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OCCURRENCE OF MULTIPLE MOLECULAR FORMS OF PORPHOBILINOGENASE IN DIVERSE ORGANISMS: THE MINIMUM QUATERNARY STRUCTURE OF PORPHOBILINOGENASE IS A PROTOMER OF ONE DEAMINASE AND ONE ISOMERASE DOMAIN

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Abstract—1. Gel filtration on Sephadex G-100 and Sepharose 4B were used to redetermine the molecular weight (MW) of porphobilinogenase, deaminase and isomerase purified from different sources, and determine the MW of these enzymes purified from *Euglena gracilis*.

2. Results reported here, indicate that porphobilinogenase can be found, into three different molecular forms, tetramers, dimers and monomers according to the source organism.

3. It is proposed that minimal functional structure of PBGase is a hybrid protomer of MW 25,000, composed by two different domains, in a ratio of 1 mol of deaminase, MW 20,000 to 1 mol of isomerase, MW 5000.

4. A model explaining the occurrence of different MW species of PBGase in nature and the possible interconversion among the various forms is postulated.

INTRODUCTION

The enzymic conversion of porphobilinogen (PBG) into uroporphyrinogens by the action of porphobilinogenase (PBGase) is one of the most exciting steps in terapyrrole biosynthesis and has always attracted considerable attention, largely because of the complicated reaction involved and its unique role in this pathway.

PBGase is a hybrid protein made up of two distinct domains, deaminase and isomerase, which can be separated by different procedures. It has also become evident, that this enzyme provides rather useful handles for studying the interaction involving the assembly of subunits in a complex enzyme.

PBGase and its components deaminase and isomerase, can be isolated from various organisms in a number of multiple forms differing in their molecular weights (MWs) (Batlle & Rossetti, 1977 and references therein).

By conventional chromatographic procedures, gel filtration, polyacrylamide electrophoresis and sucrose density gradient centrifugation there have been reports of MWs ranging from 100,000 to 25,000 for the PBGase isolated from cultured cells, animal and human sources (Llambías & Batlle, 1971a,b; Sancovich *et al.*, unpublished results). The size of deaminase corresponds to a protein of MW 40,000 (Llambías & Batlle, 1971a,b; Jordan & Shemin, 1973; Sancovich *et al.*, 1976; Kreutzer *et al.*, 1977; Higuchi & Bogorad, 1977) and there is a report of a MW 25,000 ± 5000 (Frydman & Feinstein, 1974). As far as isomerase is concerned, depending on both the source and several experimental conditions, this enzyme, once isolated, shows a great tendency to aggregation; therefore, its MW could range from 5–6,000 to 280,000 (Llambías & Batlle, 1971a,b; Sancovich *et al.*, unpublished results).

Owing to this molecular heterogeneity, we thought that PBGase can exist into large and small forms depending on a possible taxonomic ground and that there should be a minimum quaternary structure necessary for activity. This reason prompted us to undertake a survey of the MW of a range of PBGases, and its components, to redetermine the MWs of the enzymes from bovine liver, avian erythrocytes and soybean callus, as well as, investigate the molecular size characteristics of PBGase, deaminase and isomerase purified from *Euglena gracilis*.

Results here reported do show that PBGase can be found, at least, into three different molecular types, according to the source organism. We propose that its protomer is a hybrid of MW 25,000 of two different domains, in a relation of one mol of deaminase (MW 20,000) to one mol of isomerase (MW 5,000).

MATERIALS AND METHODS

All chemicals used were purchased from Sigma Chemical Co., MA and Difco, unless otherwise indicated. Sephadex and Sepharose were from Pharmacia Fine Chem., Uppsala.

Bovine liver enzymes were purified and assayed as described by Sancovich *et al.* (1969).

Avian erythrocytes enzymes were purified and assayed according to Llambías & Batlle (1971b).

Soybean callus enzymes were purified and assayed following the procedures of Llambías & Batlle (1971a).

Euglena gracilis PBGase, deaminase and isomerase were purified according to Rossetti *et al.* (1980). Source material of enzymes, growing and harvesting conditions and determination of enzymic activities were already reported by Rossetti & Batlle (1977).

