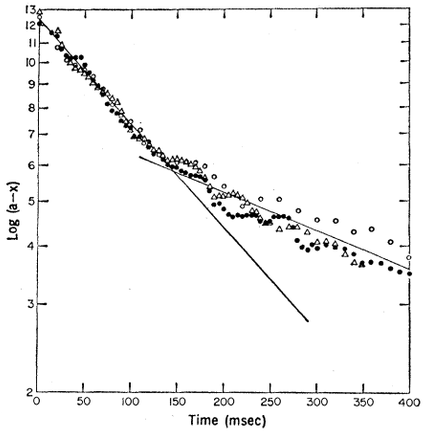

Fig. 3. First-order kinetic plot of the renaturation of acidified nuclease from *p*H 3.20 to *p*H 6.70. The value of log $(a - x)$ is plotted on the ordinate where a is the total increase in fluorescence from the beginning to the observed end of the reaction and x is the increase in fluorescence at any time.

and a half divisions on the ordinate above the base line.

The kinetics of this reaction have been analyzed by first-order plots, with the fluorescence of the acidified nuclease as the value for zero time and the final recorded value as that for infinite time. The first-order plots of three experiments at a final concentration of protein of $6 \times 10^{-5} M$ fit one line for about the first 150 msec of the reaction (Fig. 3). Subsequently, when the spectral change is more than 75 percent completed, they follow a different line. After we subtract the contribution of the second slope to the first, these lines are equivalent to processes with first-order rate constants (k_1) of 12.1 and 1.9 sec^{-1}, respectively.

This reaction has been studied at protein concentrations from 1.5 to $6.0 \times 10^{-5} M$. There was no significant change in the initial first-order rate constant.

The folding of nuclease from the extensively disrupted acidified state to the native form apparently can occur very rapidly. The kinetic analysis, by the simplest although not the only possible method, is compatible with an initial, very rapid folding, with a half time of about 55 msec (k_1 of 12.1 sec^{-1}), which is accompanied by a large change in tryptophan emission. This is followed by at least one slower conformational change, with a half time of about 350 msec (k_1 of 1.9 sec^{-1}), which causes further change in the environment of the tryptophan residue.

Most estimates of renaturation rates have been established only indirectly from the assumption of a two-state transition whose apparent equilibrium constant is the quotient of the renaturation and denaturation rate constants (*11*). However, Hauschka and Harrington (*12*) have recently directly measured the renaturation of *Ascaris* collagen, at *p*H 6.25, by optical rotation and have interpreted their data at 25°C in terms of three first-order processes, with half times of about 0.20, 2.5, and 11 minutes. Pohl (*13*) has used temperature-jump relaxation methods in the study of the reversible denaturation of chymotrypsin and trypsin. The half time values near 25°C of the renaturation of these enzymes are between about 7 and 70 seconds. The renaturation rates of staphylococcal nuclease appear significantly faster than the rates of conformational change in these proteins and faster than those in the several proteins whose renaturation rates have been estimated indirectly (*1*). The need for more than one first-order rate constant to fit our data indicates the existence of kinetic intermediates in the conformational changes of nuclease.

The rate of renaturation of nuclease is slower by several orders of magnitude than those established by temperature-jump methods for the conformational isomerizations of enzymes during their catalytic function, and many orders slower than those values established for the helix-coil transformation (*14*). These results for the renaturation of nuclease give an approximate idea of the time required for this polypeptide chain of 149 amino acids to assume a large enough subset of all possible backbone and side-chain dihedral angles so that the presumably statistical process of folding reaches the thermodynamically stable, native conformation.

The measurement of the renaturation kinetics of several spectral and hydrodynamic properties of nuclease, in addition to tryptophan fluorescence, is necessary for an understanding of the sequence of processes in folding. The correlation of these results with the structural information in the 2-Å model of nuclease offers an approach to an understanding of the mechanism of folding of this protein.

