pH 6.5. The optimal temperature was about 40 °C. Native DNA, denatured DNA, and RNA are digested by the pure enzyme. The highest activity, twice as high as with native DNA, was detected with denatured DNA. Using closed circular PM 2 DNA, a time dependent increase of the fluorescence in the presence of ethidium bromide during the action of the serum nuclease can be monitored (fig. 3). According to Paoletti et al.[19] this is due to a single endonucleolytic scission in the DNA molecule opening the closed circular DNA. From these data an endonucleolytic action of the enzyme seems probable, but the kinetics observed are nonlinear, i.e. the rate of the logarithmic change of fluorescence decreases with time (inset fig. 3). This deviation from linearity could be the result of a change in the initial velocity of the reaction or a parallel decrease in fluorescence due, for example, to concomitant exonuclease action. From the data available this cannot be decided yet.

1 Acknowledgment. This work has been supported by a research grant from Stiftung Volkswagenwerk through the Akademie der Wissenschaften und der Literatur, Mainz. We thank Mrs R. Nehrbass and Mrs C. Wolpert for technical assistance.

2 Present address: G.S., Hoechst AG, D-6230 Frankfurt/Main 80 (FRG).

3 K. Tsukada, Biochim. biophys. Acta *186*, 21 (1969).
4 M.C. Lechner, Biochem. Pharmac. *21*, 2263 (1972).
5 M.S. Duque Magalhaes and M.C. Lechner, Int. J. Biochem. *10*, 55 (1979).
6 N.B. Kurnick, Archs Biochem. *43*, 97 (1953).
7 V.M. Doctor, Int. J. Biochem. *3*, 503 (1972).
8 M. Anai, H. Haraguchi and Y. Takagi, J. biol. Chem. *247*, 193 (1972).
9 E.J. Zöllner, B. Heicke and R.K. Zahn, Analyt. Biochem. *58*, 71 (1974).
10 E.J. Zöllner, B. Heicke and R.K. Zahn, Z. klin. Chem. klin. Biochem. *12*, 459 (1974).
11 R. Hulhoven, J.P. Desager and A. Zenebergh, Bull. Soc. chim. Belg. *87*, 499 (1978).
12 J.D. Love and R.R. Hewi, J. biol. Chem. *254*, 12588 (1979).
13 S. Gupty and R.M. Herriott, Archs Biochem. Biophys. *101*, 88 (1963).
14 G.P. Frost and P.J. Lachmann, Clin. exp. Immun. *3*, 447 (1968).
15 F. Gavosto, F. Buffa and G. Maraini, Clin. chim. Acta *4*, 192 (1959).
16 R.K. Zahn, E. Tiesler, A.K. Kleinschmidt and D. Lang, Biochem. Z. *336*, 281 (1962).
17 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.S. Randall, J. biol. Chem. *193*, 265 (1952).
18 N.K. Laemmli, Nature *227*, 680 (1970).
19 C. Paoletti, J.B. Le Pecq and J.R. Lehman, J. molec. Biol. *55*, 75 (1971).

Galactose-l-phosphate uridyl transferase activity in red cells of various animal species

Sally Kelly, P. Sweeney and L. Schedlbauer

Birth Defects Institute, Division of Laboratories and Research, New York State Department of Health, Albany (New York 12201, USA), 13 August 1980

Summary. Monkey red cells were chosen as controls in tests of human red cell galactose-l-phosphate uridyl transferase, after comparing activities and isozyme patterns of the enzyme from several domestic or laboratory-bred species.

In order to estimate the day-to-day variability in an assay for galactose-l-phosphate uridyl transferase in human red cells[1], we include a 'standard' sample-either fresh cells from the same daily donor or aliquots from a sample of frozen packed cells. Monitoring the between-test reproducibility is important when the assay is used to define the transferase phenotype, e.g., to distinguish carriers of galactosemia from noncarriers. Daily donation is inconvenient, however, and the stability of the enzyme in packed cells is questionable. We therefore considered using animal red cells for the standard and assessed them as a replacement as described below.

We required a transferase with activity similar to that in human red cells, for which the assay was designed. Transferase activities have already been measured in several species, including rats[2-4], hamsters[3], rabbits[3], kangaroos and other marsupials[5-7], cats[8], sea lions[9], monkeys[3,10] and mice[11,12]. Although activities were measured by different methods, and comparisons were difficult, they appeared species-specific, with higher values when the young were tested[3-4].

In our search for a compatible source, we selected mature animals of species which were readily available. We also tested the stability of transferase in stored samples from species with the desired levels of activity.

