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EFFECT OF HEAVY WATER (^2H_2O) ON REGULATORY PROPERTIES OF PHOSPHOGLUCOSE ISOMERASE FROM *LACTOBACILLUS CASEI*

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Summary

Phosphoglucose isomerase (D-glucose-6-phosphate ketolisomerase, EC 5.3.1.9) purified to homogeneity from *Lactobacillus casei* was used for studies on ^2H_2O effects. This preparation showed distinct differences in functional properties in H_2O and ^2H_2O, respectively. Erythrose 4-phosphate which exerted sigmoidal inhibition in H_2O, acted as a competitive inhibitor in ^2H_2O. The enzyme also showed reduced rate of fructose 6-phosphate formation in ^2H_2O. The enzyme was found to be dimeric in water and monomeric in ^2H_2O. The loss of regulatory properties of the enzyme has been correlated with disaggregation of the protein in heavy water, similar to that caused by sodium dodecyl sulphate treatment.

Introduction

Several studies have shown that heavy water (^2H_2O) inhibits fundamental processes of life including growth and metabolism [1—6]. Some of these have been correlated with impaired activities of certain enzymes. Thus, in *Aspergillus niger* growth inhibition in ^2H_2O was observed as due to repression of pyruvate carboxylase [1—3]. More recent studies have suggested that ^2H_2O could also influence regulatory properties of some enzymes [1—5,7]. It has been indicated that heavy water stabilized association of the monomers of bovine glutamate dehydrogenase to form polymers, thus limiting allosteric transitions and responses to effectors [7]. The loss of regulatory properties, is often correlated

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with disaggregation of subunits in the enzyme [8—10]. The present study with phosphoglucose isomerase (D-glucose-6-phosphate ketolisomerase, EC 5.3.1.9) from *Lactobacillus casei* points to such a possibility in ^2H_2O. Purification and characterisation of the multiple forms of this enzyme from this organism have been described earlier [11]. It was observed that one of the forms had shown striking allosteric modulations in the presence of erythrose 4-phosphate. This particular preparation was chosen for the experiments reported in this paper.

Materials and Methods

Growth of culture and preparation of cell extract. The conditions for maintenance of the culture and the growth of *L. casei* ATCC 7469 have been described earlier [11,12]. The cells (20—40 g wet weight) were suspended in 150 ml of ice-cold 0.1 M phosphate buffer (pH 7.0) and were subjected to mechanical disruption in an Aminco-French pressure cell at 4000—6000 lb/inch^2. The cell debris was eliminated by centrifugation at 17 000 rev./min for 20 min and the supernatant was used as the cell-free extract. Protein estimations were carried out using Folinphenol reagent as described by Lowry et al. [13] using bovine serum albumin as standard.

Purification of phosphoglucose isomerase. The protocol employed for purification of this enzyme from *L. casei* has been outlined earlier [11]. The cell-free extract was subjected to precipitation with acetone and ethanol, respectively, dialysis in water, DEAE-cellulose chromatography and Sephadex G-100 gel filtration. The preparation (form B) thus obtained was electrophoretically homogeneous as stated earlier.

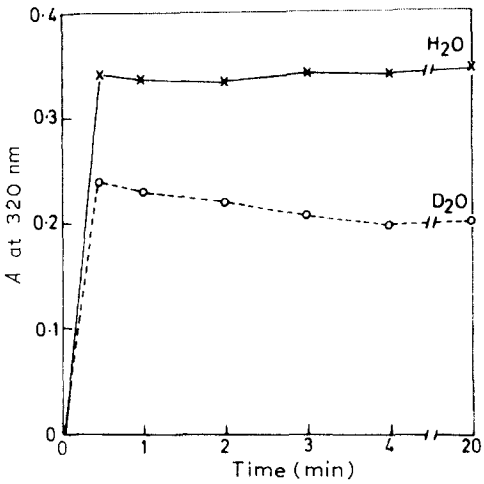
Assay of phosphoglucose isomerase. The enzyme activity was estimated by the method of Bodansky and Schwartz [14]. The reaction mixture contained in a total volume of 2.0 ml 100 μmol Tris-HCl buffer, pH 7.4/12.5 μmol glucose 6-phosphate/17 μmol sodium chloride. The reaction was stopped by adding 1.0 ml of 10% trichloroacetic acid within 2 min of incubation at 37°C. Fructose 6-phosphate formed was measured by the colorimetric procedure of Roe [15].

