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Stabilization of ribose-5-phosphate isomerase from tobacco

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The highly purified ribose phosphate isomerase from tobacco leaves is heat labile. 0.2% of various kinds of proteins stabilized isomerase activity when Mg^{++} was present. 1×10^{-3}% polyethyleneglycol 2,000 showed the same effect as the proteins did. Smaller polyethyleneglycols were less effective. Polyhydroxyl compounds showed little effect. Mn^{++} or Sr^{++} was also effective as a stabilizer instead of Mg^{++}.

Previously we reported that highly purified tobacco ribose-5-phosphate (R5P) isomerase is very heat labile, while the activity in crude extract is relatively stable (*2*). The presence of either protein or magnesium ion stabilized isomerase activity, although the respective stabilizer alone was not as effective. Proteins could be replaced by polyethyleneglycol (PEG) 6,000 but the latter was rather more effective for the stabilization of the enzyme. How can PEG, a polyether, stabilize enzyme activity? To investigate the effect of the stabilizers, the present experiments were carried out. This paper deals with the stabilization effects of various proteins, metals, polyethers and polyhydroxyl compounds on the activity of R5P isomerase.

Materials and methods

R5P isomerase.

R5P isomerase was purified from leaves of a wild tobacco, *Nicotiana sylvestris*, according to the method reported previously (*2*). The highly purified solution (1 mg/3 ml) of the isomerase which included PEG 6,000 and $MgCl_2$ was applied to a DEAE cellulose column (1.5 × 10 cm), which had been equilibrated with 0.025 M Tris-HCl buffer, pH 7.6, to remove PEG and Mg^{++}. After washing with 40 ml of 0.025 M Tris buffer (pH 7.6) including 0.05 M NaCl, isomerase was eluted with 0.3 M NaCl in the same buffer. The isomerase solution could be kept at −80°C for a month with no significant activity loss. Although Domagk et al. (*1*) reported that yeast R5P isomerase activity will be destoryed by freezing and thawing, the destruction was less than 1% in the case of tobacco R5P isomerase.

Stability determination.

The enzyme solution was diluted up to 100 μg/ml with 0.025 M Tris-HCl, pH 8.2, and used immediately in the following experiments. The enzyme solution

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(0.1 ml) was incubated at 40°C with various stabilizers such as proteins, PEG and metals in a final volume of 1 ml of 0.025 M Tris-HCl. After incubation at 40°C, the solutions were diluted 300 to 500 times with the same buffer, and isomerase activity was measured according to the method previously reported (*2*).

Results and discussion

Since R5P isomerase was relatively stable in the crude extract where many proteins and metal ions were present the first experiment was planned to examine the effects of these on the stability of the enzyme. As shown in Fig. 1, tobacco R5P isomerase without stabilizers was heat labile and lost almost all of its activity in 2 hr at 40°C. In contrast no activity loss was found with both albumin and Mg^{++} present under the same conditions. However, neither albumin nor Mg^{++} was as effective by itself as both together.

The effects of serum albumin concentration on the stability of isomerase activity is shown in Fig. 2. In the presence of Mg^{++}, 0.15% albumin could fully stabilize the activity under the experimental conditions. On the other hand, albumin alone could not prevent heat destruction of the activity even when the concentration was increased up to 6%.

In addition to serum albumin, other kinds of proteins so far examined were also effective for stabilization of enzyme activity (Table 1). Either large (Fraction I protein, 5×10^5 daltons) or small (Bacitracin, 1,450 daltons) protein has the same effectiveness as a stabilizer. Amino acids (acid hydrolyzate of Fraction I protein) or glutathione (reduced or oxidized) showed no effect. These results show that about deca or longer peptide chain is necessary for stabilization of enzyme activity.

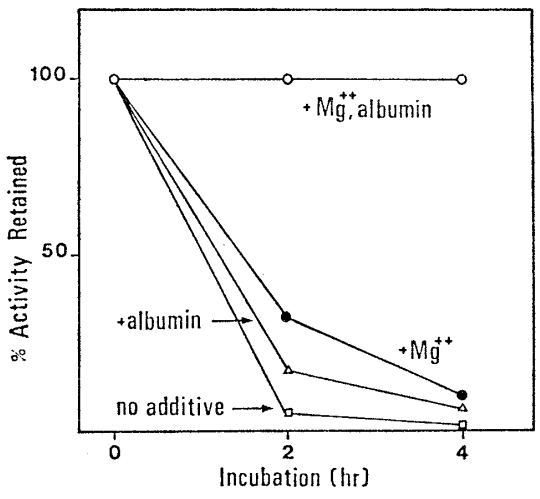

Fig. 1.