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Substrate Stabilization: Genetically Controlled Reciprocal Relationship of Two Human Enzymes

Abstract. *5-Phosphoribosyl-1-pyrophosphate, a substrate shared by adenine phosphoribosyltransferase and hypoxanthine-guanine phosphoribosyltransferase, accumulates in human erythrocytes lacking hypoxanthine-guanine phosphoribosyltransferase. 5-Phosphoribosyl-1-pyrophosphate added to purified adenine phosphoribosyltransferase stabilizes it against heat inactivation. The increased activity of adenine phosphoribosyltransferase seen in erythrocytes deficient in hypoxanthine-guanine phosphoribosyltransferase may result from substrate stabilization of this enzyme in vivo.*

The regulation of enzyme activity in mammalian cells is often discussed in terms of the mechanisms of induction,

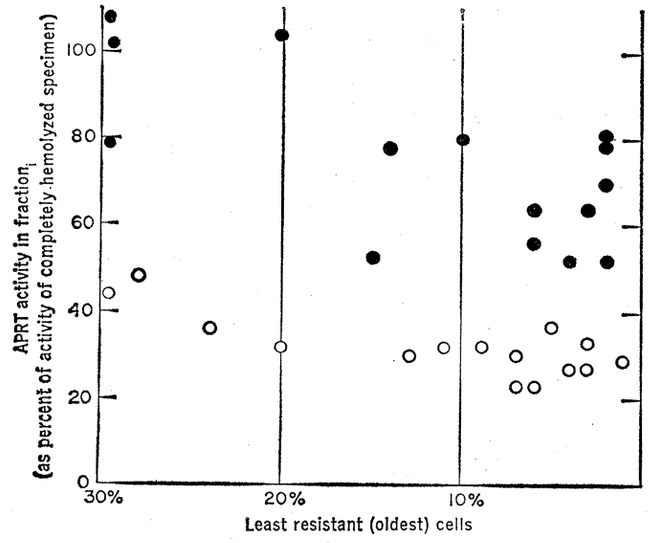

Fig. 1. Erythrocytes of heparinized blood were washed three times with two volumes of 0.154*M* KCl at 4°C. Differential osmotic lysis of the red cells was then carried out (*10*) to yield preparations of the oldest cells (those cells least resistant to hemolysis), and a hemolyzate of the entire red cell population was used as a control for each specimen. Hemolyzates were freed of cellular debris by centrifugation at 2000*g*, and the supernatant solutions were dialyzed against 0.001*M* tris buffer, *p*H 7.4, containing 0.001*M* $MgCl_2$. The APRT activity was assayed as described (*3*) and specific activity of this enzyme was expressed as nanomoles per milligram of protein per hour. The percentage of cells hemolyzed was determined by measurements of hemoglobin concentration in supernatants by the cyanmethemoglobin method before dialysis, and protein was determined by the Lowry method (*11*). ○, Normal erythrocytes; ●, erythrocytes lacking HGPRT.

repression, or derepression of genes controlling protein synthesis demonstrated in bacterial systems. We herein present evidence that a simpler mechanism, substrate stabilization, may modify the amount of enzyme activity in mammalian cells.

In man X-linked uric aciduria (Lesch-Nyhan syndrome and its variants) is associated with excessive purine synthesis (*1*) and with varying degrees of deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (E.C.2.4.2.8) (HGPRT) in erythrocytes and other tissues (*2*, *3*). In erythrocytes from patients with these disorders, the specific activity of a different enzyme, adenine phosphoribosyltransferase (E.C.2.4.2.7) (APRT) may be increased as much as 2.5 times (*4*). These phosphoribosyltransferase enzymes convert the free purine bases hypoxanthine, guanine, and adenine to their respective mononucleotides by condensing with 5-phosphoribosyl-1-pyrophosphate (PRPP). While PRPP is a shared substrate for both enzymes, they maintain a strict specificity for their purine bases.

In normal erythrocytes, the specific activity of APRT declines markedly as the cells age (*5*). In erythrocytes lacking HGPRT, the decline in APRT activity with ageing of the cells was much less marked, with the oldest cells still retaining substantial activity of APRT. In these erythrocytes lacking HGPRT, we have found increased concentrations of free PRPP, presumably reflecting diminished utilization of this compound. The APRT, purified from normal human erythrocytes, is protected by PRPP from heat inactivation. We propose that in the circulating erythrocyte which lacks HGPRT, this elevated concentration of PRPP may result in prolongation of the half-life of ARPT.