Materials. The substrate and other biochemicals were obtained from Sigma Chemical Co. (U.S.A.) and other chemicals and resins were purchased from standard sources. The heavy water (^2H_2O) used in these experiments was of 99.9% purity and was obtained from the Heavy Water Division of this Centre.

Results

Effect of heavy water on L. casei phosphoglucose isomerase. Form B of the enzyme from *L. casei* [11] was chosen for studying the effects of ^2H_2O because of greater recoveries (12%) of the protein as well as typical regulatory characteristics. The homogeneous preparation, tested on polyacrylamide gel electrophoresis [11,16] was dissolved (1 mg/ml) in ^2H_2O and water, respectively. Other solutions and buffers used in the comparative studies were also made in these two media. The profile of reaction velocity of the enzyme in H_2O and ^2H_2O is shown in Fig. 1.

In separate sets of experiments double-reciprocal plots were obtained using



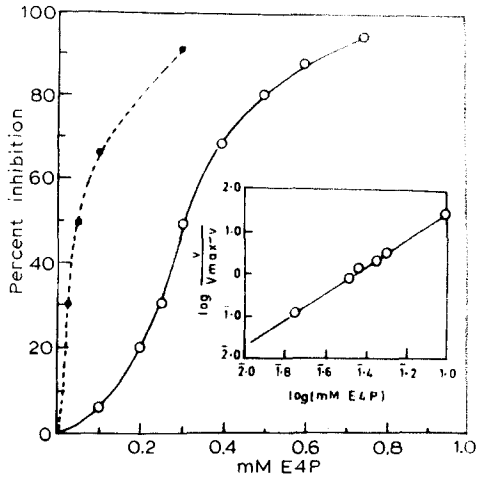


Fig. 1. Fructose 6-phosphate formation in H_2O and ^2H_2O by action of phosphoglucose isomerase. The product formed was measured by the method stated in the text.

Fig. 2. The inhibition of *L. casei* phosphoglucose isomerase by erythrose 4-phosphate (E4P). The solid line represents inhibitory responses in water, whereas the dotted line represents that in ^2H_2O. The inset shows the Hill plot with reference to varying concentrations of the inhibitor in H_2O medium.

much less enzyme, from which the apparent K_m values were calculated to be $5.0 \cdot 10^{-3}$ M in water and $7.7 \cdot 10^{-3}$ M in ^2H_2O, thus indicating similar affinities for the substrate in the two media.

Inhibitory action of erythrose 4-phosphate. With rabbit muscle phosphoglucose isomerase, it has been shown that erythrose 4-phosphate was a competitive inhibitor of the enzyme [17]. However, this compound exerted sigmoid inhibition on the present preparation obtained from *L. casei* as shown in Fig. 2. The effect was apparent only in an aqueous system, while in deuterated medium hyperbolic inhibition was observed and the enzyme was also found to be more sensitive to the inhibitor. The Hill coefficient was calculated to be 0.5 suggesting negative cooperativity as stated earlier [11].

Molecular weight and subunit composition. The molecular weight of the purified phosphoglucose isomerase determined using Sephadex G-100 was found to be 68 000 in water and 34 600 in ^2H_2O as shown in Fig. 3. The standard proteins used for calibration were bovine serum albumin (68 000), yeast hexokinase (96 000) and cytochrome *c* (12 800). The molecular weight in ^2H_2O was estimated by equilibrating the gel in ^2H_2O. Bovine serum albumin used as a standard protein did not show variation in the migration with ^2H_2O.

