.
10. Lu ZH, Zhang R and Diasio R, Dihydropyrimidine dehydrogenase activity in human peripheral blood mononuclear cells and liver: Population characteristics, newly identified deficient patients, and clinical implications in 5-fluorouracil chemotherapy. *Cancer Res* **53:** 5433–5438, 1993.
11. Wasternack C, Degradation of pyrimidines—Enzymes, localization and the role of metabolism. *Biochem Physiol Pflanz* **173:** 371–403, 1978.
12. Naguib FN, el Kouni MH and Cha S, Enzymes of uracil catabolism in normal and neoplastic human tissues. *Cancer Res* **45:** 5405–5412, 1985.
13. Podschun B, Cook PF and Schnackerz KD, Kinetic mechanism of dihydropyrimidine dehydrogenase from pig liver. *J Biol Chem* **265:** 12966–12972, 1990.
14. Fleming RA, Milano G, Thyss A, Etienne MC, Renee N, Schneider M and Demard F, Correlation between dihydropyrimidine dehydrogenase activity in peripheral mononuclear cells and systemic clearance of fluorouracil in cancer patients. *Cancer Res* **52:** 2899–2902, 1992.
15. Laemmli UK, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680–685, 1970.
16. Yokota H, Fernandez-Salguero P, Furuya H, Lin K, McBride OW, Podschun B, Schnackerz KD and Gonzalez FJ, cDNA cloning and chromosome mapping of human dihydropyrimidine dehydrogenase, an enzyme associated with 5-fluorouracil toxicity and congenital thymine uracilurea. *J Biol Chem* **269:** 23192–23196, 1994.
17. Traut T and Loechel S, Pyrimidine catabolism: Individual characterization of the three sequential enzymes with a new assay. *Biochemistry* **23:** 2533–2539, 1984.
18. Podschun B, Wahler G and Schnackerz KD, Purification and characterization of dihydropyrimidine dehydrogenase from pig liver. *Eur J Biochem* **185:** 219–224, 1989.
19. Lu ZH, Zhang R and Diasio RB, Purification and characterization of dihydropyrimidine dehydrogenase from human liver. *J Biol Chem* **267:** 17102–17109, 1992.