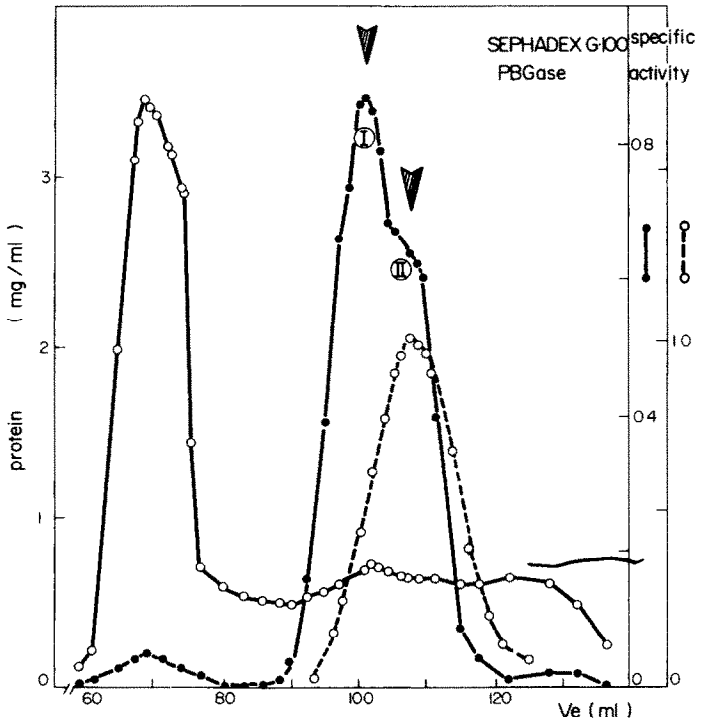

Fig. 1. Elution profile on Sephadex G-100 of: (a) A 77-fold purified PGBase preparation from *Euglena gracilis* pellet (Rossetti *et al.*, 1980) protein (O—O), activity (●——●); (b) a 73-fold purified PBGase preparation from soybean callus, activity (O- - -O). A 2.4 × 50 cm Sephadex G-100 calibrated column equilibrated and eluted with 0.05M sodium phosphate buffer pH 7.4 was used.

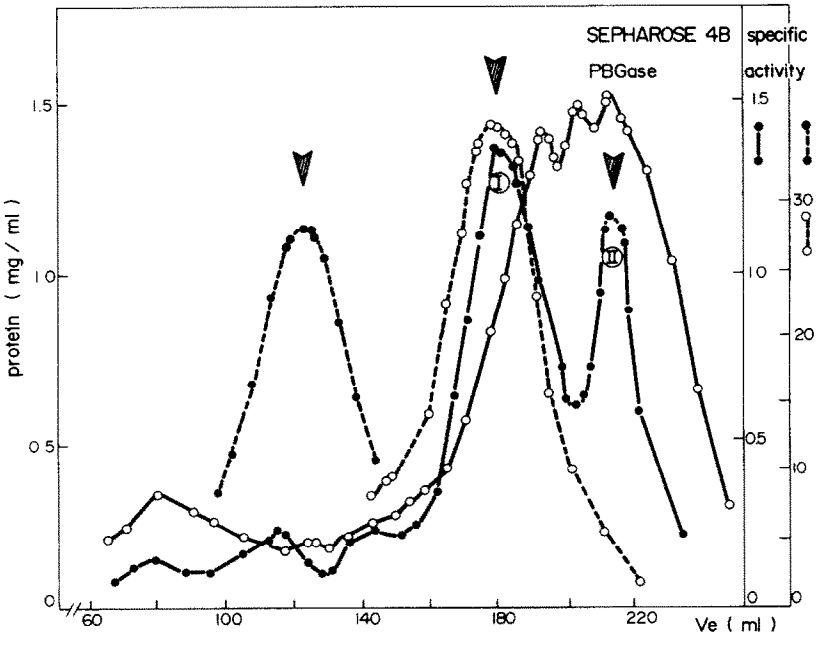

Fig. 2. Elution profile on Sepharose 4B of: (a) The same purified PBGase preparation from *Euglena gracilis* as that described in Fig. 1. Protein (O——O), activity (●——●); (b) a 334-fold purified bovine liver PBGase, activity (O- - -O) and (c) a 4900-fold purified avian erythrocytes PBGase, activity (●- - -●). A 2.4 × 50 cm Sepharose 4B calibrated column, equilibrated and eluted with 0.05 M sodium phosphate buffer pH 7.4 was used.

Proteins were determined by the method of Lowry *et al.* (1951).