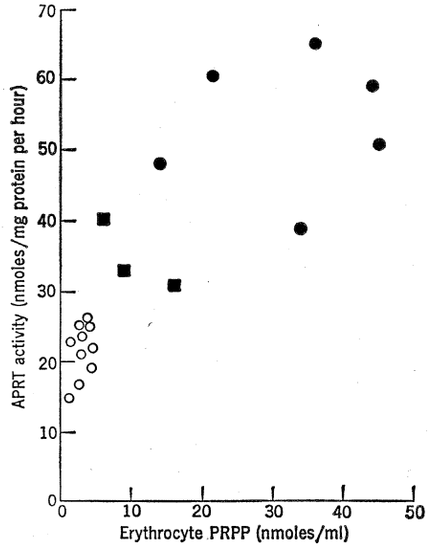

Fig. 2. The erythrocytes were washed in Krebs-Ringer buffer, *p*H 7.4, at 4°C and adjusted to a hematocrit of 2 percent. The PRPP concentration was then determined by a modification (*12*) of the method of Henderson and Khoo (*13*). Elevated PRPP concentration correlated well with elevated activity of APRT in these cells (correlation coefficient $r = 0.83$, standard error = 0.24). ○, Normal erythrocytes; ■, erythrocytes with partial deficiency of HGPRT; ●, erythrocytes with complete deficiency of HGPRT.

Since the erythrocytes lacking HGPRT had higher activity of APRT in the whole erythrocyte population than did the normal cells, results (Fig. 1) are expressed as percentage of the value found in the whole erythrocyte population of each subject. The relative specific activity of APRT was greatly reduced in the older cells of normal subjects, compared with the values obtained in the older cells from patients deficient in HGPRT.

Another erythrocyte enzyme which is known to decrease in activity with aging of the cell, glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49), was assayed as a control enzyme (*6*). The activity of this enzyme was decreased to the same degree in comparable hemolysates from both groups.

In a separate study, the intracellular PRPP concentrations of erythrocytes from normals and from subjects with HGPRT deficiency were assayed. As shown in Fig. 2, concentrations of PRPP were markedly elevated in erythrocytes lacking HGPRT, and this elevation correlated well with the elevated activities of APRT in these cells. (The correlation coefficient $r = 0.83$, standard error 0.24).

The APRT from normal human erythrocytes was purified to yield a homogeneous protein, as judged by electrophoresis on a polyacrylamide-gel and analytical ultracentrifugation (*7*). The stabilization of this purified enzyme to heat (55°C) inactivation by various substances was studied. Enzyme was incubated at 55°C in tris buffer (0.1*M*, *p*H 7.4) containing 0.005*M* $MgCl_2$ with additions of either water, 50 μM PRPP, 40 μM adenine, or 40 μM adenosine 5′-triphosphate. Portions were removed at intervals and pipetted directly into tubes containing substrates for assay of APRT (*2*) at 38°C. Results were expressed as percent of activity of control tubes containing similar additions which were not heated. After 1 minute of incubation at 55°C, the enzyme activity was unaltered from the original activity in the tubes containing 50 μM PRPP, whereas in the tubes containing other substances, activity ranged from 48 to 67 percent of control values at this time. After 4 minutes of heating at 55°C, enzyme activity was 75 percent of the control value in the tube containing PRPP but was 9 to 10 percent of control in the other tubes. After 8 minutes, 56 percent of the original activity remained with PRPP whereas with the other substances studied, values were all less than 8 percent of control values. Thus PRPP at a concentration of 50

μmole/liter protected against heat inactivation; adenine, adenosine-5′-triphosphate, and several other compounds had no effect. We have also observed that PRPP prevents loss of activity of purified APRT stored at −10°C.

Although the mechanism whereby PRPP stabilizes the adenine phosphoribosyltransferase molecule has not yet been determined, the situation may be analogous to the stabilization of rat liver tryptophan pyrrolase by its substrate, tryptophan (*8*). 2,3-Diphosphoglycerate, which alters the affinity of oxygen for deoxyhemoglobin, is present in increased amounts in erythrocytes from hypoxic individuals (*9*). Our findings provide a second example of a small, phosphorylated molecule effecting a change in the properties of an erythrocyte protein. In addition, we believe that the data are consistent with the concept that genetic abnormalities affecting one enzyme may, through accumulation of substrate, result in stabilization of a different enzyme. Although such a mechanism apparently influences the activity of APRT enzyme in mammalian cells, this conclusion does not imply that it is the only mechanism operable.