Materials and methods. Blood was drawn from mature animals of the following 8 species (table 1) bred or maintained at the Department's laboratory farm: Hartley guinea-pigs, Wistar rats, Nys: (FG) rabbits, retired riding horses, Suffolk sheep, Holstein cows, green monkeys and mixed breed goats. A few comparisons were made with young of the species, including a newborn lamb, a newborn calf, and a 2-month-old kid.

Samples were drawn by cardiac puncture from the monkeys, rats and guinea-pigs, with the rats and guinea-pigs having been immobilized by exposure to carbon dioxide, and the monkeys immobilized by intramuscular injection of phencyclidine hydrochloride (0.7 ml of a 20 mg/ml solution). Rabbits were bled from the marginal ear vein, and the sheep, cows, horses, and goats were bled by jugular puncture.

Cells were hemolyzed by freezing and thawing 0.5 ml aliquots of the blood samples 3 times in a Dry Ice-ethanol bath. 'Ghosts' were separated by centrifugation at $1400 \times g$ for 5 min.

The reaction mixture was combined in the following order just before use: 0.6 ml of 10 mM uridine-5'-diphosphoglucose (UDPG); 0.6 ml of 27 mM galactose-l-PO_4; 0.8 ml of freshly prepared 6 mM nicotinamide adenine dinucleotide phosphate (NADP); 2.0 ml of freshly prepared 0.75 M Tris-acetate buffer, pH 8.0; 0.8 ml of saturated digitonin; 0.09 ml of 27 mM disodium ethylene diaminetetraacetic acid; 0.13 ml of 0.1 M magnesium chloride; and 1.0 ml of demineralized water. The total volume was 6.0 ml (enough for 9 samples). The stock solutions were made earlier, except as noted, and freezing at −20 °C for as long as 6 weeks did not affect their stability.

The reaction mixture was divided into 5 0.2 ml portions which were warmed at 37 °C for 3-5 min. At 30-sec intervals, 20 µl of saline (0.85%) or hemolysate was added

to each tube in the following order: saline, hemolysate (3 tubes), saline. Exactly 30 min later the reactions in 50-µl aliquots were stopped in 10 ml of 0.01 M phosphate buffer, pH 7.4.

Fluorescence was measured in a Turner 110 double beam fluorometer with a Corning 7-60 primary filter and a Turner 2-A secondary filter, calibrated with a 1.34 µM solution of quinine sulfate in 0.01 M sulfuric acid and the initial blank as reference. Hemoglobin was measured at 410 nm in a Coleman, Jr, II Spectrophotometer, Model 6/20, calibrated with a didymium calibrating standard (Coleman No. C006-0-400).

Activity was expressed in µmoles UDPG consumed per h per g of hemoglobin (U) with $U = FC/_A$, where F is the fluorescence, A the absorbance at 410 nm and C the combined calibration factor for the 2 instruments[13].

C is obtained as $\frac{10 \times A_1}{F_1}$,

when F_1 is the fluorescence constant obtained with a solution of reduced nicotinamide adenine dinucleotide phosphate of known strength (determined spectrophotometrically), and A_1 is the absorbance constant obtained with a solution of hemoglobin standardized with cyanmethemoglobin.

Transferase activities of the various species were compared with those in human red cells in concurrent tests of blood from healthy laboratory workers (table 1) and also with published values[14].

The stability at −70 °C was tested in species with transferase activities similar to that of human red cells. Enzyme in whole blood (0.5 ml aliquots) and packed red cells (centrifuged at 1400 × g for 5 min and washed 3 times with 0.85% sodium chloride) was tested initially as described above and thereafter at intervals of up to 30 days.

Transferase isozymes from the various species were separated on vertical starch gels by Weitkamp's[15] method.

The effect of age on transferase activity was tested in a few representative young, as described above, and, in the human species, by comparing the levels found, during an earlier study of galactosemia phenotypes, in healthy children and adults.

Results. Mean values of red cell transferase activities in the various species ranged from 4 to 86 µm UDPG consumed/h/g Hgb (U) (table 1). Rabbit and monkey transferases closely resembled that of man, while guinea-pig and rat transferases were more active, and those of cow, horse and goat were less.

There was greater variability in the tests of rat transferase than in other species (table 1) - perhaps because the rat hemolysates appeared to form colloidal solutions. Although the method for rat transferase was modified by an extra centrifugation and the solutions cleared, reproducibility was only slightly improved.