Molecular weight measurements were performed by gel filtration. Sephadex G-100 and Sepharose 4 B columns were prepared and calibrated as described by Batlle *et al.* (1965), Batlle (1967) and Locascio *et al.* (1969). The same calibrated Sephadex G-100 and Sepharose 4B column were used throughout this study. Protein content and enzyme activity was determined in the columns eluates.

All other materials and methods not specified here or in legends to Tables and Figures were those already described by Rossetti & Batlle (1977).

RESULTS AND DISCUSSSION

Molecular sizes of PBGase, deaminase and isomerase from various sources

Redetermination of the MW of these enzymes purified from bovine liver, avian erythrocytes and soybean callus produced values consistent with those already reported. Dissociation of deaminase into species of MW 20,000 by chromatography on Sephadex G-100 columns was also confirmed. We will only illustrate here elution profiles for PBGase from these three sources (Figs 1 and 2), and later we will include in Table 2 the MW values obtained for deaminase and isomerase in the organism here examined.

Molecular weight determination of the enzymes from Euglena gracilis

Figures 1 and 2 illustrate typical elution diagrams for PBGase purified from the so-called pellet fraction (Rossetti & Batlle, 1977). We should say that in *E. gracilis* PBGase activity is distributed both in supernatant and particulate fractions, the chromatographic behavior of the enzyme purified from the cytoplasm is perhaps more complex; several species of different MW exist in equilibrium but, to the dominant peak forming 100% of uroporphyrinogen III corresponds a MW of 50,000. Fig. 1 shows that, although the main band has a MW of 50,000, a shoulder which would correspond to a form of MW 25,000 is also present. Better separation of the high and low MW species was achieved by chromatography through Sepharose 4B (Fig. 2) where we can clearly distinguish two peaks of MW 50,000 (I) and 25,000 (II). No evidence for PGBase species smaller than 25,000 MW was found at any stage along these studies for either the soluble or the particulate enzyme.

Deaminase was also purified from the *E. gracilis* supernatant and particulate fractions following different procedures, in all instances two MW species were detected, although their relative ratio varied depending on both the purification method followed and the intracellular localization; however, the high MW form was always the principal species. We will only show the elution profiles of two runs which correspond to extreme conditions. In Fig. 3, purified particulated deaminase was chromatographed through Sephadex G-100, two activity peaks were observed of MW 40,000 (I) and 20,000 (II). By gel filtration of deaminase purified from the supernatant on Sepharose 4B (Fig. 4), practically only one active band was eluted with a MW of 40,000. Obviously, resolution on Sephadex G-100 of low MW proteins is better than using Sepharose gels. Although the MW estimated for deaminase obtained from different sources is 40,000, there is also evidence of the existence of 20,000 MW forms.

Therefore, we suggest that in solution, deaminase normally exists as a dimer of two equal subunits of MW 20,000 and that in some organisms, the minimal functional unit might be the monomer.