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Glucose Production by Lamprey Meninges

Abstract. *Meningeal tissue of the lamprey larva produces glucose during incubation and contains glucose-6-phosphatase and large amounts of glycogen. Little glucose is liberated by brains from which meninges have been removed. This primitive meningeal tissue may produce glucose for use by the brain during periods of metabolic stress.*

The arachnoid portion of lamprey meninges appears to be more active metabolically than its counterpart in higher vertebrates. It is supplied with capillaries and includes columns of round cells which contain lipid droplets and considerable glycogen (*1*, *2*). Lamprey brain with intact meninges produces free glucose both *in situ* and in vitro through the breakdown of glycogen and the probable action of glucose-6-phosphatase (E.C. 3.1.3.9) (*3*). This report shows that the meninges rather than the nervous tissue is the major site of glucose production.

Larval sea lampreys (*Petromyzon marinus* L.) were anesthetized in tricane (*4*) and decapitated, and the dorsal portion of the braincase was removed under cooled Ringer fluid. The meningeal (*5*) tissue exposed at this point has a soft consistency and contains many melanocytes. It adheres to most of the external surface of the brain but is thickest lateral to the midbrain. This meningeal tissue was teased from the brain with fine forceps and incubated (*6*) or prepared for chemical analysis separately from the remaining nervous tissue.

Tissues were either directly homogenized in iced 0.03*N* HCl or frozen, weighed, and extracted by the procedure used previously (*3*). Glycogen (*7*), glucose, and glucose-6-phosphate (*8*) were measured fluorometrically through enzymatic production of reduced nicotinamide-adenine dinucleotide phosphate. Protein was measured spectrophotometrically and was used to calculate weights of directly homogenized tissues (*9*). Glucose-6-phosphatase activity is expressed as the amount of inorganic phosphate liberated by crude tissue homogenates incubated in 10 m*M* glucose-6-phosphatase at *p*H 6.9 (*10*); while maltase (α-1,4-glucosidase) (E.C. 3.2.1.20) activity was measured as the amount of glucose produced from 2 m*M* maltose in modified enzymatic glucose reagent, *p*H 7 (*11*).

Figure 1 illustrates the relative distribution of glycogen in brain and meninges as revealed by PAS (periodic acid–Schiff) staining (*12*). Meningeal cells stained dark maroon, indicating dense glycogen deposits; nervous tissue stained pink except for large neurons, which stained red. The results of the histochemical staining were confirmed by chemical measurement of glycogen in isolated meninges and the brains from which they were removed (Table 1). Values for cleaned brains approximate the highest concentrations found in brains of other cold-blooded vertebrates (*13*), while meningeal values were fourfold higher, glycogen constituting about 2 percent of the wet weight of the tissue. Glycogen contents might be higher under more favorable experimental conditions in view of the occasional samples of whole brain that showed values greater than 100 mmole of glycosyl units per kilogram (1.6 percent of wet weight). In another species of lamprey (*Lampetra fluviatilis* L.) a maximum content of glycogen in the brain of 3.7 percent has been found (*13*).

About 90 percent of the glucose produced by lamprey brain can be accounted for by the quantity released by meningeal tissue during incubation. The small amounts released by cleaned brain can be attributed to the few remaining meningeal cells and the glucose normally liberated at glycogen branch points by debranching enzyme.

Table 1. Glycogen concentration, glucose production (19° and 26°C), and glucose-6-phosphatase activity (21°C) in lamprey brain and meninges. The results are expressed as the mean ± S.D.; the number of determinations is in parentheses.

Wet weight (mg)	Glycogen (mmole of glycosyl units/kg)	Glucose production (mmole kg^{-1} hr^{-1})	Glucose-6-phosphatase (mmole kg^{-1} hr^{-1})
		Whole brain	
4.7 ± 1.0(38)	81 ± 27(24)	20 ± 2*(5)	50*
		Cleaned brain	
3.4 ± 0.7(17)	27 ± 8(11)	1.8 ± 0.7(4)	14, 17 (2)
		Meninges	
0.8 ± 0.4(18)	137 ± 33(10)	107 ± 26(4)	204 ± 56(3)

* Values previously reported (*3*).