The subunit composition of the present enzyme preparation was ascertained by treatment with 1% SDS in 0.05 M phosphate buffer, pH 6.0, as described earlier [10,16]. The single peak on a Sephadex G-100 column previously equilibrated with 1% of the detergent, indicated an apparent molecular weight of 34 600 as shown in Fig. 4. It is, therefore, apparent that the enzyme in the aqueous system was a dimer having equal sized subunits, and that it could exist only as a monomer in ^2H_2O. The identity of the subunits in the native protein was examined by treatment of the enzyme preparation with 1% SDS and elec-

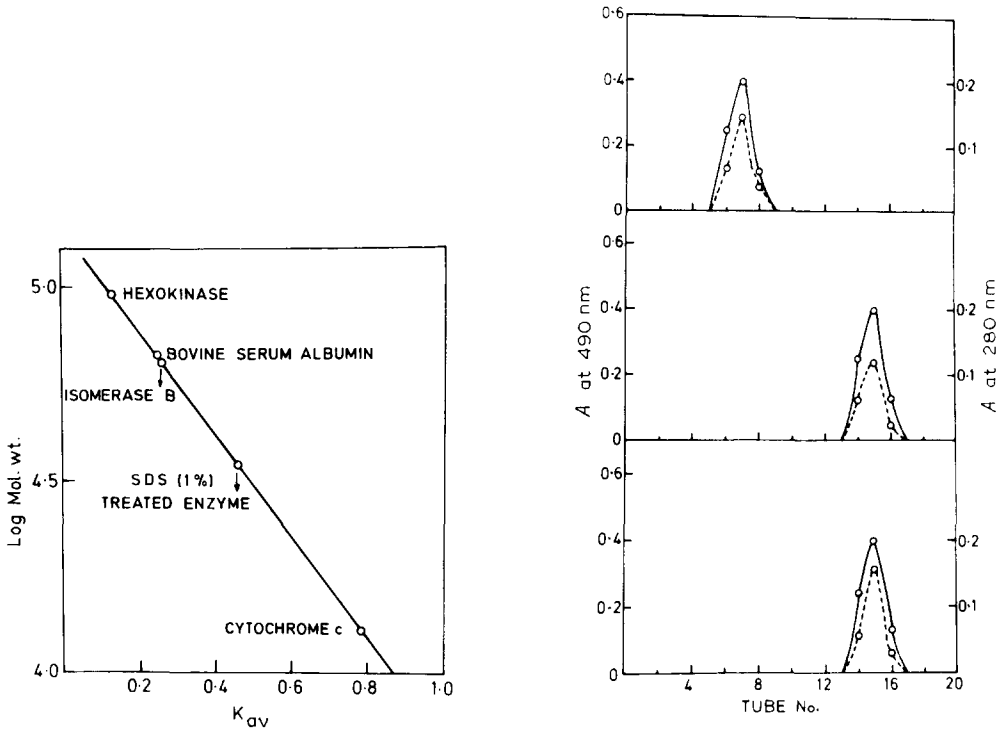


Fig. 3. Molecular weight determination of *L. casei* phosphoglucose isomerase on Sephadex G-100. The enzyme protein (1.0 mg) dissolved in 2.0 ml of 0.05 M phosphate buffer (pH 6.0) was loaded on a column (2.2 × 65 cm) previously equilibrated with the same buffer. Elution was carried out with the same buffer by collecting 5.0 ml fractions. The conditions for treatment of the enzyme with 1% SDS and the procedure followed thereafter for gel filtration have been outlined earlier (Ref. 11). The enzyme solution in ^2H_2O was also passed through the column using all the solutions made in ^2H_2O. SDS-treated enzyme and the protein in ^2H_2O eluted in the same positions. The elution volume (V_e) for each of the marker proteins and the enzyme was measured, the bed volume (V_t) and void volume (V_0) being 247 ml and 81 ml, respectively.

Fig. 4. Elution profiles of *L. casei* phosphoglucose isomerase on Sephadex G-100 with H_2O, ^2H_2O and 1.0% SDS. The protein (0.5 mg) sample was loaded on the column as described in Fig. 3. The elution was carried out with 0.05 M phosphate buffer (pH 6.0) made in H_2O (top) and ^2H_2O (bottom), respectively. Similarly, the enzyme preparation dissolved in the buffer containing 1% SDS was passed through the column previously equilibrated with the same buffer system (middle). The solid lines show the protein elution while the dotted lines show the activity.

trophoresis on polyacrylamide gel supplemented with 0.1% SDS as outlined earlier [11].