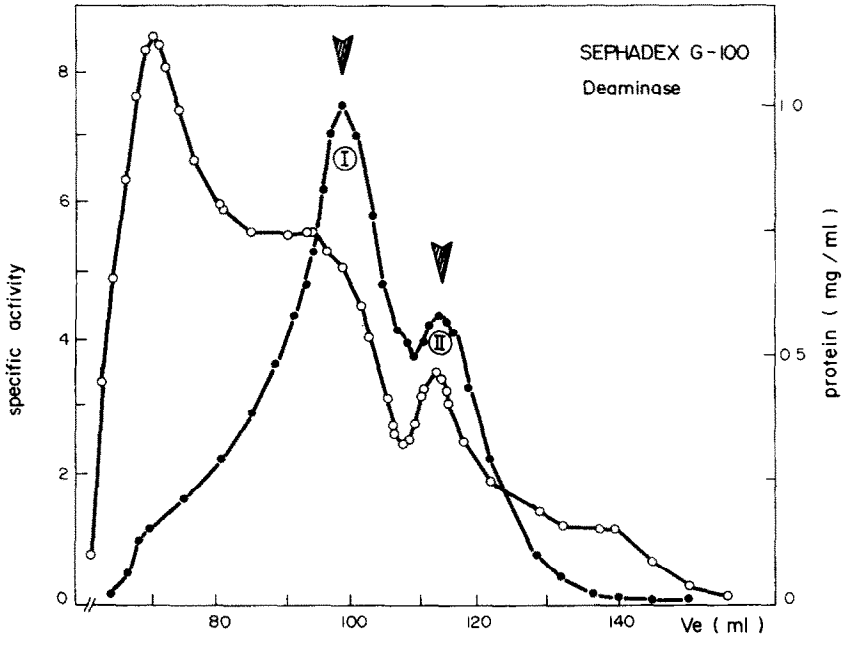

Fig. 3. Elution profile on Sephadex G-100 of a 112-fold purified deaminase prepared from *Euglena gracilis* pellet (Rossetti *et al.*, 1980). Protein (○——○), activity (●——●). The same column as that described in Fig. 1 was used.

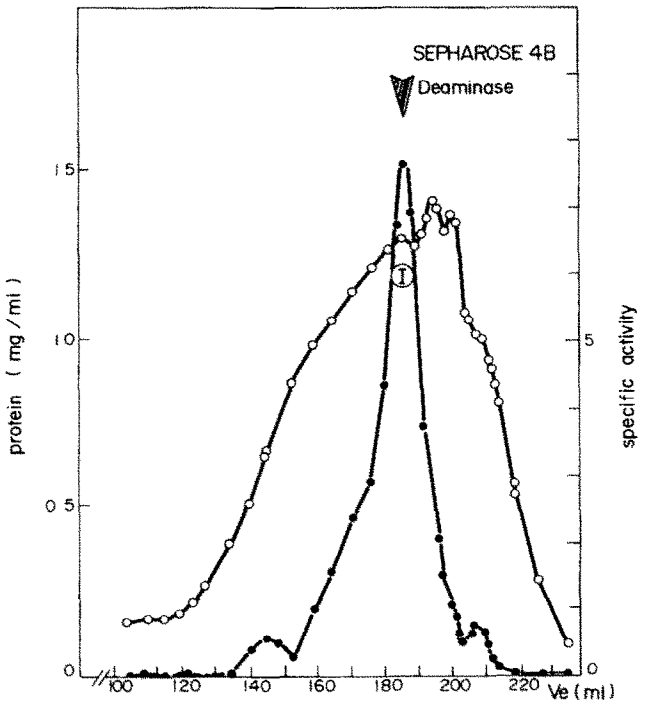

Fig. 4. Elution profile on Sepharose 4B of a 153-fold purified deaminase prepared from *Euglena gracilis* supernatant (Rossetti *et al.*, 1980). Protein (○——○), activity (●——●). The same column as that described in Fig. 2 was used.

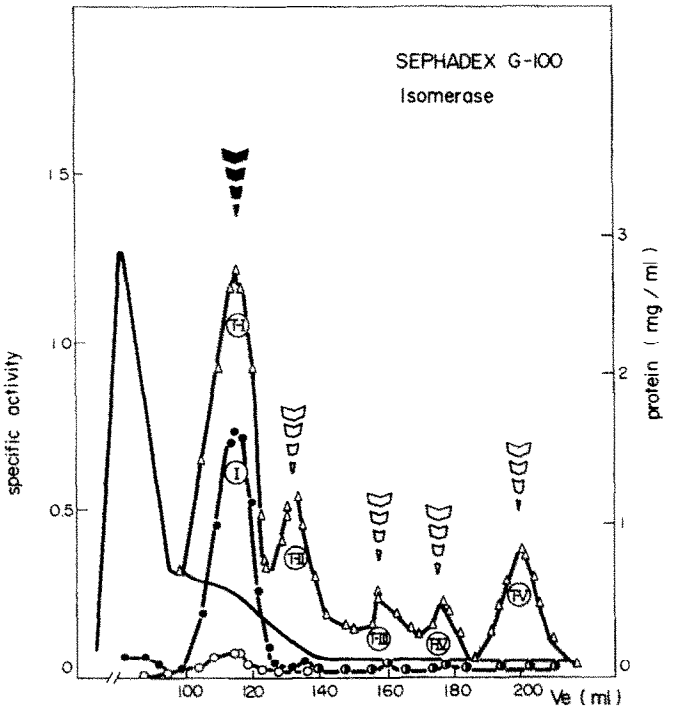

Fig. 5. Elution profile on Sephadex G-100 of a 80–90% ammonium sulphate fraction, corresponding to Step 4 of isomerase preparation from *Euglena gracilis* supernatant (Rossetti *et al.*, 1980). In all eluates, besides protein content (——), activity was measured in the absence (○——○) and presence (△,●) of a 112-fold purified *E. gracilis* deaminase, which formed 95% of uroporphyrin I. (△——△): Total activity. (●——●): Resulting activity after deducting the amount of porphyrins formed by the corresponding blanks of deaminase from the total porphyrins synthesized. The same column as that described in Fig. 1 was used.