Red cells of the 3 species tested (rabbit, monkey and man's) retained their transferase activity while stored at −70 °C (table 2). Initial activities in the packed cell preparations were lower, however, than in the corresponding whole blood sample and lessened their value as 'standards'. We limited our observations of stability, therefore, to whole blood enzyme.

The isozyme patterns of transferase from the various species were distinctive. While all the transferases migrated anodally, they did so at different rates and formed variable numbers of bands. Transferase from several species moved faster than the human enzyme and rabbit transferase moved more slowly. Enzyme from horse, cow, rabbit and rat formed 2-banded patterns, like the human red cell transferase; guinea-pig and kid patterns had 3 bands, and sheep, lamb and monkey patterns, 4 bands (fig.).

Transferase activity was greater in the young of some species. It was clearly greater (4 times) in the calf (18 vs 4 U), and 1½ times greater in the lamb (23 vs 15 U). Activity in the kid (10 U) was not different from those of mature animals. Nor were mean activities in 12 healthy infants and children under 7 years old (26 U) and 16 healthy adults

Table 1. Transferase activity in fresh whole blood from various species

Species	Age	n	Transferase activity ($\bar{x} \pm s$) (µm UDPG consumed/h/g Hgb)
Guinea-pig (male)*	6-8 weeks	10	39.8 ± 6
Rat (male)	14-16 weeks	10	71.5 ± 20
Rabbit (female)	4 months	10	29.0 ± 3
Horse	12-22 years	5	11.8 ± 2
Sheep (male castrated)	4-6 years	10	15.1 ± 3
Cow (non-lactating)	6-8 years	5	4.2 ± 1.9
Monkey	2-3 years	10	28.7 ± 4
Goat	2-6 years	5	12.0 ± 4
Man	22-45 years	10	25.7 ± 2**

* Immature. All other animals were adults. ** Normal range by this method in our laboratory: 19-29 µm UDPG consumed/h/g Hgb.

Table 2. Transferase activity in whole blood stored at −70°C

Species	Day	Transferase activity (µm UDPG consumed/h/g Hgb)		
		a	b	c
Rabbit (3)	0	25	30	31
	7	21	24	29
	21	21	25	29
Monkey (3)	0	35	39	26
	7	36	43	23
	14	-	33	26
	28	33	33	-
Man (3)	0	27	28	25
	7	29	27	23
	14	28	25	22

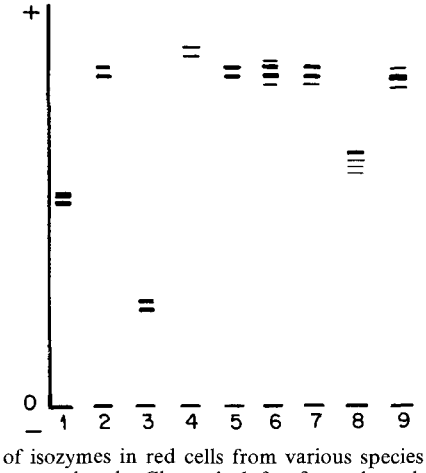

Diagram of isozymes in red cells from various species by electrophoresis on starch gels. Channels 1-9 refer to hemolysates from, respectively, man, guinea-pig, rabbit, horse, cow, sheep, rat, monkey, goat.

(23 U) significantly different, when compared at the 5% confidence level ($t < 2.06$, when $n = 26$).

Discussion. We chose red cells from monkeys as a source of 'standard' transferase for Beutler and Mitchell's[1] assay of human red cell transferase. The blood samples were readily available to us; the enzyme was approximately as active as human red cell transferase and appeared stable when cells were frozen.

The diversity of electrophoretic patterns indicates that the molecular structure of the various species' transferase protein is heterogeneous. We inferred from the different numbers of isozymes displayed that polymer structures were dimeric in some species and either trimeric or indeterminate in others[16].

Similarity of total enzyme activity among species, despite apparent structural heterogeneity, is characteristic of isozyme systems[16]. Since total activity was the parameter we sought, this feature of enzymes further validates our choice of nonhuman cells as a source of 'standard' transferase in the quantitative assay.

