Mutarotase in Erythrocytes: Isolation and Properties

Abstract. *Mutarotase was found in lysed human erythrocytes and in hemoglobin. The enzyme was partially purified by treatment with ethanol and chloroform at −15°C. It was nondialyzable and heat sensitive, and was inhibited by* D-*galactose,* L-*arabinose,* D-*ribose,* D-*xylose, and* D-*arabinose.*

Mutarotase, which catalyzes the interconversion of anomeric forms of glucose, was first found by Keilin and Hartree (*1*) in preparations of glucose oxidase. A similar enzyme has been identified in mammalian tissues (*2*), in higher plants (*3*), and in the bacterium *Escherichia coli* (*4*). However, so far there has been no evidence of the existence of mutarotase in erythrocytes (*5, 6*).

In my investigation of cerebral metabolism in mental disease (*7*), certain unusual results in some earlier experiments (*8*) prompted an attempt to develop a procedure for measuring α- and β-glucose in whole blood. The work that followed indicated that lysed human erythrocytes had mutarotase activity and that commercial preparations of hemoglobin also exhibited such action. This report deals with the separation of mutarotase from hemoglobin and with the identification and some of the properties of this enzyme.

Two methods were employed for the measurement of enzyme activity—an enzymatic method and a polarimetric one. For the assay of mutarotase activity in highly colored solutions, such as lysed erythrocytes or hemoglobin solutions, a method was evolved that made use of a coupled-enzyme system (glucose oxidase, peroxidase, and chromogen) (*9*). In the enzymatic method, the solution containing mutarotase was diluted 1:1 with freshly dissolved α-glucose (anhydrous, Calbiochem) in ethylenediaminetetraacetate (EDTA) buffer (0.01*M*, *p*H 7.0) and put into a dialysis sac. After the proper incubation time, the sac was put into a tube containing 10 ml of the coupled-enzyme reagent (*9*). The tube was shaken rapidly for 10 minutes, the reaction was then stopped by adding 2 drops of 4*M* HCl, and the optical densities were determined with a Beckman DU spectrophotometer at 400 mμ. Spontaneous mutarotation was measured by substituting EDTA buffer for the enzyme solution and proceeding as above. A linear relation was found between the amounts of β-glucose put into the sac (as equilibrium glucose) and the optical densities. Under the empirical conditions used in this method, it was established that, in the concentration range used, about 36 percent of the glucose in the sac dialyzed out into the coupled-enzyme reagent and that this method assayed β-glucose almost exclusively.

In the polarimetric method, the powdered enzyme was dissolved in EDTA buffer which was then used to dissolve the freshly weighed α-glucose. Either boiled enzyme solution (in EDTA buffer) or plain EDTA buffer was used to dissolve the α-glucose in measuring spontaneous mutarotation.

The separation of mutarotase from hemoglobin was accomplished as follows. A solution of hemoglobin was cooled to −15°C, and then cold 96 percent ethanol and cold chloroform were added slowly with stirring. After centrifugation, the alcohol layer was removed and dialyzed. The precipitated hemoglobin was extracted with cold 0.9 percent NaCl, and the saline extract was dialyzed. Both extracts were combined and lyophilized, yielding an ivory-colored powder that was about 35 percent protein.

When increasing amounts of this mutarotase preparation were incubated with the same concentration of α-glucose, the data showed a linear relation between enzyme activity and concentration by both methods of analysis. The mutarotase constant, k_e, was found by using the formula $k_e = 0.693\ t_{1/2}$ (where $t_{1/2}$ is the half-time of the reaction), and the contribution of the enzyme to the conversion was determined by subtracting k_s (spontaneous) from k_e, according to Keston (*10*).

The effect of concentration of α-glucose on the action of equine hemoglobin mutarotase is shown in Fig. 1, in which the results of the polarimetric method are shown. With this method the activity is expressed as $(\alpha_t - \alpha_e) \times 100$, where α_t represents optical rotation at time t, and α_e, optical rotation at equilibrium. Values for equilibrium were taken at periods at least ten times the reaction half-time and were checked the next day in many cases. With the use of these data, Lineweaver-Burk plots were constructed, and the Michaelis-Menten constant for this mutarotase was determined as 0.072*M*. Similar studies with the dialysis–coupled enzyme method gave a value of 0.050*M*. Thus, an average value of 0.061*M* for the K_m of this equine hemoglobin mutarotase was found.

The partially purified mutarotase, which had about ten times the enzyme activity as the original equine hemoglobin on a basis of weight, was tested with the dialysis–coupled enzyme method for inactivation by heat. Solutions of the enzyme were treated as described in Table 1, quickly cooled to 25°C, and diluted 1:1 with freshly dissolved α-glucose in EDTA buffer. The percentage

Table 1. The inactivation of partially purified equine hemoglobin mutarotase by heat as measured by the dialysis–coupled enzyme method (see text). The value for the untreated sample is given ± the standard error.