As with the preceding enzymes, isomerase has been purified from the cytoplasm and the pellet. We will report here results obtained for the soluble enzyme.

Needless to say that both the purification procedure and the assay for isomerase are cumbersome and time consuming. Many batches of *E. gracilis* had to be worked to obtain enough purified protein for its MW determination. The enzymic assay involves on the other hand, estimating the ratio uro III:uro I formed by each fraction eluted, in the presence of a constant amount of deaminase. In Fig. 5 we show the elution profile of an isomerase preparation which had practically no measurable activity in the absence of deaminase, by adding its partner enzyme and taking into account total activity, several peaks were observed. But, if we deduct the amount of porphyrins formed by blanks of deaminase in each case, a clear cut peak (I), forming 100% of uro III of MW 30,000 was the dominant species. An analysis of the total activity profile showed that peak T–I indeed corresponded to pure isomerase; peak T–II (MW 20,000) produced a mixture 1:1 of uro III to uro I; therefore isomerase was also present in that region, otherwise only uro I should have been formed, but undoubtedly deaminase was in great excesss. This same last reason is valid for explaining the appearance of peaks III, IV and V, which formed 30–40% of uro IIII, having MWs of 15,000, 10,000 and 5000 respectively, confirmed by rechromatography on a calibrated Sephadex G-75 column. From these results, the MW of *E. gracilis* isomerase appears to be 30,000 but we do know that this enzyme readily forms larger aggregates in solution, although we must have in mind that the situation *in vivo* might be probably different. We therefore, suggest that isomerase should be a polypeptide having a MW of 5000 and we will justify later this proposal.

Deaminase:isomerase ratio in the PBGase complex

Several schemes have been postulated to explain the mechanism of the reaction catalyzed by PBGase (Batlle & Rossetti, 1977 and references therein). In many of them, it is assumed that PBGase is a complex containing one unit or one mol of deaminase and one of isomerase; however, no experimental data is available to support that such combination is actually valid.

We have proposed that in most organisms, deaminase is dimeric in solution, and the MW of the monomer is 20,000. Concerning isomerase, we postulated that the domain is a polypeptide of MW 5000 which easily aggregates in solution. To obtain some insight into the molecular relation of deaminase to isomerase forming the active PBGase complex, experiments were designed, varying the ratio deaminase:isomerase. Some results are shown in Table 1. It was always observed that, when deaminase is in excess, neither the isomeric type nor the total activity were significantly altered. However, when the ratio is 1:1, the activity of the mixture was nearly twice, and practically all the isomer formed was type III. If we augmented the proportion of isomerase, uroporphyrinogen III was always produced, but the activity of the complex gradually decreased until the ratio deaminase:isomerase is 1:4, as if the latter enzyme were inhibiting the reaction, further increase of isomerase did not apparently produce other changes.

These results were not surprising, for we had evidence that *in vitro*, excess of isomerase diminished PBGase activity, acting as an inhibitor or perhaps as a regulator of the reaction. On the other hand, heating PBGase preparations have revealed that such treatment has quite different effects on the two domains of the complex, indicating that a catalytically significant interaction between these domains should occur. The target of heating is the isomerase, which is inactivated and separated as insoluble aggregates. The deaminase domain is unaffected and an apparent activation of the resulting enzyme is clearly observed, suggesting that isomerase was somehow controlling its enzymic activity.

However, from the present findings, it can also be postulated that this "regulatory" function of the isomerase domain, seems to have a maximal expression, reached when the ratio deaminase:isomerase is 1:4, indicating that isomerase could interact with deaminase at only a few sites on the protein, others than the hinge region linking the two domains.

The present studies have provided some insight into the molecular structure of this enzyme complex.