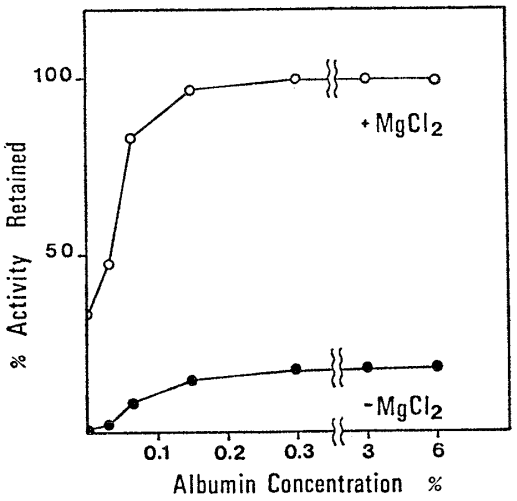

Fig. 2.

Fig. 1. *Heat lability of R5P isomerase from tobacco.* Incubation was carried out at 40°C in a medium of 0.025 M Tris-HCl buffer, pH 8.2. 0.3% serum albumin Fr.V or 1×10^{-3} M $MgCl_2$ was used in the experiment.

Fig. 2. *Effects of serum albumin concentration on the stabilization of isomerase activity.* 1×10^{-3} M $MgCl_2$ was used in the experiment.

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Table 1 *Effects of various macromolecules on the stability of R5P isomerase from tobacco leaves*

Additive	Concentration (%)	% Activity retained	
		$-MgCl_2$	$+MgCl_2$ [b]
Before incubation [a]		100	100
No addition		5	38
Fraction I protein	0.3	39	100
apo-Ferritin	0.3	41	100
γ-Globulin	0.3	35	100
Serum albumin	0.3	34	100
Ovalbumin	0.3	36	100
Myoglobin	0.3	35	100
Cytochrome C	0.3	34	100
Bacitracin	0.3	34	100
Dextran (10^5 M.W.)	10	7	100
Dextran (10^5 M.W.)	1	3	60
Dextran (10^4 M.W.)	10	3	51
Glucose	10	3	48
Glycerol	10	4	39
PEG 6,000	0.001	20	100

[a] Incubation: 40°C for 2 hr.
[b] $MgCl_2$ concentration: 1×10^{-3} M.

Although polyhydroxyl compounds have been known as effective stabilizers of protein in general (*3*), compounds such as glycerol and glucose have no effect for isomerase stabilization. Dextran with a large molecular weight had a slight effect at a very high concentration, but smaller dextran showed no effect at all.

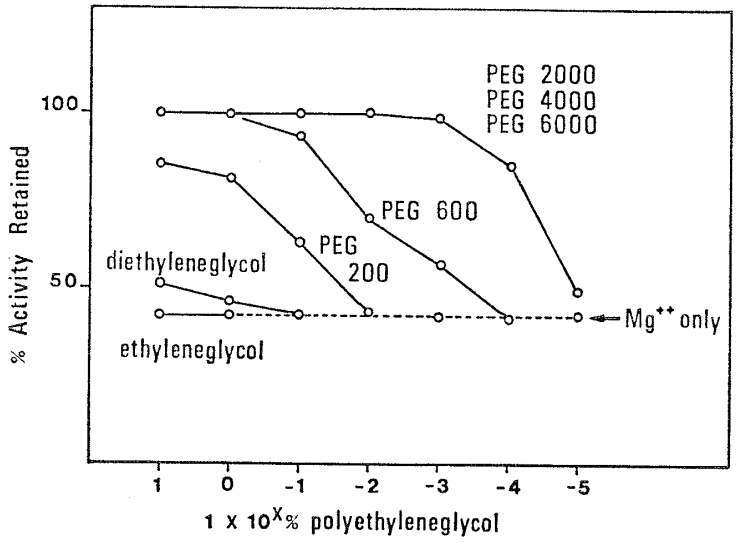

Fig. 3. *Effects of molecular weight and concentration of PEG on the stabilization of the isomerase.* Experiments were carried out under 1×10^{-3} M $MgCl_2$.