Treatment	$t_{1/2}$ (min)	Inactivation (%)
Untreated	12.9 ± .19	
42°C, 10 minutes	14.7	27
50°C, 10 minutes	17.2	56
55°C, 10 minutes	21.7	92
100°C, 30 minutes	23.1	100

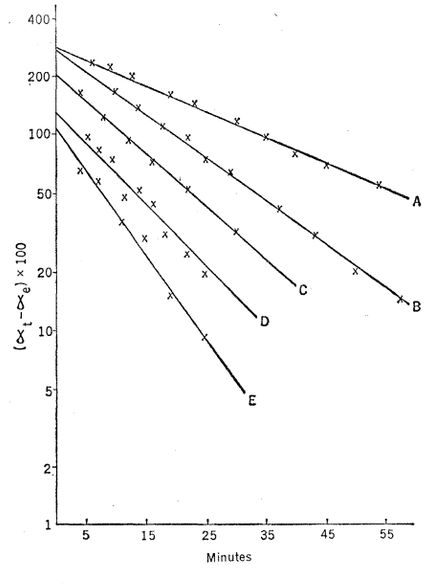

Fig. 1. Effect of the concentration of glucose on the action of equine hemoglobin mutarotase, as determined by the polarimetric method. In curve A, which demonstrates spontaneous mutarotation, the enzyme solution was held at 100°C for 30 minutes before being used to dissolve the freshly weighed α-glucose. In curves A and B the concentration of α-glucose was 0.1668*M*; in curve C, 0.1112*M*; in curve D, 0.0834*M*; and, in curve E, 0.0556*M*; α_t and α_e are optical rotation at time t and at equilibrium, respectively.

Table 2. The effect of a second sugar on the mutarotation of α-glucose by equine hemoglobin mutarotase, as determined by the dialysis-coupled enzyme method. The concentration of α-glucose was 0.02085 mole/liter; of all other sugars, 0.1 mole/liter.

Second sugar	Inhibition (%)	K_i (mole/liter)
None		.0502 (K_m)
L-Arabinose	100	
D-Galactose	100	
D-Arabinose	69	.0313
D-Ribose	66	.0365
D-Xylose	60	.0478
D-Maltose	44	.0906
D-Fructose	29	.1768
D-Lactose	26	.2020
Sucrose	0	

of inactivation was calculated from $t_{1/2}$ values, following Keston's method (*10*) for calculating fractional residual activity and percentage inhibition. The data indicate that this enzyme is quite sensitive to heat, since 92 percent of the activity could be abolished by heating the solution for 10 minutes at 55°C.

The dialysis–coupled enzyme method was also used in determining the effect of a second sugar on the mutarotase activity (Table 2). This was done by making a 0.2*M* solution of the sugar in EDTA buffer, letting it stand overnight to reach equilibrium, and using this solution to further dissolve the α-glucose for diluting the enzyme solution in the usual manner. The equine mutarotase was strongly inhibited by D-galactose, L-arabinose, D-ribose, and D-xylose but only slightly by D-fructose, lactose, and D-maltose, and not at all by sucrose. These results are similar to those reported by others when the enzyme was found in glucose oxidase preparations (*1*) and in mammalian tissues (*2*). The stereospecificity of the enzyme is demonstrated by the greater inhibition with L-arabinose (100 percent) than with D-arabinose (69 percent). In this respect, the equine hemoglobin enzyme differed somewhat from mutarotase of the eye lens which was not at all inhibited by D-arabinose, although strongly inhibited by L-arabinose (*10*). Inhibitor constants, K_i, were found by using Eq. 1 of Keston (*10*), which describes competitive inhibition in accordance with Michaelis-Menten theory.

Experiments yielding results very similar to the ones reported here with partially purified mutarotase were obtained by using lysed human erythrocytes and hemoglobin solutions with the dialysis–coupled enzyme method. Although they actually preceded the work reported here, details of these experiments cannot be given in this brief communication.

Studies with preparations of hemoglobin from five different species of mammals indicated considerable differences in mutarotase activity. Equine and porcine hemoglobin had far more activity than bovine, canine, and human hemoglobin. In those instances in which the mutarotase was separated from either hemoglobin (equine, bovine, and human) or lysed human erythrocytes and partially purified, the resulting enzyme activities were still in the same relative order.

The use of the dialysis–coupled enzyme method, which permitted assaying mutarotase activity in opaque and highly colored solutions, showed the presence of this enzyme in erythrocytes; its existence here had been suspected although not demonstrated by other investigators (*5, 6*). The separation of the enzyme from hemoglobin and its partial purification allowed the use of a conventional polarimetric method. It is thought that mutarotase participates directly in the transport of sugars (*5*) or that it may be a part of a mammalian "permease" system for sugars (*3*). The possible importance of finding mutarotase in erythrocytes is suggested by the specificity of some enzymes for anomers of glucose. The high specificity of glucose oxidase for β-D-glucopyranose was established by Keilin and Hartree (*11*). In a recent report (*12*) it was demonstrated that glucose-6-phosphate dehydrogenase from human erythrocytes is specific for β-D-glucopyranose-6-phosphate, and it was implied that mutarotase may have some metabolic function in erythrocytes.

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Human Collagenase: Identification and Characterization of an Enzyme from Rheumatoid Synovium in Culture

Abstract. *Synovial tissue from patients with rheumatoid arthritis produces lysis of gels of reconstituted collagen fibrils in culture and releases soluble collagenase when cultured in collagen-free medium. Collagen molecules in solution at neutral* pH *at 20° and 27°C are cleaved by the synovial enzyme into ¾ and ¼ length fragments. In this respect the action of synovial enzyme is similar to that of amphibian collagenase and distinct from that of bacterial collagenase. At 37°C reconstituted collagen fibrils and native fibers are attacked by the enzyme and further degraded to polypeptides of low molecular weight. These polypeptides are produced only after denaturation of the larger fragments, which occurs at temperatures near 37°C.*

The mechanism whereby collagen is degraded in human tissues under normal and pathological conditions has not been fully elucidated. In the native state collagen is relatively resistant to the action of the common proteolytic enzymes and there is no definite evidence that denaturation of the molecule precedes its breakdown in vivo. Since rheumatoid arthritis is associated with destruction of collagenous structures in and around joints we have examined