Ultracentrifugation studies. The enzyme preparation was dissolved in appropriate volumes (0.5 mg/ml) of 0.04 M phosphate buffer (pH 6.0) made in H_2O and ^2H_2O, respectively. The sedimentation pattern at different time intervals was recorded in a Beckman Model E analytical ultracentrifuge equipped with Schlieren optics [18]. Samples treated with 1% SDS in H_2O were also run similarly after the preparation was incubated with the detergent for 3 h at 37°C. The profile of the boundary movement has been presented in Fig. 5. The values of sedimentation coefficient ($s_{20,w}$) calculated for the enzyme preparation in H_2O, ^2H_2O and in presence of SDS were 4.0, 2.4 and 2.0, respectively. These

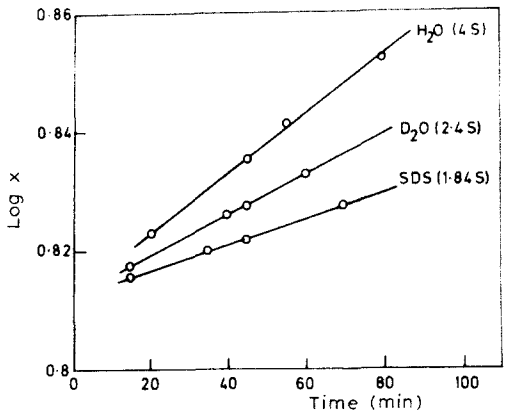


Fig. 5. Boundary movement of *L. casei* phosphoglucose isomerase in H_2O, ^2H_2O and 1% SDS in 0.05 M sodium phosphate buffer (pH 6.0) in analytical ultracentrifuge at 59 780 rev./min. Other details are stated in the text. The sedimentation coefficient ($s_{20,w}$) values were calculated from the slopes of the respective curves.

results again indicate that the subunits of the enzyme were disaggregated in ^2H_2O.

Discussion

The results presented above show that phosphoglucose isomerase from *L. casei*, existed as a dimer in H_2O while it was monomeric in ^2H_2O, though retaining the functional integrity. SDS electrophoresis, Sephadex gel filtration and ultracentrifugation studies, point to the existence of monomeric species of the enzyme in ^2H_2O. The differences observed in sedimentation coefficient of the protein in ^2H_2O and 1% SDS are, however, unclear since the elution profiles on gel filtration are otherwise similar. This apparent anomaly could perhaps be attributed to the relative unfolding of the protein, since SDS is known to bind directly to the protein, thereby altering the partial specific volume of the solution which could reflect in the variation of S values, as reported for rabbit muscle phosphoglucose isomerase [19].

The observed disaggregation of subunits of the enzyme in ^2H_2O, seems to be at variance with the observations reported for bovine glutamate dehydrogenase which was shown to be stabilized in the polymeric state in ^2H_2O, showing slower dissociation in the presence of NADH [20]. It was indicated that the inactive monomer of glutamate dehydrogenase was stabilized in ^2H_2O. On the other hand, lactic dehydrogenase showed dissociating tendencies when reassociation of subunits was attempted [21]. Alterations in the association-dissociation equilibrium of the subunit enzymes were ascribed to the strength of deuterium bonds and deuterium water bridges. The dissociation of the present enzyme was not a ligand-induced alteration and could, therefore, be an instance of hydrogen bonding between the subunits getting disturbed during the exchange with deuterium of the solvent. The observed responses with SDS causing a similar dissociation may also support this assumption.

The sigmoidal inhibition exerted by erythrose 4-phosphate on phosphoglu-

cose isomerase preparation from *L. casei* in H_2O, was also abolished in ^2H_2O. It is generally accepted that regulatory enzymes are oligomeric proteins [22,23] and the loss of regulatory properties is often accompanied by disaggregation of the protein [8—10]. The enzyme showed similar affinities for the substrate in H_2O and ^2H_2O, though the extent of product formation was reduced in the deuterated medium, which was explained on the basis of proton exchange from the medium and intramolecular transfer involved in the product formation [24]. In the monomeric state, erythrose 4-phosphate could compete with the substrate for the same binding site. The negative cooperativity observed in the H_2O medium in presence of the inhibitor and differential sensitivities to the inhibitor in H_2O and ^2H_2O may suggest that the sites may not be readily accessible to the inhibitor when the protomers are in associated state. The foregoing results nevertheless suggest that the magnitude of the effect of ^2H_2O was more on the regulatory responses than on the catalytic function of phosphoglucose isomerase from *L. casei*.

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