1 E. Beutler and M. Mitchell, J. Lab. clin. Med. *72*, 527 (1968).
2 J.H. Copenhaver, J.F. Fitzgibbons and M.J. Carver, Life Sci. *9*, 617 (1970).
3 E.L. Tallman, Physiol. Chem. Physics *1*, 131 (1969).
4 S. Kelly and L. Desjardins, Proc. Soc. exp. Biol. Med. *138*, 545 (1971).
5 T. Stephens, S. Irvine, P. Mutton, J.D. Gupta and J.D. Harley, Nature *248*, 524 (1974).
6 T. Stephens, C. Crollini, P. Mutton, J.D. Gupta and J.D. Harley, Aust. J. exp. Biol. med. Sci. *53*, 233 (1975).
7 B.J. Richardson, B. Inglis, W.E. Poole and B. Rolfe, Aust. J. exp. Biol. med. Sci. *57*, 43 (1979).
8 B.E. Rabinow, P.W.K. Wong, E.R. Maschgan and S. Natelson, Clin. Chem. *22*, 2010 (1976).
9 C.K. Mathai, M.E.Q. Pilson and E. Beutler, Proc. Soc. exp. Biol. Med. *123*, 603 (1966).
10 S. Bissbort, H. Ritter and J. Schmitt, Humangenetik *26*, 139 (1975).
11 G. Bulfield, E.A. Moore and H. Kacser, Genetics *89*, 551 (1978).
12 S. Rogers, S. Kirsch and S. Segal, Life Sci. *24*, 2159 (1979).
13 E. Beutler, Red Cell Metabolism, p.84. Grune and Stratton, New York 1971.
14 E. Beutler, M. Baluda, P. Sturgeon and R. Day, Lancet *1*, 353 (1965).
15 L.R. Weitkamp, personal communication (1978).
16 C.L. Markert, in: Isozymes, Current Topics in Biological and Medical Research, vol. 1, p.6. Ed. M.C. Rattazzi, J.G. Scandalios and G.S. Whitt. Alan R. Liss, Inc., New York 1977.

Ammonia assimilatory enzymes in a nif^{-III} mutant of *Azotobacter chroococcum*

S. Sadasivam and G. Gowri

Department of Biochemistry, Tamil Nadu Agricultural University, Coimbatore-641 003 (India), 21 October 1980

Summary. The levels of ammonia assimilatory enzymes have been studied in a nif^{-III}mutant and a wild strain of *Azotobacter chroococcum* grown in media containing different nitrogen sources. It is suggested that the nif^{-III}mutant may be defective in transport or incorporation of molybdenum.

Studies on nif$^-$ mutants in nitrogen fixing organisms have thrown light on the mechanism and control of biological nitrogen fixation[1,2]. We report here a hitherto nondocumented nif^{-III}mutant of *Azotobacter chroococcum*, possibly defective in the transport or incorporation of molybdenum.

Materials and methods. Azotobacter chroococcum isolated from the root zone of *Cyprus rotandus*, CYP3, obtained from the Advanced Centre for Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, was used as the wild type. This strain was grown in Burk medium[3], and the cells were collected at the exponential phase (56 h), and resuspended in 0.2 M phosphate buffer (pH 7.0) to a final concentration of 10^7 cells/ml. The cell suspension (5 ml) was treated with 300 µg/ml of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), and mutagenesis was carried out according to Adelberg et al.[4]. 4 mutants (nif^{-I}-nif^{-IV}) were identified by scoring out the colonies grown on agar plates with ammonium acetate. *Azotobacter* strains were maintained in Burk agar medium plus 1.1 mg/ml ammonium acetate for the nif$^-$strains or without ammonium for the wild strain[5].

The wild and the nif^{-III} strains were grown in 100 ml of Burk liquid medium in 250 ml Erlenmeyer flasks and also in media supplied with either ammonium acetate, gluta-

Table 1. Nitrogenase and ammonia assimilatory enzymes in wild and nif^{-III} mutant of *A. chroococcum* grown on different nitrogen sources

Nitrogen	No nitrogen		Ammonium acetate		Glutamate		Glutamine		Aspartate	
Enzymes	wild	nif^{-III}	wild	nif^{-III}	wild	nif^{-III}	wild	nif^{-III}	wild	nif^{-III}
Nitrogenase[a]	70.40	No growth	8.20	Not detected	4.24	Not detected	Traces	Not detected	1.50	Not detected
GS[b]	1000	No growth	360	100	300	60	200	90	300	50
GOGAT[c]	300	No growth	110	130	130	120	70	40	250	10
GDH[c]	Not detected	No growth	180	120	70	20	20	20	20	20
GOT[d]	80	No growth	90	160	130	80	150	150	140	100

[a] nmoles of C_2H_4 produced/cell/h; [b] nmoles of γ-glutamyl hydroxamate formed/min/mg protein; [c] nmoles of NADPH oxidized/min/mg protein; [d] nmoles of pyruvate formed/min/mg protein.