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Table 2 *Effects of various metal ions on the stability of R5P isomerase from tobacco leaves*

Additive	% Activity retained	
	−Albumin	+Albumin [b]
Before incubation [a]	100	100
No addition	2	20
Mg^{++} [c]	38	100
Mn^{++}	34	98
Sr^{++}	38	99
Ca^{++}	10	75
Ni^{++}	9	40
Co^{++}	1	20
Zn^{++}	1	16
K^{+}	2	20

[a] Incubation: 40°C for 2 hr.
[b] Albumin concentration: 0.3%.
[c] Concentration of metals: 1×10^{-3} M.

These results show that the effectiveness of the large dextran is not due to its hydroxyl-groups but its long chain structure or perhaps its ether bonds.

Contrary to dextran, PEG 6,000, a polyether, was highly effective for the stabilization of the enzyme. Only 0.001% PEG 6,000, a concentration 300 times less than that of protein, fully stabilize the enzyme activity. However, the effectiveness of PEG was highly dependent on its chain length. As shown in Fig. 3, PEG 2,000 or PEG 4,000 showed the same effectiveness as PEG 6,000 at the same concentration. However, PEG 600 (average molecular weight; 600) could only stabilize enzyme activity at under 1% concentration. PEG 200 was less effective than PEG 600, and ethylene glycol and diethyleneglycol showed little effect on the stabilization of enzyme activity.

It is known that serum albumin prevents the denaturation of enzymes. This

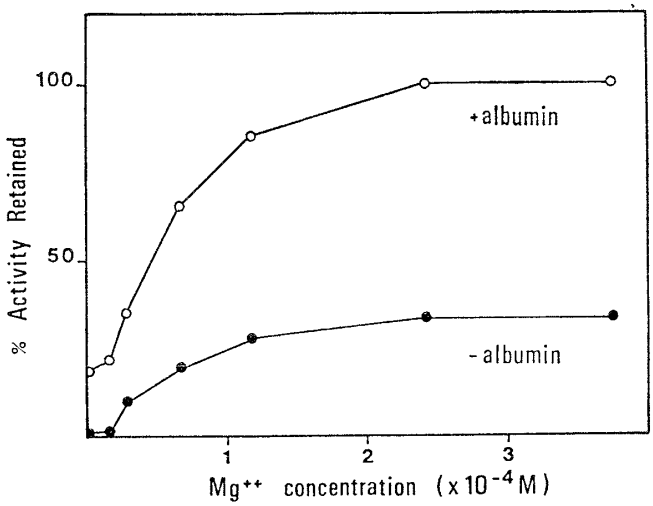

Fig. 4. *Effects of Mg^{++} concentration on the stabilization of isomerase activity.* 0.3% albumin was used in the experiment.

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phenomenon may be due to coating of the enzyme by albumin molecules (*4*). PEG may have similar properties for coating the enzyme, R5P isomerase, to those of proteins. Besides, it may be suspected that usually protein have structures effective both helping and hindering the coating of an enzyme. On the contrary, the structure of PEG may only be effective for coating and therefore a small amount is enough for the stabilization of R5P isomerase.

To obtain maximum stabilization by albumin or PEG, the presence of Mg^{++} was always necessary. The effect of Mg^{++} concentration on the stability of isomerase activity is shown in Fig. 4. In the presence of 0.3% albumin, 2.5×10^{-4} M Mg^{++} could fully stabilize activity under the experimental conditions. On the other hand, Mg^{++} alone could not prevent heat destruction of activity even when the concentration was increased up to 1×10^{-2} M.

Several divalent cations such as Mn^{++} and Sr^{++} could be substituted for Mg^{++} (Table 2). Ca^{++} and Ni^{++} were less active than Mg^{++}. Co^{++}, Zn^{++} and monovalent cation K^{+} had no effect at all. These results show that broad but not unlimited specificity for metal ions is included in the stabilization process. These metals may serve for mediating between the enzyme molecule and long chain stabilizers.

References

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