Table 1. Deaminase:isomerase ratio

Incubation system	Ratio D:I mg:mg	Ratio D:I mol:mol	Specific activity	Isomeric type % I	Isomeric type % III
1. D	—	—	1.120	90	10
2. I	—	—	0.014	—	—
3. D + I	4:0.5	2:1	1.140	80	20
4. D + I	4:1	1:1	2.016	5	95
5. D + I	4:2	1:2	1.176	10	90
6. D + I	4:3.4	1:3.4	0.887	—	100
7. D + I	4:4.0	1:4	0.742	—	100
8. D + I	4:4.5	1:4.5	0.741	—	100
9. D + I	4:6.7	1:6.7	0.745	—	100
10. D + I	4:8	1:8	0.743	—	100

Deaminase and isomerase were purified from *E. gracilis* supernatants as described by Rossetti *et al.* (1980). Considering that the MW of the deaminase monomer is 20,000 and that of the isomerase monomer 5000, increasing amounts of the latter enzyme were added to 4 mg of deaminase as indicated, so as to vary the deaminase:isomerase (D:I) ratio from 2:1 to 1:8.

Table 2. Summary of molecular weight reports

Source	Molecular weight PBGase	Deaminase	Isomerase	Reference
Bovine liver	50,000	40,000	30,000	Sancovich *et al.* (1976)
Ox liver			(70,000–210,000)	and this paper
Avian erythrocytes	—	280,000	—	Kreutzer *et al.* (1977)
	100,000	40,000	70,000	Llambías & Batlle, 1971b
		(20,000)	(280,000)	
Human erythrocytes	—	25,000 + 5000	—	Frydman & Feinstein (1974)
Spinach leaf	—	40,000	—	Higuchi & Bogorad (1975)
Wheat germ	—	40,000	60,000	
Soybean cellus	25,000	40,000	210,000	Llambias & Batlle, 1971a
		(20,000)	(6000–12000–24,000)	
R. spheroides	—	40,000	—	Jordan & Shemin (1973)
Euglena gracilis	50,000	40,000	30,000	This paper
	(25,000)	(20,000)	(20,000–15,000–10,000–5000)	

In parenthesis are indicated the MW species found in equilibrium (and lower percentage), with the main species.

We propose that the smallest catalytical unit of PBGase, the protomer or monomer is a hybrid protein of MW 25,000 composed by two separate and different domains, in a ratio of 1 mol of deaminase (MW 20,000) and 1 mol of isomerase (MW 5000).

We have already stated that these enzymes have been isolated from various sources in a number of multiple molecular forms (Table 2). A model explaining the occurrence of these different MW species of PBGase in nature and the possible interconversions among the various forms is proposed (Fig. 6).

Results reported here, clearly indicate that PBGase can be found at least, into three molecular species, let us call them tetramers, dimers and monomers according to the source organism. In some tissues the monomer would be the catalytic species, as it is the case of soybean callus PBGase. In other organisms, addi on of 2 or 4 units of this protomer would be necessary for activity, suggesting that enzyme activity might be in part controlled by association of monomers to dimers or tetramers.

In some organisms the dominant species can be present in equilibrium with certain amounts of other low or high MW forms; however their relative ratio vary markedly between different tissues and even in the same cells or tissues as a function of the mode of solubilization as well as the intracellular localization.

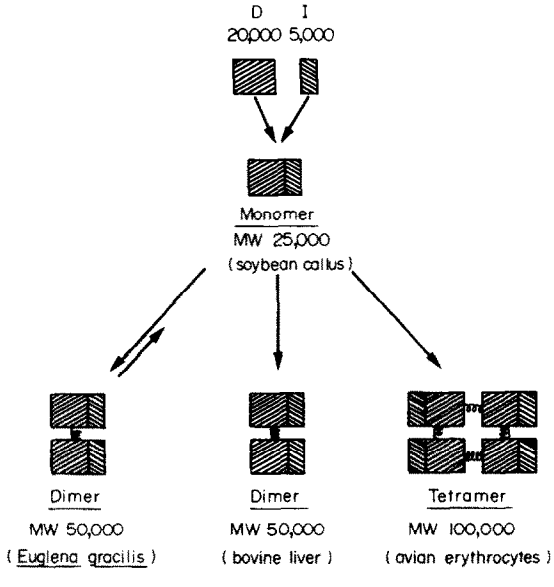

Fig. 6. Postulated model for explaining the occurrence of multiple MW forms of PBGase in different organisms, and the possible interconversions among monomers, dimers and tetramers. D: Deaminase (■). I: Isomerase (▩).

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